Enhancing immune responses against *Schistosoma mansoni* cathepsin B through the use of various vaccination platforms

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Abstract

Schistosomiasis is caused by a helminthic parasite and affects over 250 million of the world's population. Nearly one billion individuals, especially women and children, are at risk of infection each year through contact with fresh water. Schistosomiasis causes debilitating chronic infections leading to growth defects and mental deficits; depending on the species it can result in liver failure or bladder cancer. Most pathology is attributed to granuloma formation around parasitic eggs that become embedded into host tissue. Currently, schistosomiasis is controlled by combinations of water sanitation and hygiene programs, hygiene education, and mass drug administration (MDA) of one first line drug: praziquantel (PZQ). While these measures are effective, they have not been sufficient to eliminate the problem of schistosomiasis. Unfortunately, PZQ does not reverse current pathology and MDA is suboptimal. Moreover, neither treatment nor natural infection confers sterilizing immunity; thus, re-infection is common in endemic regions. A vaccine, even a partially effective one, is a necessary tool to eradicate schistosomiasis by the World Health Organization (WHO) 2030 target. Our lab has developed several anti-schistosome vaccines that target the parasitic gut enzyme *Schistosoma mansoni* cathepsin B (SmCB), addressing the most widespread species causing schistosomiasis. The overall goal of this thesis was to confirm protective efficacy of this antigen when formulated within various vaccine platforms and elucidate protective immune responses. Our first aim combined recombinant SmCB with novel adjuvants: sulfated lactosyl archaeol archaeosomes (SLA), or AddaVax™ (AddaVax). We found that SmCB/SLA led to T_H1 and inflammatory immune responses whereas SmCB/AddaVax led to T_H2 and anti-inflammatory responses. While both vaccines induced robust SmCB-specific antibody responses, SmCB/AddaVax gave a parasite burden reduction (PBR; reduction of adult worms, and hepatic and intestinal eggs compared to control animals) of 83% whereas SmCB/SLA gave a PBR of 56.6%. Next, we decided to express SmCB using a human adenovirus serotype 5 (Ad) vector (AdSmCB:SmCB) and combine it with boosting immunizations of recombinant protein. This unadjuvanted vaccine uses a cost-effective platform that is readily scalable for global production. AdSmCB:SmCB triggered robust activation of T_H1 and T_H2 arms of immunity, inducing polyfunctional CD4+ T cells, IFNγ, and IL-5 expression of systemic lymphocytes, and antibody responses. This vaccine led to a PBR of 72% and reduced parasite associated pathology significantly. To enhance this PBR, we decided to adjuvant this vaccine with AddaVax, due to its

efficacy in our first aim. Our final formulation involved AdSmCB followed by boosting immunizations of SmCB adjuvanted with AddaVax (AdSmCB:SmCB/AddaVax). This vaccine displayed similarities from both its predecessors: SmCB/AddaVax and AdSmCB:SmCB; namely, strong immunogenicity with increased systemic expression of T_H1 cytokines (IFNγ), T_H2 cytokines (IL-5), and anti-inflammatory cytokines (IL-10). AdSmCB:SmCB/AddaVax also stimulated the production of highly avid IgG and IgA and led to a PBR of 54% when the challenge was delayed from three weeks post final immunization to six. AdSmCB:SmCB/AddaVax was also found to target female schistosomes and increase soluble worm antigen antibodies reducing egg deposition and possibly preventing *S. mansoni* re-infection. Through this work we have demonstrated the efficacy of SmCB as a schistosomiasis vaccine target through many vaccination platforms, each eliciting unique immune effectors for protection. The results of this thesis strongly promote the use of SmCB as a vaccine to help aid the elimination of schistosomiasis and emphasize the importance of both T_H1 and T_H2 arms of immunity in protection.

Résumé

La schistosomiase est causée par un parasite helminthique et affecte plus de 250 millions de personnes dans le monde. Chaque année, près d'un milliard d'individus, sont susceptibles d'être infectés lors d'un contact avec de l'eau douce. La schistosomiase provoque des infections chroniques débilitantes qui entraînent des retards de développement et des déficits intellectuels; selon l'espèce, elle peut provoquer une insuffisance hépatique ou un cancer de la vessie. La plupart des pathologies sont imputables à la formation de granulomes autour des œufs parasitaires qui s'incrustent dans les tissus de l'hôte. À ce jour, la schistosomiase est contrôlée par des programmes combinés d'assainissement de l'eau et d'hygiène, d'éducation à l'hygiène et d'administration massive d'un médicament de première ligne: le praziquantel. Si ces mesures sont efficaces, elles n'ont pas suffi à éliminer le problème de la schistosomiase. Un vaccin est un moyen indispensable pour éliminer la schistosomiase d'ici 2030, objectif fixé par l'Organisation mondiale de la santé. Notre laboratoire a développé plusieurs vaccins anti-schistosomes qui ciblent l'enzyme intestinale parasitaire *Schistosoma mansoni* cathepsin B (SmCB). Cette thèse avait pour objectif principal de confirmer l'efficacité protectrice de cet antigène lorsqu'il est formulé dans différentes plateformes vaccinales et d'élucider les réponses immunitaires protectrices. Notre premier objectif consistait ainsi à combiner SmCB avec des adjuvants: les lactosyls sulfatés archéols archéosomes (LSA) ou AddaVax^{MD} (AddaVax). Les résultats ont montré que SmCB/LSA entraînait des réponses immunitaires inflammatoires et T_H1, tandis que SmCB/AddaVax entraînait des réponses anti-inflammatoires et T_H2. Si les deux vaccins ont induit de solides réponses anticorps spécifiques à SmCB, SmCB/AddaVax a permis une réduction de la charge parasitaire (RCP; réduction des vers adultes et des œufs hépatiques et intestinaux par rapport aux animaux témoins) de 83% par rapport à SmCB/LSA, qui a produit une RCP de 56,6%. Nous avons ensuite décidé d'exprimer SmCB à l'aide d'un vecteur adénovirus humain de sérotype 5 (Ad) et de l'associer à des immunisations de rappel de la protéine recombinante (AdSmCB:SmCB). Ce vaccin utilise une plateforme économique facilement transposable pour une production à l'échelle mondiale. AdSmCB:SmCB a provoqué une forte activation des composantes T_H1 et T_H2 de l'immunité, induisant des cellules T-CD4$^+$ polyfonctionnelles, l'expression d'INFγ et d'IL-5 par les lymphocytes systémiques et des réponses anticorps. Ce vaccin a permis d'obtenir une RCP de 72% et de réduire sensiblement la pathologie associée au parasite. Pour améliorer ce résultat, nous avons décidé de

mettre en place un adjuvant à ce vaccin avec AddaVax, en raison de son efficacité dans le cadre de notre premier objectif. Notre formulation finale comprenait AdSmCB suivi d'immunisations de rappel SmCB adjuvanté avec AddaVax (AdSmCB:SmCB/AddaVax). Ce vaccin présentait des similitudes avec ses deux prédécesseurs: à savoir une forte immunogénicité avec une hausse de l'expression systémique des cytokines T_H1, T_H2 et anti-inflammatoires. En outre, AdSmCB:SmCB/AddaVax a stimulé la production d'IgG et d'IgA fortement avides et a permis d'obtenir une RCP de 54% lorsque l'infection a été retardée de six semaines après l'immunisation finale. On a aussi constaté que AdSmCB:SmCB/AddaVax ciblait les schistosomes femelles et augmentait les anticorps solubles contre l'antigène du parasite, diminuant ainsi le dépôt d'œufs et prévenant éventuellement la réinfection par *S. mansoni*. Grâce à ces travaux, nous avons démontré l'efficacité de SmCB en tant que cible vaccinale contre la schistosomiase par le l'entremise de nombreuses plateformes de vaccination. Les résultats de cette thèse encouragent fortement l'utilisation de SmCB comme vaccin pour éliminer la schistosomiase et soulignent l'importance des composantes T_H1 et T_H2 de l'immunité dans la protection.

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Contributions of Authors

This candidate has chosen to present a manuscript-based thesis. This thesis contains three manuscripts and is in accordance with the "Guidelines for Thesis Preparation," provided by the Faculty of Graduate and Postdoctoral Studies of McGill University. The candidate, Dilhan Perera, is recognized as the principal author and to have performed the majority of the work of the manuscripts presented. The specific contributions of authors are as follows:

Chapter 1:

The completed entirety of Chapter 1 was compiled and written by DJP and revised by MN.

Section 1 contains extracts from **Perera DJ**, Koger-Pease C, Daoudi M, Paulini K, Ndao M. *Schistosoma* co-infections: when pathogens get friendly, do they get deadly?

Extracts from this review are adapted in this thesis. This review has been submitted to *Clinical Microbiology Reviews*. This manuscript was written by DJP, CKP, MD, and KP. MN revised the manuscript.

Section 4 contains **Perera DJ**, Ndao M. Promising Technologies in the Field of Helminth Vaccines. Front Immunol. 2021 Aug 19;12:711650.

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Chapter 2:

Perera DJ, Hassan AS, Jia Y, Ricciardi A, McCluskie MJ, Weeratna RD, Ndao M. Adjuvanted *Schistosoma mansoni*-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model. Front Immunol. 2020 Nov 16;11:605288.

This manuscript has been reprinted from the journal *Frontiers in Immunology* under CC BY 4.0 licensing. DJP was involved in all aspects of this manuscript including study design, data collection, and manuscript writing. ASH assisted in the design of the snail housing facility and the infection model. YJ was responsible for created vaccine formulations with SLA. MJM and RDW contributed to study design, and MJM, RDW, and AR contributed to

manuscript revision. MN supervised all parts of the study and contributed to manuscript editing.

Chapter 3:

Perera DJ, Hassan AS, Liu SS, Elahi SM, Gadoury C, Weeratna RD, Gilbert R, Ndao M. A low dose adenovirus vectored vaccine expressing *Schistosoma mansoni* Cathepsin B protects from intestinal schistosomiasis in mice. EBioMedicine. 2022 Jun;80:104036.

This manuscript has been reprinted from the journal *eBioMedicine* under Crown Copyright. DJP was involved in all aspects of this manuscript including study design, data collection, and manuscript writing. ASH assisted in the design of the snail housing facility and the infection model. SSL assisted in data collection. SME contributed to adenoviral vector design and manuscript revision. CG contributed to vaccine production. RDW and RG contributed to study design and manuscript revision. MN supervised all parts of the study and contributed to manuscript editing.

Chapter 4:

Perera DJ, Liu SS, Koger-Pease C, Labrie L, Gilbert R, Weeratna RD, Ndao M. Enhancing an Adenovirus-based Schistosomiasis Vaccine Using Boosting Immunizations of Recombinant *Schistosoma mansoni* Cathepsin B Dispersed into AddaVax™.

Manuscript prepared for submission to *Science Translational Medicine.* DJP was involved in all aspects of this manuscript including study design, data collection, and manuscript writing. SSL contributed to data collection and analysis. CKP assisted in data collection. LL contributed to data collection. RDW and RG contributed to study design and manuscript revision. MN supervised all parts of the study and contributed to manuscript editing.

Chapter 5:

The general discussion was written by DJP and revised by MN.

Contributions to Original Knowledge

The work presented in this thesis contributes original knowledge to the fields of schistosomiasis vaccine development and correlates of immunity in protection from schistosomiasis. Specific contributions of knowledge to these fields are as follows:

1. Adjuvanting SmCB with SLA or AddaVax both significantly reduce parasite burden (worms, hepatic eggs, intestinal eggs, pathology) in a murine model of schistosomiasis.
2. SmCB/SLA tended to induce T_H1 immune responses; SmCB/AddaVax stimulated T_H2 and anti-inflammatory immune responses.
3. A heterologous prime-and-boost *S. mansoni* vaccine could be developed using human adenovirus serotype 5 vectoring.
4. Adenoviral vectoring of SmCB induced strong T_H1 immunity and boosting with SmCB protein led to maintenance of antigen specific T_H2 immunity.
5. SmCB expressed by an adenovirus followed by boosting immunizations of protein resulted in significant protection from schistosomiasis.
6. Combining AddaVax into an adenoviral vectored vaccine regimen stimulated strong immune responses associated with both adjuvant (specific to AddaVax) and viral vectored vaccine strategies.
7. This combined vaccine strategy protected immunized animals from schistosomiasis challenge and protection could be extended up to six weeks after final immunization.

List of Abbreviations

Abbreviation	Definition
Ad	Human adenovirus serotype 5
AD	Anno Domini, the year of our Lord
ADAD	Adjuvant adaptation
ADCC	Antibody dependent cellular cytotoxicity
AddaVax	Addavax™
AdSmCB	Human Adenovirus Serotype 5 expressing *Schistosoma mansoni* cathepsin B
AIDS	Aquired immunodeficiency syndrome
ANOVA	Analysis of Variance
APC	Antigen presenting cell
ART	Antiretroviral therapy
BC	Before Christ
B_{REG}	Regulatory B-helper
BSA	Bovine serum albumin
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
CCL	C-C motif chemokine ligand
CDC	Centers for Disease Control and Prevention
CHR	Cercarien hullen reaction
CMI	Cell mediated immunity
CMV	Cytomegalovirus
CNS	Central nervous system
COPT	Circumoval precipitin test
CR	Cure rate
CXCL	C-X-C motif chemokine ligand
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
ELISA	Enzyme-linked immunosorbant assay

ELISPOT	Enzyme-linked immunosorbant spot
EN	Endemic normal
ERR	Egg reduction rate
ES	Excretory-secretory
FABP	Fatty acid binding protein
FDA	Food and Drug Administration
FECT	Formalin-ether concentration technique
FGS	Female genital schistosomiasis
Fh15	*E. coli*-expressed *F. hepatica* fatty acid binding protein
Fh15b	Baculovirus-expressed *F. hepatica* fatty acid binding protein
FIA	Freund's incomplete adjuvant
fRPMI	Fancy RPMI
GLA	Glucopyranosyl lipid adjuvant
GLA-SE	Stable emulsion of glucopyranosyl lipid adjuvant
GMP	Good manufacturing practice
hAdV5	Human adenovirus serotype 5
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDM	House dust mite
HEK293A	Immortalized human embryonic kidney cells
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HPV	Human papilloma virus
HRP	Horseradish peroxidase
HSC	Hepatic stellate cells
IFNγ	Interferon gamma
Ig	Immunoglobulin
IHA	Indirect hemagglutination assay
IL	Interleukin
ILC2	Type 2 innate lymphoid cells
IM	Intramuscular

IN	Intranasal
INFγ	Interféron gamma
IPSE	IPSE/α-1
ISA 720	Montanide ISA 720 VG
IU	Infectious unit
KK	Kato-Katz
KS	Katayama syndrome
LAMP	Loop-mediated isothermal amplification
LC	Langerhans cell
LF	Lymphatic filariasis
LMIC	Low- and middle- income country
LSA	Lactosyls sulfatés archéols archéosomes
MDA	Mass drug administration
MGS	Male genital schistosomiasis
MHC	Major histocompability complex
MHC-1	Major histocompability complex 1
MoDC	Monocyte-derived dendritic cell
mRNA	Messenger ribonucleic acid
NDV	Newcastle disease virus
NET	Neutrophil extracellular trap
ng	Nanogram
NHP	Non-human primate
NK	Natural killer
NK T	Natural killer T
NO	Nitric oxide
NOD	Non-obese diabetic
NRCC	National Research Council of Canada
OMS	Organisation Mondiale de la Santé
OXA	Oxamniquine
PAH	Pulmonary arterial hypertension
PBMC	Peripheral blood mononuclear cell

PBR	Parasite burden reduction
PBS	Phosphate buffered saline
PBS-BSA	Phosphate buffered saline-bovine serum albumin
PBS-T	Phosphate buffered saline-tween
PCR	Polymerase chain reaction
PFU	Plaque forming unit
PG	Prostaglandin
POC	Point-of-Care
PRV	Pseudorabies virus
PZQ	Praziquantel
RA	Radiation attenuated
RA vaccines	Irradiated schistosome vaccines
RAG-1	Recombination activating gene 1
RANTES	Regulated on activation, normal T expressed and secreted
RI-MUHC	Research Institute of the McGill University Health Centre
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
RRID	Research resource identifiers
rSmCB	Recombinant *Schistosoma mansoni* cathepsin B
SAC	School-age children
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SC	Subcutaneous
SEA	Soluble egg antigen
SEM	Standard error of the mean
Sj26GST	*Schistosoma japonicum* 26 kDa glutathione-S-transferase
SjTOR	*Schistosoma japonicum* tetraspanning orphan receptor
SLA	Sulfated lactosyl archaeol archaeosomes
Sm-p80	*Schistosoma mansoni* large subunit of calpain
Sm28GST	*Schistosoma mansoni* 28 kDa glutathione-S-transferase
SMAF	*Schistosoma mansoni* apoptosis factor
SmCB	*Schistosoma mansoni* cathepsin B

SmCKBP	*Schistosoma mansoni* chemokine binding glycoprotein
SmKI-1	*Schistosoma mansoni* kunitz type protease inhibitor
SmTSP2	*Schistosoma mansoni* tetraspanin 2
SOP	Standard operating protocol
SRB	Senegal River basin
STEG	Schistosomula tegument
STH	Soil transmitted helminth
SWA	Soluble worm antigen
SWAP	Soluble worm antigen preparation
T_{EM}	Effector memory T
T_{FH}	T follicular helper
TGF-β	Transforming growth factor beta
TGR	Thioredoxin glutathione reductase
T_H1	T-helper type 1
T_H17	T-helper type 17
T_H2	T-helper type 2
T_H9	T-helper type 9
TLR	Toll-like receptor
TMB	3,3',5,5'-tetramethyl benzidine
TNFα	Tumor necrosis factor alpha
T_{REG}	Regulatory T-helper
TRPM	Transient receptor potential melastatin
Ts-MCD-1	*T. spiralis* multi-cystatin-like domain protein 1
TsMIF	*T. spiralis* macrophage migration inhibitory factor
UCP-LF	Up-converting phosphor-lateral flow
US	United States
USA	United States of America
USD	United States dollar
UTI	Urinary tract infection
UV	Ultra-violet
VIT	Vaccine induced thrombocytopenia

WASH	Water, Sanitization, and Hygiene
WHO	World Health Organization
wpi	Weeks post infection
μg	Microgram
μL	Microlitre
μm	Micrometre
ω1	Omega-1

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Chapter 1

Literature Review and Research Objectives

1.1 Schistosomiasis

Schistosomiasis, also known as Bilharzia, is caused by trematodes of the genus *Schistosoma.* This parasitic helminth is spread through contact with freshwater and has been described as the most important parasitic disease behind only malaria, in terms of socioeconomic impact[1]. There are over 250 million individuals infected with schistosomiasis yearly, with over 700 million more living in endemic areas at risk of infection[2]. Most human infections are caused by *S. haematobium* (causing urogenital disease), and *S. mansoni* and *S. japonicum* (causing intestinal/hepatic disease). Since 1970, this parasite has been managed by snail vector control, increasing access to clean water, and a single FDA approved drug, praziquantel (PZQ)[3]. While schistosomiasis control efforts have been effective in certain regions, global prevalence of disease has not significantly diminished over time[4].

1.1.1 History

Despite being officially discovered by German parasitologists Theodor Maximilian Bilharz and Carl Theodor Ernst in 1851 during an autopsy in Cairo, Egypt[5], schistosomiasis is an ancient disease. In fact, the earliest evidence for schistosomiasis dates back more than 6000 years; terminal spined schistosome eggs were found in pelvic sediments from an early settlement in northern Syria (5800-4000 years before Christ (BC))[6]. Since then, schistosomiasis-like diseases have been described in several Egyptian medical papyri (1500 BC)[7], Assyrian medicine, and even the Bible[8,9]; while schistosomiasis itself, has been reported in Egyptian mummies of the twentieth dynasty (1200-1000 BC)[10] and within those from Sudanese Nubia (350-550 AD)[11]. Interestingly, modern molecular biology techniques have been used to identify *Schistosoma* DNA from tissue samples of the Nekht-Ankh and Khnum-Nakht mummies[12], dating back to the 12th dynasty.

Schistosomiasis is thought to have been spread from Syria to Egypt due to the importation of monkeys and slaves during reign of the 5th dynasty of pharaohs[13]. To thrive in a geographic location, *Schistosoma* spp. require an intermediate snail vector and it is believed that these snails

originated from Ethiopia and were naturally carried along the Nile River[13]. With the help of travelling Egyptians, this parasite likely spread to the region of the African great lakes. Known as the 'cradle' of schistosomes, the African great lakes are an area where both the parasite and the snail intermediate host are in an active state of evolution[14].

Africa was not the only continent afflicted by schistosomiasis. In fact, schistosomiasis-like diseases have been recorded in ancient Chinese writings as early as 4700 years ago[15]. There is little information on the arrival of schistosomiasis in Asia; however, the first confirmed cases of schistosomiasis were in 1904 in Japan[16], in 1905 in China[17], and in 1906 in the Philippines[18].

There is limited evidence to suggest schistosomiasis existed in South America before the arrival of Christopher Columbus[19,20] and it is believed that its spread here was in large part due to the slave trade. However, other factors such as colonization, trade, and subsequent travel may have played a role.

While Europe is not considered endemic for schistosomiasis, there have been outbreaks of the disease over the years. Moreover, *S. mansoni* eggs were found as early as the 15th century within a latrine in France[21], and *S. haematobium* eggs were found during the 19th century[22]. Since Europe does not have a suitable intermediate host for *Schistosoma* spp., maintaining endemicity is unlikely. Here too, it is thought that infection was introduced through the slave trade and/or the infection of foreigners or returning travellers[21].

1.1.2 Epidemiology and global spread

Humans contract schistosomiasis when they come into contact with freshwater containing infectious parasites. The prevalence of schistosomiasis in a community is dictated by the amount of human waste which contaminates local bodies of water such as rivers and lakes. As rural regions, and regions of poverty, often have limited access to sanitation and clean freshwater, schistosomiasis thrives in them; thus, making it a neglected tropical disease.

Globally there are approximately 250 million people infected with schistosomiasis yearly, with another 700 million at risk of infection[2]. Yet, due to low intensity cases and those which go undiagnosed in remote areas, these numbers are thought to be underestimated. An individual of any age can be infected with schistosomiasis, though children and women are at higher risk of

infection. This is because children spend time swimming, and women do domestic chores, in water containing infectious cercariae[23].

The six species of *Schistosoma* which are found to infect humans are *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis* (previously considered synonymous with *S. intercalatum*)[24]. While these parasites are found in tropical and sub-tropical climates (Figure 1.1), regions where each species is found is dependent on species-specific cognate snails being present in the environment.

S. mansoni is the most geographically widespread of the species infecting humans and can be found distributed throughout Africa, in South America, and even in the Caribbean, although risk here is low. *S. haematobium* is commonly found throughout Africa and in the Middle East. *S. japonicum* is contained to the continent of Asia; it is found in countries such as Indonesia and parts of China. *S. mekongi* is restricted to the Mekong River basin of Cambodia and Laos while *S. intercalatum* and *S. guineensis* are found in regions of Central and West Africa[25]. Interestingly, there is recent evidence of hybridization between the human *S. haematobium*, and the cattle *S. bovis*, *S. curassoni*, or *S. mattheei* resulting in hybrid schistosomes that can infect humans[26]. These hybrids have been found within Africa[27,28], but also recently in Corsica, France[29,30].

As snail habitats change, so does the geographical distribution of infection. That is to say that disease prevalence can be altered by water resource development and climate change, among other factors. In the early 1990s, two dams were built in the region of the Senegal River basin (SRB). At this time, *S. mansoni* was not present in the SRB, and cases of *S. haematobium* were low[31,32]. Within two years of the dam opening, an outbreak of *S. mansoni* occurred in the lower SRB, and within six years, both species were widespread through the Lac de Guiers region which is connected to the Senegal River by an intake canal[32–34]. Moreover, climate change affects the availability of parasite-snail survival spaces. Both *Schistosoma* spp. and their snail vectors thrive within a specific temperature range. As that range shifts in a locale, their ability to survive is also modified. Global warming has created the potential for schistosomiasis to reach its upper temperature limits in regions at lower altitudes in Africa; however, this has also led to species like *S. haematobium* resurfacing in cooler climates, as seen in Europe.

With increased travel and immigration, schistosomiasis may not stay a disease of the tropical and sub-tropical world. In fact, although North America is not endemic for this disease in humans, cases from foreigners are already compounding on the public health burden. Refugees

and asylum claimants may enter North America asymptomatically; however, data shows that up to 56% of them test positive for schistosomiasis, indicating a past or current infection[35–39]. Although all cases of human schistosomiasis to date in North America are imported, the region is known to have species of avian schistosomiasis and non-primate mammal schistosomiasis[40,41]. These species are capable of penetrating human skin; however, they do not mature and they usually die here[42]. Human skin penetration from avian schistosomes can result in an allergic reaction called cercarial dermatitis, or "swimmer's itch", and is commonly reported around the world (e.g., in Canada[43,44], USA[40], Denmark[45], Hungary[46], and New Zealand[47]). Climate change, increased travel to and from endemic regions, and the presence of avian schistosomes (which share similar intermediate hosts) may create a melting pot of factors increasing the risk of schistosome hybridization. Such hybridization may give rise to new species capable of causing human schistosomiasis.

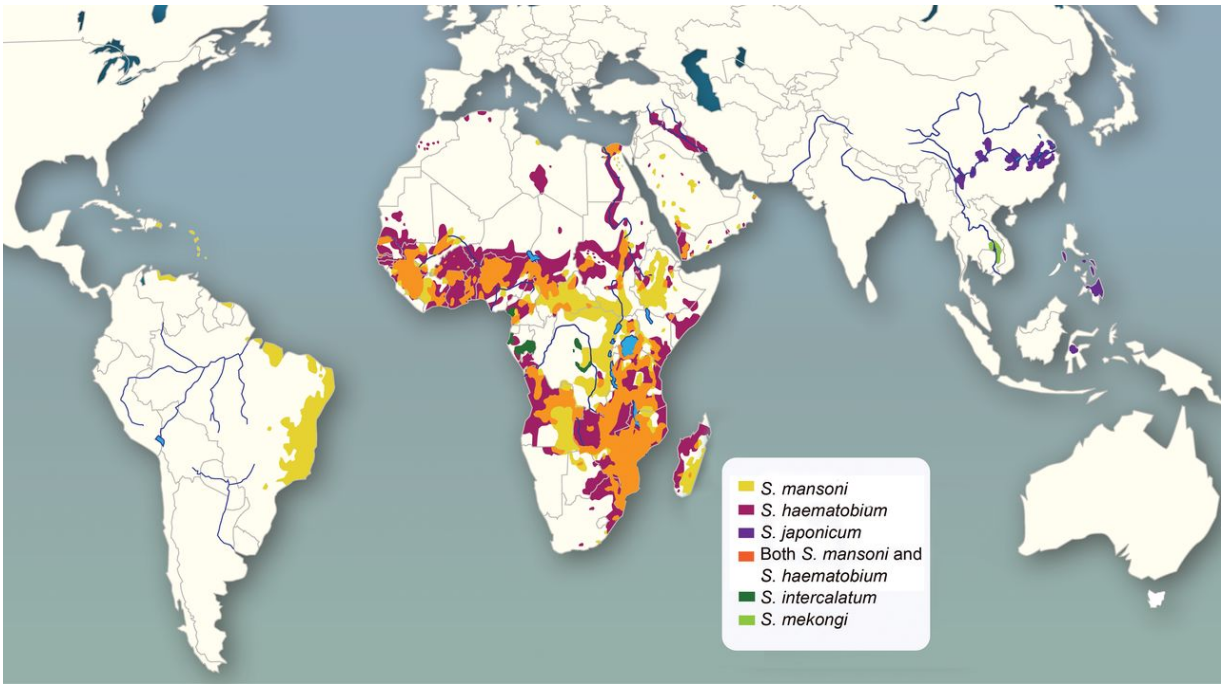

Figure 1.1 Geographical distribution of *Schistosoma* spp.

The following map depicts the geographical distribution of various *Schistosoma* spp. *S. mansoni* is the only species present in South America and is also found in a large portion of Africa and in the Middle East; many regions co-endemic for *S. haematobium*. Differently, *S. japonicum* is restricted to the continent of Asia, primarily located in China and the Philippines. *Reproduced with permission from Weerakoon KG, Gobert GN, Cai P, McManus DP. Advances in the Diagnosis of Human Schistosomiasis. Clin Microbiol Rev. 2015 Oct;28(4):939-67. doi: 10.1128/CMR.00137-*

14. PMID: 26224883; PMCID: PMC4548261., Copyright American Society for Microbiology and Elsevier.

1.1.3 Lifecycle

The lifecycle of *Schistosoma* spp., first described by Robert Thomson Leiper[48], begins when parasitic eggs are released into freshwater (Figure 1.2). From each egg, the first larval stage of the parasite hatches and will swim through water to find its intermediate vector, the snail. Each species of *Schistosoma* has a different snail intermediate vector, for instance, *S. mansoni* will infect *Biomphalaria* spp., *S. haematobium* will infect *Bulinus* spp., and *S. japonicum* will infect *Oncomelania* spp. Within the snail, *Schistosoma* will undergo several rounds of asexual reproduction giving rise to another larval stage of the parasite, and the infectious stage to mammals, cercariae. Cercariae too, are free-swimming organisms which actively penetrate host skin upon contact with infectious water. As a cercaria enters the skin, it loses its tail-end, transforming into a schistosomulum, the final larval stage of the parasite. Schistosomula spend a few days within the skin and then travel through the circulation to the lungs where they spend approximately one week, lengthening and narrowing, to allow for passage through smaller blood vessels. At this point, schistosomula follow the circulation to the liver where they will mature into juvenile worms and pair into male and female couples. These couples travel together to their final site of residence which is the mesenteric venules around the intestines for most species, or the venous plexus around the bladder for *S. haematobium*, and mature into adult worms. Here, adult female worms will lay upwards of 300 eggs/day (*S. mansoni*) and more in the cases of other species (Table 1.1); approximately half of laid eggs will be released with faeces (*S. mansoni*, and other species causing intestinal schistosomiasis), or urine (*S. haematobium*), and propagate the lifecycle upon contact with freshwater, while remaining eggs will become trapped in host tissues, causing pathology. Although the lifespan of an adult worm is 3-5 years, they can live up to 40 years in definitive hosts causing severe morbidity over time[49].

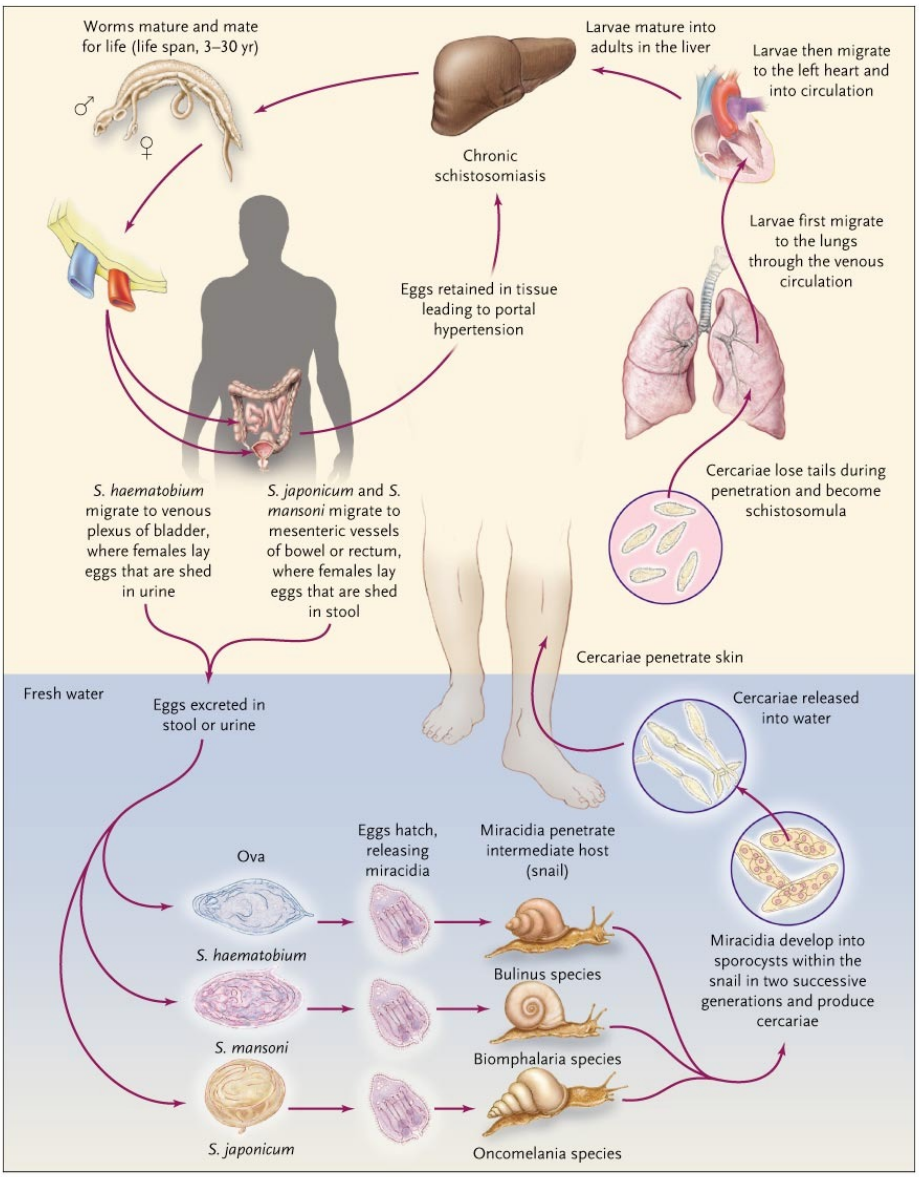

Figure 1.2 *Schistosoma* spp. lifecycle

Schistosoma eggs released into freshwater hatch into miracidia, the infectious larval stage to the snail. Within snails, miracidia develop into cercariae, the infectious stage to mammals. Cercariae swim and actively penetrate host skin, travelling through the lung and to the venous plexus of the bladder or intestinal mesentery, depending on the species. Here, adult female worms lay eggs which continue the life cycle, though when trapped in host tissues cause pathology. *Reproduced with permission from King CH. Toward the elimination of schistosomiasis. N Engl J Med. 2009 Jan 8;360(2):106-9. doi: 10.1056/NEJMp0808041. PMID: 19129524., Copyright Massachusetts Medical Society.*

Table 1.1 Eggs laid by *Schistosoma* spp.

***Schistosoma* species**	**Number of eggs laid per day**	**Amount of eggs shed in feces (%)**	**Reference**
S. mansoni	>300 in murine models	30	50
S. haematobium	100-200 in human models	N/A	50
S. japonicum	1000-2500 in murine models	30-50	50

1.1.4 Disease

1.1.4.1 Acute schistosomiasis

Besides causing local hypersensitivity reactions in the skin, as a temporary urticarial rash, schistosomiasis may also present with a systemic hypersensitivity reaction known as Katayama fever. Katayama fever, or Katayama syndrome (KS), was first described in Japan[51] and is related to immune responses to the migration of schistosomula within the body. Symptoms onset quickly with fever, fatigue, myalgia, eosinophilia, and patchy infiltrates on chest radiography. Most of these symptoms resolve spontaneously within 2-10 weeks; however, in some patients, symptoms progress into weight loss, diarrhoea, hepatosplenomegaly, and widespread rash[52].

While KS is commonly found in infections of *S. japonicum*[53] and in travellers, tourists, or those exposed to a high infection of *S. mansoni* and *S. haematobium*, KS is rarely seen in populations chronically exposed to intestinal schistosomiasis[52]. It is possible that endemic locals are exposed to *Schistosoma* antigens and/or antibodies in utero, causing an altered immune response upon exposure to infection[54].

1.1.4.2 Chronic schistosomiasis

Curiously, most pathology to schistosomiasis is not caused by adult worms[24], but to oviposition marking the start of chronic infection. To continue the lifecycle, schistosome eggs must

undergo peri-vesical or peri-intestinal migration to pass from the circulation, through the tissue, to the lumen of the bladder or intestines. During this process, eggs can get trapped and/or embolized in these tissues as well as non-specific organ tissues (e.g., spleen, liver, lungs, cerebrospinal system). *Schistosoma* eggs release proteolytic enzymes that provoke eosinophilic, inflammatory, and granulomatous reactions, which become replaced by fibrotic deposits[52] to protect the host from these molecules[55] (Figure 1.3).

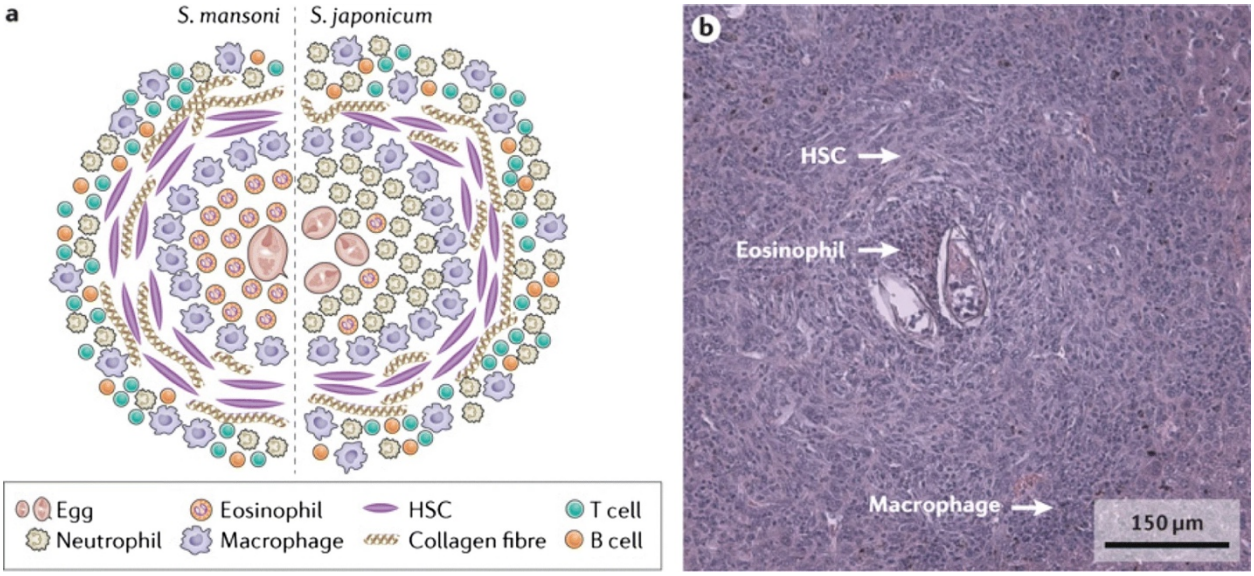

Figure 1.3 Granulomatous response around *Schistosoma* eggs

Schistosoma eggs which become trapped in host tissues release hepatotoxins and other parasitic antigens which mediate a delayed type hypersensitivity response and subsequent granuloma formation. (A) A graphical depiction of the components found in *S. mansoni* and *S. japonicum* granulomas made up of neutrophils, eosinophils, hepatic stellate cells (HSC), and lymphocytes. (B) Hematoxylin and eosin staining of a liver section containing two *S. mansoni* eggs and granuloma formation surrounding them. *Reproduced with permission from McManus DP, Bergquist R, Cai P, Ranasinghe S, Tebeje BM, You H. Schistosomiasis-from immunopathology to vaccines. Semin Immunopathol. 2020 Jun;42(3):355-371. doi: 10.1007/s00281-020-00789-x. Epub 2020 Feb 19. Erratum in: Semin Immunopathol. 2020 Jun 9;: PMID: 32076812; PMCID: PMC7223304. Copyright Springer Nature and Elsevier.*

1.1.4.3 Intestinal schistosomiasis

Most *Schistosoma* spp. cause intestinal schistosomiasis as adult worms reside in the mesentery around the intestines. Schistosome eggs migrate through the intestinal wall causing granuloma formation, pseudopolyposis, microulcerations, and superficial bleeding[56]. The most common symptoms of intestinal schistosomiasis are chronic abdominal pain, discomfort, loss of appetite, and diarrhoea[57,58]. Generally, the severity of these symptoms is associated with the intensity of infection.

1.1.4.4 Hepatic schistosomiasis

Hepatic schistosomiasis is caused by *S. mansoni*, *S. japonicum*, and *S. mekongi*, but not *S. intercalatum* or *S. guineensis*[59]. When *Schistosoma* ova become trapped in the presinusoidal periportal spaces of the liver, it causes inflammation leading to hepatomegaly in children and adolescents[60]. This type of hepatomegaly is common in children (up to 80%); it is less common or severe in adults[61].

This infection can progress into fibrotic schistosomiasis, usually in young adults with longstanding infections and immunogenetic predispositions[62,63]. Fibrotic schistosomiasis is the result of massive collagen deposition into the periportal spaces leading to Symmer's pipestem fibrosis[56], appearing as white plaques on the liver surface. This can lead to portal vein occlusion, splenomegaly, gastrointestinal varices, and bleeding from gastro-oesophageal varices resulting in hepatic failure and death if left untreated. This process is long (5-15 years) for *S. mansoni*[56,63]; however, in cases of *S. japonicum*, progression can be more rapid[64], maybe due to the increased number of eggs laid by adult female worms.

1.1.4.5 Urogenital schistosomiasis

Urogenital schistosomiasis is caused by eggs laid by *S. haematobium* worms residing within the veins draining the bladder, uterus, and cervix. Continuous inflammatory reactions to eggs trapped in nearby tissues of the organs listed prior leads to fibrosis, granuloma formation, and fibrotic nodules called sandy patches[65]. Common early signs of urogenital schistosomiasis include dysuria, proteinuria, and haematuria. In endemic regions, haematuria is so common it is considered

a natural sign of puberty in adolescents[66]. Chronic fibrosis of the urinary tract, presenting as obstructive uropathy, can result in bacterial superinfection and renal dysfunction[67].

S. haematobium eggs can also become trapped within the reproductive organs of both females and males resulting in female genital schistosomiasis (FGS) and male genital schistosomiasis (MGS), respectively. FGS is estimated to affect roughly 40 million females, especially in sub-Saharan Africa[68,69], and it is caused by eggs becoming trapped in the uterus, cervix, vagina, or vulva and results in contact pain and bleeding, infertility, ectopic pregnancy, and an increased risk of human immunodeficiency virus (HIV) acquisition[70–73]. MGS is caused by egg migration within the male genital tract and its prevalence is up to 53% depending on the region[74]; symptoms of MGS are pelvic, coital, and ejaculatory pain and haemospermia, among others[75,76]. While most cases of FGS and MGS are caused by *S. haematobium* there are some reported to be caused by *S. mansoni*[70,74,77].

S. haematobium is one of three helminth species classified as Group 1 Definitive Biological Carcinogenic Agents[78]. In fact, the association of urinary schistosomiasis and bladder cancer has been studied for years[79]. An estimated 3-4 patients out of 100 000 with *S. haematobium* develop bladder cancer each year[80]. The mechanisms of cancer development due to schistosomiasis is unclear; however, it is generally accepted that while urogenital schistosomiasis contributes to, alone it is insufficient for, oncogenesis. Instead, the concurrent presence of additional carcinogens, environmental exposures (e.g., smoking, diet), genetic predispositions, and other pathogens (e.g., HPV) may accelerate the development of bladder cancer[81].

1.1.4.6 Neuroschistosomiasis

Although rare when compared to its counterpart diseases, neuroschistosomiasis is not uncommon and is underreported. While eggs of *S. mansoni* and *S. haematobium* are large and may become entrapped in the spinal cord, *S. japonicum* eggs are small and may become encephalitic[82,83] during retrograde venous flow in the Batson venous plexus. Similar to eggs trapped in other tissues, the presence of eggs in the central nervous system (CNS) induces a cell-mediated CD4+ T-helper driven granulomatous reaction. The effect of these eggs within the brain and spinal cord results in symptoms of intracranial pressure, radiculopathy, and subsequent clinical sequelae[84]. Neuroschistosomiasis rarely occurs during the acute phase of infection; neurological symptoms at

this stage are likely due to eosinophil-mediated toxicity causing vasculitis and small-vessel thrombosis[85,86]. Upon chronic infection and egg deposition, fibrotic changes can cause severe tissue damage and large egg clusters will calcify within the CNS.

1.1.4.7 Pulmonary schistosomiasis

Due to portacaval shunting, venous blood can bypass the liver carrying *Schistosoma* eggs to the lung capillaries inducing granulomas in the perialveolar area. Eggs in this area may cause mechanical obstructions, elicit immune responses driving vascular remodelling, or increase stress on pulmonary vasculature from opening portosystemic shunts leading to pulmonary arterial hypertension (PAH). PAH occurs in 5-10% of hepatosplenic schistosomiasis patients, largely due to *S. mansoni*[87].

1.1.5 Immunopathology

Upon oviposition, there is a significant shift in immune response from T-helper 1 (T_H1) to T-helper 2 (T_H2) responses, mediated by soluble egg antigens (SEA)[88] (Figure 1.4). These T_H2 responses induce cytokines such as IL-4 and IL-13 contributing to fibrosis. Mice infected with *S. mansoni* and *S. japonicum* demonstrate elevated levels of IL-13 in the serum and liver[89,90]. IL-13Rα2 is a soluble high affinity antagonist of IL-13 and increases during schistosomiasis after the onset of the fibrotic response[91]. *S. mansoni* infected IL-13Rα2 knock-out mice showed a distinct exacerbation in hepatic fibrosis, which could be reversed by administering IL-13Rα2-Fc[92], showing its contributions to fibrosis control.

T-helper 17 (T_H17) responses have been implicated in schistosomiasis pathology, yet its contributions are still unclear. IL-17 was elevated in the serum of *S. mansoni* infected patients[93], and mice infected with *S. japonicum* showed elevated expression of IL-17 from hepatic lymphocytes[94]. When granulocytes from *S. mansoni* infected patients were supplemented with IL-17 and SEA, granuloma formation was accelerated, and granulocyte function was inhibited. This could be ameliorated by the addition of IL-22[93], a cytokine which has been demonstrated to prevent SEA-induced granuloma formation *in vitro*[95] and protect against severe liver disease in chronically infected *S. japonicum* and *S. mansoni* patients[96]. These data, describing the effects of IL-13, IL-17, and IL-22, illustrate that these cytokines are subject to tight regulation in schistosomiasis.

While granulomas are the major cause of pathology in the host, they protect the host from death; thus, promoting the parasites' ability to survive. Eggs, especially in *S. mansoni* infections, release proteases and antigens which are hepatotoxic causing inflammation leading to lasting damage to host tissues[97]. Along with T cell-dependent antibodies, granulomatous lesions act to prevent schistosome egg toxins from reaching hepatocytes. Mouse models of infection have shown that immunocompromised mice, such as T cell deficient mice, are more susceptible to mortality due to the inability to mount granulomatous responses[98].

Indeed, while hepatic fibrosis was impaired in *S. mansoni* infected IL-4Rα knock-out mice, it was ablated in both mice treated with IL-13Rα2-Fc and those which are IL-13 deficient[89,99,100]. IL-13 and IL-4 work in combination to express arginase in macrophages by M2 polarization. Arginase and another enzyme convert L-arginine to proline, an amino acid which is crucial in collagen production and fibrosis development[101]. The presence of M2 macrophages within granulomas provides a constant supply of proline to fibroblasts resulting in collagen synthesis. Mice which are IL-4Rα deficient, especially on macrophages and neutrophils, do not develop M2 macrophages and these mice experienced mortality after infection with *S. mansoni*[102]; T cell derived IL-4 and IL-13 have also been shown to be necessary for protection from *S. mansoni*[103], demonstrating the importance of Type 2 immunity.

On the contrary, IL-10 and IL-12 deficient mice, polarized to Type 2 immunity, experienced significant mortality by 12-15 weeks after infection[104]. Moreover, all mice that were polarized to Type 1 immunity (IL-10 and IL-4 deficient) also succumbed to infection within 9 weeks[104]. These data demonstrate that polarized immune responses, both T_H1 and T_H2, can be detrimental to the host.

Regulatory T (T_{REG}) cell responses limit collateral damage by managing polarizing immune responses. It has been shown that there is a significant expansion of CD4+CD25+Foxp3+, natural T_{REG} cells, after infection with *S. mansoni* which accumulates in the liver and spleen[105,106]. Inducible T_{REG} cells, CD4+CD25+Foxp3-, also develop during schistosomiasis[107] and are potent contributors of IL-10[108]. When these two cell types were depleted in an animal model (adoptive transfer of CD25-depleted CD4+ T cells into RAG-1 deficient mice), the animals experienced increased weight loss, hepatotoxicity, and mortality[107]. These observations suggest that regulatory pathways are necessary for managing liver pathology in schistosomiasis.

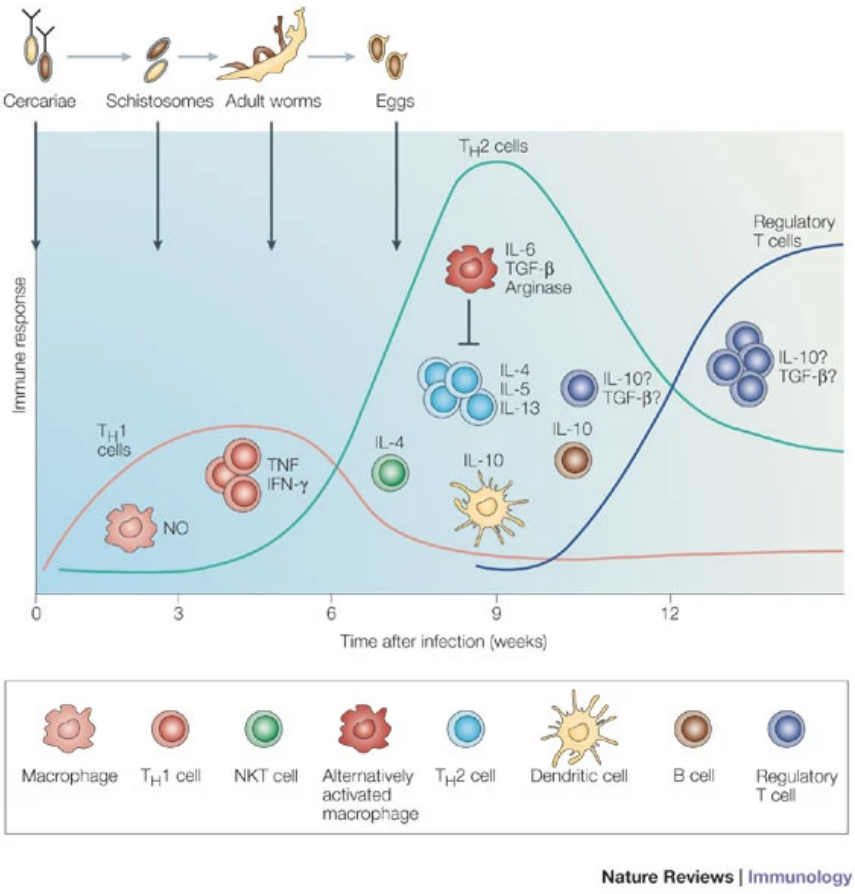

Figure 1.4 Immune responses to the schistosome lifecycle

Immune responses to *Schistosoma* are dynamic and change over time. Infection begins with T_H1 mediation which shifts towards T_H2 responses upon oviposition. To protect the host, T_{REG} responses arise as the infection progresses into chronicity. *Reproduced with permission from Dunne DW, Cooke A. A worm's eye view of the immune system: consequences for evolution of human autoimmune disease. Nat Rev Immunol. 2005 May;5(5):420-6. doi: 10.1038/nri1601. PMID: 15864275. Copyright Springer Nature.*

1.1.6 Polyparasitism

Adapted from submission to *Clinical Microbiology Reviews*

Perera DJ, Koger-Pease C, Daoudi M, Paulini K, Ndao M. *Schistosoma* co-infections: when pathogens get friendly, do they get deadly?

Due to the prevalence of schistosomiasis and its broad geographical distribution[23], co-infections of *Schistosoma* spp. and other pathogens are common[109] (Figure 1.5). As this parasite is common in low- and middle-income countries (LMICs), affected individuals may be unaware of their co-infections and/or have limited access to treatment for them.

Although classified as a helminth, *Schistosoma* spp. carry unique traits which separate them from the rest. Schistosomes are non-hermaphroditic trematodes which reside in the vascular system, rather than the gut lumen. In fact, correlates of immunity from schistosomiasis are proposed to be more complicated than that of their helminth counterparts, as their modulation of the immune system shifts according to their life cycle[110]. For these reasons it is worth exploring the effect schistosomes have on co-infecting pathogens and vice versa. These effects ultimately lead to altered immune responses on all pathogens present, increases disease complexity, and may change diagnosis, treatment, and prognosis.

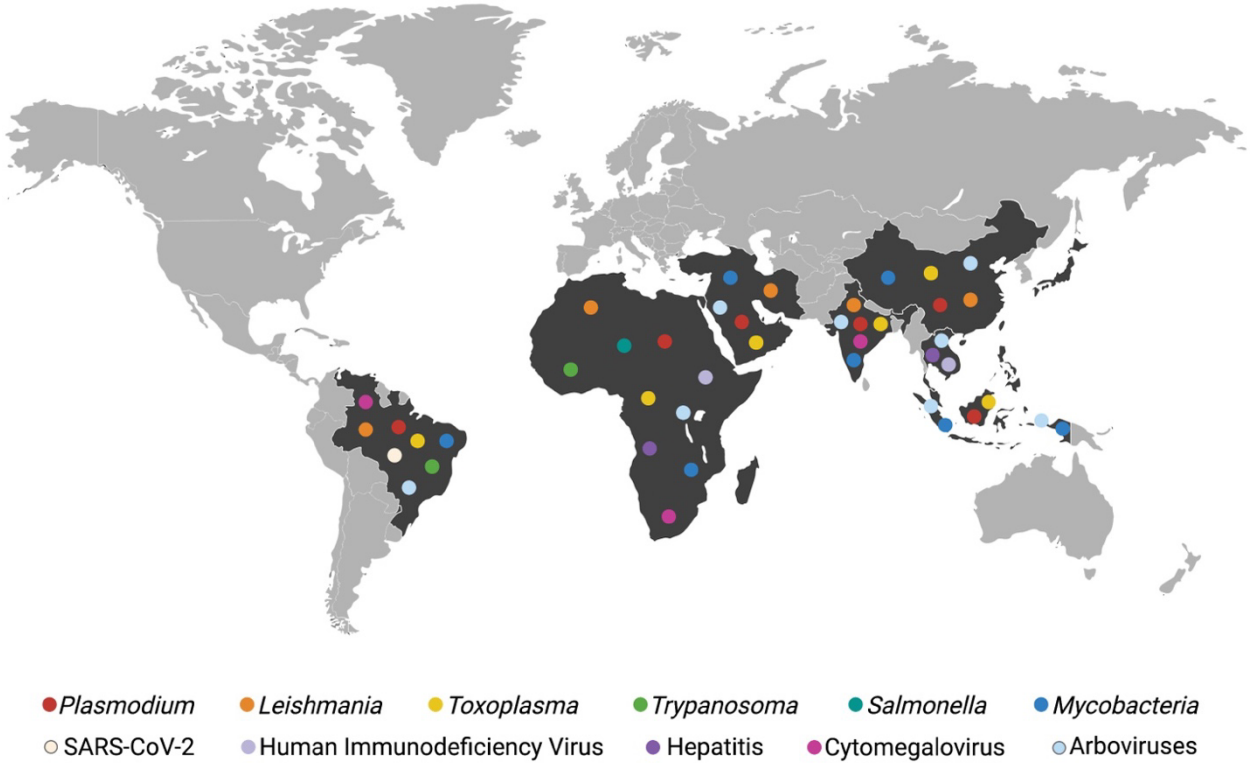

Figure 1.5 *Schistosoma* and co-infections world map

The global spread of *Schistosoma* spp. endemicity can be seen depicted in the portion of the graph shaded in dark grey. Within these regions, co-endemicity with the pathogens discussed in this paper have been denoted by coloured dots. Dots have been placed within generalized geographical regions across the map. Intestinal protozoa have been omitted as they can be found in all regions. Other pathogens which can be similarly found in all regions (*Salmonella,* SARS-CoV-2, Cytomegalovirus) have been denoted in the regions where their prevalence is the greatest. Created with BioRender.com. *Reproduced from Perera DJ, Koger-Pease C, Daoudi M, Paulini K, Ndao M. Schistosoma co-infections: when pathogens get friendly, do they get deadly? Submitted to Clinical Microbiology Reviews.*

1.1.6.1 *Schistosoma* spp.

Two of the three most common human-infecting *Schistosoma* species, *S. mansoni* and *S. haematobium*, have significantly overlapping endemic areas. Given that most *Schistosoma* infections occur in co-endemic sub-Saharan Africa[23], interspecies co-infection is a significant, yet understudied and underreported, risk[111]. While research is lacking on the epidemiology and morbidity of co-infections at a large scale (country/global), there is a growing body of work at the local scale (village/region), notably in Senegal[113–118], Cameroon[112,113], Nigeria[114], Ghana[115], Kenya[116], Niger[117], and Mali[117,118]. In many of the areas investigated, co-infection appeared to be the norm[111,115,119], with over half of infected individuals harbouring multi-species infections.

One common indicator of co-infection with *S. mansoni* and *S. haematobium* is ectopic egg elimination, which refers to *S. haematobium* eggs eliminated in the stool and/or *S. mansoni* eggs eliminated in the urine. This phenomenon can sometimes be attributed to unusual worm localization or "spill over" from typical egg laying sites in mono-infections, particularly in the case of high parasite burdens[112]. More typically, however, ectopic egg elimination is associated with *Schistosoma* x *Schistosoma* co-infections and occurs due to heterospecific mating between the different schistosome species[112,115,120]. When worms of both species are present in an individual, heterospecific mating can occur in the hepatic portal vein, after which the species of the male will determine where the couple migrates and lays eggs[121].

Multiple studies have found that higher infection intensities occur in co-infection versus mono-infection. A study in Niger found an association between high infection burdens and mixed

infections[122], which was further validated in later studies in Niger, Mali, and Senegal[117], the SRB[111], and Ghana[115]. Interestingly, hepatic and splenic pathologies appear to decrease in co-infections while bladder pathologies tend to increase. In Cameroon, it was found that hepatomegaly and splenomegaly (assessed by physical exam) were less common in co-infections than in *S. mansoni* mono-infections[112].

Further complicating the picture, co-infections with multiple schistosome species can result not only in parallel infections, but in hybridization between the different species. Hybridization has important implications for host range (vertebrate and snail), pathology, and epidemiology[123,124]. The most discussed hybrids are between *S. haematobium* and the bovine species *S. bovis*, which have been identified in West Africa as well as the outbreak in Corsica, France[123]. Still, the effects of these hybridization events remain to be seen.

1.1.6.2 Soil transmitted helminths

Soil transmitted helminths (STHs) are a group of intestinal parasites infecting up to a quarter of the world's population. There are three main types of STH: the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*). Additionally, the threadworm (*Strongyloides stercoralis*) is a STH but is often excluded from analyses and control efforts due to differences in diagnosis and treatment compared to the other three STHs[125]. Generally, STHs and schistosomiasis endemicity patterns are similar; however, because schistosomes require the presence of freshwater bodies and snail hosts, their epidemiology is more focal than STHs, which can occur anywhere suitably moist soil and faecal contamination intersect[126].

Co-infections with STHs and schistosomes are well established to have different infection burdens than mono-infections (higher or lower, depending on various factors), as elaborated in reviews (see[109,127]). However, in addition to changes in worm burdens, co-infections can result in pathology changes. In mice, co-infection with *Heligmosomoides polygyrus* (model organism for hookworm) two days prior to *S. mansoni* infection resulted in lower schistosome induced hepatic egg pathology[109,128]. In contrast, studies investigating the effect of whipworm infection on schistosomiasis egg pathology have found that this co-infection led to significantly increased pathology[109,129,130]. One recent study compared schistosomiasis in baboons already chronically

infected with *T. trichiura* to those mono-infected with *S. mansoni.* The authors found that while worm and egg burdens were similar between the groups, the co-infected group had significantly larger liver granulomas; the average size was nearly double that of the mono-infected group. RNA sequencing analysis corroborated these data, showing enrichment in pathways predictive of liver damage and hepatic injury in the co-infected group compared to the mono-infected group[130].

Given the increases of IgE in helminth infections and the links between helminths and allergy[131], another area of investigation is the effect of co-infection on this association. In a region of Uganda co-endemic for hookworm and *S. mansoni* it was shown that simultaneous treatment of PZQ and albendazole in school age children (SAC) resulted in increased anti-schistosome adult worm IgE production, which was associated with a reduced susceptibility to *S. mansoni* re-infection; however, the same immunity was not developed for hookworm[132]. Histamine release was found increased after treatment, in response to *S. mansoni* and hookworm adult worm antigens, but curiously, also to environmental allergen house dust mite (HDM)[133], demonstrating that helminthic co-infection may suppress basophil histamine release and in turn, allergy. This study presents preliminary evidence supporting the hygiene hypothesis in hookworm/*S. mansoni* co-infections; albeit there are not yet sufficient data to definitively conclude that co-infection with these two worms can prevent allergy.

1.1.6.3 *Plasmodium* spp.

Plasmodium spp. are protozoan parasites responsible for malaria. Helminth and *Plasmodium* co-infection is common due to their geographical overlap. A recent study in Cameroon demonstrated that most co-infected SAC had light *S. haematobium* infections and low-density *Plasmodium* infections compared to the singly infected children[134]. The authors hypothesize that this is a consequence of the early T_H1 response elicited by schistosomiasis that helps control the *Plasmodium* infection[135] , though it was not determined which infection came first.

In most studies, the rate of anaemia was higher in co-infected patients compared to *Schistosoma* or *Plasmodium* single infections[134,136–140]. A study in Cameroon looked at the most common symptoms of *Schistosoma* and *Plasmodium* co-infection in SAC and found them to be haematuria, microcytosis, and fever[134]. High levels of haematuria were also seen in SAC in Nigeria

where 57.1% of children were infected with *S. haematobium* and *P. falciparum* and 63.8% of coinfected individuals had haematuria compared to 52.2% in the *S. haematobium* group and 43.7% in the *P. falciparum* group[141].

Despite the increases in pathological events in co-infected humans, in non-human primates (NHPs), chronic schistosomiasis protected animals from severe *P. knowlesi* infection, and they had reduced levels of *Plasmodium* parasite. These co-infected animals had lower levels of IFNγ compared to *Plasmodium* only infections and compared to PZQ treated animals. Similarly, in C57BL/6 mice infected with *S. japonicum* and *P. berghei*, co-infected mice had lower levels of IFNγ but higher levels of IL-4, IL-5, IL-13, and TGFβ when compared to *P. bergehi* single infections. Akin to natural malaria immunity which is believed to be developed due to anti-sporozoite and anti-merozoite IgG antibodies[142], schistosomiasis T_H2 responses may result in increased humoral antibodies developed against *Plasmodium* parasites.

1.1.6.4 Urinary tract infection causing agents

Due to their global prevalence, urinary tract infections (UTIs) can be easily found in locations endemic for schistosomiasis[143], and have been reported as co-infections in Cameroon[144], Ghana[145], Niger[146], and Nigeria[147], to name a few.

Interestingly, while females are more commonly infected with UTIs, field data demonstrate that males are more often infected with *S. haematobium*[148–150]. When both UTIs and schistosomiasis data were compared together, it was found that co-infection in Nigeria was age and gender dependent and more common in young males[151]. Further, most groups conclude that *S. haematobium*[147,152–160] and *S. mansoni*[153,159,161] infections increased the incidence of concomitant UTIs.

The most found pathogen co-infecting the urinary tract with schistosomiasis was *Escherichia coli;* though groups also found significant amounts of *Klebsiella* spp., *Staphylococcus aureus*, *S. saprophyticus, Proteus vulgarius, Pseudomonas aeruginosa*, *Streptococcus* spp. and *Candida albicans*[148,150,154,156,162] in urine specimens. *Trichomonas*[150,163,164], *Neisseria gonnorrhea*[164], and *Salmonella* Paratyphi A[165] have also been reported to infect the urinary tract of individuals with schistosomiasis.

Some have postulated that infection with schistosomiasis poses a risk factor for UTIs due to egg spines causing damage to urinary tissues, providing bacterial entry sites and blood which acts as a bacterial culturing medium[166,167]. Animal studies using models of co-infection demonstrate that *S. haematobium* eggs increase susceptibility to uropathogenic *E. coli* bacteriuria in BALB/c mice due to an increased expression of IL-4[168] and a reduction of invariant natural killer T cell-mediated bacterial clearing[169]. Additionally, administration of *S. haematobium* IPSE (an egg-derived protein) reduced anti-microbial peptides in the bladder and may work in concert with IL-4 to promote the establishment of bacterial infection, despite showing no changes in bacterial cfu[170].

1.1.6.5 Human immunodeficiency virus

There were approximately 38.4 million people across the globe living with HIV in 2021[171]. Geographical overlap between HIV and schistosomiasis is concerning, particularly in sub-Saharan Africa[172] as schistosomiasis appears to be a cofactor in the spread and progression of HIV/AIDS.

People with chronic schistosomiasis may be more likely to become infected with HIV Type-1 (HIV-1) and be impaired in viral control once infected[173] due to immunological changes. It has been shown that schistosomiasis-induced pathology (such as inflammation and damage to the mucosal lining of genital and rectal areas) can increase the shedding of HIV and facilitate its transmission during sexual contact[174]. Therefore, PZQ treatment may be beneficial in preventing HIV susceptibility[175,176]. Several studies show that schistosomiasis, especially female genital schistosomiasis (FGS) among adolescents and young adults, may be associated with a higher risk of HIV acquisition[176,177]. In cases of MGS near Lake Malawi, it was found that MGS could be associated with low level HIV-1 RNA shedding in semen, potentially increasing the risk of HIV transmission[178].

Co-infections of HIV and FGS cause mucosal changes in the intra-vaginal epithelium of the cervix, fornices, and vagina, as well as infertility when the upper genital tract is involved[179]. In addition to *Schistosoma* induced immunological changes, mast cells have been described as a reservoir of HIV-1 that can be induced by schistosome infections[180]. Though it has been demonstrated that schistosomiasis may increase the risk of HIV-1, it is unclear what effect HIV-1 infection has on susceptibility to schistosomiasis[172].

It has been found that individuals co-infected with HIV-1 and *S. japonicum* exhibited a lower frequency of CD4+ T cells and a higher frequency of CD8+ T cells[181]. Individuals co-infected with HIV-1 and *S. mansoni* displayed an extra enlarged left hepatic lobe which suggests that co-infection may be associated with severe hepatosplenic disease[182]. Other liver ailments have also been attributed to HIV-1 and schistosomiasis including periportal fibrosis[183], and higher hepatotoxicity in co-infected patients on antiretroviral therapy (ART)[184]. Interestingly, in sub-Saharan Africa, it has been shown that HIV-1 infection reduced egg excretion in individuals infected with *S. mansoni*[172,185,186], suggesting that *Schistosoma* diagnosis by faecal microscopy may not be ideal in co-endemic regions. As many populations lack access to MDA of PZQ and ART, *Schistosoma*/HIV co-infections may synergize to increase pathology and HIV transmission to uninfected individuals.

1.1.6.6 Hepatitis viruses

Schistosomiasis and hepatitis co-infection is reported in many countries, notably in Egypt[187]. Here, the prevalence of schistosomiasis in patients with Hepatitis C virus (HCV) infection was reported to be between 27.3% and 50% across two studies[188,189]. Co-infection of Hepatitis B virus (HBV) and *S. mansoni* ranged from 19.6 to 33.0%[190]. It is interesting to note that a high prevalence was also demonstrated in China, where about 58.4% of the patients with chronic *S. japonicum* had HBV[191] and in Hubei, specifically, where the prevalence of HBV in schistosomiasis patients was much higher (25.37%) compared to those without schistosomiasis (0.62%)[192].

The clinical course of illness once an individual was co-infected with *Schistosoma* and either HBV or HCV was typically more severe than those which were mono-infected. For both viruses, co-infection resulted in detrimental pathologies which resulted in higher mortality[193], likely because both hepatitis viruses and *S. mansoni* result in significant liver damage.

Schistosomiasis, being a chronic infection, is often found co-infecting with other pathogens. While some have been discussed here, there are a myriad of other pathogens which can co-infect people, increasing the complexity of disease. Further, co-infections with schistosomiasis are underreported, understudied, and undertreated to an even greater extent than neglected tropical

disease mono-infections. To properly assess the burden of schistosomiasis on health and to determine effective public health interventions, co-infections must be considered.

1.1.7 Diagnosis

Schistosomiasis transmission control is the only way to reach WHO goals of schistosomiasis elimination. This is mainly done through diagnosis, treatment, and vector control. In endemic regions, severe morbidity and mortality occur due to chronic infections and repeated exposures over time, but this can be avoided through transmission control including early screening and treatment[194].

Despite the massive and extensive integrated control programs launched over the past years[18,195,196], schistosomiasis continues to be a persistent problem in the Global South. This may be due to a lack of consistent and accurate diagnostic tools for screening communities in endemic regions.

1.1.7.1 Microscopy

Direct parasitological observation was the first means of schistosomiasis diagnosis involving visualization of schistosome eggs in the faeces (for intestinal schistosomiasis) or in the urine (for urinary schistosomiasis). Stool can be assessed by direct faecal smear or by the formalin-ether concentration technique (FECT), followed by microscopic analysis; however, both techniques have low sensitivity for helminth infections, especially *S. mansoni*[197,198].

In 1972 the Kato-Katz technique (KK) was developed by Drs. Fumitaka Kato and Yuzo Katz and is still considered by the WHO to be the gold standard of intestinal schistosomiasis diagnosis[199]. Briefly, KK involves pressing fresh stool through a sieve and viewing it under a microscope after a clearing time of 30 minutes to, ideally, 24 hours. It can also be used to identify infections of other STHs based on egg morphology[200]. The KK technique is the preferred method of diagnosis for intestinal schistosomiasis since it is relatively easy, cost-effective, and can detect moderate to high level infections[200]; thus, making it a simple choice for use in the field. However, the sensitivity of this diagnostic decreases in low level infections (approximately 20-50 eggs per gram of stool). When KK was used to assess *S. japonicum* schistosomiasis in three low-endemic regions of China, it was found to miss an average of 83% of infections when a single smear was

examined[201]. KK sensitivity also decreases after treatment with PZQ[202], which is likely due to a lower burden of worms producing eggs; also, KK is dependent on a consistent release of eggs with each bowel movement, which is unrealistic due to daily fluctuations in egg counts[203].

Another technique, FLOTAC, was developed for veterinary use and validated for human diagnosis of nematodes and trematodes, including *Schistosoma* spp[197]. This method involves the homogenization of one gram of faeces in formalin, its filtration, and the addition of a flotation solution, allowing helminth eggs to float. These eggs can then be visualized through microscopy. While a large amount of faeces can be processed, FLOTAC requires a large bucket centrifuge which may not be available in endemic regions with limited access to lab resources. To overcome this hurdle, the mini-FLOTAC was created. The mini-FLOTAC maintains a low limit of detection (approximately 10 eggs per gram of faeces)[198], has a quicker processing time compared to KK (18 min versus >34 min), and acts as a closed system making it safe with no risk of contamination. More importantly, this method was found to be more accurate than direct faecal smear, FECT, and KK when tested along Lake Victoria, Tanzania[204].

While stool samples are assessed for intestinal schistosomiasis, urine filtration or urine sedimentation can be done to diagnose the presence of *S. haematobium* eggs from urine samples[205]. Generally, syringe filtration is preferred to centrifugation since it is simpler and requires less machinery. In addition to egg detection, urinalysis can include other tests including, for example, macro- and micro-haematuria detected by rapid detection reagent strips[206]. These data can be useful to monitor the effectiveness of treatment within communities as was seen in Nigeria[207]. Despite being able to use a large volume of urine and an absence of the solid materials found in stool, egg visualization through these techniques is also prone to low sensitivity[208].

These diagnostics are simple to perform which is why they are still used today; however, they have limitations when it comes to light intensity infections and need to be run several times to account for the day-to-day variation in egg output. Furthermore, since egg deposition occurs at the onset of chronic infection, this diagnostic method is not capable of identifying early-stage infections.

1.1.7.2 Serology

Since schistosome eggs cause the majority of pathology, if patients are screened and treated before the maturation of egg-laying adults, the progression of schistosomiasis into severe disease might be circumvented. Moreover, in situations where microscopy results are unclear, serological diagnostics can be used as confirmation. These tests are commonly used to determine if an individual has been previously exposed to schistosomiasis. In many cases, anti-schistosome antibodies will develop within 6-8 weeks of infection and can be detected before eggs can be found in the stool/urine; albeit early infections can still be missed[52]. As infection continues, the sensitivity of antibody tests increases; however, these tests do not allow for the discrimination between species or between *Schistosoma* and some helminths, due to cross-reactivity[209,210]. Further, antibody tests fail to distinguish between active infections and past infections as antibodies can remain in the serum for long periods after schistosomiasis has been treated and cleared[211]. While these tests may not be helpful in highly endemic communities, they may be useful in detecting children, early in infection before the egg stage, who may need special care[212] and in travellers returning from endemic countries[213].

The circumoval precipitin test (COPT) and cercarien Hüllen reaction (CHR) involve mixing patient serum with lyophilized schistosome eggs (mainly used for *S. japonicum*), and live cercaria, respectively. These methods are complex, time consuming, and since they are based on antibodies they can result in false negatives in early stages of infection and false positives in already cleared infections[214,215]. Indirect haemagglutination assays (IHAs) with *S. mansoni* worm antigens (SWA) and enzyme-linked immunosorbent assays (ELISAs) using *S. mansoni* SEA and SWA have also been developed[216]. Even schistosomula have been used for antibody-based diagnosis, using crude antigens obtained through parasite homogenization, allowing for a sensitivity and specificity of 96.67% and 86.67%, respectively[217]. In fact, several other ELISA-based antibody diagnostics have been developed; though they are not standardized among companies and users, leading to varying accuracies.

Differently, serologic testing can also involve looking for parasitic antigens in circulation or in urine. One example is of the circulating anodic antigen (CAA) which is found in worm vomitus and can be detected through epitopes on its O-linked glycans[218]. ELISA detection of CAA is useful as it only detects active infections; however, in cases of low-endemicity it is not

significantly more sensitive than KK[219]. Since its initial creation these tests have been altered to increase sensitivity, by using up-converting phosphor-lateral flow (UCP-LF) reporter technology, for example[220]. When tested in a non-endemic setting the UCP-LF CAA assay was able to capture positive diagnoses as early as 4 weeks after infection, in some cases this was earlier than anti-schistosome antibody tests became positive[221]. This UCP-LF system can be used for serum but also non-invasively for urine tests, and it is 10-fold more accurate than a triplicate KK smear in detecting *S. japonicum*[222].

Like CAA, circulating cathodic antigen (CCA) is a similar adult worm antigen which has been used in the development of ELISAs and rapid chromatographic strip tests. CCA strip tests have been used to detect *S. mansoni* infections in children before eggs can be found in stool and antibodies in serum[212]. A commercially available point-of-care (POC) urine cassette test has been developed and can accurately detect not only *S. mansoni* in several populations, but also *S. japonicum* and *S. mekongi*[222–225]. While not as sensitive as the UCP-LF CAA system[226], the POC-CCA is a convenient test for mass use in areas of medium to high-endemicity.

1.1.7.3 Molecular techniques

For clinical analysis, DNA and RNA techniques have been developed for schistosomiasis diagnosis. Studies demonstrate that these assays result in almost 100% specificity, while sensitivity ranges from equal to or greater than that of KK. When detecting DNA in urine, sensitivity can be increased by concentrating the sample by urine sedimentation[227]. Unlike other diagnostic techniques, DNA and RNA detection can also be used to detect genital schistosomiasis through the analysis of seminal and vaginal lavages[228].

In endemic settings, molecular techniques can easily determine infecting *Schistosoma* spp. and even type strains. This has been done to identify the natural interaction between *S. haematobium* and *S. bovis* resulting in hybrid species[121,229].

Ultra-sensitive blood-based diagnostics are important to identify early-stage infections especially in travellers. Ideally these assays will become positive before serology and microscopy. Detectable levels of *Schistosoma* DNA have been shown using polymerase chain reaction (PCR) in the plasma and serum of patients during acute infections[230,231], however these levels do not

decrease for months after treatment[230–232]. It is unclear if prolonged detectable DNA is a result of the continuous release of DNA from tissue trapped eggs.

While PCR-based diagnosis is common in the Global North due to its high sensitivity and specificity, these methods are rarely used in the field as they require expensive laboratory equipment and skilled workers[233]. Recently, loop-mediated isothermal amplification (LAMP) technology has been used to bridge this gap and can be adapted to environments with limited resources. The sensitivity of a LAMP assay to detect *S. mansoni* in the urine and stool was 14% and 78.3%, respectively[234]. Sensitivities seem to be diverse and are likely dependent on DNA targets, as a study from an endemic region of Brazil showed a specificity of 12% from stool samples[235]. While specificities of LAMP assays are high, they have some work to go to raise levels of sensitivity, which may be possible by increasing the duration of the assay[236]. Additionally, a study from *S. japonicum* serum samples demonstrated LAMP assays share the limitation of remaining positive after treatment for up to nine months in some individuals[237].

1.1.8 Treatment

Discovered over 50 years ago, the most effective and widely used drug to treat schistosomiasis is PZQ[238]. A meta-analysis demonstrated that the cure rate (CR) and egg reduction rates (ERR) of PZQ for *S. mansoni* are 76.7% and 86.3%, respectively. This drug is also effective against other species of schistosomiasis; CR and ERR for *S. haematobium* are 77.1% and 94.1%, respectively, and for *S. japonicum* are 94.7% and 95%, respectively[239]. While PZQ never provides a 100% CR, its general efficacy reaches between 80-90% for all species, including *S. intercalatum*[240] and *S. mekongi*[241], when administered at the WHO recommended dose of 40 mg/kg[242]. In fact, PZQ is also effective against other helminths[243] which can be advantageous in endemic regions where polyparasitism with helminths is common. PZQ is effective as quickly as one hour after ingestion and almost 80% of the drug undergoes biotransformation in the liver into metabolites which are excreted through the urine. Attempts have been made to increase the dose of PZQ to >60mg/kg and a study conducted in Ugandan children suggests that higher doses are more efficacious[244]. If the dosage of PZQ is increased, it is important to note that the active form of the drug is excreted in small amounts within breast milk[245]. Although it is unlikely that a

newborn will experience adverse events, it is recommended that breast-feeding individuals do not breastfeed for up to 72 hours after treatment.

Currently, PZQ can be used to treat children over 4 and pregnant females after their first trimester[246]. In fact, school going children and other risk groups such as these, are the focus of many mass drug administration (MDA) programs[247]. An average treatment for schistosomiasis is estimated to be approximately 0.25 United States dollars (USD). Through a donation from Merck Serono to the WHO, PZQ is now available to high-endemic countries of sub-Saharan Africa for free, and to other selected countries through the Department for International Development and the United States Agency for International Development[242].

PZQ is formulated as two enantiomers, a racemic mixture of *R*-PZQ (active anthelminthic) and *S*-PZQ (less active)[248,249]. The functional mechanism of PZQ has been said to be generally not well understood; however, in the last decade, the transient receptor potential melastatin (TRPM) ion channel of *S. mansoni* (Sm.TRPM$_{PZQ}$) was discovered to be the primary target. PZQ activates Sm.TRPM$_{PZQ}$ and TRPM homologs, causing Ca^{2+} entry into cells, inducing paralysis and changes in parasitic tegument architecture. As quickly as 11 seconds, PZQ can cause visible contracture of the helminthic musculature, and tegument damage like surface blebbing can be seen as early as 15 minutes[250].

While PZQ continues to be the mainstay for schistosome treatment, it isn't without its limitations. Importantly, PZQ has little effect on juvenile worms and schistosomula and cannot prevent re-infection[251,252]. This has great impact on regions of high parasite burden, some of which have shown reduced susceptibility to PZQ[253]. In Egypt, viable eggs are released from individuals after PZQ treatment[254] and low cure rates have been observed in Senegal[255]. Additionally, in individuals with schistosomiasis and concurrent cysticercosis, PZQ can induce seizures, cerebral infarction, and permanent eye lesions because of severe inflammation in response to dying *Taenia solium* worms[256]. Finally, the overuse of PZQ, as the single drug used to treat recurring schistosomiasis, has led to the fear of eventual drug resistance. While there are no reports of PZQ resistance in the field to date, there are regions of Africa which show reduced CR after treatment[254,257–259] and several cases of PZQ resistance established in laboratory settings[260–266]. Furthermore, there is evidence to show that continued MDA may reduce population immunity in the long term and, if stopped, will result in a rebound of increased *S. haematobium* parasite burden[267,268].

Monitoring drug efficacy and resistance is an ongoing effort; to alleviate the limitations of PZQ, many groups are researching novel treatments for schistosomiasis. When PZQ was reformulated using nanotechnology and encapsulated into niosomes, which are lipid carriers made up of cholesterol and non-ionic surfactant vesicles, its efficacy was re-established in mice infected with *S. mansoni* with a reduced susceptibility to PZQ[269]. Before the introduction of PZQ, oxamniquine (OXA) was used in South America to treat intestinal schistosomiasis; however, OXA is only active against *S. mansoni*, and resistance has been observed in Brazil[270]. Interestingly, some anti-malarial drugs have shown efficacy in treating schistosome infections. In co-endemic regions, patients were given artemether-lumfantrine, which cured all cases of schistosomiasis[271]. Further, in the Democratic Republic of Congo (DRC), a study found that 47% of children were co-infected with *Plasmodium* and *S. mansoni*. When treated with artesunate-amodiaquine, 74.4% of these children experienced full cure, 22% partial cure, and only 3.6% had no change in schistosomiasis parasite burden[272]. In 2021, a clinical trial was conducted in Senegalese children, for the efficacy of artesunate-mefloquine as a treatment for *S. mansoni* and *S. haematobium*, though data for this study has yet to be released[273]. While this may be a promising avenue for the treatment of two diseases with one drug, there is a growing concern that the overuse of antimalarials will lead to resistant *Plasmodium* spp., as observed in the DRC[274]. In pre-clinical studies, a compound of acridanone-hydrazones was shown to be highly effective in killing *S. mansoni* schistosomula, in the skin of mice (24 hours after infection) and in the circulation of Cebus monkeys (7 days after infection)[275]. Furoxan, an oxadiazole nitric oxide (NO)-releasing molecule which targets thioredoxin glutathione reductase (TGR), has also been shown to effectively cure murine schistosomiasis when given at any point after infection[276]. Furoxan has been fused with PZQ and shown *in vitro* to be antiparasitic[277] and similar oxadiazole derivatives have been discovered which have even greater inhibition of *S. japonicum* TGR than furoxan[278]. Continued research and exploration of existing and novel drugs to treat schistosomiasis will be of great value to supplement the short comings of MDA with PZQ.

1.1.9 Schistosomiasis control

MDA continues to be a keystone in schistosomiasis control and its importance has been demonstrated through the indirect effects of COVID-19 on schistosomiasis transmission. With

social distancing and the halting of PZQ mass drug administration, Brazil has seen a reduced amount of people tested for schistosomiasis; thus, underestimated positivity rates.

Aside from drug treatment, schistosomiasis is currently controlled by a myriad of other techniques such as global water, sanitization, and hygiene (WASH) efforts and vector control, diminishments of which have also been detrimental to schistosomiasis elimination. COVID-19 closures allowed the snail vector of *S. japonicum*, *Oncomelania hupensis,* to return to its natural habitat in Wuhan, China, increasing the risk of schistosomiasis spread[279].

Mathematical modelling has been used to approximate the effects of COVID-19-caused postponement of MDA and other mitigation strategies on the elimination plans of schistosomiasis. For *S. mansoni* and *S. haematobium*, elimination plans have been estimated to be delayed by up to two years in both moderate- and high-transmission areas[280]. To reduce schistosomiasis infections and to achieve WHO elimination goals, these mitigation strategies must be reinstated and maintained through future healthcare emergencies.

1.1.9.1 Vector control

Schistosomiasis intermediate hosts can also be targeted to control parasite transmission. In combination with PZQ treatment, snail control allowed for the elimination of schistosomiasis in Japan by 1994. These control measures included: cementing irrigation canals, drying wetlands, and applying molluscicides[281]. The most widely used molluscicide since the 1960s is niclosamide, which can kill snails for 24 hours after application. The concentrations of niclosamide deployed should be non-toxic to vertebrates (e.g., fish and humans); however, its uneven distribution has been shown to affect freshwater life and raise health concerns[282,283].

Modifying snail habitats can also lead to both positive and negative outcomes within the context of schistosomiasis. Vegetation removal, land reclamation, cementing canals, and even hydrological interventions to increase or alter stream flow have been used in numerous countries to control schistosomiasis[281,284,285]. In contrast, habitat changes linked to dam construction and irrigation expansion, without schistosomiasis consideration, have led to unintentional parasite outbreaks[286,287].

Curiously, snails can also be controlled by their natural predators (e.g., crustaceans, birds, fish). While the introduction of these predators is sometimes effective, if not carefully considered,

it can lead to collateral impacts and non-target effects where these predators are not native[288]. A successful example of this control measure is the introduction of competitor snails in the Caribbean. These snails, which are not competent hosts for schistosomiasis, displaced the host snails responsible for transmitting the disease[289,290].

1.1.9.2 Water, sanitization, and hygiene

WASH efforts vary greatly suggesting that their impact on schistosomiasis is highly setting specific. However, it has been shown that when people have access to safe water and adequate sanitation, they had a significantly lower risk of *Schistosoma* infection[291]. Importantly, these efforts must also be combined with population education as the transmission of schistosomiasis is deeply connected to social-ecological systems, and cultural factors need to be examined. As schistosomiasis is spread through contact with water, most people are infected through daily activities such as swimming, fishing, and doing laundry[292]. Although unrealistic, if human contact with these waters were to stop, transmission of the parasite would also stop.

With the combination of these schistosomiasis control programs several countries (namely Japan, China, St. Lucia and other Caribbean islands, Morocco, Tunisia, and Mauritius) have shown that progression to schistosomiasis elimination is possible[293].

1.2 Anatomy and immunomodulation

Schistosomes possess many innate characteristics which allow them to survive in the circulation despite being in constant contact with the immune system. Each of these mechanisms have been evolutionarily crafted to best protect the parasite at each stage of its lifecycle. The following section will revisit the *Schistosoma* lifecycle and will discuss how this parasite has adapted to progress each lifecycle stage into the next.

1.2.1 Miracidia: The external larva I

As eggs contact freshwater the boundary layer between the eggshell and the outer envelope disintegrates and eggs hatch giving rise to free-swimming miracidia. In *S. japoncium* eggs, the parasite hatches through a shell rupture, allowing for an inflow of water and muscular activity of

the larva. After rupture, miracidia exit through the split but remain within a sac from which it must escape to enter the water [294]. An enzyme, leucine aminopeptidase, in addition to light, osmolarity, and temperatures around 28°C are suggested to help in the process of hatching[294–296].

Once hatched, miracidia must swim and infect snail hosts of a specific genus. When miracidia are exposed to specific snail-derived peptides and excretory-secretory (ES) attractants they change in behaviour and demonstrate aggregation around the attractants, and chemoklinokinesis (the random movement in proximity to a chemical, including slowdown and turning)[297,298].

1.2.2 Breaching the snail

Miracidia are larva covered in cilia which they use to swim through freshwater. When they have found a suitable snail host, they undergo a process of repeated investigation followed by attachment and penetration[299]. In *S. japonicum* more than half of invading miracidia will do so through natural openings such as the branchial cavity, mouth, and rectum[300]. To the contrary, *S. mansoni* miracidia preferentially penetrate the tegument, near the tentacles[301].

Once inside the snail, miracidia transform into primary sporocysts covered by an extracellular matrix which protects them from components of the snail immune system: hemocytes and fibrous cells[301–303]. Sporocysts will undergo several rounds of asexual reproduction into daughter sporocysts which release cercaria that exit the snail through the body wall[304].

1.2.3 Cercariae: The external larva II

Cercariae are the infectious form of the parasite to mammals. Similar to miracidia, they swim through the water in response to chemoattractants; however, cercariae possess a forked tail which they use to row themselves through the water[305]. The most transcriptionally active portion of the cercaria is the head, as this section is what transforms into a schistosomulum upon host penetration. Cercariae possess six pre- and four post-acetabular gland cells which secrete enzymes and other molecules which mediate skin penetration[306].

1.2.4 Dermal entry: A silent invader

As cercariae penetrate the skin they change in both physiology and morphology, shedding both their tail and osmo-protective glycocalyx becoming schistosomula. Tissue remodelling also occurs as they obtain a new tegument surface complex[307]. Schistosomula spend approximately 2-3 days within the skin and during this time they modulate the immune system to encourage infection establishment.

Exposure to a small number of cercariae generally results in brief localized skin inflammation[308], but repeated exposure, or exposure in schistosomiasis naïve individuals, can result in cercarial dermatitis. *S. mansoni* and *S. haematobium* skin-stage schistosomula elicit IL-1 receptor antagonist, IL-10, and TNFα, while *S. japonicum* will induce these mediators among others[309].

IL-10, a regulatory cytokine, seems to be a key of schistosomal entry. This cytokine is also found to be released after other pro-inflammatory cytokines such as IL-12 and IL1-β suggesting it controls their effects[310]. In fact, radiation attenuated (RA) cercariae, which produce a high level of immunity in mice when used as a vaccine, either do not induce IL-10 production[308] or do so much later than wild-type cercariae[310]. Schistosome-induced prostaglandin (PG) E_2 functions to help the production of IL-10[308], while schistosome-derived PGD_2 works independently of IL-10 to inhibit Langerhans cell (LC) migration[311]. Further, a significant component of cercarial ES products is *S. mansoni* apoptosis factor (SMAF). SMAF specifically promotes apoptosis in the CD4+ lymphocyte population via Fas-FasL interaction[312]. Interestingly, SMAF is not expressed in RA cercariae providing another explanation why they are more efficient at stimulating host immunity.

1.2.5 Schistosomula: Induction of the T_H1 response

As schistosomula travel through the skin and into the circulation, they are carried by the bloodstream to the lungs where they reside for approximately a week. At this stage, larvae may be less susceptible to macrophage-mediated killing[313]. Here, 18 μm wide larvae narrow and elongate to bypass thin walled, 6 μm wide lung capillaries[314]. To evade immune attack, lung stage larvae coat themselves in host antigens such as blood group antigens and major histocompatibility (MHC) proteins to block the binding of anti-schistosomula antibodies[315–317]. By acquiring host decay

accelerating factor, schistosomula also prevent the assembly of complement convertases used in both the classical and alternative pathways for complement activation[318].

Fascinatingly, during lung migration, schistosomula do not appear to expand T_{REG} populations despite IL-10 expression being maintained[319]. Rather, within the first 5 weeks of infection, schistosomes induce T_H1 immune responses[320]. Schistosomula antigens, when used to stimulate peripheral blood mononuclear cells (PBMC) from Ugandans infected with *S. mansoni,* induced an increased expression of T_H1 and pro-inflammatory cytokines[321]. As schistosomula passage through the circulation they employ various mechanisms such as enolases and other plasminogen binding proteins which prevent blood clotting around them[322,323] before they arrive in the liver, maturing into adult worms.

1.2.6 Adult worms: Making a home

The term schistosomiasis is coined from the combination of two Greek words: “schistos” meaning split, and “soma” meaning body. This parasite was named by David Friedrich Weinland in 1858 based on the morphology of male worms, since they possess a gynecophoric canal which holds adult female worms.

Like schistosomula, adult worms also incorporate host antigens onto their surface to mask from immune recognition[324–328]. In addition, schistosome worms utilize molecular mimicry where they produce molecules which have significant resemblance to host signalling molecules. Some examples include their production of adrenocorticotropic hormones which can be processed by human leukocytes into molecules which inactivate them, and their production of substances with significant homology to morphine and codeine, which may also decrease leukocyte activation[329–331].

The adult worm tegument contains many immunomodulatory factors which promote the worm’s persistence. *S. japonicum* worms express a tetraspanning orphan receptor (*Sj*TOR) which can bind complement C2 and prevent complement mediated cell lysis[332]. In *S. mansoni*, the tegument contains the Kunitz type protease inhibitor (*Sm*KI-1), a serine protease inhibitor which targets neutrophil elastase and is crucial for parasite survival in mice[333]. Sm200 is an additional tegument protein which has been seen to increase levels of IL-10, regulating immune activation[334].

Additionally, schistosome worms express a large variety of redox enzymes which are thought to be central to their ability to neutralize phagocyte-generation, and toxic oxygen- and nitrogen-based metabolites[335–338]. These are released along with other ES proteins which are capable of immunomodulation (e.g., suppressing exogenous antigen presentation, inducing M2 macrophage generation and anti-inflammatory cytokines: TGFβ and IL-10, and regulating immune cell function)[339–341].

1.2.7 Eggs: Shifting to T_H2 responses

Fully mature adult schistosome worms live *in copula*, releasing eggs into the vasculature. Schistosome eggs are characteristic of the species from which they come from, and the morphology of the egg is used for distinction during diagnosis (Figure 1.6). Of note, *S. mansoni* eggs present a lateral spine, *S. haematobium* eggs have a terminal spine, and *S. japonicum* eggs show a small bump as a spine or no bump at all[342–344]. Interestingly, parasite eggs vary in size not only between species but also within the same species[345]. These eggs are surrounded by an eggshell which protects the embryo while it develops and is resistant to chemical and physical impacts.

Schistosome embryogenesis takes place within the reproductive tract of adult female worms as well as within host tissues after egg deposition. Adult worms produce eggs by sexual reproduction and after formation of the eggshell, eggs move into the uterus and are released through the female genital pore. Released eggs contain immature embryos which mature over 7 days while receiving nutrients from vitelline cells and from the host[346], at this point eggshells will contain a fully formed larva capable of muscle contraction and possessing a beating flame-cell.

The characteristic T_H2 polarization observed in helminths is only dramatically clear following egg deposition and can be recreated in animal models with the injection of schistosome eggs or egg extracts[88,347,348]. This includes robust expression of type 2 markers, and IgE responses. Different from the other life stages of *Schistosoma* found in the definitive host, eggs are not hidden from the immune system and become engorged in immune cells forming granulomas. Recruitment of immune cells and driving of the T_H2 response are mediated by components released from the eggs. SEA is the crude, soluble, schistosome egg fraction which has been studied due to its ability to promote basophil degranulation and induce IL-4, IL-13, and histamine release[348].

SEA has been well examined to determine which specific molecules induce polarizing immune responses and granuloma formation around eggs. Mature eggs secrete a highly immunogenic protein, IPSE/α1 (IPSE). IPSE is a hepatotoxin[349]; but it induces the production of IL-4 from basophils, by binding immunoglobulin (Ig) E[350,351]. Omega-1 (ω1) is a protein expressed in *S. mansoni* eggs and SEA. *In vitro* ω1 has been found to drive monocyte-derived dendritic cells (MoDC) to prime T_H2 polarized responses from CD4+ T cells[352]. This protein is expressed in mature eggs and, at lower levels, in immature eggs[346]. Of interest, in a model of murine diabetes, when ω1 was injected subcutaneously into C57BL/6 or NOD mice, the percentage and number of FoxP3+CD4+ T cells in the draining lymph nodes increased[353], demonstrating its ability to induce the expansion of T_{REG} cells. Curiously, ω1 too, is considered a hepatotoxin[354]. Kappa-5 and Sm chemokine binding glycoprotein (SmCKBP) are also found in *S. mansoni* egg ES. Kappa-5 is expressed in the subshell of the egg and has been suggested to prevent major fibrosis within the granuloma, thereby preventing eggs from becoming trapped within the tissue[355], whereas SmCKBP neutralizes IL-8, CCL2, CCL3, CCL5, CXCL1 and inhibits neutrophil migration[356]. Neutrophils are found in early granulomas, though granuloma composition changes over time.

Intestinal granulomas are composed largely of macrophages, eosinophils, T cells, B cells, neutrophils, and basophils which are typically replaced by fibroblasts as the granuloma matures and/or the egg is released[55]. There are several theories as to why granulomas are formed, with the prevailing one being that they are instrumental in pushing parasite eggs through the tissue[55]. Hepatic granulomas differ from intestinal granulomas due to their inability to release schistosome eggs and so they become fibrotic over time. As infection progresses into chronicity, liver granuloma sizes decrease and stabilize from 20 weeks post infection (wpi) until at least 52 wpi[357]. While hepatic granulomas are unable to resolve like intestinal granulomas, they play a vital role in protecting hepatocytes from egg-derived toxins, namely ω1[55].

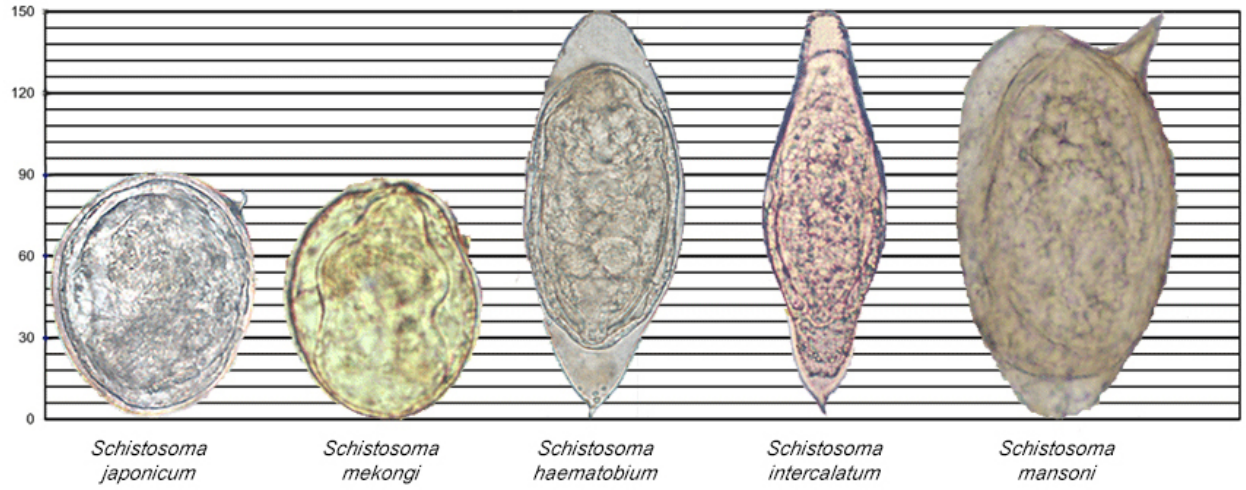

Figure 1.6 *Schistosoma* egg morphology

The following image demonstrates the visual differences between the eggs of *Schistosoma* spp. The y-axis shows a ruler in μm. *S. mansoni* possesses a distinct lateral spine. *S. haematobium* and *S. intercalatum* have demonstrable apical spines. *S. japonicum* and *S. mekongi* have small inconspicuous spines and are the smallest eggs of the genus. *Image sourced from the CDC and is publicly available on DPDx.*

1.2.8 Chronic illness: T_{REG} responses create homeostasis

Without treatment, schistosomiasis enters chronic infection. Here, granulomas become fibrotic and without immune regulation, T_H2, T_H17, even T follicular helper (T_{FH}) and T_H9 mediators can promote pathogenesis[358].

Within the granuloma, there is an increase of regulatory cells which modulate inflammation[107,359]. This includes macrophages which produce IL-10 in response to SEA, controlling liver damage and managing antifibrotic processes[360–362]. Additionally, there were dramatic increases in the expression of FoxP3 mRNA, as well as CD103, GITR, OX40, and CTLA-4 (all common phenotypic markers of T_{REG} cells) mRNA, in the spleens of *S. mansoni* infected mice[106,363,364]. Schistosome infection also stimulates CD5+ B cells, which can induce IL-10, FasL, regulatory antibodies, among other regulatory modulators[365,366].

In the circulation, schistosomiasis induces an overproduction of IgG4 in humans. This antibody is known for its regulation of inflammation and may act as an IgE blocking antibody or down-regulate the immune system in the excess of antigen[367–369].

As is evident, *Schistosoma* spp. tightly regulate host immune responses. By carefully promoting specific immunosuppression, they can limit host pathology and prevent host death, allowing their chronic persistence for decades. Schistosome induced immune modulation even extends beyond schistosomiasis, as chronically infected individuals have muted immune responses to vaccinations and other pathogenic co-infections[370].

1.3 Host immunity

1.3.1 Acquired immunity

It has been shown that some protective immunity to schistosomiasis develops in endemic areas albeit very slowly[52,371,372]. It is believed that this immunity may be driven by stimulation of protective immune responses to cryptic antigens which become exposed after the death of adult worms[373,374]. But in general, this immunity has been shown to be age-dependent, as children under the age of eleven are more susceptible to infection and re-infection than adults[375,376].

Cryptic antigens can be released through natural worm death over time or repeated treatments with PZQ. As worms die, they release immunogens which cross-react with antigens from migrating larvae and stimulate protective IgE responses. Multiple sources indicate that production of parasite-specific IgE, eosinophils, and T_H2 cytokines: IL-4 and IL-5 are required for protection from *Schistosoma* re-infection in humans[369,377–381]. In addition, soluble IgE receptors, as well as those expressed on B cells and eosinophils, mediate resistance to re-infection[382]. Aside from IgE, IgA and IgG antibody isotypes have also been associated with resistance to re-infection. In Kenya, IgA specific to *S. mansoni* 28 kDa glutathione-S-transferase (Sm28GST) was associated with an age dependent-decrease in egg excretion in individuals infected with *S. mansoni*[383], and in Senegal, IgG specific to a 37 kDa larval surface antigen was also associated with resistance[384].

In contrast, induction of IgG4 has been linked with susceptibility to schistosomiasis[385]. After treatment with PZQ, the level of parasite specific IgG4 in adults decreases while IgE levels are sustained but in children the ratio of IgE/IgG4 decreases[386]. IgG4 is produced by IL-10

producing regulatory B (B_{REG}) cells in humans and IL-10 has been demonstrated in mice to hinder the development of schistosomiasis resistance to re-infection[387].

Differently, it was shown that IL-33 displayed an inverse relationship with schistosomiasis pathology in a group of children from Cameroon[388]. IL-33 plays important roles in type-2 innate immunity by activating eosinophils, basophils, mast cells, and innate lymphoid type 2 cells (ILC2)[389]. Helminth infections commonly expand ILC2 populations expressing both IL-4 and IL-13[390]; ILC2 are also capable of upregulating GATA3 expression and driving T_H2 polarization of T cells[391]. In humans, it seems as though development of immunity to schistosomiasis is a long and arduous process maintained by the balancing of T_H2 responses and IgE expression over T_{REG} and B_{REG} responses producing IL-10 and IgG4.

1.3.2 Natural immunity

Of particular interest is a population living in an endemic region of Siqueira, Minas Gerais, Brazil who appear to be naturally resistant to *Schistosoma mansoni* and were termed endemic normal (EN). Despite living in this endemic region for five years and never being treated with anthelminthics, these residents had no eggs in their stools after repeated examinations[392,393]. Surprisingly, these individuals mount different protective immune responses than those which have developed immunity slowly over time. Where acquired immunity is generally regarded as T_H2, EN individuals demonstrate the additional involvement of T_H1 based immunity[392–395].

In this group, anti-adult worm IgE levels were comparable to those of infected individuals or those with acquired resistance. However, there was a significantly higher amount of schistosomula tegument (STEG) specific IgE in EN; STEG-IgE was only seen in patients who became resistant after treatment and was not found in susceptible individuals[392,396]. As EN populations never exhibit egg excretion it is reasonable to deduce that these antibodies were developed early after initial exposure. In concert with IgE, IgG specific to *Schistosoma* paramyosin was found to be elevated in EN individuals and was also associated with a lack of eggs in stool[395].

When PBMCs from EN individuals were stimulated with a preparation of schistosome antigen there was a robust secretion of IFNγ produced by CD4+ T cells[393,397]. In the same vein that EN individuals produce schistosomula-specific IgE, this IFNγ may act primarily in the lungs to activate macrophages and cytotoxic T cells, which can kill lung-residing schistosomula which are

susceptible to oxidative burst, NO, and the release of perforin and granzymes[398–400]. The significance of IFNγ, as suggested by the data collected from the EN population, is not mirrored in the population that has developed resistance over time, suggesting the possibility that T_H1 immunity may serve as an additional benefit.

There are many mechanisms which have been put forward to explain why EN individuals exhibit this natural resistance. Some examples include: protection passed from mother to child, single sex schistosome infections incapable of egg laying, aborted or destroyed infections before worm maturation, light infections that are unable to be detected by stool examination, worms incapable of reproducing, and self-cure. In many of these cases, development of a protective immune response is still necessary and seems to be rooted in the promotion of both T_H1 and T_H2 immunity.

1.3.3 Concomitant immunity

Although WASH efforts and hygiene education have been initiated in some locales, many still lack access to uncontaminated freshwater. And as such, people living in endemic regions are constantly exposed to infectious cercariae, yet they do not develop super-infection. In 1967, Smithers and Terry demonstrated their hypothesis of concomitant immunity delivered from adult worms[401]. When adult worms were transferred into naïve rhesus macaques, following challenge with cercariae, these monkeys were almost completely protected from new schistosomes, though they were unable to clear the transferred worms. This began the theory that already established *Schistosoma* worms convey protection to the host from incoming larvae and super-infection, thereby also protecting themselves[402]. Since then, concomitant immunity has also been observed in mouse models[403–405] and this protection could be passively transferred between mice through serum transfers from infected animals[406], suggesting antibodies may play a role.

It was initially shown that concomitant immunity was only conferred when infections were made up of male and female worms and that unisexual primary infection was not able to deliver detectable resistance[407], indicating that eggs, instead of worms may be providing protection. Almost 30 years later, contrary data were published showing that five successive unisexual infections with schistosomes did in fact provide significant protection from re-infection[408]. Schistosome worms, however, are not hermaphroditic and unisexual infections do not lead to

oviposition. This recent data, in addition to studies showing that eggs alone are unable to provide resistance to schistosomiasis[409], brings the focus back to adult worms.

Adult worms may provide stimulus to produce antibodies against an array of antigens, especially those found in ES products, which are shared between life cycle stages. Pre-existing adult worms are already coated in host molecules for protection whereas there is a brief time where incoming larvae are uncoated and susceptible to binding of adult worm induced antibodies. In fact, it has been shown that sera from infected humans and animals can recognize schistosomula surface antigens[410,411].

Using *in vitro* schistosomula killing assays some mechanisms of concomitant protection have been discovered. When exposed to adult worms, macrophages from *Schistosoma* infected mice became activated and were subsequently able to kill schistosomula *in vitro*[412], possibly through TNFα. TNFα, a cytokine elevated during schistosomiasis[413,414], has been implicated in protection. It has been proposed that in schistosomiasis, TNFα results from responses to worm antigens or bacterial endotoxins which leak from the lumen into the vasculature, as intestinal eggs are released[414]. It was shown that TNFα mediated larvicidal activity at high concentrations and when combined with IFNγ it was larvicidal at low concentrations[415]. Confirming this, schistosomula experienced direct toxicity when recombinant TNFα was applied; this toxicity was then abrogated when schistosomula were cultured with activated macrophages and anti-TNFα antisera[412,415]. Differently, IL-4 knock-out mice were used to show that IL-4 immunity too, was imperative for preventing super-infection[403]. Although the idea of concomitant immunity was discovered decades ago, there is limited research regarding the mechanisms behind it; however, akin to natural immunity in EN individuals, both T_H1 and T_H2 factors seem to play a role.

1.4 Promising technologies in the field of helminth vaccines

Perera DJ, Ndao M. Promising Technologies in the Field of Helminth Vaccines. Front Immunol. 2021 Aug 19;12:711650. doi: 10.3389/fimmu.2021.711650. PMID: 34489961; PMCID: PMC8418310.

Like other helminth infections, schistosomiasis is commonly resolved using drug therapy and prevented by various methods including vector control, health education, and WASH programs. These efforts have been notable in cases such as STH in China[416], and filariasis in Thailand[417] and Sierra Leone[418], among others. Yet in many affected regions, despite MDA and WASH programs, helminths remain a problem[419,420] due to low drug efficacies, reinfection, and a lack of other control measures. Additionally, as many helminths are treated with a limited number of drugs, resistance to anthelmintics is emerging for several species[421,422]. Unlike in the case of guinea worm[423], where cases have dropped from 3.5 million in 1986 to 27 in 2020[424] by community-based education, the elimination of many other helminths can't be accomplished using singular control measures alone. To reach WHO goals of helminth elimination, various tools (MDA, vector control, education, etc.) should be combined; vaccines, making an important addition to this multipronged strategy.

Vaccination has been essential to the excision of several pathogens[425], yet to date there are no anti-helminth vaccines licensed for human use. Helminths are complex eukaryotic organisms possessing many characteristics which make their targeting by vaccination methods difficult. Helminths are multicellular invertebrates, which exhibit complex life cycles with different life stages, often occupying vectors for transmission, and infecting multiple hosts both intermediate and definitive. Through the study of paleoparasitology, we know that helminths, similar to other parasites, have co-evolved with humans[426] and have undergone unique adaptations that allow them to evade the immune system[427] often co-living undetected. These immune evasion techniques are essential to their ability to establish chronic infections.

Fortunately, the world of helminth vaccinology is not so dire. Research has shown protection among individuals, although many of these correlates remain unelucidated. In the case of lymphatic filariasis (LF), caused by species *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*, mathematical modeled studies suggest the emergence of herd immunity in endemic communities[428]. More recently, evidence has been demonstrated that prevalence of infection with LF shares a negative correlation with age. It was found that younger individuals are more susceptible to infection[429], speculating that protective immunity may be developed with time. In a similar vein, protection from *Schistosoma* spp. has been witnessed over multiple rounds of praziquantel therapy and in EN populations, as previously discussed.

Numerous studies and models have additionally shown the phenomenon of concomitant immunity where adult parasites will prevent re-infection to avoid super-infection, deleterious to both the host and themselves. Penetration from *Echinococcus* oncospheres is immunogenic and leads to significant acquired resistance against egg re-infection[430]. Similar protection has also been demonstrated in calves[431], supporting the findings that *Dictyocaulus viviparus* larvae are able to induce protective immunity from homologous re-infection[432] limiting host parasite burden. Even female LF worms, which are subcutaneously implanted, can partially protect from super-infection in animal models[433–435].

For these reasons, and the discovery and publishing of other correlates of immunity, it is our belief that there is a strong rationale for the development of effective helminth vaccines. Three vaccines for livestock are currently commercially available against *D. viviparus* (bovine lung worm), *Haemonchus contortus* (barber's pole worm), and *E. granulosus*[436]. These multi-dose vaccines are effective and reduce parasite burden up to 98%, 94%, and 100% respectively. Vaccines for human use are currently in development, with promising constructs in clinical and pre-clinical trials.

1.4.1 Immune response to helminths

The dominant immune response to helminths is widely accepted to be T_H2[437], through interactions between the innate immune system, antigen presenting cell (APC) and T helper cell complexes, and the combination of IL-4 and IL-33. This feature is reflected in many helminth species regardless of their biological niche in the body (i.e.: vasculature, intestinal lumen, subcutaneous sites, lymphatic system, etc.), and in most cases coordinated T_H2 responses have been demonstrated to protect from parasitic worms[437]. Increased levels of classical T_H2 cytokines: IL-4, IL-5, and IL-13 have been associated with lower parasite burdens[438,439] by activating eosinophils, mast cells, alternatively activated macrophages, and antibody defenses like IgE[440–442]. Additional cytokines IL-6 and IL-9 have also been implicated in immunity to filariasis, and human whipworm *Trichuris trichiura*[443–445].

Most helminths also invoke immunoregulatory responses by upregulating TGFβ production or releasing parasite-derived TGFβ mimics, to expand T_{REG} cell differentiation and promote their persistence. Macrophages and leukocytes have been implicated in IL-10 and TGFβ

production, downregulating parasite clearing T cell responses and cytokine production[363,446,447]. These regulatory elements enhance parasite survival by leading to the increase of regulatory dendritic cells (DC), B_{REG} cells, and alternatively activated macrophages – permitting the development of chronic infections. At first glance the reduction of immune activation may seem to be solely at the cost of the host, yet this IL-10 pathway also moderates destructive immune responses protecting the host from self-damage[448,449].

The upregulation of T_H2 and T_{REG} responses in helminth infections leads to a "modified T_H2" response[450], simplified in Figure 1.7. This suppresses T_H1 immunity and is further complicated by T_H17 function. Helminths which lead to liver disease tend to increase T_H17 cytokines and worsen inflammatory pathologies[451]. DC derived IL-6, TGFβ, and IL-23 cause naïve CD4+ T cell differentiation into T_H17 cells[452]. This differentiation however is dampened by both helminth-induced T_H2 and T_{REG} responses.

Although it is acknowledged that T_H2 responses are important effectors of helminth protection, it is unclear which specific immune mechanisms must be rescued by vaccination. As such, through various vaccination strategies, the search for the ideal anti-helminth immune response continues.

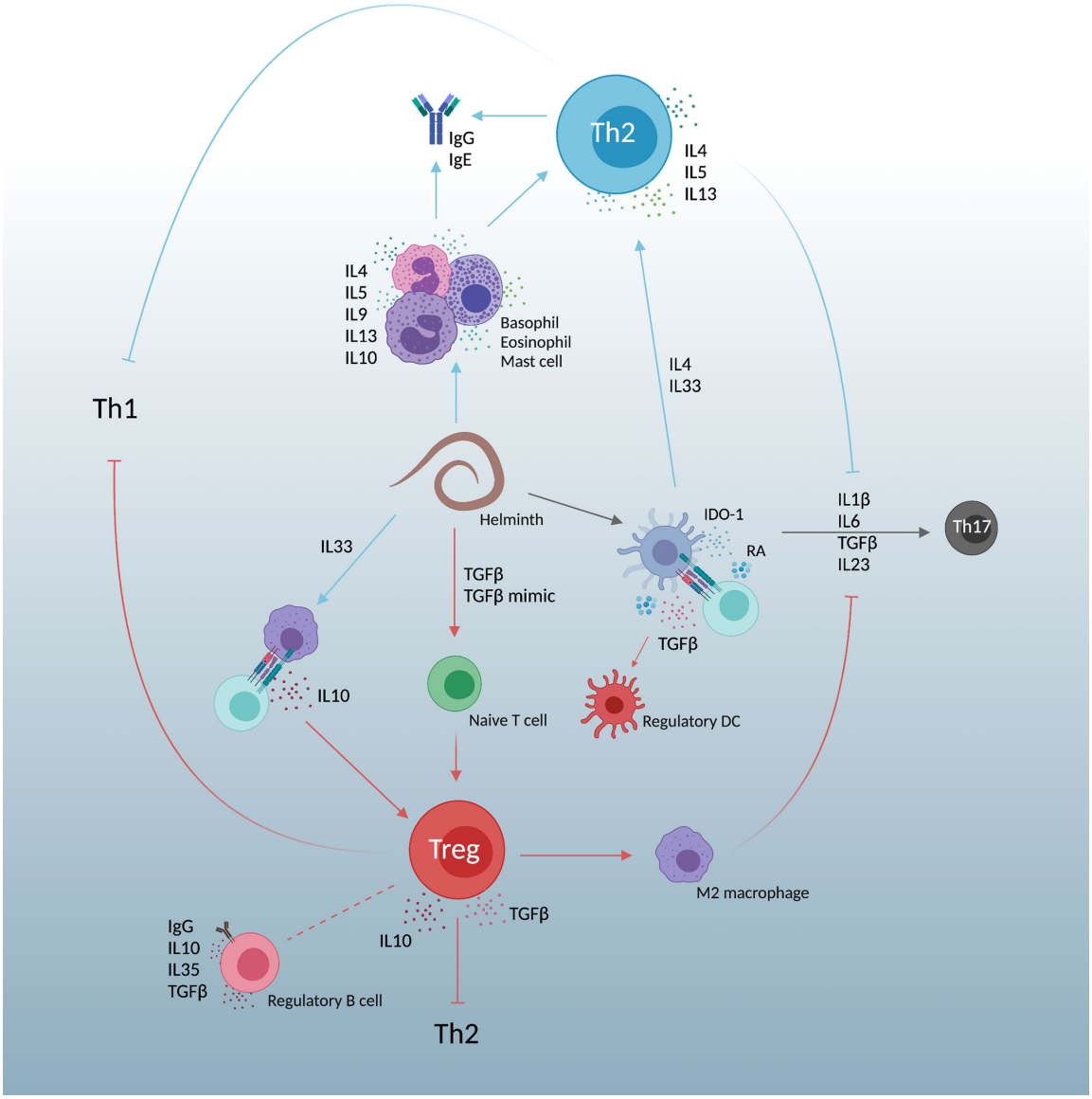

Figure 1.7 A simplified view of the "modified T_H2" response created by helminth infections

Responses are heavily T_H2, promoting IL-4, IL-5, and IL-13. Simultaneous expansion of regulatory T cell immunity by host TGFβ and parasite TGFβ mimics dampen T_H2 skewing, allowing parasite persistence and decreasing both T_H1 and T_H17 responses. This combinatory T_H2/T_{REG} response has been termed a modified T_H2 response. Created with BioRender.com. *Reproduced from Perera DJ, Ndao M. Promising Technologies in the Field of Helminth Vaccines. Front Immunol. 2021 Aug 19;12:711650. doi: 10.3389/fimmu.2021.711650. PMID: 34489961; PMCID: PMC8418310., under CC BY 4.0 licensing, Frontiers in Immunology.*

1.4.2 Irradiated helminth vaccines

Similar to attenuated viral vaccines, among the first proposed vaccines for helminths were radiation attenuated. First tested in the 1950s, and leading to the commercial vaccine Dictol®, was the live attenuated vaccine against *D. viviparus* in cattle. This vaccine is an oral vaccination with irradiated larvae that are unable to mature into adult worms but survive long enough to stimulate protective immune responses. Sprouting from this research came various vaccine constructs developed using X-ray, γ-ray, UV, and even microwave irradiation against a diverse collection of helminths including**,** but not limited to: lymphatic filarial worms[453–456], amphistomes[457], STH[458–463], *Fasciola* spp.[464–467], *Toxocara canis*[468,469], *Trichinella* spp.[470,471], *Onchocerca volvulus*[472–474], *Clonorchis sinensis*[475], and *E. granulosus*[476].

Perhaps the most widely researched irradiated helminth vaccines are against *Schistosoma* spp. Onwards of 1962, researchers tested irradiated schistosome vaccines (RA vaccines) in animal models of the most clinically relevant species: *S. mansoni*[477–479], *S. haematobium*[480–483], and *S. japonicum*[484,485]. This vaccination strategy has been referred to as the "gold standard" for years, as it consistently generates high levels of protection against challenge.

Most groups show that protection increases with additional boosting doses of irradiated parasite[460,471], although the amount of radiation is controversial and may be species specific. In 1986, a group showed that increased levels of γ radiation afforded increased protection from *Fasciola gigantica*[467]. In this study, metacercariae were irradiated with 3- and 20-krad and were used to immunize zebu calves. These vaccinated groups gave protection from adult parasites of 77% and 88% respectively, with irradiated parasites from immunizations only developing into adult worms in the 3-krad group. In contrast, Harrison et al. showed that against *S. haematobium,* cercariae that were given 2-3 doses of 20-krad radiation were more effective than doses of 3-krad, 60-krad, and much more effective than a single vaccination[483].

The correlates of immunity provided by these vaccines are debated within the literature. In the case of RA vaccines, an importance has been placed on the expression of IFNγ and T_H1 immune responses[399,486–488], despite the protection delivered by T_H2 responses for most helminths[455,462,473,489].

Although effective, these vaccines pose challenges as human vaccines for several reasons. They are composed of live parasites which require vectors and experimental models to be grown

and maintained. This makes culturing large amounts of helminths impractical. In the same vein, the heterogenous nature of eukaryotic worms makes batch manufacturing of a homogenous vaccine impossible. Additionally, in some instances these vaccines can contain viable parasites, leading to patent infections in vaccinated individuals. Due to logistical and ethical reasons it is unlikely that an irradiated helminth vaccine will be developed for human use. However, the protection mediated by these vaccines further rationalizes the ability to induce protective immunity from parasitic worms, and this research has been used to give insight on the development of promising subunit vaccines.

1.4.3 Subunit vaccines and antigen selection

To ameliorate some of the challenges of developing radiation attenuated helminth vaccines, many recent strategies utilize subunit vaccines. In this case the most promising components, or antigens, which best stimulate the immune system are administered. By carefully selecting appropriate antigens, vaccine development can be targeted to enhance protection and minimize possible side effects[490]. Antigens are habitually identified by proteomic analysis on crude homogenates of helminths, and then chosen based on immunogenicity and their ability to stimulate an immune response. In recent years, new technologies have allowed the identification and prediction of antigens using immunoinformatics[491,492] and *in silico* approaches which use computer software to anticipate T cell epitopes given pathogen genome analysis[493]. These antigens can then be isolated from parasites, produced as recombinant proteins, or delivered using innovative vaccine strategies.

1.4.4 Combining recombinant proteins with novel adjuvants

To better enhance immune responses, several vaccine efforts have employed the use of novel adjuvants - some of which have not yet been approved for human use. Antigens used as vaccine targets often lack immunogenicity. Helminth antigens, capable of stimulating immune responses, have been known to possess inbuilt adjuvanticity[494,495]. Nevertheless, adjuvants can be used to augment or skew immunogenicity to enhance protective effects. The most common adjuvants used in US vaccines include: formulations of aluminum, AS04, MF59, $AS01_B$, and CpG dinucleotides[496]. These adjuvants are effective and have demonstrated protective capacities,

explaining their use in clinical applications; however, the field of adjuvant discovery is expanding, and several innovative adjuvants are in pre-clinical trials with promising results.

Many helminth vaccines in clinical development utilize the synthetic toll like receptor (TLR) 4 agonist, glucopyranosyl lipid adjuvant (GLA). Naturally, some pre-clinical efforts have followed the same path. A recent effort to protect from *S. mansoni* involved adjuvanting the parasitic large subunit of calpain (Sm-p80) with a stable emulsion of GLA (GLA-SE). This vaccine, tested in baboons, demonstrated a female worm specific reduction of 93.45%, with an overall protection of 65.9%[497]. Although adult worm reduction is enticing, perhaps a more important metric is the reduction of tissue eggs which, in support of this vaccine, is 89.95%. These measures greatly surpass the 40% vaccine standard set forth by the WHO, and almost reach a 70% vaccine standard which may be more appropriate considering recent vaccine efforts[498]. GLA-SE has also provided protection from LF in a *B. malayi* mouse model. In this case, a tetravalent vaccine was prepared using the following recombinant antigens: heat shock protein 12.6, abundant larval transcript-2, tetraspanin large extracellular loop, and thioredoxin peroxidase (rBm-HAXT). After the three-dose immunization schedule, administered subcutaneously, vaccinated animals displayed high titers of antigen specific IgG in serum and peritoneal fluid, predominating in IgG1 with expansions of IgG2. A significant protection of 88.05% was obtained, compared to 79.47% and 78.67% given when rBm-HAXT was adjuvanted with conventional alum and mannosylated chitosan (MCA), respectively. All vaccines tested increased the percentage of central memory T cells (T_{CM}) in the spleen, with an increased expression of IFNγ specifically in those cells from the GLA-SE vaccine arm[499]. Unfortunately, when this vaccine was tested in a non-human primate (NHP) model, despite adding another boosting immunization, vaccine efficacy dropped drastically to 57.14% protection[500]. Interestingly, in this different animal model, the immune landscape conferred by this vaccine seems to be more balanced T_H1/T_H2 versus the mouse model where the T_H1 bias was apparent in splenocyte expressed cytokines IFNγ and IL-2, among others. In the NHP study, T_{CM} cells were found to be expressing more IL-4, while effector memory T cells (T_{EM}) were found to be contributing the IFNγ. This balanced response was seen in PMBC cytokine expression of IFNγ, IL-12p70, IL-4, IL-5, and TNFα, among others. Despite the drop in parasite burden reduction, antibodies developed in the NHP study were able to mediate protection via antibody dependent cellular mediated cytotoxicity (ADCC) by recognizing, covering, and killing L3 larvae *in vitro.*

ADCC has been a proposed mechanism of protection in other helminth vaccines including our work using an adjuvanted *S. mansoni* cathepsin B (SmCB). The larval stage of *S. mansoni* passes through the lungs and is vulnerable to antibody and cell mediated effectors. When we combined SmCB with Montanide ISA 720 VG (ISA 720), lung stage protection was reported to be provided by ADCC, specifically through macrophages, natural killer (NK) cells, and CD4+ and CD8+ T cells[398]. By conducting *in vitro* larval killing assays with and without cells and sera from immunized mice it was shown that CD4+ T cells and NK cells were able to significantly increase killing only in the presence of immune sera. The necessity of cells and sera from immunized animals for larval killing makes an argument that protection is mediated by ADCC. In the context of a SmCB and ISA 720 immunized lung, CD4+ T cells and NK cells are key players in parasitic killing, aided by CD8+ T cell and macrophage killing dependent on antibodies. The resulting protection from this vaccine was approximately 60% with a mixed T_H1/T_H2 based immune response[501]. Seppic-produced Montanide series adjuvants are produced with good manufacturing practice (GMP); and ISA 720 as well as Montanide ISA 51 VG have been developed as human therapeutics. In addition to these are many other Montanide adjuvants which are used in veterinary vaccines. ISA 720 has also been used in a vaccine against *Ascaris suum* although protection was higher when the target antigen, As16, was formulated in alum[502]. Despite the protection afforded by the T_H1/T_H2 skewing *S. mansoni* vaccine, in the case of *A. suum,* protection was increased with T_H2 responses. Other Montanide based vaccines have been developed against *Fasciola* spp.[503–506]*, S. japonicum*[507]*, T. spiralis* [508,509]*,* and LF[510], to name a few.

S. mansoni vaccines have been in development for over 30 years and recent work has been conducted using the adjuvant adaptation (ADAD) system originally developed in 2004 for *F. hepatica*[511]. This vaccination system involves two subcutaneous injections. The first "adaptation" immunization contains a combination of synthetic aliphatic diamine and saponins emulsified in a non-mineral oil. Five days later a second immunization is given with the same elements including antigen. A three-dose vaccine developed using predicted B and T cell epitopes of a *S. mansoni* kunitz-type serine protease inhibitor was tested using the ADAD system. Both epitopes used conferred protection; however, the T cell epitopes delivered a slightly higher parasite burden reduction of 91% in female adult worms than the B cell epitope vaccine (89% reduction). Interestingly, both these vaccines acted specific to worm sex and male adult worms were unaffected. Contrary to the lower reduction in worms, the B cell epitopes delivered a higher

reduction in gut liver eggs (77-81%) compared to the T cell epitope vaccine (57-77%); still, both had a reduced number of egg-induced granulomas[512]. A second vaccine was also tested against *S. mansoni* using the ADAD vaccination system. Two vaccines were developed comparing recombinant protein expression systems, both of them two-dose, targeting *F. hepatica* fatty acid binding protein (Fh15 (*E. coli* expressed), and Fh15b (baculovirus expressed)) which show a 44% identity to Sm14, an antigen in clinical trials as a *S. mansoni* vaccine target. This study demonstrated that when expressed in *E. coli*, Fh15 delivered higher protection from schistosomiasis than when expressed by baculovirus. This finding supports the hypothesis that post-translational modifications by different expression systems can impact the immune response elicited by recombinant antigens, as this group found that delivering a baculovirus expressed Fh15 resulted in an impairment of the humoral response[513]. The *E. coli* expressed Fh15 vaccine reduced parasite burden by 64%, 69%, 58%, 67%, 61%, and 77% in adult worms, female worms, male worms, hepatic lesions, and eggs per gram of liver and intestines, respectively. Despite this protection being lower than the protease inhibitor ADAD vaccine, the reality of a schistosome vaccine targeting a *Fasciola* antigen could mean cross protection from both helminths which is incredibly alluring for co-endemic regions.

Although the ADAD system seems to be effective, the advancement of this vaccine strategy may prove to be infeasible as each immunization consists of two injections five days apart. The first vaccine described would result in six necessary injections, and the second would be four. In endemic regions, vaccine compliance and the lack of infrastructure will challenge the ability to properly vaccinate the population. Current helminth vaccines use three immunizations as a standard, however effective vaccine strategies requiring fewer boosting immunizations should be explored.

1.4.5 Nucleic acid vaccines

In recent years, the push for nucleic acid vaccines have become more prominent. To our knowledge, as of 2023, there were no RNA vaccines developed against helminthic infections; however, DNA vaccines have been tested since the early 2000s. Genetic vaccines deliver antigen RNA or DNA which are then translated within the host for *in vivo* antigen expression. Internal delivery of antigens using DNA is compelling as it is easy to manufacture and has been

demonstrated to induce both humoral and cell-mediated immune responses in animals[514,515]. DNA is also stable at ambient temperatures[516] which is highly practical for use in endemic and rural regions.

A common obstacle of DNA vaccines seems to be a lack of immunogenicity when used in humans, which groups have been attempting to ameliorate by using molecular adjuvants and advanced DNA vector design[517,518]. DNA vaccines may also pose safety risks associated with biodistribution and persistence, as well as the potential for plasmid-based vaccines to be integrated into the microbiome genome. To subside some of these fears, Liu et al. showed that although their *S. japonicum* plasmid-based DNA vaccine can be found in every tissue site tested, it was successfully cleared by day 120[519]. Additionally, after vaccination with their plasmid containing a hygromycin resistance gene, they plated the intestinal microflora on hygromycin containing plates and saw no growth, demonstrating that the microbiome of vaccinated animals was not found to uptake the plasmid. To confirm, they also ran a PCR for the hygromycin gene and their gene of interest, in DNA extracted from intestinal and excremental samples and obtained negative results. To date there are no DNA vaccines approved for human use, although four are licensed for veterinary use[520] and there are over 600 clinical trials that focus on DNA vaccination registered in the USA.

Several efforts have been made to produce a DNA vaccine against *T. spiralis*. In 2013, Tang et al. published their data of a vaccine which reduced parasite burden by 37.95%[521]. This vaccine encoded two antigens (*T. spiralis* macrophage migration inhibitory factor (TsMIF) and multi-cystatin-like domain protein 1 (Ts-MCD-1)) and was delivered in two doses. They found their vaccine to stimulate T_H1 responses, increasing IFNγ with no significant changes to IL-4 and IL-5 expression similar to the results of a *S. mansoni* DNA vaccine which only reduced parasite burden by 30%[522]. In the same year, a DNA vaccine delivered in 3 doses alongside recombinant protein (Ts87) was able to increase parasite burden reduction to 43.8%[523]. This vaccine increased both T_H1 and T_H2 immune responses with cytokine expression increases of IL-2, IL-4, IL-6, and IFNγ. It is interesting to note that optimal antigen selection is crucial for protective immunity from helminths. Although the TsMIF+Ts-MCD-1 vaccine was only partially protective, a DNA vaccine expressing *T. spiralis* 43 kDa and 45 kDa glycoproteins was able to confer protection of 75.9%[524]. This vaccine also deployed a mixed T_H1/T_H2 immune response alike the Ts87 vaccine, without the need for additional recombinant proteins. The Ts43+Ts45 vaccine showed increases of IFNγ, IL-4, and

IL-10, but more striking was the increase in the percent of B220+ B cells when compared to both single antigen DNA vaccines and the PBS control. This data supports the idea that both T_H1 and T_H2 arms of immunity can work synergistically to protect from parasitic worms.

The most significant parasite burden reductions afforded by DNA vaccines can be seen in their use against *B. malayi* and LF. Gupta et al. has demonstrated the protective efficacy of two DNA vaccines, both of which use a heterologous DNA prime and recombinant protein boost strategy targeting a myosin gene. An initial endeavor gave two immunizations of DNA followed by two protein boosts adjuvanted by Freund's incomplete adjuvant (FIA). This vaccine reduced parasite burden by 75.3% and showed a 78.5% reduction in microfilarial density in the blood[525]. Antibody responses were shown to kill L3 larvae, and cytokines IL-2, IFNγ, TNFα, IL-12, IL-4 and IL-10 were increased after immunization and maintained through challenge. This immunogenicity was increased when their DNA vaccine was delivered along with CpG dinucleotides, and in replacement of FIA. This 4-dose vaccine was now found to reduce parasite burden by 84.5% with similar cytokine expression, and the additional proliferation of CD4+ T cells, CD8+ T cells, and CD19+ cells. Likely, the increase of DC activating, and T cell associated markers (CD40, CD80, CD86) observed on DCs from vaccinated animals combatted the APC dysfunction and lack of T cell responsiveness common in filarial infection[526,527].

DNA vaccines typically promote T_H1 immune responses. Although these show promise in models of LF, other helminths may be better targeted by alternative methods of vaccination. This can be seen more explicitly in the Sm-p80 vaccine which has been tested in baboons against *S. mansoni* delivered both by DNA vaccines and as adjuvanted protein. The Sm-p80 vaccine has been in development for over a decade, optimizing through various vaccine platforms and adjuvant combinations[528]. The most significant protection data from the Sm-p80 DNA vaccines were obtained when a DNA vaccine was boosted by recombinant protein adjuvanted with CpG dinucleotides which reduced parasite burden by 47.34%[528]. This reduction was less enticing than that of their adjuvanted recombinant protein vaccine using GLA-SE (65.9%)[497]. Zhang et al. conducted RNA-sequencing on PBMCs, spleen and lymph node cells from baboons vaccinated with their various Sm-p80 vaccines to determine functional immune profiles from each. The DNA vaccine presented a relative T_H1-mediated immune response with 8.41% of its differentially expressed genes in PBMCs relating to the TLR9 signalling pathway. This was reflected by T_H2 pathways in spleen cells, and iCOS-iCOSL signaling in PBMCs and spleen cells predicted to be

deactivated. This downregulation of T_H2 immunity may have diminished protective correlates, as in previous reports Sm-p80-mediated protection seems to be enhanced by antibody responses[497,529].

Supported by a hypothesis by Versteeg et al.[530], an interesting avenue of research will be the development of RNA vaccines for helminths which, given the efficacy of their COVID-19 counterparts, can be expected in the near future. Their versatility and simple means of production made mRNA vaccines a top contender for prophylactic SARS-CoV-2 vaccines and were exploited by the two earliest vaccines made available by the FDA for COVID-19 emergency use[531,532]. RNA vaccines are shown to stimulate potent and safe immune responses in animals[533] and humans[534–536]. Although their stability requires a cold-chain, mRNA vaccines have a low-cost manufacturing process and unlike DNA vaccines, they remain outside the host cell nucleus making them an attractive vaccine vector worth exploring.

1.4.6 Viral vectored vaccines

Since the discovery of vaccines and the efficacy of live attenuated virus vaccines to protect from their wildtype counterparts, the concept of using viruses to fight other infectious agents has evolved over time. Around the introduction of nucleic acid vaccines like DNA and RNA vaccines, delivering genetically modified viruses arrived as another vaccine platform. Viral vectored vaccines utilize the natural infectivity of viruses and their "life" cycle, using host machinery to translate their own genetic elements and incorporated antigens. By using infectious agents to deliver vaccine targets potent cellular responses can be elicited, specifically CD8+ cytotoxic T cells. As in the case of nucleic acid vaccines, since vaccine encoded genes are expressed intracellularly, antigens can be processed and presented on class I MHC (MHC-I) of APCs. These approaches may be favoured for diseases where cell-mediated immunity can significantly enhance protective responses afforded by humoral immunity or in those cases where antibody production alone is insufficient.

Viral vectored vaccines have been developed against Lassa fever[537], HIV[538], malaria[539], taeniasis[540], and countless others. As each viral vectored vaccine can utilize a different virus, each of their mechanisms of antigen presentation and immune stimulation will vary according to the nature of the virus used. Commonly used viral vectors are adenoviruses, pox viruses, and vaccinia

viruses as they are well characterized, each with their own unique features. While there are many other viral vectors that have been used, we will only touch on some of them.

One of the largest limitations to viral vectoring is neutralizing antibodies to the vector from previous exposure. Vaccine priming doses can also produce neutralizing antibodies which may render boosting immunizations useless. Despite groups showing robust T cell responses regardless of the presence of neutralizing immunity[541] to circumvent this problem, heterologous prime-boost strategies, varying vaccine immunization routes, and the use of viruses which do not circulate in target populations, have been employed.

Several groups have utilized the antigen EG95, a protective vaccine target for *E. granulosus*, demonstrating its expression using goatpox virus[542], morbillivirus[543], and on the surface of orf virus[544,545]. Protective benefits of only one *E. granulosus* viral vectored vaccine has been published to date, and this was in a vaccinia virus vector[546]. In this model, mice were immunized with 10^8 plaque forming units (PFU) of recombinant vaccinia virus, intranasally. Some groups of mice were given a boosting immunization of either recombinant virus or recombinant EG95 prepared with alum and delivered intraperitoneally. The reverse was also tested, where adjuvanted antigen was the priming immunization for a recombinant virus boost. Analysis of EG95-antibody responses showed the highest titers of Ig in mice which received virus prior to adjuvanted protein, followed by the group administered the reverse. Protective capacity was demonstrated *in vitro* using an oncosphere killing assay. In this experiment, mouse anti-sera were applied to oncospheres and the highest dilution in which killing was observed was reported. The group in which the highest dilution of sera provided killing was the group first immunized by virus followed by adjuvanted protein. The trend follows that the amount of antigen specific immunoglobulin may be correlated with protection, as those groups with higher titers observed killing at higher dilutions.

The earliest viral vector for *Schistosoma* spp. developed for *S. mansoni* showed no antigen specific antibody response or protective efficacy[547]; however, since then, several have been deployed as a vector for *S. japonicum.* In 2010, data were published on a pseudorabies virus (PRV) expressing both *S. japonicum* fatty acid binding protein (FABP) and 26 kDa glutathione-S-transferase (Sj26GST)[548]. This vaccine was administered in two doses and increased IL-2 expression from stimulated splenocytes when compared to the negative control. The virus expressing both antigens even significantly increased IFNγ production higher than either viral

vaccine expressing single antigens. The protection they observed in mice (39.3% reduction of worms) was less than that of sheep (48.5% reduction of worms) inferring the importance of the animal model used.

Attenuated pseudorabies viruses have been developed against many infectious diseases and have been comprehensively reviewed[549]. Although in some cases PRV vectored vaccines have been effective, groups have shown faster antibody responses and recruitment of cell-mediated immunity using adenoviral vectors[550]. This schistosomiasis protection delivered by PRV was inferior to another group which expressed *S. japonicum* triosephosphate isomerase using a human adenovirus serotype 5 vector (hAdV5).

An initial study in mice looked at the immune responses and protective efficacy elicited by various administration routes of a recombinant adenoviral vector: including intramuscular (IM), subcutaneous (SC), and oral administration. Vaccines were administered in three doses, each giving 10^8 PFU of virus. Oral immunization did not result in a humoral response or significant protection. Although robust antibody titers to antigen were observed at the study endpoint in the IM and SC routes of administration, the question of neutralizing antibodies was not addressed, and humoral responses were not measured throughout immunization. Interestingly, the route of administration caused a shift in the isotype of IgG expressed. In the case of SC immunization, IgG1 was significantly expanded over the IgG2c which was promoted in the case of IM administration. This immune skew was reflected in ELISPOT data from stimulated splenocytes. Splenocytes from mice in the SC vaccinated group had a higher proportion of IL-4 secreting cells to IFNγ, and in the IM group the opposite was observed. Although both routes delivered protection from infection, the IM or T_H1 skewed response may be more desirable giving 54.92% reduction compared to the SC 37.50%[551]. Protection was further increased when their recombinant adenovirus was delivered in a heterologous prime boost strategy using recombinant protein adjuvanted with FIA. This vaccination method promoted expansion of both antigen specific IgG1 and IgG2c isotypes and reduced parasite burden by 72.09% in adult worms, compared to the recombinant adenovirus alone and adjuvanted protein arms which reduced adult worms by 50.59% and 26.67%, respectively[552].

This wave of viral vectoring in vaccinology has been used in many pre-clinical applications and has expanded into human use in the past years[553–555]. Other viral vectors of interest which may be considered include CMV, vesicular stomatitis virus, and measles virus due to their large carrying

capacities[556], and Newcastle disease virus due to the lack of pre-existing virus immunity in human populations[557]. Although their use in helminthology is lean, viral vectored vaccines allow for enhanced immunogenicity when compared with other genetic vaccine vectors and should be further investigated.

1.4.7 Helminth vaccines in clinical trials

To date, the only helminth vaccines in clinical trials have been developed using recombinant protein technology, generally with adjuvants to enhance immunogenicity as summarized in Table 1.2. These vaccines showed promising data in pre-clinical studies and have been largely safe and immunogenic in humans. The one exception being the Na-ASP-1 vaccine for human hookworm. Due to the generalized urticaria witnessed with this vaccine[558], hookworm vaccine efforts have shifted to other antigens such as Na-GST-1 and Na-APR-1.

Currently, the only completed phase 3 trial with reported efficacy data is the vaccine for urinary schistosomiasis. Unfortunately, this 4-dose vaccine regimen proved to be ineffective in providing sufficient protection from *S. haematobium* when looking at the delay in reinfection between experimental groups[559]. The authors hypothesize that an expansion of antigen specific IgG4 hinders protective responses of IgG3. They also explain that their efficacy readout was suboptimal, as they were unable to visualize differences in infection intensity in the vaccine group versus the control. Despite this lack of efficacy, the confirmed safety of the Sh28GST vaccine in humans and the availability of other promising vaccine constructs in pre-clinical testing, suggest the probability of a protective helminth vaccine in the foreseeable future.

Table 1.2 Helminth vaccines in human clinical trials

Target Antigen	Adjuvant	Doses	Pathogen	Phase	Ref
Glutathione-s-transferase (Na-GST-1)	Alhydrogel Alhydrogel + CpG Alhydrogel + GLA-AF	3	Hookworm	1; complete	560–562
Aspartic protease (Na-APR-1)	Alhydrogel Alhydrogel + GLA-AF	3	Hookworm	1; complete	563
Na-GST-1 + Na-APR-1	Alhydrogel + GLA-AF	3	Hookworm	1; complete	564,565
L3 larvae		3	Hookworm	N/A; complete	566
Ancyclostoma-secreted protein (Na-ASP-2)	Alhydrogel	3	Hookworm	1; complete and halted	558,567–569
Glutathione-s-transferase (Sh28GST)	Alhydrogel Alum	4 2,3	*Schistosoma haematobium*	3; complete 1; complete	559,570 571,572
Sm14	GLA-SE	3	*Schistosoma mansoni*	2/3; ongoing 2; complete 1; complete	573 574,575 576,577
Tetraspanin (Sm-TSP-2)	Alhydrogel Alhydrogel + AP 10-701 Alhydrogel + GLA/AF	3	*Schistosoma mansoni*	½; recruiting 1; complete 1; complete	578 579 580

1.4.8 Helminth vaccine induced protection

The central dogma around helminth protection has encompassed T_H2 immunity for decades. This includes cytokines IL-4, IL-5, Igs, and eosinophils. IL-4 is an important mediator of T_H2 cell differentiation and the activation of the class switching mechanism of B cells to produce IgE, whereas IL-5 is a potent growth and survival signal for eosinophils. Eosinophils are correlated with helminth infections[581] and although their protective effects are unclear[582,583], *in vitro* studies have demonstrated helminth killing in models of *Schistosoma*[584,585] and *Strongyloides*[582]. Yet recent efforts are finding effector mechanisms of cell mediated immunity providing protection from various helminth species. Although the T_H2 response clearly drives protection in many models, several of the most promising vaccine candidates mentioned here tend to elicit both T_H1 and T_H2 arms of immunity, as summarized in Table 1.3.

Helminths are complicated eukaryotes which have evolved to promote regulatory T cell responses, dampening T_H2 type immunity, and allowing their chronic persistence. While vaccines may strive to boost T_H2 responses, they should maintain T_{REG} responses as regulation plays a vital role in reducing parasite driven pathology; this is especially important in schistosomiasis where T_{REG} responses protect from immunopathological damage[586–588].

The contribution of the type 1 response may be underappreciated and underestimated, but vaccines targeting helminths, especially at vulnerable larval stages, may enhance this immunity. In the case of *Echinococcus*, T_H1 responses were shown to be protective[589]. Additionally, a key cytokine of the T_H1 response, IFNγ, plays in important role in protection from filariasis[590]. Although in natural infection models, the immunity afforded by nonspecific T_H1 responses is diminutive, protection in vaccine models is more pronounced through the expansion of focused cell mediated immunity and T_H1 cytokines. It is possible that a carefully balanced immune response, eliciting multiple immune mechanisms directed at vital parasitic molecules, could be the key to protection from helminthic infections.

As worms mature through multiple life cycle stages within the host it is also reasonable that these various facets of immunity can act at different points in time. Where T_H2 immunity is often highly effective against adult worms, T_H1 and innate immunity may better target juvenile stages of worms travelling through skin and mucosal sites.

Other innate immune cells which may provide direct protection from helminths are neutrophils and NK cells. Neutrophil extracellular traps (NETs) are web like chromatin structures that are known to protect against large pathogens[591]. Research into NETs in helminthic contexts has shown that they negatively impact the fitness of hookworm larvae after skin penetration[592]. NETs are also able to trap *Strongyloides* larvae *in vivo*, potentially making them vulnerable to the neutrophil, eosinophil, and macrophage killing seen *in vitro*[593]. Neutrophils may be an interesting target for other helminths which spend time passaging through skin sites such as *Schistosoma*, and *Onchocerca*. NK cells are not well studied in helminth vaccines, and despite their relation to the innate immune system, there is evidence that these cells can be long lived and acquire antigen specific memory[594,595]. Previous work using recombinant *O. volvulus* ASP-1 induced a dominant IFNγ response, likely produced by activated NK cells[596]; albeit NK cells can also act through ADCC and kill via degranulation. NK cells may recognize antibody-sequestered parasite and release perforin, granzyme, and granulysin, the latter two of which are upregulated in vaccinated animals against *Ostertagia*[597].

It is difficult to concretely attribute immune effectors of helminth protection provided by vaccines although a collection of some protective responses can be seen in Figure 1.8. Despite many studies seeking to identify protective mechanisms in infection models, most helminth vaccine studies give only broad descriptions of the conferred immune landscape, and systemic immune cell responses after vaccination. A few groups have looked more in detail at vaccine induced immune mechanisms, however, more emphasis should be placed on immune effector knock outs and passive transfer experiments. To find the essentials for helminth protection, we must not only look at general immunogenicity but also on specific responses critical for helminth killing.

Table 1.3 Summary of promising helminth vaccines

Vaccine Platform	Parasite	Target Antigen	Doses	Parasite Reduction	Animal Model	Immune Skew	Ref
Adjuvant (QuilA)	*Fasciola hepatica*	Cathepsin L1 mimotopes	2	79.5% worms	Goats	T_H1/ T_H2	598
Adjuvant (cholera toxin B subunit) intranasal	*Trichinella spiralis*	Serine protease	3	71.1% worms 62.1% muscle larvae	BALB/c mice	T_H1/ T_H2/mucosal IgA	599
Adjuvant (GLA-SE)	*Schistosoma mansoni*	Sm-p80	4	65.9% worms 91.4% liver eggs 88.8% intestinal eggs	Baboons	T_H2	497
Adjuvant (ADAD)	*Schistosoma mansoni*	B-cell epitope of Serine protease inhibitor	3	89% female worms only 77% intestinal eggs 81% liver eggs	BALB/c mice	T_H2	512
Adjuvant (AddaVax)	*Schistosoma mansoni*	Cathepsin B	3	86.8% worms 78% liver eggs 83.4% intestinal eggs	C57BL/6 mice	T_H1/ T_H2/ anti-inflammatory	600
Adjuvant (GLA-SE)	*Brugia malayi*	Tetravalent fusion protein	3	88.1% larvae	BALB/c mice	T_H1/ T_H2	499
DNA/Adjuvant (CpG) prime, Protein/Adju	*Brugia malayi*	Heavy chain myosin	4	84.5% larvae	BALB/c mice	T_H1	601

vant (CpG) boost							
DNA	*Trichinella spiralis*	Co-administered Ts43 and Ts45	3	75.9% muscle larvae	BALB/c mice	T_H1/ T_H2	524
DNA	*Schistosoma japonicum*	2 Co-expressed bivalent fusion proteins (tetravalent)	1	70.8% worms 60.7% liver eggs	BALB/c mice	not determined	602
Adenovirus prime, Protein/Adjuvant (Freund's incomplete) boost	*Schistosoma japonicum*	Triosephosphate isomerase	4	72.1% worms 72.1% liver eggs	BALB/c mice	T_H1/ T_H2	552

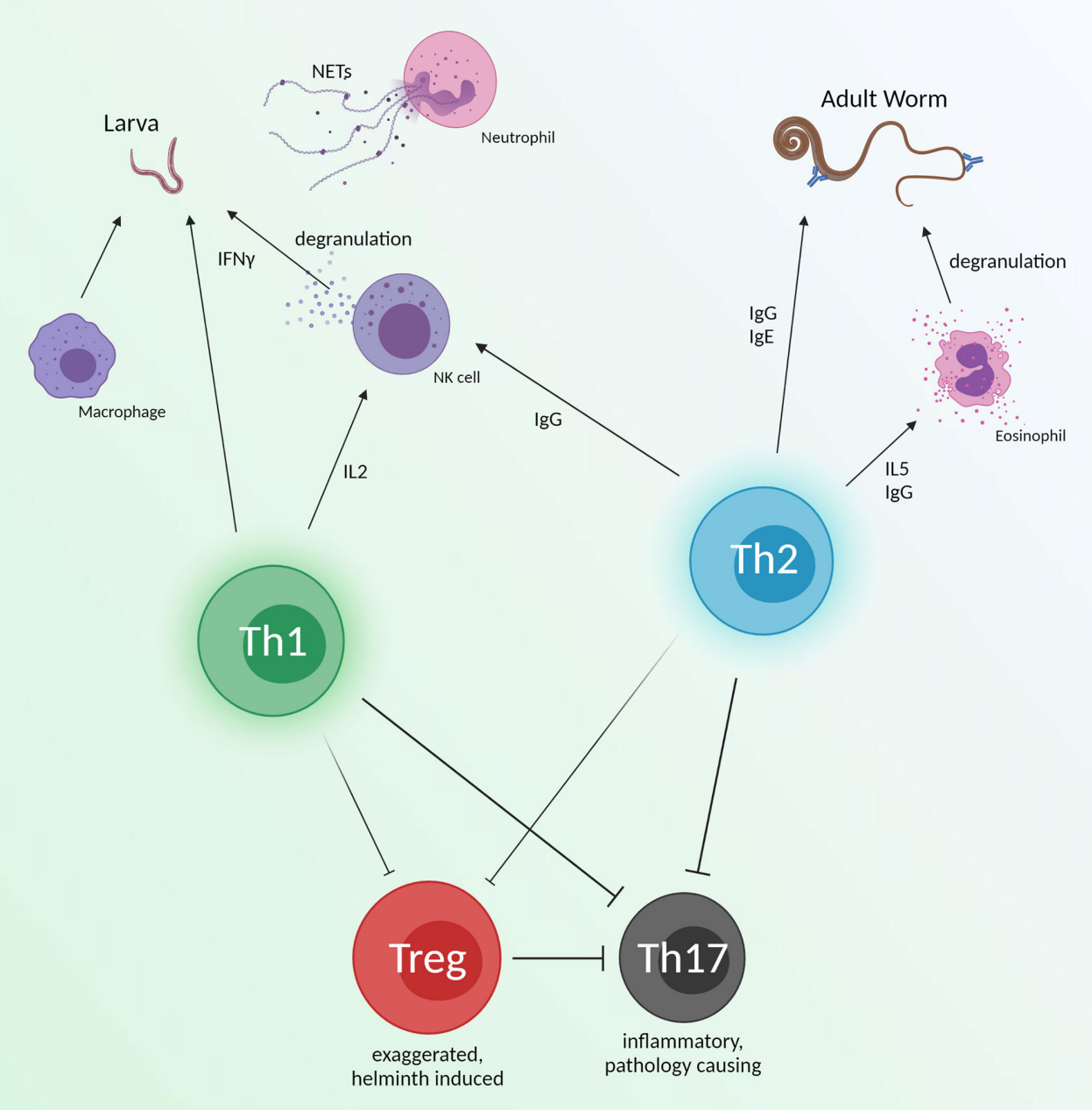

Figure 1.8 An overview of immune effectors which have shown helminth killing

These responses are broad and could be an ideal immune response for vaccines to emulate. T_H1 and T_H2 responses may work synergistically with innate immunity to directly target juvenile and adult worms. Simultaneously, helminth induced T_{REG} responses will be diminished, but still prevent inflammation from T_H17 function. Created with BioRender.com. *Reproduced from Perera DJ, Ndao M. Promising Technologies in the Field of Helminth Vaccines. Front Immunol. 2021 Aug 19;12:711650. doi: 10.3389/fimmu.2021.711650. PMID: 34489961; PMCID: PMC8418310., under CC BY 4.0 licensing, Frontiers in Immunology.*

1.4.9 A vaccine for schistosomiasis

Each vaccine platform discussed brings forth its own set of positive and negative attributes; each of them, when combined with an antigen may elicit unique features of the immune system. Some commonly observed advantages and disadvantages of the platforms discussed are described in Table 1.4, considering each platform independently. Adjuvanted recombinant protein vaccines are well studied and have been shown to be highly effective in animal models. However, recombinant protein expression systems and adjuvants can be expensive which may not be ideal for a vaccine that is geared for use in the Global South. With the authorization of vaccines utilizing mRNA-based and viral vectored technology, catalyzed by the 2019 coronavirus pandemic, new avenues of vaccine development have been opened, ones which are arguably easier to develop and more cost-effective. To ameliorate disadvantages in these platforms some groups have employed heterologous prime boost strategies to help carve a desired immunotype, thereby increasing protective responses. To this end, there is a solid foundation to explore novel vaccination strategies which can be applied to helminths like schistosomiasis.

Table 1.4 Advantages and disadvantages of various vaccine platforms

Vaccine Platform	Advantages	Disadvantages	Key Features
Irradiated Parasite	• Strong protection, especially with increasing doses	• Heterogenous vaccine • Parasite life-cycle dependent for development • Unethical as some parasite may mature to adult stage	• Mimic natural infection
Adjuvanted Protein	• Various adjuvants will give different	• Can be expensive to produce	• Immune response varies

	immune responses • Shown to be effective • Can be used in populations with weakened immune systems		depending on adjuvant used
Nucleic Acid (DNA based)	• Quick and simple design • Thermostable • Cost effective	• Low immunogenicity in humans • Enters host cell nucleus	• Mostly cell mediated immune responses
Viral Vectored	• Specific delivery of antigen to target cells • High antigen expression • Gene expression can be short or long term	• Neutralizing immunity • Off target virus shedding	• Humoral responses • CD8+ T cell responses • T_H1 dominant CD4+ T cell responses

1.5 Rationale and research objectives

The WHO has targeted the elimination of schistosomiasis by 2030 due to the severity of disease and the number of people affected and at risk. MDA of PZQ while effective, has yet to effectively curb schistosomiasis transmission. Even when combined with other control measures such as vector control and WASH programs, the burden of schistosomiasis continues. To help eliminate this parasite at a worldwide scale, the development of a prophylactic vaccine has been proposed by several. This includes 50 experts who have ranked the top 10 diseases for which vaccines are urgently needed, schistosomiasis ranking #7, the highest-ranking parasitic infection on the list[603]. In fact, mathematical modelling has provided support to the hypothesis that an effective schistosomiasis vaccine does not even need to provide sterilizing immunity; a partially protective vaccine would contribute to reducing infection and interrupt endemic transmission[252,604,605]. The following chapters describe the formulation of schistosomiasis vaccines utilizing *S. mansoni* cathepsin B (SmCB) and various vaccination platforms.

SmCB is an essential *S. mansoni* gut peptidase used for host blood macromolecule digestion and nutrient acquisition (Figure 1.9). It is expressed by migrating schistosomula and adult worms; targeting this enzyme creates an opportunity to abrogate the infection before sexually mature worms begin laying pathology causing eggs. Although SmCB is immunogenic on its own, to alter immune responses to this peptide we coupled it with several adjuvants to identify which would provide the highest protection from *S. mansoni* (Chapter 2).

Alternative vaccine platforms include vaccine vectors which utilize attenuated pathogens to express target antigens. A commonly used vector in vaccinology is hAdV5 due to its large carrying capacity, limited side effects, and low cost for development. Cost is an important consideration when developing a vaccine for schistosomiasis since most at risk of infection live in regions of poverty and have limited financial resources. Viral vectoring also provides unique immune responses which may not be achievable using an adjuvant alone. We then used hAdV5 to express SmCB to determine if protection could be increased using this platform (Chapter 3).

The success of our previous vaccine strategies led us to believe that combining these efforts may create a synergy between their unique mechanisms of action, amplifying immune responses and protection. Helminth vaccines are typically three doses to provide optimal and durable immunity. In this final chapter, we created a heterologous recombinant hAdV5 prime and

adjuvanted SmCB boost vaccine, to integrate the efficacies of both strategies (Chapter 4). The work contained in this thesis describe multiple schistosomiasis vaccine endeavours, each of which holds the potential for advancing into clinical trials and contributes to the existing body of knowledge regarding correlates of immunity associated with schistosomiasis protection.

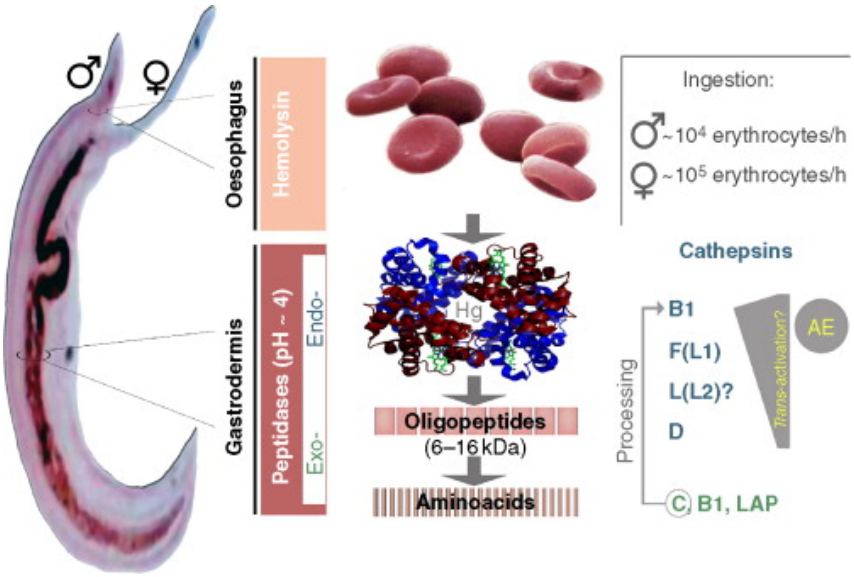

Figure 1.9 *Schistosoma mansoni* cathepsin B

Adult worms express several protein peptidases which process blood macromolecules for nutrient acquisition. *Reproduced with permission from Kasný M, Mikes L, Hampl V, Dvorák J, Caffrey CR, Dalton JP, Horák P. Chapter 4. Peptidases of trematodes. Adv Parasitol. 2009;69:205-97. doi: 10.1016/S0065-308X(09)69004-7. PMID: 19622410., Copyright Elsevier.*

Chapter 2

Adjuvanted *Schistosoma mansoni*-Cathepsin B with Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model

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Perera DJ, Hassan AS, Jia Y, Ricciardi A, McCluskie MJ, Weeratna RD, Ndao M. Adjuvanted *Schistosoma mansoni*-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model. Front Immunol. 2020 Nov 16;11:605288. doi: 10.3389/fimmu.2020.605288. PMID: 33304354; PMCID: PMC7701121.

2.1 Preface

Schistosoma mansoni cathepsin B (SmCB) is the most abundant cysteine peptidase expressed by *S. mansoni* trematodes and has been demonstrated to be essential for nutrient acquisition and worm maturity. Our group has previously assessed this protein as a vaccine target when formulated with CpG dinucleotides and Montanide ISA 720 VG, adjuvants which skew the immune system towards T_H1 and mixed T_H1/T_H2, respectively. There are several other adjuvants commercially available for use, each of which elicit unique components of the immune system.

We sought to optimize this recombinant protein vaccine by combining it with various adjuvants to determine which formulation would yield the highest immune responses and protection. The following chapter describes the various vaccine formulations we tested in our well-established mouse model of *S. mansoni* infection. We subsequently characterized vaccine-induced immune responses and protection from *S. mansoni* pathology.

2.2 Abstract

Schistosomiasis threatens 800 million people worldwide. Chronic pathology manifests as hepatosplenomegaly, and intestinal schistosomiasis caused by *Schistosoma mansoni* can lead to liver fibrosis, cirrhosis, and blood in the stool. To assist the only FDA approved drug, praziquantel, in parasite elimination, the development of a vaccine would be of high value. *S. mansoni* cathepsin B (SmCB) is a well documented vaccine target for intestinal schistosomiasis. Herein, we test the increased efficacy and immunogenicity of SmCB when combined with sulfated lactosyl archaeol (SLA) archaeosomes or AddaVax™ (a squalene based oil-in-water emulsion). Both vaccine formulations resulted in robust humoral and cell mediated immune responses. Impressively, both formulations were able to reduce parasite burden greater than 40% (WHO standard) with AddaVax™ reaching 86.8%. Additionally, SmCB with both adjuvants were able to reduce granuloma size and the amount of larval parasite hatched from feces which would reduce transmission. Our data support SmCB as a target for *S. mansoni* vaccination; especially when used in an adjuvanted formulation.

2.3 Introduction

Schistosomiasis (Bilharzia) is an underestimated parasitic disease for which over 800 million people are at risk[1]. This blood fluke spreads through fresh water in tropical and sub-tropical regions. Adult worms cause little to no pathology[2], however, female worms lay hundreds to thousands of eggs per day depending on the species of *Schistosoma*, some of which exit with the feces or urine, and others become trapped in host tissues causing chronic pathology.

Praziquantel (PZQ) used for the treatment of schistosomiasis has a reported efficacy of 86-93%[3,4], however, it does not protect individuals from reinfection or remove pre-existing egg deposition. To aid the interruption of schistosomiasis a vaccine is pertinent[5]. In the 1990s,

independent testing of six candidate *S. mansoni* antigens underwent protective studies organized by a UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR/WHO) committee. Although these trials resulted in protection, the WHO goal of 40% or greater protection was not met, headlining the need for possible adjuvanted formulations[6].

S. mansoni cathepsin B (SmCB) is the most abundant cysteine protease found in schistosomula and adult worm gut and somatic extracts. This protein is used for host blood molecule degradation and nutrient acquisition[7,8]. RNA interference studies demonstrate that when cathepsin B transcript levels are suppressed resulting worms show significant growth retardation compared to control parasites[9]. By targeting cathepsin B, reduced egg fitness has been demonstrated by our group[10], and parasite anti-fecundity has also been seen in other flukes[11].

Our lab has exploited the immunogenic gut peptidase SmCB as a vaccine target, which reduces worm parasite burden by 59% and 60% when adjuvanted with CpG dinucleotides, and Montanide ISA 720 VG, respectively[12,13].We believe that by combining this antigen with novel adjuvants, we will be able to increase parasite burden reduction and develop a more promising anti-schistosomiasis vaccine. Herein, we evaluate the immunogenicity and protective capability of our recombinant SmCB (rSmCB) adjuvanted by two additional adjuvants namely sulfated lactosyl archaeol (SLA) archaeosomes, and AddaVax™ (AddaVax).

2.4 Methods

2.4.1 Ethics statement

All animal procedures were performed in accordance with Institutional Animal Care and Use Guidelines approved by the Animal Care and Use Committee at McGill University (Animal Use Protocol 7625).

2.4.2 SmCB recombinant protein preparation

S. mansoni cathepsin B was prepared and purified as we previously described[12]. Briefly, the PichiaPink™ system (Thermo Fisher Scientific, Waltham, MA, USA) was used and recombinant yeast cells were cultured in a glycerol medium. After three days of growth, yeast cells were resuspended in a methanol induction medium to allow expression of recombinant protein.

Recombinant protein purification was performed by Ni-NTA chromatography (Ni-NTA Superflow by QIAGEN, Venlo, Limburg, Netherlands). The eluted protein was analyzed by Western Blot using antibodies directed at the His-tag.

2.4.3 Immunization protocol

Six- to eight-week-old female C57BL/6 mice were bred from mice obtained from Charles River Laboratories (Senneville, QC). Four groups of mice (n = 10-13) were immunized for humoral and cytokine assessment. Four groups of mice, (n = 10) were immunized and subsequently infected for parasite burden assessment. Group 1: control: mice were injected with phosphate-buffered saline (PBS) (Wisent Bioproducts, St. Bruno, QC). Group 2: positive control: mice were immunized with 20µg of recombinant SmCB (rSmCB) and 35µL of Montanide ISA 720 VG (SEPPIC Inc., Fairfield, NJ). Group 3: mice were immunized with 20µg rSmCB admixed with 1mg of pre-formed empty SLA archaeosomes (NRC, Ottawa, Canada). Group 4: mice were immunized with 20µg rSmCB and 25µL of AddaVax™ (InvivoGen, San Diego, CA). Each mouse was immunized at weeks 0, 3, and 6 intramuscularly in the thigh with 50µL of vaccine.

2.4.4 *Schistosoma mansoni* challenge

Biomphalaria glabrata snails infected with the Puerto Rican strain of *S. mansoni* were provided by NIAID Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD). Three weeks after the final immunization mice were challenged with 150 cercaria via tail exposure for one hour and sacrificed seven weeks later for parasitological measures. Images of mouse livers were taken during dissection using a Galaxy S8 cell phone camera (Samsung Group, Seoul, South Korea). Adult worms were perfused from the hepatic portal system and counted manually[13]. Liver sections were suspended in 10% buffered formalin phosphate (Fisher Scientific) and processed for histology as previously described[10]. Remaining liver and intestines were weighed and digested overnight in 4% potassium hydroxide. The following day, the eggs present in these tissues were counted by microscopy and adjusted per gram of tissue. Burden reductions were calculated as previously described[12–14]:

$$Percent\ of\ worms\ or\ eggs\ reduction = \left(1 - \frac{mean\ number\ of\ worms\ or\ eggs\ recovered\ in\ immunized\ mice}{mean\ number\ of\ worms\ or\ eggs\ recovered\ in\ PBS\ control\ mice}\right) \times 100\%$$

2.4.5 Serum total SmCB-specific IgG

SmCB-specific serum IgG was assessed by ELISA as described elsewhere[10]. Briefly, high binding 96-well plates (Greiner Bio-One, Frickenhausen Germany) were coated with rSmCB (0.5 μg/mL) in 100 mM bicarbonate/carbonate buffer (pH 9.6) overnight at 4°C. After blocking plates with 2% bovine serum albumin (BSA; Sigma Aldrich) in PBS-Tween 20 (PBS-T: 0.05%; Fisher Scientific, Ottawa, ON, Canada) (blocking buffer) serum samples were added to the plates in duplicate. Plates were incubated for one hour at 37°C then washed with PBS (pH 7.4) and horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Sigma Aldrich) was diluted 1:20 000 in blocking buffer and applied. Again, plates were washed with PBS and 3,3',5,5'-Tetramethyl benzidine (TMB) substrate (Millipore, Billerica, MA) was used for detection followed by the addition of H_2SO_4 (0.5M; Fisher Scientific). Optical density (OD) was measured at 450 nm with an EL800 microplate reader (BioTek Instruments Inc., Winooski, VT), and concentration of SmCB specific IgG was calculated by extrapolation from the IgG standard curve.

2.4.6 Serum SmCB-specific IgG1 and IgG2c

SmCB-specific serum IgG1, and IgG2c were assessed by ELISA as described elsewhere[10]. Briefly, Immulon 2HB flat-bottom 96-well plates (Thermo Fisher) were coated with recombinant SmCB (0.5 μg/mL) in 100 mM bicarbonate/carbonate buffer (pH 9.6). The plates were washed with PBS-T and blocking buffer was applied for 90 minutes. A serial dilution of serum was applied to plates in duplicate and incubated for 2 hours at 37°C. Plates were washed again with PBS-T, and goat anti-mouse IgG1-HRP (Southern Biotechnologies Associates, Birmingham, AL) or goat anti-mouse IgG2c-HRP (Southern Biotechnologies Associates) was applied to plates for one hour at 37°C. After a final wash, TMB was added followed by H_2SO_4. Again, OD was measured as above. IgG1 and IgG2c endpoint titers were calculated as the reciprocal of the highest dilution which gave a reading above the cut-off. The endpoint titer cut-off was statistically established as described elsewhere [15] using the sera of PBS immunized, unchallenged mice.

2.4.7 Serum total IgE

Total IgE was assessed by ELISA using the BD OptEIA™ Set Mouse IgE Kit (BD, San Diego, CA) following manufacturer's guidelines. Briefly, high binding 96-well plates (Grenier Bio-One) were coated with anti-mouse IgE capture antibody diluted in 100 mM bicarbonate/carbonate buffer (pH 9.6) 250-fold. Plates were washed and then blocked using PBS with 10% fetal bovine serum (Wisent Bio Products) for one hour at room temperature. Plates were again washed. Samples were diluted in assay diluent then added to plates with standards and incubated for two hours at room temperature. Plates were washed again, and biotinylated anti-mouse IgE antibody and streptavidin-horseradish peroxidase were added together for one hour at room temperature. Plates were then washed a final time before TMB was added for 30 minutes protected from light. Lastly, 50 µL of H_2SO_4 was added to wells and absorbance was read at 450 nm within 30 minutes.

2.4.8 Cell-mediated immune responses

Three weeks after the last immunization, mice were sacrificed, spleens collected, and splenocytes isolated as previously described[16] with the following exceptions: splenocytes were resuspended in RPMI-1640 supplemented with 10% fetal bovine serum, 1 mM penicillin/streptomycin, 10 mM HEPES, 1X MEM non-essential amino acids, 1 mM sodium pyruvate, 1 mM L-glutamine (Wisent Bioproducts), and 0.05 mM 2-mercaptoethanol (Sigma Aldrich) (fancy RPMI, fRPMI). These cells were then used in the following assays:

2.4.9 Proliferation assay by BrdU

Cell proliferation was measured by using the Roche chemiluminescent kit, following manufacturer's guidelines. Splenocytes were seeded in black 96-well flat bottom plates at 200 000 cells per well. Each sample was seeded unstimulated, stimulated with rSmCB (2.5 µg/mL), and stimulated with concavalin A (2.5 µg/mL) as a positive control. Briefly, cells were incubated for 48 hours at 37°C and 5% CO_2. At this time 20 µL BrdU- 70 -abellingg reagent was added to each well after being diluted 1:100 in fRPMI, and cells were incubated again for another 24 hours. Cells were resuspended in 200 µL PBS as a wash step, and then dried for one hour in a 60°C

hybridization oven (Thermo Fisher). Carefully 200 µL FixDenat was added to each well for 30 minutes at room temperature, before 100 µL of Anti-BrdU-POD working solution (1:100 in antibody dilution solution) was added for an additional 90 minutes. Plates were washed three times with washing solution and 100 µL/well of substrate solution was added. The plate was protected from light and shaken for three minutes before light emission was measured using a Tecan Infinite® 200 PRO (Tecan, Switzerland) within 10 minutes.

2.4.10 Cytokine production by multiplex ELISA

Splenocytes were incubated at 300 000 cells in 200 µL with rSmCB in fRPMI (2.5 µg/mL recombinant protein). After 72 hours at 37°C + 5% CO_2, plates were centrifuged and supernatant collected and stored at -80°C until analysis. Cell supernatants were assessed for the presence of 16 cytokines and chemokines (IL1-a, IL1-b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, IFNy, TNFa, CCL2 (MCP-1), CCL3 (MIP-1a), CSF2 (GM-CSF), and CCL5 (RANTES) using Q-plex Mouse Cytokine – Screen (16-plex) multiplex ELISA following the manufacturer's guidelines (Quansys Biosciences, Logan, UT, USA). Samples were run in singlet.

2.4.11 T cell-mediated cytokine secretion by flow cytometry

Splenocytes were seeded into 96-well U-bottom plates (BD Falcon) at 10^6 cells in 200 uL/well. Duplicate cultures were stimulated with or without rSmCB in fRPMI (2.5 µg/mL) for 24 hours at 37°C + 5% CO_2. For the last 6 hours of incubation, protein transport inhibitor was prepared according to the manufacturer's guidelines (BD Science, San Jose, CA) and added to all samples. Cells stimulated with phorbol 12-myristate 13-acetate and ionomycin were processed as positive controls. Plates were then processed for flow cytometry as described elsewhere[17]. Briefly, splenocytes were washed twice with 200 µL of cold PBS, and fixable viability dye eFluor 780 (Affymetrix ebioscience, Waltham, MA) was applied at 50 µL/well diluted at 1:300 and incubated for 20 minutes at 4°C protected from light. Cells were washed as above with PBS 1% BSA (PBS-BSA), and Fc block (BD Science) diluted 1:50 was added for 15 minutes. All surface stains were diluted 1:50 in PBS-BSA and 50 µL/well of extracellular cocktail was applied for 30 minutes at 4°C protected from light. The following antibodies made up the extracellular cocktail: CD3-FITC (Clone 145-2C11, Affymetrix ebioscience), CD4-V500 (RM4-5, BD Bioscience) and CD8-PerCP-

Cy5 (Clone:53-6.7, BD Science). Cells were then washed as above with 1X fixation buffer (BD Science) and left overnight at 4°C in the dark. Plates were washed as before with 1X permeabilization buffer (BD Science) and stained with an intracellular cocktail of antibodies diluted 1:50 in PBS-BSA applied as 50 µL/well for 30 minutes at 4°C protected from light. The intracellular cocktail was made up of: IL-2-Pe-Cy5 (Clone: JES6;5H4, Biolegend, San Diego, CA), IFNγ-PE (Clone: XMG1.2, BD Science), and TNFα-efluor450 (Clone: MP6-XT22, Affymetrix ebioscience). After staining, cells were resuspended in PBS and analyzed on BD LSRFortessa X-20 (BD Science) using Flowjo software (version 10.0.8r1). Our gating strategy is shown in Supplemental Figure 2.1.

2.4.12 Histology and egg granuloma quantitation

Liver sections in 10% buffered formalin phosphate were stained using hematoxylin and eosin to assess granuloma size and egg morphology. Granuloma area was measured using Zen Blue software (version 2.5.75.0; Zeiss) as previously reported[10,18–21]. Briefly, while working at 400x magnification, the pointer was used to trace the perimeter of 37-41 granulomas per experimental group with a clearly visible egg which the software converted into an area. Hepatic eggs were classified as abnormal if their internal structure was lost or the perimeter of the egg was crenelated. Fifteen different fields of vision were assessed per experimental group over two independent experiments. Abnormal eggs were counted and reported as a percent of the total eggs counted per field of vision.

2.4.13 Miracidia hatching experiments

Miracidia hatching was optimized and adapted from a protocol as described elsewhere[22]. Briefly, one gram of feces from each experimental group was collected twice, one day before and at sacrifice seven weeks post infection. Feces from each time point were assessed individually. Feces were resuspended in distilled water and transferred into an Erlenmeyer flask/conical tube. The flask/tube was then wrapped in tin foil to protect from light and was topped up with distilled water so that only 3 mm under the lid was exposed to light. Tin foil wrapped flasks were placed inside of a cardboard box, with a hole the same diameter as a lamp, through which light was shone on them for three hours. After this time, water samples were collected from the exposed fraction

of water and miracidia were counted. An image of the set up, and further detailed methodology can be seen in Supplemental Figure 2.2.

2.4.14 Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 software (La Jolla, CA). Data were analyzed by Kruskal Wallis one-way ANOVA with Dunn's multiple comparisons tests. Flow cytometry data were analyzed by a two-way ANOVA and Dunnett's multiple comparisons tests. If present, outliers were calculated using GraphPad QuickCalcs and removed. P values <0.05 were considered significant.

2.5 Results

2.5.1 Humoral response to vaccination

No mice had detectable SmCB-specific IgG antibodies at baseline, and the PBS control remained negative throughout the study (Figure 2.1A). Mice receiving adjuvanted rSmCB developed SmCB-specific IgG after a single immunization. At week 3, groups adjuvanted with Montanide and AddaVax had significantly higher titers than with SLA, however this difference was no longer significant post first boost. Antigen specific IgG titers in vaccinated mice rose until week 6 before plateauing.

Endpoint titers were calculated for antigen specific IgG1 and IgG2c at the time of infection (Figure 2.1B). Each experimental group elicited a robust mixed IgG1/IgG2c response, although mice vaccinated with antigen and SLA or AddaVax had much higher IgG1 (3.84e6 ± 1.13e6 and 2.69e6 ± 9.24e5 respectively than IgG2c titers (6.60e4 ± 3.21e4 and 7.88e4 ± 2.38e4 respectively). Mice immunized with rSmCB/Montanide had a balanced IgG1/IgG2c response with titers of 8.00e5 ± 1.09e5 and 2.03e5 ± 9.75e4 respectively. At this time, and at the second study endpoint (post-challenge) mouse serum was also analyzed for total IgE (Figure 2.1C). At baseline, mice had little to no detectable IgE. In comparison to PBS controls, there was a greater increase in total IgE levels post immunization in groups receiving rSmCB adjuvanted with SLA (~3 fold) or AddaVax (~3.5 fold) than in the group immunized with rSmCB adjuvated with Montanide which saw no

increase. Upon parasite challenge, the total IgE titers increased in all groups including the PBS controls with no significant differences between groups.

2.5.2 Lymphoproliferation, splenocyte cytokine and chemokine production in response to vaccination

Enhanced SmCB-specific lymphoproliferation was seen in in *ex vivo* stimulated splenocytes from immunized compared to control mice. However, no statistical differences in the magnitude of lympho-proliferation was observed between immunized groups (Figure 2.2A). Differences in functionality of antigen-specific lymphocytes were further assessed by measuring cytokine and chemokine concentrations in culture supernatants.

For many of the cytokines and chemokines tested, adjuvanted formulations generated elevated levels above the PBS control (Supplemental Figure 2.3). However, differences can be seen in the cytokine milieus between experimental groups. The fold change expression of each cytokine/chemokine from the PBS control is depicted in a radar plot (Figure 2.2B) indicating each vaccine formulation favours a slightly different immune phenotype. Montanide has an increased T_H17 immune profile, SLA an inflammatory, T_H1, T-cell associated, and myeloid proliferating profile, and AddaVax a T_H2 and anti-inflammatory profile.

2.5.3 T cell T_H1 response to vaccination

Flow cytometry was used to enumerate splenic CD4+ and CD8+ T cell expression of IFNγ, IL-2, and TNFα in response to SmCB. Overall, an increase cytokine expression were observed in CD4+ (Figure 2.3A) and CD8+ (Figure 2.3B) T cells in groups immunized with adjuvanted rSmCB over PBS controls. Mice immunized with rSmCB adjuvanted with SLA showed a significant increase in CD4+ IL-2 expression whereas mice immunized with rSmCB adjuvanted with AddaVax showed a significant increase in CD4+ IL-2 and IFNγ expression compared to PBS control mice. All groups receiving adjuvanted rSmCB showed a significant increase in CD8+ IFNγ expression.

2.5.4 Protection from infection upon immunization with adjuvanted rSmCB

To determine the protective potential of the vaccines, a three-dose immunization regiment was tested. The average amount of worms collected from PBS control mice was 31 ± 7 worms over two independent experiments. Parasite burden reductions were calculated in reference to the PBS control mice within the same experiment to keep consistency within batches of infections. Parasite burden reductions were then combined and compared. All vaccine formulations significantly reduced parasite burden over PBS control with percent reduction in worm burden of 70.9 ± 3.9%, 60.5 ± 6.3% and 86.8 ± 4.0% in groups adjuvanted with Montanide, SLA and AddaVax respectively (Figure 2.4A). There were no statistical differences in burdens between the three formulations.

Pathology in schistosomiasis is caused by parasite eggs which become trapped in host tissues. Egg burdens in the liver (Figure 2.4B) and intestines (Figure 2.4C) were also calculated. Hepatic eggs in the PBS control group varied between 1250 and 14525 eggs/gram liver tissue. Similarly, intestinal eggs ranged between 1660 and 16973 eggs/gram intestine. rSmCB/Montanide reduced parasite burden by 70.3 ± 7.4% and 71.3 ± 8.4% in hepatic and intestinal eggs, respectively. The formulation of rSmCB/SLA reduced parasite burden by 49.8 ± 9.9% and 59.4 ± 8.8%, while rSmCB/AddaVax reduced parasite burden the most significantly, 78.0 ± 7.2% and 83.4 ± 6.6%, in hepatic and intestinal eggs respectively.

2.5.5 Liver pathology

During mouse dissection, images were taken of gross liver sections as pathology was clearly visible (Figure 2.5A). Livers from PBS control mice had many granulomas (visualized as white circular formations) that covered the surface of the liver due to heavy egg deposition, while vaccinated mice in all groups had less granuloma formation compared to PBS controls. By visual examination, mice immunized with rSmCB adjuvanted with Montanide or AddaVax had the least granuloma formation. Microscopic examination of liver tissue stained with hematoxylin and eosin stain (Figure 2.5B) revealed the presence of *S. mansoni* eggs within granulomatous formations. Granulomas were large, and well formed in PBS control mice, and eggs in granulomas were intact with normal appearances. Upon vaccination with adjuvanted rSmCB, granuloma sizes dropped from approximately 30000 μm^2 to below 20000 μm^2 (Figure 6A). Mean granuloma sizes in rSmCB

formulated with Montanide and SLA were 17541 ± 1991 μm^2 and 16185 ± 2070 μm^2 respectively. Although granulomas were smallest in the group adjuvanted with AddaVax (13637 ± 1398 μm^2) there were no statistical differences between vaccinated groups. Eggs in vaccinated animals were also abnormal in appearance (ie: internal structure was lost or compromised, edges were crenellated and incomplete) (Figure 2.6B). A percentage of 47.7 ± 9.5% eggs were found to be abnormal in mice immunized with rSmCB/Montanide. When rSmCB was adjuvanted with SLA and AddaVax, 39.9 ± 7.0% and 42.9 ± 5.3% of eggs were found to be abnormal, again differences between vaccinated groups were not significant.

2.5.6 Egg hatching

To assess whether our vaccine formulations could interrupt the transmission of schistosomiasis we tested whether eggs retrieved from feces were able to give rise to larvae. Feces from PBS control mice gave rise to 76.3 ± 10.0 miracidia (Figure 2.7). Feces from experimental groups saw significant reductions in miracidia: 15.4 ± 0.4, 36.2 ± 3.7, and 13.6 ± 1.7 miracidia hatched from Montanide, SLA, and AddaVax groups respectively, with no statistical significance between them.

2.6 Discussion

Our group has previously shown the protective capabilities of SmCB, when adjuvanted with CpG dinucleotides[12] and Montanide ISA 720 VG[13]. In this work we evaluated the protective capabilities of two new adjuvants: sulfated lactosyl archaeol (SLA) archaeosomes and AddaVax, a squalene-based oil-in-water emulsion similar to MF59. When used as an adjuvant, SLA has been shown to activate strong humoral and cell-mediated responses against multiple antigens by increasing local cytokine production, immune cell trafficking, and antigen uptake at the injection site, leading to increased protection in murine models of infectious disease and cancer[23–25]. In this study, we used a novel admixed formulation which provides a simple ready to mix adjuvant formulation with no loss of antigen during the formulation process[26]. AddaVax alternatively, is a squalene-oil based emulsion structurally similar to MF59, which acts by stimulating local cytokine and chemokine production, attracting immune cells to the injection site and increasing antigen trafficking and presentation[27].

SmCB, is a gut cysteine peptidase necessary for parasite growth and maturity. Although immunogenic and capable of protecting from *S. mansoni* infection when used alone[28], our lab has shown that adjuvants enhance its immunogenicity and protective efficacy[12,13,29], the highest protection seen with Montanide[13].

In a series of preliminary studies, we tested a variety of other adjuvant formulations (including: AddaVax, aluminum hydroxide (alum), alum/CpG dinucleotides, alum/monophosphoryl lipid A, and SLA, alongside Montanide and PBS as controls) in combination with rSmCB for immunogenicity and protection from parasite challenge, to determine the most efficacious (Supplemental Figure 2.4). Of the adjuvant formulations tested, the most significant impact on reducing the parasite burden was seen when rSmCB was adjuvanted with SLA or AddaVax. Therefore, the present study was conducted to further elucidate the immune mechanisms behind this protection. The two adjuvanted formulations in this study were able to surpass the WHO schistosomiasis vaccine threshold of 40% protection[6], similar to our previous efforts. SLA reduced adult worms, liver eggs, and intestinal eggs by 60.5%, 49.8%, and 59.4% respectively, while AddaVax reached 86.8%, 78.0%, and 83.4% in the same readouts (Figure 2.4).

Eggs trapped in host tissues release soluble egg antigens triggering granuloma formation, leading to liver cirrhosis and other fatal morbidities[30]. Both emulsion-based vaccines (Montanide and AddaVax) were able to visibly reduce granuloma size, and parasite pathology to the liver (Figure 2.5). Granuloma formation is initiated by T_H2 immune responses however when mice mount extreme T_H1 polarization responses, liver pathology is severe[31]. This was shown in mice immunized with schistosome egg antigens (SEA) and complete Freunds adjuvant[32], and again in mice that lack both IL-10 and IL-4 which reached 100% mortality upon infection with schistosomiasis[33]. Although SmCB is not expressed by eggs trapped in host tissue, it is a secreted protein of *S. mansoni* adult flukes which reside in venules in and around the liver and intestines. It is possible that SmCB specific lymphocyte reactivation is causing the expression of T_H1 and inflammatory cytokines that are indirectly contributing to the deleterious liver pathology seen in SLA vaccinated animals. Despite a greater number of eggs found in SLA liver tissues than Montanide and AddaVax, granulomas around these eggs were equally reduced in size.

Eggs released in feces into freshwater, will hatch miracidia, the first larval stage of the parasite. To our knowledge, we are the first group to test *S. mansoni* vaccine efficacy in reducing hatched parasite from fecal samples, although others have demonstrated hatching from liver

deposited eggs[34,35]. We found that one gram of feces led to a reduced number of hatched miracidia in animals vaccinated with rSmCB and Montanide or AddaVax. As shown in previous work[9,36], targeting a digestive enzyme may lead to a suppression of metabolic activities necessary for proper reproduction, leading to the lowered fertility and egg fitness demonstrated by our vaccines. Despite the fact that our results do not account for the variability in fecal egg shedding from day-to-day[37,38], we believe the reduction in hatched parasite observed in vaccinated animals would mean reduced schistosomiasis transmission.

Immunogenicity studies suggest that the protection mediated by our vaccine formulations could be explained by a robust humoral and cellular mediated immunity (CMI) and it is likely that both these responses contribute to protection from schistosomiasis.

Several groups have shown a positive correlation between IgG antibody titer and protection from schistosomiasis suggesting a necessity for the humoral response. This response was seen to mediate antibody mediated cellular cytotoxicity (ADCC) and activate complement as an attack against schistosomula[29,39,40] in vitro. By this mechanism or due to another, high IgG titers have been found in vaccinated animals with reduced adult worm burdens. Interestingly, a study in rhesus macaques not only showed a reduction in worm burden correlated to IgG, but collected worms were morphologically stunted with degenerated reproductive systems[41]. As our vaccine formulations produced robust IgG titers, they all showed promise for a protective vaccine. Learning from the failed hookworm vaccine[42], we wanted to ensure our vaccine formulations did not cause IgE hypersensitivity, as IgE is a trademark of helminth infections like *S. mansoni*[43]. We saw slight increases in total IgE levels after immunization using SLA and AddaVax which were not present in Montanide adjuvanted groups or the PBS control. However, post challenge, total IgE levels were similar in all groups including unvaccinated controls (Figure 2.1C). Thus, detrimental effects associated with vaccine induced IgE responses are unlikely.

Ex vivo re-stimulation of splenocytes with rSmCB showed significant lymphoproliferation in all vaccinated groups, so we were curious to see what cell mediated immunity was being elicited by our different vaccines. Although all vaccine groups increased cytokine expression, there were subtle differences in their cytokine milieus between different adjuvant formulations (Figure 2.2B). When combined with SLA, SmCB was broadly stimulating increasing inflammatory cytokines, T_H1 and T-cell associated cytokines, as well as the myeloid proliferation cytokine IL-3, whereas

with Montanide and AddaVax, SmCB led to increased T_H17, and T_H2/Anti-inflammatory cytokines respectively.

From the creation of the *S. mansoni* radiation-attenuated cercaria vaccine, it has been the consensus that IFNγ and TNFα play pivotal roles in protection[44–46]. It is a promising feature that when CD4+ and CD8+ T cells from vaccinated animals were stimulated ex vivo with rSmCB we observed increases of IFNγ, with trends of increased TNFα. Although the percentage increases observed are small, the number of cells that they represent specific to our antigen is significant. Interestingly, our multiplex ELISA data show significant production of IFNγ by all vaccinated groups, which is not fully reflected in our T cell expression as seen by flow cytometry. Future studies could prove useful to identify which other cell types are contributing to IFNγ expression, especially for mice vaccinated with Montanide and SLA.

As previously mentioned, both SLA and AddaVax have been shown to activate the immune system[23–25,27] and it is due to this quality that they have been exploited as vaccine adjuvants. This study sought to assess the increased efficacy of SmCB when combined with these compounds, however further studies should be conducted to elucidate the possible protective effects of the adjuvants themselves in schistosomiasis infection.

Adjuvanting schistosomiasis vaccines is not a new concept. Previous work has shown 70% reduction in worm burden with a Sm-p80 tegument vaccine administered by DNA prime and boosted with protein and oligodeoxydinucleotides[47], and 57% reduction in worm burden with a Sm-Tsp-2 tetraspanin vaccine adjuvanted with Freund's incomplete adjuvant[48], among others. However, to our knowledge we are the only group to test SLA archaeosomes and AddaVax in the presence of SmCB and are reducing adult worm burden the most significantly of all the recombinant protein vaccines in pre-clinical trials. Our data support the hypothesis that *Schistosoma mansoni* cathepsin B is a strong candidate for an anti-schistosome vaccine and can be readily formulated with multiple different types of adjuvants including oil-in-water and water-in-oil emulsions, archaeosomes and TLR9 agonists. Future directions include conducting dose response experiments on tested adjuvants, as a single dose level of SLA was tested and AddaVax was formulated as per the manufacturer's guidelines. Additionally, it would be useful to conduct more in-depth immunological and mechanistic studies to further elucidate the correlates of protection being elicited by our vaccines.

2.7 Acknowledgements

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2.8 References

1. Schistosomiasis (Bilharzia). *World Health Organization* https://www.who.int/health-topics/schistosomiasis#tab=tab_1 (2020).
2. El-Garem, A. A. Schistosomiasis. *Digestion* **59**, 589–605 (1998).
3. Barda, B. *et al.* Efficacy and Safety of Moxidectin, Synriam, Synriam-Praziquantel versus Praziquantel against Schistosoma haematobium and S. mansoni Infections: A Randomized, Exploratory Phase 2 Trial. *PLoS Negl Trop Dis* **10**, e0005008 (2016).
4. Zwang, J. & Olliaro, P. L. Clinical Efficacy and Tolerability of Praziquantel for Intestinal and Urinary Schistosomiasis—A Meta-analysis of Comparative and Non-comparative Clinical Trials. *PLoS Negl Trop Dis* **8**, e3286 (2014).
5. Siddiqui, A. A. & Siddiqui, S. Z. Sm-p80-Based Schistosomiasis Vaccine: Preparation for Human Clinical Trials. *Trends in Parasitology* **33**, 194–201 Preprint at https://doi.org/10.1016/j.pt.2016.10.010 (2017).

6. McManus, D. P. & Loukas, A. Current status of vaccines for schistosomiasis. *Clinical Microbiology Reviews* **21**, 225–242 Preprint at https://doi.org/10.1128/CMR.00046-07 (2008).
7. Sajid, M. *et al.* Functional expression and characterization of Schistosoma mansoni cathepsin B and its trans-activation by an endogenous asparaginyl endopeptidase. *Mol Biochem Parasitol* **131**, 65–75 (2003).
8. González, A. Y., Sulbarán, G. S., Ballen, D. E. & Cesari, I. M. Immunocapture of circulating Schistosoma mansoni cathepsin B antigen (Sm31) by anti-Sm31 polyclonal antibodies. *Parasitol Int* **65**, 191–195 (2016).
9. Correnti, J. M., Brindley, P. J. & Pearce, E. J. Long-term suppression of cathepsin B levels by RNA interference retards schistosome growth. *Mol Biochem Parasitol* **143**, 209–215 (2005).
10. Hassan, A. S., Zelt, N. H., Perera, D. J., Ndao, M. & Ward, B. J. Vaccination against the digestive enzyme Cathepsin B using a YS1646 Salmonella enterica Typhimurium vector provides almost complete protection against Schistosoma mansoni challenge in a mouse model. *PLoS Negl Trop Dis* **13**, e0007490 (2019).
11. Norbury, L. J. *et al.* Intranasal delivery of a formulation containing stage-specific recombinant proteins of Fasciola hepatica cathepsin L5 and cathepsin B2 triggers an anti-fecundity effect and an adjuvant-mediated reduction in fluke burden in sheep. *Vet Parasitol* **258**, 14–23 (2018).
12. Ricciardi, A., Dalton, J. P. & Ndao, M. Evaluation of the immune response and protective efficacy of Schistosoma mansoni Cathepsin B in mice using CpG dinucleotides as adjuvant. *Vaccine* **33**, 346–353 (2015).
13. Ricciardi, A., Visitsunthorn, K., Dalton, J. P. & Ndao, M. A vaccine consisting of Schistosoma mansoni cathepsin B formulated in Montanide ISA 720 VG induces high level protection against murine schistosomiasis. *BMC Infect Dis* **16**, (2016).
14. Hassan, A. S., Zelt, N. H., Perera, D. J., Ndao, M. & Ward, B. J. Vaccination against the digestive enzyme Cathepsin B using a YS1646 Salmonella enterica Typhimurium vector provides almost complete protection against Schistosoma mansoni challenge in a mouse model. *PLoS Negl Trop Dis* **13**, e0007490 (2019).

15. Frey, A., Di Canzio, J. & Zurakowski, D. A statistically defined endpoint titer determination method for immunoassays. *J Immunol Methods* **221**, 35–41 (1998).
16. Yam, K. K. *et al.* AS03-adjuvanted, very-low-dose influenza vaccines induce distinctive immune responses compared to unadjuvanted high-dose vaccines in BALB/c mice. *Front Immunol* **6**, (2015).
17. Hodgins, B., Pillet, S., Landry, N. & Ward, B. J. A plant-derived VLP influenza vaccine elicits a balanced immune response even in very old mice with co-morbidities. *PLoS One* **14**, (2019).
18. Cronan, M. R. *et al.* An explant technique for high-resolution imaging and manipulation of mycobacterial granulomas. *Nat Methods* **15**, 1098–1107 (2018).
19. Ebenezer, J. A. *et al.* Periostin as a marker of mucosal remodelling in chronic rhinosinusitis. *Rhinology journal* **55**, 234–241 (2017).
20. Tang, C. *et al.* Effect of Cytotoxic T-Lymphocyte Antigen-4 on the Efficacy of the Fatty Acid-Binding Protein Vaccine Against Schistosoma japonicum. *Front Immunol* **10**, 1022 (2019).
21. Hagen, J. *et al.* Omega-1 knockdown in Schistosoma mansoni eggs by lentivirus transduction reduces granuloma size in vivo. *Nat Commun* **5**, 1–9 (2014).
22. Jurberg, A. D., De Oliveira, Á. A., Lenzi, H. L. & Coelho, P. M. Z. A new miracidia hatching device for diagnosing schistosomiasis. *Mem Inst Oswaldo Cruz* **103**, 112–114 (2008).
23. Stark, F. C. *et al.* Archaeal glycolipid adjuvanted vaccines induce strong influenza-specific immune responses through direct immunization in young and aged mice or through passive maternal immunization. *Vaccine* **37**, 7108–7116 (2019).
24. Akache, B. *et al.* Effect of Different Adjuvants on the Longevity and Strength of Humoral and Cellular Immune Responses to the HCV Envelope Glycoproteins. *Vaccines (Basel)* **7**, (2019).
25. Stark, F. C. *et al.* Simplified Admix Archaeal Glycolipid Adjuvanted Vaccine and Checkpoint Inhibitor Therapy Combination Enhances Protection from Murine Melanoma. *Biomedicines* **7**, (2019).
26. Jia, Y. *et al.* A comparison of the immune responses induced by antigens in three different archaeosome-based vaccine formulations. *Int J Pharm* **561**, 187–196 (2019).

27. O'Hagan, D. T., Ott, G. S., De Gregorio, E. & Seubert, A. The mechanism of action of MF59 - An innately attractive adjuvant formulation. *Vaccine* **30**, 4341–4348 Preprint at https://doi.org/10.1016/j.vaccine.2011.09.061 (2012).
28. Tallima, H. *et al.* Protective immune responses against Schistosoma mansoni infection by immunization with functionally active gut-derived cysteine peptidases alone and in combination with glyceraldehyde 3-phosphate dehydrogenase. *PLoS Negl Trop Dis* **11**, e0005443 (2017).
29. Ricciardi, A., Zelt, N. H., Visitsunthorn, K., Dalton, J. P. & Ndao, M. Immune mechanisms involved in Schistosoma mansoni-Cathepsin B vaccine induced protection in mice. *Front Immunol* **9**, (2018).
30. McManus, D. P. *et al.* Schistosomiasis. *Nat Rev Dis Primers* **4**, (2018).
31. La Flamme, A. C., Patton, E. A. & Pearce, E. J. Role of gamma interferon in the pathogenesis of severe Schistosomiasis in interleukin-4-deficient mice. *Infect Immun* **69**, 7445–7452 (2001).
32. Rutitzky, L. I., Hernandez, H. J. & Stadecker, M. J. Th1-polarizing immunization with egg antigens correlates with severe exarcebation of immunopathology and death in schistosome infection. *Proc Natl Acad Sci U S A* **98**, 13243–13248 (2001).
33. Hoffmann, K. F., Cheever, A. W. & Wynn, T. A. IL-10 and the Dangers of Immune Polarization: Excessive Type 1 and Type 2 Cytokine Responses Induce Distinct Forms of Lethal Immunopathology in Murine Schistosomiasis. *The Journal of Immunology* **164**, 6406–6416 (2000).
34. Tedla, B. A., Pickering, D., Becker, L., Loukas, A. & Pearson, M. S. Vaccination with Schistosoma mansoni cholinesterases reduces the parasite burden and egg viability in a mouse model of schistosomiasis. *Vaccines (Basel)* **8**, (2020).
35. Le, L. *et al.* Schistosoma egg-induced liver pathology resolution by Sm-p80-based schistosomiasis vaccine in baboons. *Pathology* **50**, 442–449 (2018).
36. Morales, M. E. *et al.* RNA interference of Schistosoma mansoni cathepsin D, the apical enzyme of the hemoglobin proteolysis cascade. *Mol Biochem Parasitol* **157**, 160–168 (2008).

37. Engels, D., Sinzinkayo, E., De Vlas, S. J. & Gryseels, B. Intraspecimen fecal egg count variation in Schistosoma mansoni infection. *American Journal of Tropical Medicine and Hygiene* **57**, 571–577 (1997).
38. Degarege, A., Legesse, M., Medhin, G., Teklehaymanot, T. & Erko, B. Day-to-day fluctuation of point-of-care circulating cathodic antigen test scores and faecal egg counts in children infected with Schistosoma mansoni in Ethiopia. *BMC Infect Dis* **14**, 210 (2014).
39. Ramalho-Pinto, F. J., Rossi, R. De & Smithers, S. R. Murine Schistosomiasis mansoni: anti-schistosomula antibodies and the IgG subclasses involved in the complement- and eosinophil mediated killing of schistosomula in vitro. *Parasite Immunol* **1**, 295–308 (1979).
40. Khalife, J. *et al.* Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of Schistosoma mansoni. *J Immunol* **142**, 4422–7 (1989).
41. Wilson, R. A. *et al.* Elimination of Schistosoma mansoni Adult Worms by Rhesus Macaques: Basis for a Therapeutic Vaccine? *PLoS Negl Trop Dis* **2**, e290 (2008).
42. Diemert, D. *et al.* Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: implications for the development of vaccines against helminths. *J Allergy Clin Immunol* **130**, 169-176.e6 (2012).
43. Maizels, R. M. & McSorley, H. J. Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology* **138**, 666–675 (2016).
44. Wilson, R. A. & Coulson, P. S. Immune effector mechanisms against schistosomiasis: looking for a chink in the parasite's armour. *Trends in Parasitology* **25**, 423–431 Preprint at https://doi.org/10.1016/j.pt.2009.05.011 (2009).
45. Wilson, R. A., Coulson, P. S. & Mountford, A. P. Immune responses to the radiation-attenuated schistosome vaccine: What can we learn from knock-out mice? in *Immunology Letters* **65**, 117–123 (1999).
46. Hewitson, J. P., Hamblin, P. A. & Mountford, A. P. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunology* **27**, 271–280 Preprint at https://doi.org/10.1111/j.1365-3024.2005.00764.x (2005).
47. Ahmad, G. *et al.* Prime-boost and recombinant protein vaccination strategies using Sm-p80 protects against Schistosoma mansoni infection in the mouse model to levels previously attainable only by the irradiated cercarial vaccine. *Parasitol Res* **105**, 1767–1777 (2009).

48. Pearson, M. S. *et al.* Enhanced Protective Efficacy of a Chimeric Form of the Schistosomiasis Vaccine Antigen Sm-TSP-2. *PLoS Negl Trop Dis* **6**, e1564 (2012).

2.9 Figures and legends

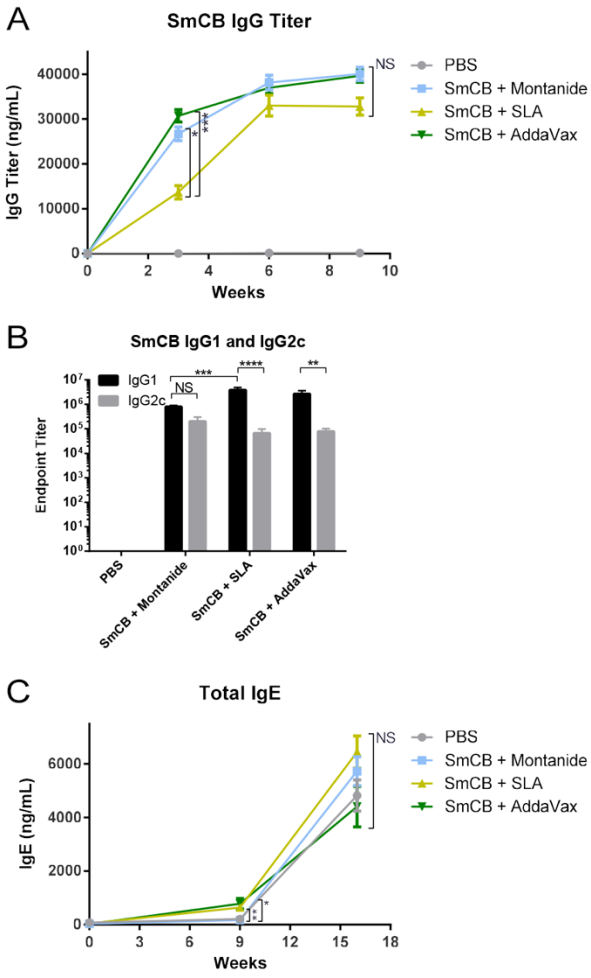


Figure 2.1 Humoral response

Production of SmCB specific total IgG n=20 from four independent experiments (A), Antigen specific IgG1 and IgG2c n=10 from two independent experiments (B) in immunized mice. Production of total IgE n=10 from two independent experiments (C) in immunized and challenged mice. Graphs A and C show antibody titers for PBS control mice, rSmCB and Montanide, SLA, and AddaVax. Graph B shows the endpoint titer of SmCB specific IgG1 and IgG2c at week 9, in

black and gray respectively. Serum from individual mice was analyzed by ELISA. Means and SEM are shown. NS=not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

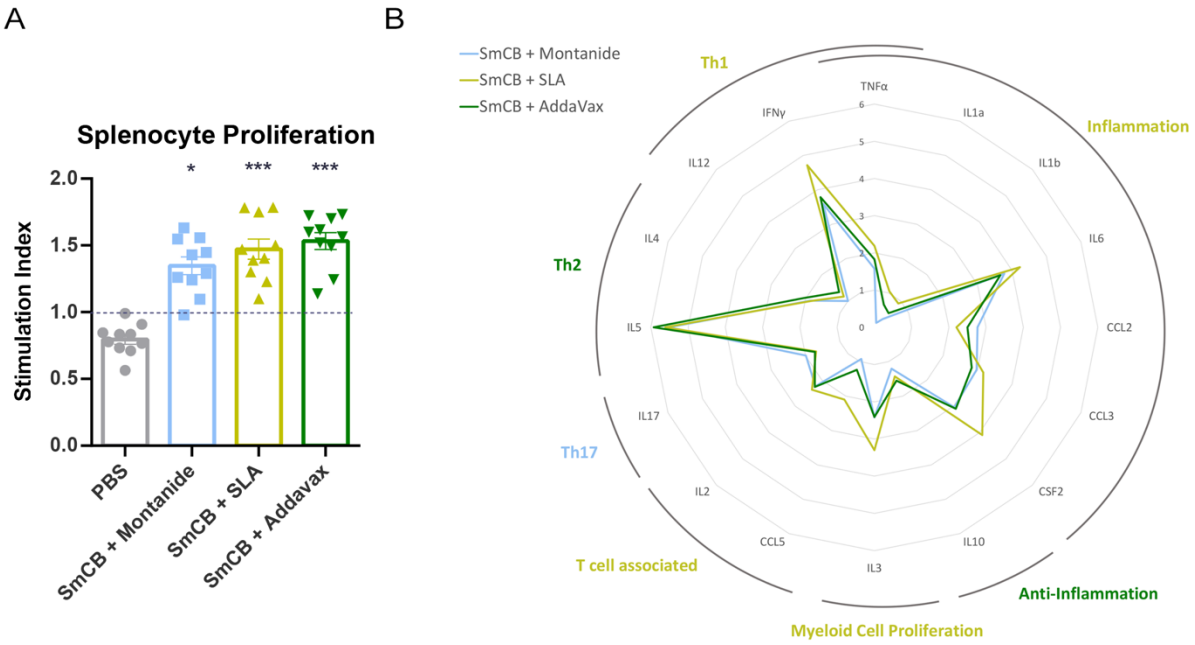


Figure 2.2 Lymphoproliferation, cytokines and chemokines

Splenocyte proliferation shown as stimulation index in response to rSmCB restimulation *ex vivo* (A). Means are shown with SEM. Significance is calculated against the PBS control. Mean levels of cytokine and chemokine expression were also reported as the fold change above the PBS control group and depicted in the radar plot in (B) with the axis in the natural log. Cytokines and chemokines have been grouped according to general functionality and labelled accordingly. Labels are coloured reflecting the experimental group expressing the most amount of their cytokines/chemokines. N=10 from two independent experiments. *P<0.05, ***P<0.001.

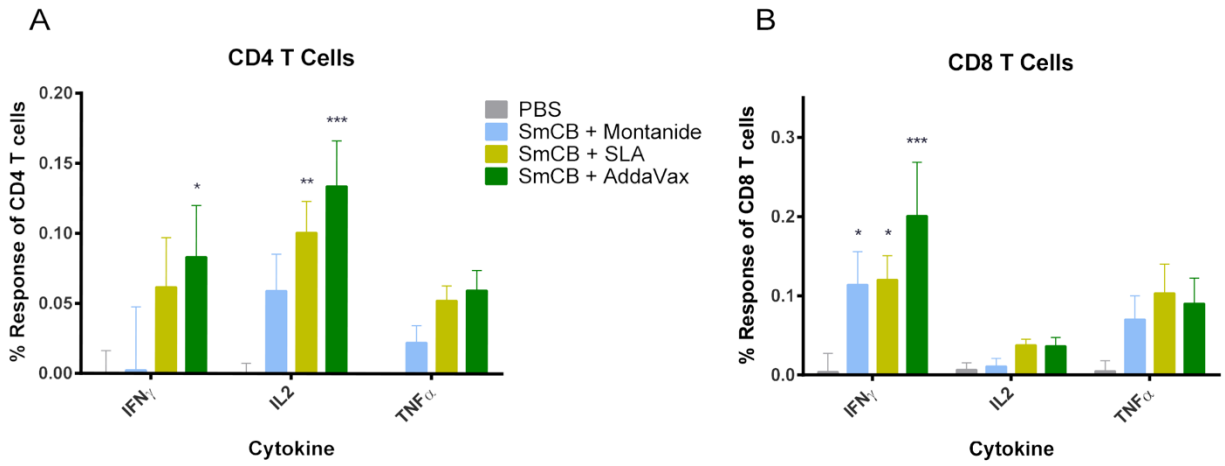


Figure 2.3 CD4+ and CD8+ T cell response

Splenocytes were restimulated with rSmCB *ex vivo* and CD4+ (A) and CD8+ T cells (B) were assessed for their expression of IFNγ, IL-2, and TNF-α. Means and SEM of subtractive data are shown (stimulated cells – unstimulated cells). The PBS control group is shown in gray, Montanide, SLA, and AddaVax groups are shown in blue, light green, and dark green respectively. Significance is calculated against the PBS control. N=13 from three independent experiments. *P<0.05, **P<0.01, ***P<0.001.

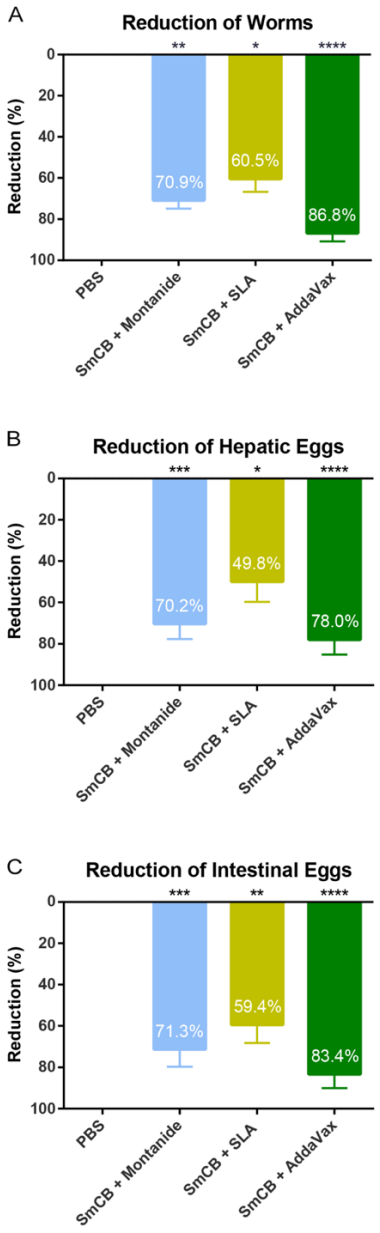


Figure 2.4 Parasitological outcomes

Seven weeks after challenge, mice were euthanized, and worms and eggs were counted for parasite burden. Parasite burden reductions are shown as mean and SEM for adult worms (A), hepatic eggs (B), and intestinal eggs (C), eggs adjusted per gram of tissue. Significance is calculated against the PBS control. N=10 from two independent experiments. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

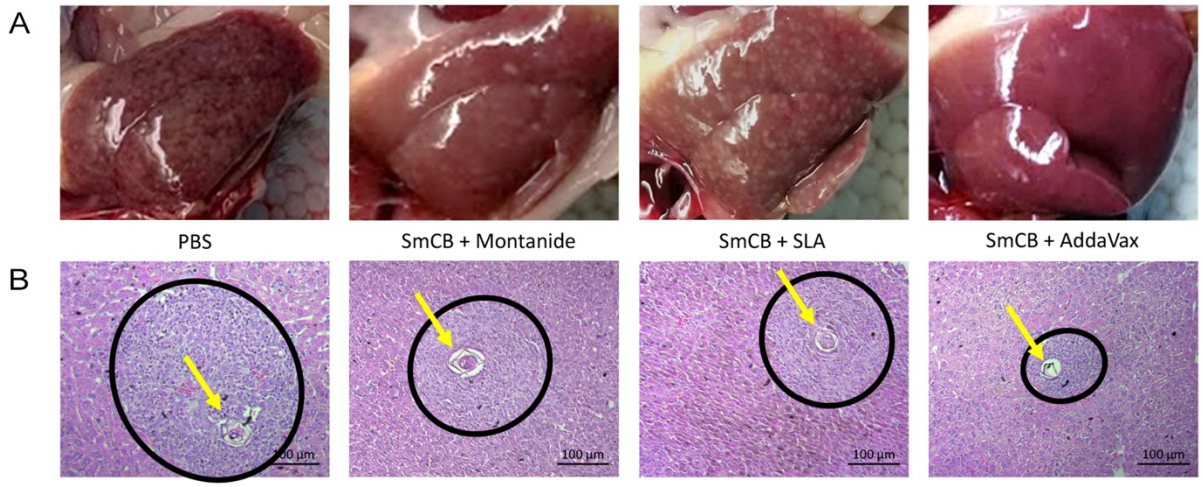


Figure 2.5 Liver pathology

Images of gross livers were taken (A), and liver sections were stained by H&E (B). In A, representative liver images from two independent experiments are shown for the PBS control on the left, and the experimental groups from left to right: Montanide, SLA, and AddaVax. Below, in B, H&E staining of hepatic tissue shows a *S. mansoni* egg (pointed to with a yellow arrow) within a granulomatous formation (within a black circle). H&E stained slides were viewed at 400X.

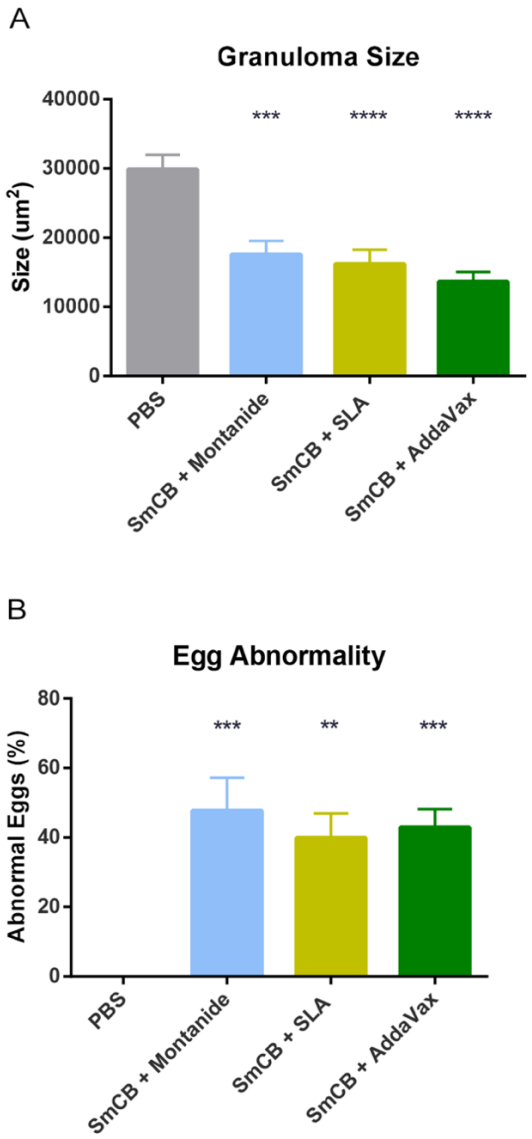


Figure 2.6 Egg granuloma size and egg abnormality

Using Zen Blue software, 37-41 granulomas were measured per group of vaccinated animals from two independent experiments and the mean and SEM of their size is shown in (A). Of these granulomas, when they were visualized in groups (15 groups of eggs were assessed per experimental group over two independent experiments) a percentage of abnormal eggs was calculated, and the mean and SEM of abnormality is shown in (B). Significance was calculated against the PBS control. **P<0.01, ***P<0.001, ****P<0.0001.

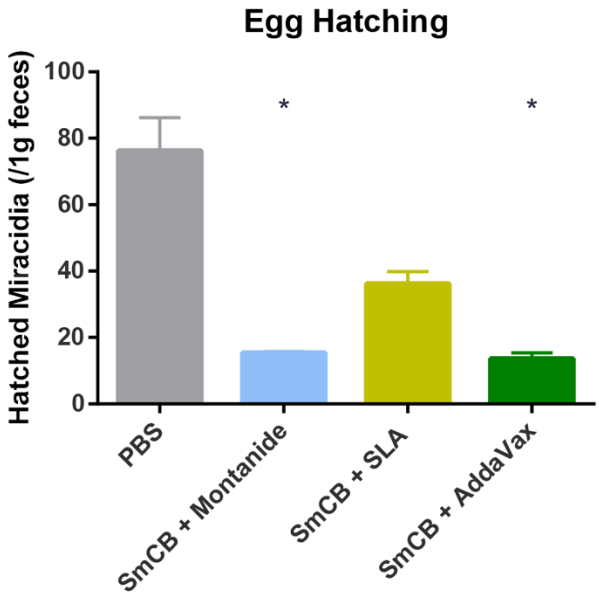
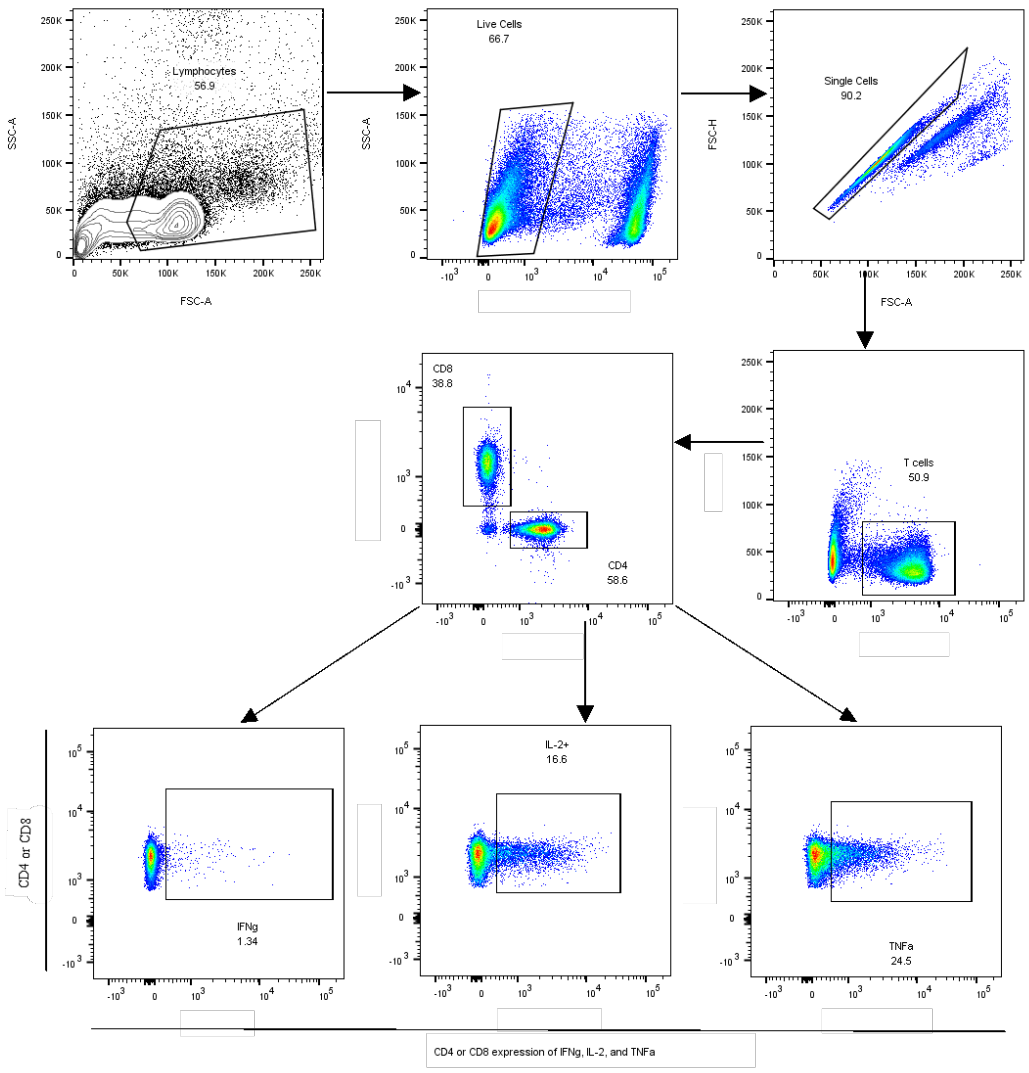


Figure 2.7 Egg hatching

Seven weeks after challenge, feces from mice were collected and hatched in water. The number of resulting miracidia was counted and adjusted to one gram of feces, and the mean and SEM are shown. Feces were collected from two independent mouse experiments, at two separate time points each. Significance is calculated against the PBS control. *P<0.05.

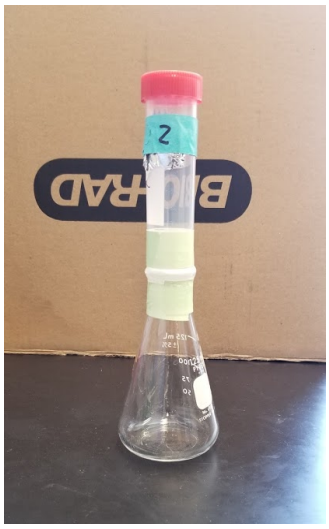
2.10 Supplemental data



Supplemental Figure 2.1 Gating strategy for flow cytometry analysis

IFNg=IFNγ.

A)

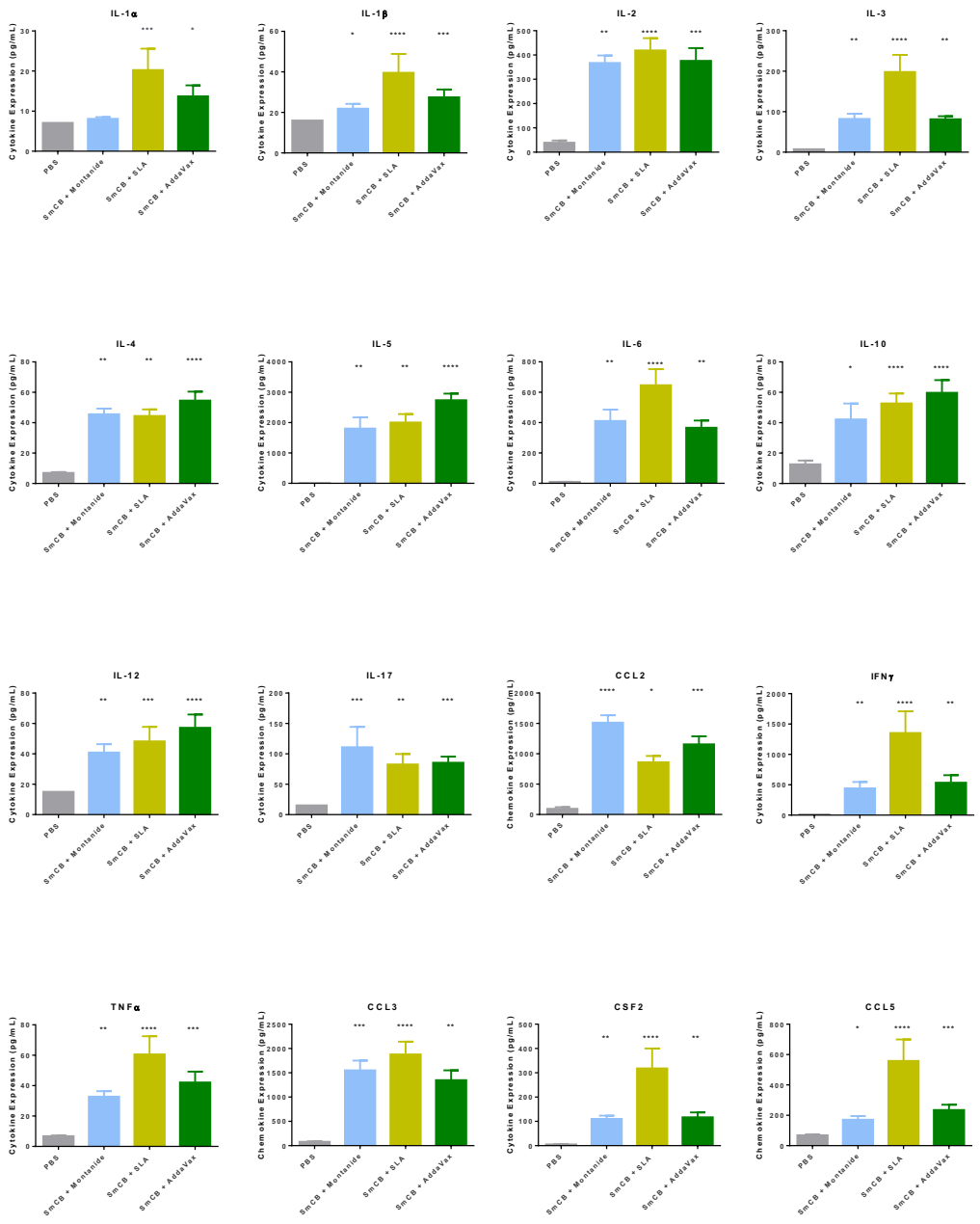


B)

C)

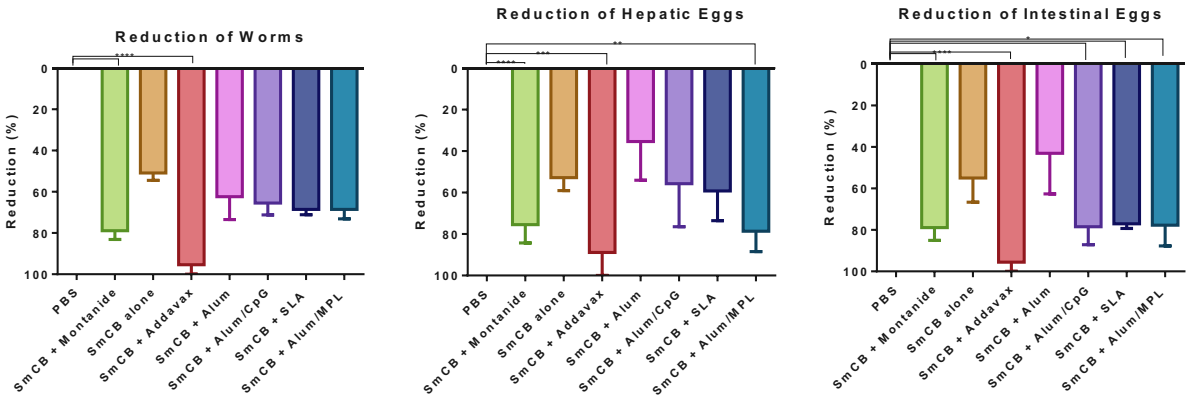
Supplemental Figure 2.2 Egg hatching set up

Seven weeks post challenge, one gram of feces was put into conical tubes with distilled water. Fecal samples were homogenized then transferred to 125 mL Erlenmeyer flasks. The bottom sections of 50 mL conical tubes were removed using an exacto knife and attached tightly to the top of each Erlenmeyer flask using parafilm and tape (A). The Erlenmeyer flask and tube were covered in tin foil to protect from light, except for the top 3 mm of the tube under the twist-on cap (B). The flask and tube were then filled with distilled water to the top and the cap was attached. These light-protected chambers were put inside of a cardboard box, to further protect from light. A hole was cut into the cardboard box and a lamp was shone into it to direct light at the small sections of unprotected tube (3 mm under the cap) (C). After 3 hours, the top 3 mm of water was removed from the tube using a pipette gun and ejected into a 12-well plate. Hatched miracidia were then counted using a dissecting microscope.



Supplemental Figure 2.3 Cytokine and chemokine production

Splenocyte supernatants were run on a multiplex-ELISA for 16 cytokines and chemokines: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, MCP-1, IFNγ, TNFα, MIP-1α, RANTES, and GM-CSF. Supernatant expression of these molecules can be seen as mean along with standard error of the mean. Significance is calculated against the PBS control. N=10. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Supplemental Figure 2.4 Pilot study comparing SmCB vaccine formulations

Six- to eight-week-old female C57BL/6 mice were immunized for parasite burden reduction. Group 1: control: mice were injected with phosphate-buffered saline (PBS) (Wisent Bioproducts, St. Bruno, QC) n=15. Group 2: positive control: mice were immunized with 20 µg of recombinant SmCB (rSmCB) and 35 µL of Montanide ISA 720 VG (SEPPIC Inc., Fairfield, NJ) n=15. Group 3: mice were immunized with 20 µg rSm-CB n=5. Group 4: mice were immunized with 20 µg rSmCB and 25 µL of AddaVax™ (InvivoGen, San Diego, CA) n=5. Group 5: mice were immunized with 20 µg rSmCB and 40 µg of aluminum hydroxide (alum; Alhydrogel; Brenntag BioSector A/S, Frederikssund, Denmark) n=5. Group 6: mice were immunized with 20 µg rSm-CB and 40 µg of aluminum hydroxide and 10 µg CpG dinucleotides (Hycult Biotechnology B.V., Netherlands) n=5. Group 7: mice were immunized with 20 µg rSmCB admixed with 1 mg of pre-formed empty SLA archaeosomes (NRC, Ottawa, Canada) n=5. Group 8: mice were immunized with 20 µg rSmCB and 40 µg of aluminum hydroxide and 10 µg monophosphoryl lipid A (List Biological Laboratories, California) n=5. Each mouse was immunized at weeks 0, 3, and 6 intramuscularly in the thigh with 50 µL of vaccine. Mice were infected at week 9, and at week 16 parasite burden was assessed. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Chapter 3

A Low Dose Adenovirus Vectored Vaccine Expressing *Schistosoma mansoni* Cathepsin B Protects from Intestinal Schistosomiasis in Mice

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3.1 Preface

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic brought the widespread testing and development of novel vaccination platforms (i.e., mRNA and viral vectored vaccines) for human use. These strategies were preferred as they were rapid and economical to develop at a global scale. Arguably, the most used viral vaccine vector in pre-clinical work are adenoviral vectors, such as human adenovirus serotype 5 (hAdV5). HAdV5 is a well-

documented, double-stranded DNA virus, known to cause self-limiting illness in humans. An attenuated strain of this virus has become popular in vaccine development as it does not integrate into the host genome and can safely express exogenous antigens *in vivo* resulting in robust and durable immune responses. In this chapter, we describe the development of a SmCB-expressing hAdV5 (AdSmCB) and its use as a vaccine for schistosomiasis. To avoid the limitation of neutralizing antibodies, our vaccination schedule consisted of a heterologous AdSmCB-prime and boost strategy with recombinant SmCB protein as boosting immunizations. Immune responses following immunization were assessed and parasite burden and associated pathology after infection was described.

3.2 Abstract

Background:

Schistosomiasis is an underestimated neglected tropical disease which affects over 236.6 million people worldwide. According to the CDC, the impact of this disease is second to only malaria as the most devastating parasitic infection. Affected individuals manifest chronic pathology due to egg granuloma formation, destroying the liver over time. The only FDA approved drug, praziquantel, does not protect individuals from reinfection, highlighting the need for a prophylactic vaccine. *Schistosoma mansoni* cathepsin B (SmCB) is a parasitic gut peptidase necessary for helminth growth and maturation and confers protection as a vaccine target for intestinal schistosomiasis.

Methods:

A SmCB expressing human adenovirus serotype 5 (AdSmCB) was constructed and delivered intramuscularly to female C57BL/6 mice in a heterologous prime and boost vaccine with recombinant protein. Vaccine induced immunity was described and subsequent protection from parasite infection was assessed by analysing parasite burden and liver pathology.

Findings:

Substantially higher humoral and cell-mediated immune responses, consisting of IgG2c, T_H1 effectors, and polyfunctional CD4+ T cells, were induced by the heterologous administration of AdSmCB when compared to the other regimens. Though immune responses favoured T_H1 immunity, T_H2 responses provided by SmCB protein boosts were maintained.

This mixed T_H1/T_H2 immune response resulted in significant protection from *S. mansoni* infection comparable to other vaccine formulations which are in clinical trials. Schistosomiasis associated liver pathology was also prevented in a murine model.

Interpretation:

Our study provides missing pre-clinical data supporting the use of adenoviral vectoring in vaccines for *S. mansoni* infection. Our vaccination method significantly reduces parasite burden and its associated liver pathology – both of which are critical considerations for this helminth vaccine.

Funding:

This work was supported by the Canadian Institutes of Health Research, R. Howard Webster Foundation, and the Foundation of the McGill University Health Centre.

3.3 Research in context

Evidence before this study:

There are currently no helminth vaccines approved by the FDA to date. Vaccines for schistosomiasis which are in clinical trials have demonstrated varying protective capacity reaching a maximum of 70% when tested in animals. These vaccines utilize a homologous vaccination strategy, consisting of 3 doses of adjuvanted recombinant protein, to confer protection that has yet to be validated in human trials. Adenovirus vectored vaccines are generally tested in high doses though cell-mediated responses can be delivered by lower doses.

Added value of this study:

This low dose adenovirus vaccine is effective in mounting a robust immune response against a helminth antigen and conferring protection from schistosomiasis and its resulting pathology to the liver. The protection we demonstrated is higher than most in human clinical trials and provided by one less recombinant protein dose, without the need of an adjuvant.

Implications of all the available evidence:

The development of a low-dose adenoviral vectored vaccine administered with less protein boosts which are unadjuvanted would greatly ease its production for the global population. This would increase vaccine availability while lowering both cost and potential adverse events. With the nearly one billion individuals at risk of schistosomiasis infection, the threat of resistance to

chemotherapy, and lethal prognoses to affected individuals due to increased susceptibility to coinfections, the need for a preventative vaccine is urgent.

3.4 Introduction

Schistosomiasis is a neglected tropical disease affecting over 236.6 million individuals in over 70 countries worldwide[1]. The impact of this disease has been estimated by the CDC to be the second most devastating parasitic infection behind malaria. Of the species that affect humans, the most widespread cause of schistosomiasis is *Schistosoma mansoni,* from which egg deposition in digestive tissues causes chronic disability and morbidity in endemic regions.

Current control strategies rely mainly on chemotherapy with praziquantel (PZQ). Although effective, PZQ does not protect from reinfection and drug resistance is a rising concern[2,3], justifying the development of vaccines for this parasite. *S. mansoni* cathepsin B (SmCB) is a cysteine peptidase predominantly found in adult worms and migratory larvae and is involved in the digestion of host blood macromolecules for nutrient acquisition. We have previously described the protective efficacy of SmCB both as an adjuvanted protein[4–6] and when expressed by a *Salmonella* vector[7,8].

Adenovirus vectored vaccines have been developed for multiple infectious diseases and tested in both healthy[9–11] and immunocompromised patients[12–14] showing strong induction of cellular and humoral immune responses despite the presence of pre-existing immunity to the vector[12,15,16]. A positive safety profile in immunocompromised patients becomes more important in areas where *S. mansoni* is endemic due to co-infections with other pathogens (e.g., HIV[17,18], hepatitis[19], malaria[20], tuberculosis[18,21], etc.). In response to the 2019 COVID-19 pandemic, several vaccines using adenovirus technology were engineered since they are cost effective, and production can scale up easily to meet the needs of a global disease[22].

In this study we describe the construction and pre-clinical evaluation of a replication-incompetent recombinant human adenovirus serotype 5 (Ad) expressing SmCB (AdSmCB) when delivered in a heterologous prime-boost method with recombinant SmCB. The development of an efficacious anti-schistosome vaccine would aid in the elimination of this parasite, protecting nearly one billion individuals at risk of infection[23].

3.5 Methods

3.5.1 Ethics statement

All animal procedures were performed in accordance with Institutional Animal Care and Use Guidelines approved by the Animal Care and Use Committee at McGill University (Animal Use Protocol 7625). Mouse housing, husbandry, and environmental enrichment can be found within McGill standard operating procedures (SOP) #502, #508, and #509. Animals were monitored for adverse events for three days post vaccination and weekly until the end of each experiment. Humane intervention points were monitored according to McGill SOP #410. All animals were humanely sacrificed at endpoint by anaesthesia with isoflurane before euthanasia by carbon dioxide asphyxiation followed by pneumothorax and blood collection by cardiac puncture.

3.5.2 Cell lines and reagents

Cell lines were obtained from commercial sources, passed quality control procedures, and were certified and validated by the manufacturer. SF-BMAd-R cells were validated for identity, as human derived[24]. All reagents were validated by the manufacturer and/or has been cited previously in the literature. For most reagents RRID tags have been listed in text.

3.5.3 Generation of AdSmCB vector

The AdSmCB was developed following a similar protocol as described[25]. Briefly, the SmCB gene cassette combined a Kozak sequence with the full length of SmCB (Genbank accession number M21309.1) followed by a proline-linked 6X histidine tag and the poly-A signal "AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTG" (GenScript, Piscataway, NJ, USA) (Supplemental Figure 3.1). The full cassette was synthesized and codon optimized to mouse and human expression by Integrated DNA Technologies (Coralville, IA, USA) and cloned into the vector, pShuttle-CMV-Cuo[26]. The plasmid containing our recombinant non-replicating human adenovirus serotype 5 (E1 and E3 genes removed (ΔE1-, ΔE3-); 1st generation) encoding the *S. mansoni* cathepsin B gene was made through homologous recombination in

AdEasier-1 cells (strain), a gift from Bert Vogelstein (Addgene plasmid #16399) (Addgene, Watertown, MA, USA)[27]. It was then linearized with *PacI* and transformed into HEK293A cells (RRID:CVCL_6910). Our recombinant adenovirus was then amplified using SF-BMAd-R cells[24], purified by ultracentrifugation on CsCl gradients as described previously[28], and titrated using the Adeno-X RapidTiter Kit (Clontech, Mountain View, CA, USA). A second human adenovirus serotype 5 (ΔE1-, ΔE3-; 1st generation), lacking a gene cassette, was used as a negative control.

3.5.4 Western blot assays

Western blot analysis to determine protein expression of SmCB by AdSmCB was performed after infection of HEK293A cells. Briefly, cells were infected at a multiplicity of infection of 5 particles per cell and incubated for 48-72 hours followed by the lysis of cells using Lysis Buffer (0.1M Tris, 10 µL EGTA, 50 µL Triton-100, 0.1M NaCl, 1mM EDTA, 25 µL 10% NaDeoxycholate, 1X protease inhibitor, in ddH_2O). Cell supernatants and lysates were resolved on an SDS-PAGE gel under reducing conditions followed by transfer onto a nitrocellulose membrane. The membrane was subsequently blocked in phosphate buffered saline (PBS) with 0.05% Tween 20 (PBS-T) and (Fisher Scientific, Ottawa, ON, Canada) 5% milk (Smucker Foods of Canada Corp, Markham, ON, Canada) (PBS-TM). The membrane was then incubated with mouse monoclonal anti-polyHistidine (RRID:AB_258251) antibody diluted 1:5 000 in PBS-TM overnight at 4°C. The membrane was then washed in PBS-T before incubation with horseradish peroxidase (HRP)-conjugated anti-mouse IgG (IgG-HRP) (Sigma Aldrich) diluted 1:20 000 in PBS-T for one hour at room temperature. After incubation the membrane was washed again and developed using SuperSignal West Pico Plus Chemiluminescent Substrate (ThermoFisher Scientific, Waltham, MA, USA).

3.5.5 *S. mansoni* cathepsin B recombinant protein preparation

S. mansoni cathepsin B was prepared and purified as previously described[6]. Briefly, the PichiaPink™ system (Thermo Fisher Scientific) was used, and recombinant yeast cells were cultured in a glycerol medium. After three days of growth, yeast cells were induced in a methanol medium to allow expression of recombinant protein. Recombinant protein was purified by Ni-NTA

chromatography (Ni-NTA Superflow by QIAGEN, Venlo, Limburg, Netherlands), eluted, and dialysed into PBS. Recombinant SmCB was analysed by Western Blot using antibodies directed at the His-tag (RRID:AB_258251).

3.5.6 Animals and immunization protocol

Six- to eight-week-old female C57BL/6 mice were bred from mice purchased from Charles River Laboratories (RRID:IMSR_CRL:027) (Senneville, QC, Canada). Four groups of mice (n = 8) were immunized for humoral and cell-mediated immunity assessment. Another four groups of mice (n = 8) were immunized and subsequently infected for parasite burden assessment. Each mouse was immunized at weeks 0, 3, and 6 (Supplemental Figure 3.2) by intramuscular injection in the thigh in a total volume of 50µL. Group 1 (PBS): mice were injected with PBS (Wisent Bioproducts, St. Bruno, QC, Canada). Group 2 (SmCB): mice were immunized with 20µg of recombinant SmCB three times. Group 3 (AdNeg:SmCB): mice were immunized with 10^5 infectious units (IU) of an empty adenovirus containing no gene cassette, followed by two boosts of 20µg SmCB. Group 4 (AdSmCB:SmCB): mice were immunized with 10^5 IU of AdSmCB, followed by two boosts of 20µg SmCB. A fifth group was included in the challenge study as a control for non-specific protection from the empty adenovirus vector. Group 5 (AdNeg): mice were immunized with 10^5 IU of an empty adenovirus containing no gene cassette, followed by two injections of PBS. Mice were bled from the saphenous vein at weeks 0, 3, and 6. Mice immunized for humoral and cell-mediated immunity assessment were euthanised three weeks after the final vaccination and blood and spleens were collected.

3.5.7 *Schistosoma mansoni* challenge

Biomphalaria glabrata snails infected with the Puerto Rican strain of *S. mansoni* were provided by NIAID Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD, USA). At week 9, *S. mansoni* cercariae were shed from snails and experimental groups immunized for the challenge study were blinded and challenged with 150 parasites via tail exposure for one hour. Seven weeks post infection, animals were euthanised to assess parasite burden. Images of mouse livers were taken during dissection using a Galaxy S10 cell phone camera

(Samsung Group, Seoul, South Korea). Adult worms were perfused from the hepatic portal system and counted manually[4,6]. Liver sections were suspended in 10% buffered formalin phosphate (Fisher Scientific) and processed for histology as described before[6,29]. Remaining liver and intestines were weighed and digested overnight at 37°C in 4% potassium hydroxide. The following day, eggs present in these tissues were counted by microscopy and adjusted per gram of tissue. Burden reductions were calculated as previously described[6,29]:

$$Percent\ of\ worms\ or\ eggs\ reduction = \left(1 - \frac{mean\ number\ of\ worms\ or\ eggs\ recovered\ in\ immunized\ mice}{mean\ number\ of\ worms\ or\ eggs\ recovered\ in\ PBS\ control\ mice}\right) \times 100\%$$

3.5.8 Serum Total SmCB-specific IgG, IgG avidity, IgM, IgE, and IgA

SmCB-specific serum IgG was assessed by ELISA as described elsewhere[29]. Briefly, high binding 96-well plates (Greiner Bio-One, Frickenhausen, Germany) were coated with recombinant cathepsin B (0.5 µg/mL) in 100 mM bicarbonate/carbonate buffer (pH 9.6) overnight at 4°C. Then plates were blocked with 2% bovine serum albumin (BSA; Sigma Aldrich) in PBS-T (blocking buffer) before serum samples were added in duplicate. When running serum for IgG, an additional set of serum samples were run in duplicate to determine IgG avidity. Plates were incubated for one hour at 37°C then washed with PBS (pH 7.4). IgG avidity assessment: 10M urea was added to one set of samples while blocking buffer was added to the other set and the standard curve. Plates were covered and incubated for 15 minutes at room temperature, washed four times, then blocked again with blocking buffer for one hour at 37°C. Next, plates were washed with PBS and anti-mouse IgG-HRP (Sigma Aldrich) was diluted 1:20 000 in blocking buffer and applied. For other immunoglobulins, the same protocol was followed without the additional avidity steps and the appropriate HRP-conjugated antibody was applied. HRP-conjugated anti-mouse IgM (RRID:AB_2794240, SouthernBiotech, Birmingham, AL, USA) or IgE (RRID:AB_2868311, Thermofisher) was diluted 1:6 000 in blocking buffer and applied. For IgA, HRP-conjugated anti-mouse IgA (Sigma Aldrich) was diluted 1:10 000 in blocking buffer and applied. Plates were washed a final time with PBS and 3,3',5,5'-Tetramethyl benzidine (TMB) substrate (Sigma Aldrich) was added to each well. The reaction was stopped after 10 minutes using H_2SO_4 (0.5M; Fisher Scientific) and the optical density (OD) was measured at 450 nm with an EL800 microplate

reader (BioTek Instruments Inc., Winooski, VT, USA). Concentrations of SmCB specific IgG and IgA were calculated by extrapolation from respective standard curves. IgG avidity indices were calculated by dividing the IgG titre in the urea condition by the IgG titre in the non-treated condition. IgM and IgE were reported as OD values.

3.5.9 Serum SmCB-specific IgG1, and IgG2c

SmCB-specific serum IgG1, and IgG2c were assessed by ELISA as described elsewhere[6,29]. Briefly, Immunolon 2HB flat-bottom 96-well plates (Thermofisher) were coated with recombinant SmCB (0.5 μg/mL) in 100 mM bicarbonate/carbonate buffer (pH 9.6). Plates were washed with PBS-T and blocking buffer was applied for 90 minutes. A serial dilution of serum was applied to plates in duplicate and incubated for 2 hours at 37°C. Plates were washed again with PBS-T, and goat anti-mouse IgG1-HRP (RRID:AB_2794426, SouthernBiotech) or goat anti-mouse IgG2c-HRP (RRID:AB_2794462, SouthernBiotech) was applied to plates for one hour at 37°C. After a final wash, TMB was added followed by H_2SO_4. Again, OD was measured as above. IgG1 and IgG2c endpoint titres were calculated as the reciprocal of the highest dilution which gave a reading above the cut-off. The endpoint titre cut-off was statistically established as described elsewhere[30] using the sera of PBS immunized, unchallenged mice.

3.5.10 Cell-mediated immune responses

Three weeks after the last immunization, mice were euthanised, spleens were collected, and splenocytes were isolated as previously described[6]. Splenocytes for multiplex ELISA assay were resuspended in RPMI-1640 supplemented with 10% foetal bovine serum, 1 mM penicillin/streptomycin, 10 mM HEPES, 1X MEM non-essential amino acids, 1 mM sodium pyruvate, 1 mM L-glutamine (Wisent Bioproducts), and 0.05 mM 2-mercaptoethanol (Sigma Aldrich) (fancy RPMI, fRPMI). Splenocytes for flow cytometry were resuspended in RPMI-1640 supplemented with 10% foetal bovine serum, 1 mM penicillin/streptomycin, and 10 mM HEPES (complete RPMI, cRPMI).

3.5.11 Cytokine production by multiplex-ELISA

Splenocytes were incubated at 10^6 cells in 200 µL with SmCB in fRPMI (2.5 µg/mL recombinant protein). After 72 hours at 37°C + 5% CO_2, plates were centrifuged, and supernatant was collected and stored at -80°C until analysis. Cell supernatants were assessed for the presence of 16 cytokines and chemokines (IL1α, IL1β, IL2, IL3, IL4, IL5, IL6, IL10, IL12p70, IL17, IFNγ, TNFα, MCP-1 (CCL2), MIP-1α (CCL3), GM-CSF (CSF2), and RANTES (CCL5)) using Q-plex Mouse Cytokine – Screen (16-plex) multiplex ELISA following the manufacturer's guidelines (Quansys Biosciences, Logan, UT, USA). Samples were run in singlet.

3.5.12 T cell-mediated cytokine secretion by flow cytometry

Splenocytes were seeded into 96-well U-bottom plates (BD Falcon) at 10^6 cells in 200 uL/well. Duplicate cultures were stimulated with or without SmCB in cRPMI (2.5 µg/mL) for 18 hours at 37°C + 5% CO_2. For the last 6 hours of incubation, protein transport inhibitor was prepared according to the manufacturer's guidelines (RRID:AB_2869014, BD Science, San Jose, CA, USA) and added to all samples. Cells stimulated with phorbol 12-myristate 13-acetate (Thermofisher) and ionomycin (Thermofisher) were processed as positive controls. Plates were then processed for flow cytometry as described elsewhere[31]. To minimize spectral overlapping: single stain, fluorescence minus one, and unstained controls were also included. All staining and fixation steps took place at 4°C protected from light. Briefly, splenocytes were washed twice with cold PBS, and stained with 50 µL/well fixable viability dye eFluor 780 (Thermofisher) diluted at 1:300 for 20 minutes. Cells were washed twice using PBS with 1% BSA (PBS-BSA), and then blocked with Fc block (RRID:AB_394656, BD Science) diluted 1:50, for 15 minutes at 4°C protected from light. All surface stains were diluted 1:50 in PBS-BSA and 50 µL/well of extracellular cocktail was applied for 30 minutes. The following antibodies made up the extracellular cocktail: CD3-FITC (RRID:464883, Thermofisher), CD4-V500 (RRID:AB_1937327, BD Bioscience) and CD8-PerCP-Cy5 (RRID:AB_394081, BD Science). Cells were then washed as before and fixed with 1X fixation buffer (RRID:AB_2869005, BD Science) overnight. The next day, plates were washed twice with 1X permeabilization buffer (perm buffer) (RRID:AB_2869011, BD Science) and stained with an intracellular cocktail of antibodies diluted 1:50 in perm buffer applied as 50

μL/well for 30 minutes. The intracellular cocktail was made up of: IL-2-Pe-Cy5 (RRID:AB_2123674, Biolegend, San Diego, CA, USA), IFNγ-PE (RRID:AB_395376, BD Science), and TNFα-efluor450 (RRID:AB_1548825, Thermofisher). After staining, cells were washed once with perm buffer, once with PBS-BSA, and resuspended in PBS-BSA and acquired on a BD LSRFortessa X-20 (BD Science). Flow data were analysed using Flowjo software (version 10.0.8r1) (Treestar, Ashland, OR, USA) and SPICE software (version 6.1)[32]. Our gating strategy is shown in Supplemental Figure 3.3.

3.5.13 Histology, egg granuloma assessment, and fibrotic area measurements

Liver sections in 10% buffered formalin phosphate were processed for histopathology and stained using haematoxylin and eosin to assess granuloma size and egg morphology and Masson's trichrome to measure fibrotic area. Granuloma sizes were measured using Zen Blue software (version 2.5.75.0; Zeiss) as previously reported[6,29,33–36]. Briefly, while working at 400X magnification, the pointer was used to trace the perimeter of 24-32 granulomas in an exudative-productive stage with a clearly visible egg per experimental group, which the software converted into an area. Hepatic eggs were classified as abnormal if their internal structure was lost or the perimeter of the egg was crenelated. Abnormal eggs were counted and reported as a percent of the total eggs counted per field of vision. Eighteen to 32 different fields of vision were assessed per experimental group over two independent experiments. Slides stained with Masson's trichrome were imaged using the Aperio AT Turbo digital whole slide scanning system (Leica Biosystems, Concord, ON, Canada) at 20X magnification. Twenty-five to 37 single egg formed granulomas per group were delimited and the area of visible blue was measured using QuPath 0.3.0[37].

3.5.14 Statistical analysis

Experimental units are defined as individual animals. Sample size determination: Sample sizes (n=7) were calculated using G*Power (3.1.9.3)[38] based on the means and standard deviation of preliminary data to achieve at least 90% power and allow for a five percent type I error. One mouse was added (n=8) to each group to compensate for a possible attrition rate of 10%. To minimise potential confounders, mice were matched for age, sex, and body weight.

Randomisation: Mice were randomised into experimental groups before the start of each study. Blinding: For challenge experiments, staff performing infections and sample harvesting were blinded to groups, and unblinded after data analysis. Inclusion/Exclusion: No animals were excluded. For the assessment of granuloma size and fibrotic area around single eggs, outliers were calculated using the ROUT method (Q=1) and if present, they were excluded.

Statistical analysis was performed using GraphPad Prism 9 software (La Jolla, CA, USA). Data were assessed for normality using Shapiro-Wilk tests. Non-parametric data were analysed by Kruskal-Wallis tests with Dunn's multiple comparisons. When appropriate, one-way and two-way ANOVAs were employed with Tukey's multiple comparisons. P values <0.05 were considered significant.

3.5.15 Role of funders

Funding agencies did not have a role in the study design, data collection, data analyses, interpretation, or writing of this manuscript.

3.6 Results

3.6.1 Vaccination with AdSmCB:SmCB results in robust humoral responses

Humoral responses were determined throughout the immunization schedule. No mice had detectable SmCB specific IgG at baseline, and the PBS control remained negative throughout the study. Mice receiving SmCB developed IgG antibody titres by week 3, whereas mice receiving recombinant Ad as a primary immunization showed detectable IgG titres only after immunization with a protein boost. However, by week 6 IgG titres between SmCB and AdSmCB:SmCB groups were no longer significantly different and at the end of the immunization period (week 9) AdSmCB:SmCB produced significantly higher titres than the AdNeg:SmCB group (p=0.0261, Kruskal-Wallis test) (Figure 3.1a). We also sought to determine antigen-specific IgG avidity (Figure 3.1b) and IgG subtypes at the time of infection. All vaccinated animals produced highly avid IgG antibodies. Although there was no significant difference in IgG avidity between groups SmCB and AdSmCB:SmCB, mice which received the AdSmCB prime showed significantly

greater avidity compared to the group which was primed with the empty Ad vector followed by 2x SmCB protein (AdNeg:SmCB) (p=0.0371, one-way ANOVA). There were no statistical differences between the amount of IgG1 produced by any of the experimental groups (Figure 3.1c), however AdSmCB:SmCB significantly increased the production of SmCB specific IgG2c (3.66e5 ± 1.39e5) when compared to both the SmCB (9.64e3 ± 7.78e3) (p=0.0017) and AdNeg:SmCB groups (3.05e3 ± 9.04e2) (p=0.0037, Kruskal-Wallis test) (Figure 3.1d). Finally, when compared to the SmCB group, the ratio of IgG1 to IgG2c was significantly reduced in mice first given a priming immunization of recombinant adenovirus (p=0.0046, Kruskal-Wallis test) (Figure 3.1e). Throughout the immunization schedule, all vaccinated animals saw a trend of increasing antigen-specific IgM, however this trend was not significant. Additionally, no animals developed antigen-specific IgE or IgA in response to vaccination (Supplemental Figure 3.4).

3.6.2 AdSmCB:SmCB enhances cytokine and chemokine expression

To determine the immune landscape of lymphocyte responses created by vaccination, we ran a multiplex ELISA on the supernatants of stimulated splenocytes. For many of the cytokines and chemokines tested, the AdSmCB:SmCB group generated elevated levels of molecular signals as shown in the radar plot (Figure 3.2a). Each vaccine formulation can be seen to produce a unique cytokine and chemokine signature. Notably, AdSmCB:SmCB maintains the significant expression of IL5 (p=0.0100) also seen in the SmCB group (p=0.0308, Kruskal-Wallis test) (Figure 3.2b), while enhancing expression of IFNγ from both PBS (p=0.0009) and AdNeg:SmCB groups (p=0.0152, Kruskal-Wallis test) (Figure 3.2c), and RANTES (CCL5) compared to SmCB alone (p=0.0100, Kruskal-Wallis test) (Figure 3.2d), among others (Supplemental Figure 3.5).

3.6.3 AdSmCB:SmCB increases IFNγ+ T cell frequency and promotes CD4+ T cell polyfunctionality

IFNγ is a key contributor of protection in *Schistosoma* radiation attenuated vaccine models, so we were interested in its increased expression in mice vaccinated with our vectored vaccine. Since AdSmCB:SmCB immunized animals also displayed elevated levels of RANTES, a T-cell associated chemokine, we used flow cytometry to determine if T cells could be responsible for

IFNγ production. Indeed, when splenic T cells were stimulated *ex vivo* with SmCB, we observed an increased frequency of CD4+ T cells (Figure 3.3a) expressing IFNγ in mice vaccinated with AdSmCB:SmCB (0.141 ± 4.27e-2%) compared to PBS (0.013 ± 8.40e-3%) (p=0.0032), SmCB (0.015 ± 8.86e-3%) (p=0.0040), and AdNeg:SmCB (0.026 ± 8.00e-3%) (p=0.0105, two-way ANOVA). The percent of CD4+ T cells expressing IL2 was also increased in the recombinant adenovirus group (0.098 ± 5.85e-2%) when compared to the PBS control (0.001 ± 7.89e-4%) (p=0.0429, two-way ANOVA). Using Boolean and SPICE analyses, we then assessed the polyfunctional profiles of our experimental groups. Figure 3.3b shows the distribution of CD4+ T cell populations expressing one, two, and three cytokines. We saw that our recombinant adenovirus elicited a larger repertoire of polymorphic CD4+ T cells than the recombinant protein and empty viral vector groups, with an emergence in triple positive cells (IFNγ+TNFα+IL2+), as well as IFNγ+TNFα+, and IL2+TNFα+ cells. Since these pie charts are not to scale, we included a heat map which graphically represents the frequencies of cells in each polymorphic category. Each category depicts a different cytokine expression profile which has been established in the legend to the right of the heat map. By the increased intensity of red observed in our recombinant adenovirus group, we saw that AdSmCB:SmCB has a larger proportion of each CD4+ T cell type (categories 1-6) except for those expressing only TNFα (category 7) which was higher in the SmCB vaccinated mice. While there was a marked increase in cells expressing IFNγ alone (category 4) and IL2 alone (category 6), the increased percentage of CD4+ T cells expressing more than one cytokine can be easily visualized (categories 1-3, and 5) within the heat map. When looking at the proportion of AdSmCB:SmCB CD8+ T cells expressing IFNγ (0.158 ± 6.68e-2%) we again see a striking increase when compared to all other groups: PBS (0.021 ± 1.29e-2%) (p=0.0026), SmCB (0.041 ± 1.41e-2%) (p=0.0137), and AdNeg:SmCB (0.014 ± 1.10e-2%) (p=0.0014, two-way ANOVA) (Figure 3.3c). We saw similar trends of increased TNFα expression from both CD4+ and CD8+ T cells in groups SmCB and AdSmCB:SmCB; however, these were not significant. Although Boolean analysis and pie chart depictions of each vaccine resulted in a unique CD8+ T cell signature (Figure 3.3d), the differences between groups were far less drastic than in the case of the CD4+ T cells, as seen in the corresponding heat map. In summary, our polymorphic T cell analysis nicely corroborated the striking expression of IFNγ and IL2 witnessed in our AdSmCB:SmCB vaccinated animals.

3.6.4 AdSmCB:SmCB significantly reduces parasite burden

To determine the protective efficacy of our Ad vaccine, immunized animals were infected with *S. mansoni* and assessed for adult worms, hepatic eggs, and intestinal eggs. A fifth group of mice, vaccinated with an empty adenovirus vector without protein boosts, was included to control for any non-specific protective capacity of the vector itself. The average amount of adult worms collected from control mice was 37 ± 7 worms over two independent experiments. Relative reduction was calculated against the PBS control group within the same experiment to reduce batch discrepancy between infections. The AdNeg vector group was unable to significantly reduce adult worm burden from the PBS control (Supplemental Figure 3.6). However, when this empty vector was boosted twice with recombinant protein, protection increased to 24.2 ± 8.1% (p=0.0380, one-way ANOVA) (Figure 3.4a). Worm burden was further reduced in animals vaccinated with 3 doses of recombinant protein, and those initially primed with our recombinant Ad by 42.8 ± 4.2% (p=0.0001) and 71.7 ± 7.8% (p<0.0001, one-way ANOVA), respectively. The main cause of pathology in schistosomiasis is egg deposition by adult worms. Therefore, egg burden reductions in both livers (Figure 3.4b) and intestines (Figure 3.4c) were also calculated. Hepatic eggs averaged 14 096 ± 3 953 eggs per gram and intestinal eggs averaged 15 327 ± 4 705 eggs per gram of tissue in the PBS control. Similar to worm reduction, AdNeg alone was unable to confer any significant protection from egg deposition. When boosted twice with recombinant protein, mice initially immunized with the AdNeg vector had liver and intestinal egg reductions of 22.3 ± 7.1% (p=0.0245) and 22.4 ± 7.4% (p=0.0798, one-way ANOVA), respectively. Animals immunized with recombinant protein alone reduced liver and intestinal eggs by 42.9 ± 4.8% (p<0.0001) and 41.6 ± 5.4% (p=0.0004), respectively, whereas animals immunized with AdSmCB:SmCB were protected from liver and intestinal eggs by 68.6 ± 5.8% (p<0.0001) and 75.7 ± 8.7% (p<0.0001, one-way ANOVA), respectively.

3.6.5 Liver pathology is markedly reduced in vaccinated animals

During animal dissection, images were taken of whole livers. Visual analysis showed an increased number of granulomas (white formations) and hepatomegaly in infected PBS mice.

Despite the presence of granuloma formation, livers in all vaccinated animals showed reduced pathology post infection, which was marked in groups SmCB and AdSmCB:SmCB (Figure 3.5a). Microscopic examination of liver tissue stained by haematoxylin and eosin or Masson's trichrome was used to assess granuloma formation (Figure 3.5b) and egg-induced liver fibrosis (Figure 3.5c), respectively. Granulomas in control mice were large and well formed with an average size of 69 982 ± 7 636 μm^2 harbouring intact eggs with normal appearances. When compared to both the PBS control and the AdNeg:SmCB groups, recombinant protein (SmCB) and recombinant adenovirus (AdSmCB:SmCB) groups were able to reduce granuloma sizes to 38 902 ± 2 954 μm^2 (p=0.0095, p=0.0003) and 37 796 ± 4 189 μm^2 (p=0.0089, p=0.0004, Kruskal-Wallis test), respectively (Figure 3.5d). Larger and more developed granulomas were found to have collagen deposition within them. Only animals vaccinated with AdSmCB:SmCB displayed a reduction in egg-induced fibrotic areas compared to control mice, from 114 815 ± 13 575 μm^2 to 64 891 ± 7 146 μm^2 (p=0.0159, Kruskal-Wallis test) (Figure 3.5e). Through microscopic visualization, we also determined the amount of liver eggs which were abnormal in structure. Although the number of abnormal eggs increased in all mice which received recombinant protein, only the AdSmCB:SmCB group showed a significantly increased proportion (Supplemental Figure 3.7), reaching 32 ± 4% compared to the 14 ± 4% of the PBS control (p=0.0136) and the 12 ± 4% (p=0.0135, Kruskal-Wallis test) of the SmCB group.

3.7 Discussion

Schistosomiasis continues to be a major public health problem despite ongoing control efforts. The emergence of drug resistant strains and high reinfection rates after drug therapy highlight the need for additional anti-schistosome tools[39–41]. The development of an effective vaccine against *Schistosoma* is of global importance. Although many pre-clinical efforts are in the pipeline, none yet have been approved for human use. Current *Schistosoma* vaccine strategies include recombinant protein and DNA-based vaccines; however, recent work has demonstrated protection via pathogen-vectored vaccines, for example *Salmonella* YS1646[7,8]. With the development, proposed safety, and wide distribution of adenoviral vectored vaccines during the SARS-CoV-2 pandemic, we decided to develop our own vaccine using this technology. Only a single other adenoviral vectored vaccine has been tested in models of schistosomiasis[42,43]. In our

work, we targeted the most widespread species causing human infection, *S. mansoni.* Our group has previously demonstrated the protective efficacy of recombinant SmCB[4–6] which acts primarily through T_H2 mediated immunity[44]. Therefore, in our present study, we focused on increasing this protection through the use of viral vectoring and heterologous prime-boosting.

Based on preliminary dose response studies, we found that contrary to the common delivery of high doses of viral vector in the literature (>10^7 IU), our AdSmCB elicited similar T cell cytokine expression and higher protective capacity at very low doses (10^5 IU) (Supplemental Figure 3.8), which may be preferable to prevent vaccine related adverse events. Our vaccine strategy, using a recombinant viral vector prime followed by protein boosts, offered protection from *S. mansoni* infection, well surpassing the WHO 40% threshold indicating significance, and practically reaching the 75% threshold proposed at a National Institute of Allergy and Infectious Diseases schistosomiasis vaccine meeting[45]. Parasite burden reduction seems to be dependent on priming with a SmCB-expressing adenovirus followed by recombinant protein boosts as protection was lower in mice which received homologous immunizations of SmCB protein alone and abrogated in animals which received either an empty adenovirus vector alone or the empty vector boosted by SmCB.

Humoral responses and antibodies targeting secreted proteins, such as the abundantly expressed SmCB, have been suggested to play a key role in cure from schistosomiasis[46]. Intramuscular immunization with our recombinant adenovirus vectored vaccine resulted in trends of increased antigen-specific IgM and significant expansions of highly avid antigen-specific IgG. The role of IgM in schistosomiasis is not well defined. Although some groups have shown putative effects of IgM hindering protection mediated through other antibody isotypes[47,48] it has also been shown to recognize *Schistosoma* epitopes, kill larvae *in vitro*, and provide passive protection *in vivo*[49]. We hypothesize that pentameric IgM may play a helping role in our vaccine by broadly sequestering the peptidase activity of SmCB contributing to parasite starvation, prior to specialized antibody isotype switching. More solidly, the protective effects of IgG have been demonstrated numerous times[50–52]. Although antibody production was delayed in contrast to mice which received recombinant protein alone, mice primed with AdSmCB exhibited comparable levels of antigen-specific IgG by the time of infection. Antibodies produced by both AdSmCB:SmCB and SmCB vaccinated mice displayed high avidity, likely due to boosting immunizations of antigen, and given the T_H1 skewing nature of adenoviral vectors, we were not surprised to see a dramatic

increase in antigen-specific IgG2c antibodies. Despite overall humoral responses skewing towards T_H1 immunity, our AdSmCB:SmCB vaccine maintained analogous levels of antigen-specific IgG1 when compared with the recombinant protein alone group. IgG1 has been explicitly correlated with protection from schistosomiasis in animal models[53,54]; protection by IgG2 antibodies has also been described[55,56]. Additionally, we assessed antigen-specific IgA and IgE, which did not seem to be elicited by our vaccines. Although total IgE has shown protection from many parasitic worms[48,57–59], the lack of vaccine-induced antigen-specific IgE is a promising feature of AdSmCB:SmCB to avoid allergy-type hypersensitivity reactions which have been detrimental to helminth vaccine safety[60].

Splenocyte memory responses to antigen revealed an increased level of cytokine and chemokine expression from our recombinant adenovirus vaccine superior to both protein alone and AdNeg:SmCB. Splenocytes from mice vaccinated with recombinant protein alone showed significant production of IL5, a key mediator of eosinophil activation and differentiation. Several early studies have demonstrated schistosomula killing dependent on eosinophils[61–63]. SmCB is expressed as early as the schistosomula stage and its suppression by RNA interference resulted in growth retardation[64]. We saw that our recombinant adenovirus vaccine also increased IL5, which may mean targeting lung-stage larvae before their maturity into egg-laying adult worms.

A hallmark of schistosomiasis protection, which was brought to light during the evaluation of radiation attenuated schistosome vaccines, is IFNγ[65,66]. This cytokine was only increased in those mice which received our adenovirus vectored vaccine. Since the production of RANTES (CCL5) was also increased, even compared to the SmCB group, we were curious to determine if T cells could be responsible for IFNγ expression. We found that not only was the frequency of AdSmCB:SmCB T cells expressing IFNγ elevated compared to all other groups, but CD4+ T cells expressing IL2 was also increased. Of note, when we assessed memory responses from restimulated T cells, we found varying functionalities between vaccine groups. While CD8+ T cell phenotypes were similar between groups we saw a marked increase in the polyfunctionality of CD4+ T cells when mice were vaccinated with our recombinant adenovirus. Although the role of polyfunctional T cells in schistosomiasis protection remains elusive, their contributions have been described in models of yellow fever[67,68] and influenza as functionally superior cells which exhibit increased degranulation and expression of CD40L and T_H1 cytokines: IFNγ, IL2, and TNFα[69,70]. Data from both murine and human studies corroborate polyfunctional cell protection from

influenza lethality and disease severity respectively[71,72]. Interestingly, these cells have also been identified as key players in immunity conferred by vectored vaccines. A smallpox vaccine elicited polyfunctional T cells specific to vaccinia virus which extended to vaccine expressed HIV gene products[73]. Further, triple positive CD4+ T cells (IFNγ+IL2+TNFα+), a subset of cells which were increased only in our AdSmCB:SmCB group, delivered protection in a parasite infection model where an adenovirus vectored vaccine was tested against *Leishmania major*[74].

Schistosomiasis pathology is caused by the induction of T_H2 responses by the release of soluble egg antigens from eggs trapped in host tissue. As SmCB expression is continued into the adult worm life cycle stage, we hypothesize that steady pressure on a worms' ability to acquire nutrients will lead to a reduction in its fitness and, in turn, that of the eggs it produces. In support, when we evaluated visual fields of egg clusters under a microscope, there was a significantly larger proportion of eggs which were crenelated with a loss of internal structures in mice vaccinated with AdSmCB:SmCB. Through vaccination we were able to prevent many manifestations of liver pathology, including granuloma size and fibrotic area, normally caused by *S. mansoni* infection. We also witnessed visual protective effects on gross livers in those mice which were vaccinated with SmCB alone or our recombinant adenovirus prior to challenge.

Though encouraging, there are limitations to our current study. The use of adenovirus based vaccines has been criticised due to neutralising antibodies to the vector and the induction of vaccine related adverse events[75]. Although some research has shown antigen-specific immune responses despite pre-existing anti-adenovirus immunity, these responses may be futile if adverse events are inherent of adenoviral vectors. To circumvent these issues, we are exploring the expression of our target antigen from other viral vectors. A second limitation is the use of the mouse model for testing *S. mansoni* vaccine efficacy. It has been proposed that, due to physiological features of the murine pulmonary system, vaccine efficacy in mouse models may be over-exaggerated (caused by non-specific, vaccine-induced systemic T cell activation and cytokine levels being maximal at the time schistosomes passage through the lungs)[76]. Despite mice being the most feasible animal model for screening schistosomiasis vaccines, future studies will be needed to determine if protection can be replicated in other animals (e.g., non-human primates) and when parasite challenge is delayed. Finally, this vaccine was tested in a prophylactic capacity without drug intervention, which is not fully reflective of endemic areas where many cases go

undiagnosed and individuals are likely already infected. Future directions include testing our adenovirus vectored vaccine in therapeutic models and in reinfection models after chemotherapy.

Protective correlates of immunity for helminthic infections are widely debated, thus we broadly assessed immune responses (including immunoglobulins, cytokines, and chemokines). However, the careful balancing of targeted T_H1 and T_H2 responses has been proposed[77]. Due to the complex nature of parasitic infections and their inherent modulation of the host immune system, we expect that a multipronged immune response would be necessary for cure. Our data suggest that the use of adenovirus as a vector alters the natural T_H2 skewing of the immune system to SmCB, facilitating enhanced cell-mediated immunity without hindering protection offered by the humoral response. We believe our heterologous strategy could be improved by adjuvanting protein boosts to further augment immune responses thereby increasing protection.

In summary, our findings describe a viral vectored vaccine which prophylactically protects from schistosomiasis, at levels comparable to others in pre-clinical work and those currently in clinical trials, through a platform which has been widely used in humans and can be easily up scaled for global production. Our adenovirus vectored vaccine elicits strong humoral immunity and cellular effectors, balancing T_H2 and T_H1 arms of immunity to target SmCB-expressing larvae and adult worms. More importantly, parasite burden reduction by our vaccine led to a prevention of pathology caused by *S. mansoni* egg deposition, which is crucial to alleviating chronic morbidities and may significantly aid regions where coinfections make liver pathologies lethal.

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3.9 References

1. Schistosomiasis. *World Health Organization* https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (2022).
2. McManus, D. P. *et al.* Schistosomiasis—from immunopathology to vaccines. *Seminars in Immunopathology* **42**, 355–371 (2020).
3. Vale, N. *et al.* Praziquantel for schistosomiasis: Single-drug metabolism revisited, mode of action, and resistance. *Antimicrobial Agents and Chemotherapy* **61**, (2017).
4. Ricciardi, A., Visitsunthorn, K., Dalton, J. P. & Ndao, M. A vaccine consisting of Schistosoma mansoni cathepsin B formulated in Montanide ISA 720 VG induces high level protection against murine schistosomiasis. *BMC Infectious Diseases* **16**, (2016).
5. Ricciardi, A., Dalton, J. P. & Ndao, M. Evaluation of the immune response and protective efficacy of Schistosoma mansoni Cathepsin B in mice using CpG dinucleotides as adjuvant. *Vaccine* **33**, 346–353 (2015).
6. Perera, D. J. *et al.* Adjuvanted Schistosoma mansoni-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model. *Frontiers in Immunology* **11**, (2020).
7. Hassan, A. S., Zelt, N. H., Perera, D. J., Ndao, M. & Ward, B. J. Vaccination against the digestive enzyme Cathepsin B using a YS1646 Salmonella enterica Typhimurium vector provides almost complete protection against Schistosoma mansoni challenge in a mouse model. *PLOS Neglected Tropical Diseases* **13**, e0007490 (2019).
8. Hassan, A. S., Perera, D. J., Ward, B. J. & Ndao, M. Therapeutic activity of a Salmonella-vectored Schistosoma mansoni vaccine in a mouse model of chronic infection. *Vaccine* **39**, 5580–5588 (2021).
9. Zhu, F. C. *et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet* **396**, 479–488 (2020).

10. Baden, L. R. *et al.* Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. *The Lancet HIV* **7**, e688–e698 (2020).
11. Cicconi, P. *et al.* First-in-Human Randomized Study to Assess the Safety and Immunogenicity of an Investigational Respiratory Syncytial Virus (RSV) Vaccine Based on Chimpanzee-Adenovirus-155 Viral Vector–Expressing RSV Fusion, Nucleocapsid, and Antitermination Viral Proteins in Healthy Adults. *Clinical Infectious Diseases* **70**, 2073–2081 (2020).
12. Balint, J. P. *et al.* Extended evaluation of a phase 1/2 trial on dosing, safety, immunogenicity, and overall survival after immunizations with an advanced-generation Ad5 [E1-, E2b-]-CEA(6D) vaccine in late-stage colorectal cancer. *Cancer Immunology, Immunotherapy* **64**, 977–987 (2015).
13. Morse, M. A. *et al.* Novel adenoviral vector induces T-cell responses despite anti-adenoviral neutralizing antibodies in colorectal cancer patients. *Cancer Immunology, Immunotherapy* **62**, 1293–1301 (2013).
14. Covid-19 Vaccine Response in Immunocompromised Haematology Patients. *Clinicaltrials.gov* https://clinicaltrials.gov/study/NCT04805216.
15. Osada, T. *et al.* Optimization of vaccine responses with an E1, E2b and E3-deleted Ad5 vector circumvents pre-existing anti-vector immunity. *Cancer Gene Therapy* **16**, 673–682 (2009).
16. Smaill, F. *et al.* A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. *Science Translational Medicine* **5**, (2013).
17. Furch, B. D., Koethe, J. R., Kayamba, V., Heimburger, D. C. & Kelly, P. Interactions of Schistosoma and HIV in Sub-Saharan Africa: A Systematic Review. *The American Journal of Tropical Medicine and Hygiene* **102**, 711–718 (2020).
18. McLaughlin, T. A. *et al.* Schistosoma mansoni Infection Is Associated With a Higher Probability of Tuberculosis Disease in HIV-Infected Adults in Kenya. *Journal of acquired immune deficiency syndromes (1999)* **86**, 157–163 (2021).
19. Abruzzi, A., Friedx, B. & Alikhan, S. B. Coinfection of Schistosoma Species with Hepatitis B or Hepatitis C Viruses. *Advances in Parasitology* **91**, 111–231 (2016).

20. Getie, S. *et al.* Prevalence and clinical correlates of Schistosoma mansoni co-infection among malaria infected patients, Northwest Ethiopia. *BMC Research Notes* **8**, 1–6 (2015).
21. McLaughlin, T. A. *et al.* CD4 T Cells in Mycobacterium tuberculosis and Schistosoma mansoni Co-infected Individuals Maintain Functional TH1 Responses. *Frontiers in Immunology* **11**, 127 (2020).
22. Mendonça, S. A., Lorincz, R., Boucher, P. & Curiel, D. T. Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. *npj Vaccines* **6**, 1–14 (2021).
23. Lago, E. M. *et al.* Phenotypic screening of nonsteroidal anti-inflammatory drugs identified mefenamic acid as a drug for the treatment of schistosomiasis. *EBioMedicine* **43**, 370–379 (2019).
24. Gilbert, R. *et al.* Establishment and validation of new complementing cells for production of E1-deleted adenovirus vectors in serum-free suspension culture. *J Virol Methods* **208**, 177–188 (2014).
25. Haq, K. *et al.* Evaluation of recombinant adenovirus vectors and adjuvanted protein as a heterologous prime-boost strategy using HER2 as a model antigen. *Vaccine* **37**, 7029–7040 (2019).
26. Mullick, A. *et al.* The cumate gene-switch: a system for regulated expression in mammalian cells. *BMC Biotechnology* **6**, 1–18 (2006).
27. He, T.-C. *et al.* A simplified system for generating recombinant adenoviruses. *Proceedings of the National Academy of Sciences* **95**, 2509–2514 (1998).
28. Oualikene, W., Lamoureux, L., Weber, J. M. & Massie, B. Protease-Deleted Adenovirus Vectors and Complementing Cell Lines: Potential Applications of Single-Round Replication Mutants for Vaccination and Gene Therapy. *Hum Gene Ther* **11**, 1341–1353 (2004).
29. Hassan, A. S., Zelt, N. H., Perera, D. J., Ndao, M. & Ward, B. J. Vaccination against the digestive enzyme Cathepsin B using a YS1646 Salmonella enterica Typhimurium vector provides almost complete protection against Schistosoma mansoni challenge in a mouse model. *PLoS neglected tropical diseases* **13**, e0007490 (2019).
30. Frey, A., Di Canzio, J. & Zurakowski, D. A statistically defined endpoint titer determination method for immunoassays. *Journal of Immunological Methods* **221**, 35–41 (1998).

31. Hodgins, B., Pillet, S., Landry, N. & Ward, B. J. A plant-derived VLP influenza vaccine elicits a balanced immune response even in very old mice with co-morbidities. *PLoS ONE* **14**, (2019).
32. Roederer, M., Nozzi, J. L. & Nason, M. C. SPICE: Exploration and analysis of post-cytometric complex multivariate datasets. *Cytometry Part A* **79A**, 167–174 (2011).
33. Cronan, M. R. *et al.* An explant technique for high-resolution imaging and manipulation of mycobacterial granulomas. *Nature Methods* **15**, 1098–1107 (2018).
34. Hagen, J. *et al.* Omega-1 knockdown in Schistosoma mansoni eggs by lentivirus transduction reduces granuloma size in vivo. *Nature Communications* **5**, 1–9 (2014).
35. Ebenezer, J. A. *et al.* Periostin as a marker of mucosal remodelling in chronic rhinosinusitis. *Rhinology journal* **55**, 234–241 (2017).
36. Tang, C. *et al.* Effect of Cytotoxic T-Lymphocyte Antigen-4 on the Efficacy of the Fatty Acid-Binding Protein Vaccine Against Schistosoma japonicum. *Frontiers in Immunology* **10**, 1022 (2019).
37. Bankhead, P. *et al.* QuPath: Open source software for digital pathology image analysis. *Scientific Reports* **7**, 1–7 (2017).
38. Faul, F., Erdfelder, E., Lang, A. G. & Buchner, A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods* **39**, 175–191 (2007).
39. Melman, S. D. *et al.* Reduced Susceptibility to Praziquantel among Naturally Occurring Kenyan Isolates of Schistosoma mansoni. *PLOS Neglected Tropical Diseases* **3**, e504 (2009).
40. Zacharia, A., Mushi, V. & Makene, T. A systematic review and meta-analysis on the rate of human schistosomiasis reinfection. *PLoS ONE* **15**, (2020).
41. Woldegerima, E., Bayih, A. G., Tegegne, Y., Aemero, M. & Zeleke, A. J. Prevalence and Reinfection Rates of Schistosoma mansoni and Praziquantel Efficacy against the Parasite among Primary School Children in Sanja Town, Northwest Ethiopia. *Journal of Parasitology Research* **2019**, (2019).
42. Dai, Y. *et al.* Construction and evaluation of replication-defective recombinant optimized triosephosphate isomerase adenoviral vaccination in Schistosoma japonicum challenged mice. *Vaccine* **32**, 771–778 (2014).

43. Dai, Y. *et al.* Enhancement of Protective Efficacy through Adenoviral Vectored Vaccine Priming and Protein Boosting Strategy Encoding Triosephosphate Isomerase (SjTPI) against Schistosoma japonicum in Mice. *PLOS ONE* **10**, e0120792 (2015).
44. El Ridi, R. *et al.* Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. *PloS one* **9**, (2014).
45. Mo, A. X. & Colley, D. G. Workshop report: schistosomiasis vaccine clinical development and product characteristics. *Vaccine* **34**, 995–1001 (2016).
46. Amaral, M. S. *et al.* Rhesus macaques self-curing from a schistosome infection can display complete immunity to challenge. *Nature Communications* **12**, 1–17 (2021).
47. Yi, X. Y., Omer-Ali, P., Kelly, C., Simpson, A. J. & Smithers, S. R. IgM antibodies recognizing carbohydrate epitopes shared between schistosomula and miracidia of Schistosoma mansoni that block in vitro killing. *The Journal of Immunology* **137**, (1986).
48. Capron, A. & Dessaint, J.-P. Immunologic aspects of schistosomiasis. *Annu Rev Med* **43**, 209–227 (1992).
49. Jwo, J. & LoVerde, P. T. The ability of fractionated sera from animals vaccinated with irradiated cercariae of Schistosoma mansoni to transfer immunity to mice. *The Journal of parasitology* **75**, 252–260 (1989).
50. Mangold, B. L. & Dean, D. A. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against Schistosoma mansoni in mice. *The Journal of Immunology* **136**, (1986).
51. Wilson, R. A. *et al.* Elimination of Schistosoma mansoni Adult Worms by Rhesus Macaques: Basis for a Therapeutic Vaccine? *PLOS Neglected Tropical Diseases* **2**, e290 (2008).
52. Zhang, W. *et al.* Fifteen Years of Sm-p80-Based Vaccine Trials in Nonhuman Primates: Antibodies From Vaccinated Baboons Confer Protection in vivo and in vitro From Schistosoma mansoni and Identification of Putative Correlative Markers of Protection. *Frontiers in Immunology* **11**, 1246 (2020).
53. Molehin, A. J. *et al.* Cross species prophylactic efficacy of Sm-p80-based vaccine and intracellular localization of Sm-p80/Sm-p80 ortholog proteins during development in Schistosoma mansoni, Schistosoma japonicum and Schistosoma haematobium. *Parasitology research* **116**, 3175 (2017).

54. Delgado, V. & Mclaren, D. J. Evidence for enhancement of IgGl subclass expression in mice polyvaccinated with radiation-attenuated cercariae of Schistosoma mansoni and the role of this isotype in serum-transferred immunity. *Parasite Immunology* **12**, 15–32 (1990).
55. Hewitson, J. P., Hamblin, P. A. & Mountford, A. P. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunology* **27**, 271–280 (2005).
56. Lam, H. Y. P. *et al.* Heat-killed Propionibacterium acnes augment the protective effect of 28-kDa glutathione S-transferases antigen against Schistosoma mansoni infection. *Acta Tropica* **222**, 106033 (2021).
57. Rihet, P., Demeure, C. E., Bourgois, A., Prata, A. & Dessein, A. J. Evidence for an association between human resistance to Schistosoma mansoni and high anti-larval IgE levels. *European Journal of Immunology* **21**, 2679–2686 (1991).
58. Jiz, M. *et al.* Immunoglobulin E (IgE) responses to paramyosin predict resistance to reinfection with Schistosoma japonicum and are attenuated by IgG4. *Infection and Immunity* **77**, 2051–2058 (2009).
59. Wu, L. C. & Zarrin, A. A. The production and regulation of IgE by the immune system. *Nature Reviews Immunology* **14**, 247–259 (2014).
60. Diemert, D. J. *et al.* Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: Implications for the development of vaccines against helminths. *Journal of Allergy and Clinical Immunology* **130**, 169-176.e6 (2012).
61. Caulfield, J. P., Lenzi, H. L., Elsas, P. & Dessein, A. J. Ultrastructure of the attack of eosinophils stimulated by blood mononuclear cell products on schistosomula of Schistosoma mansoni. *The American Journal of Pathology* **120**, 380 (1985).
62. Jong, E. C., Chi, E. Y. & Klebanoff, S. J. Human Neutrophil-Mediated Killing of Schistosomula of Schistosoma Mansoni: Augmentation by Schistosomal Binding of Eosinophil Peroxidase. *The American Journal of Tropical Medicine and Hygiene* **33**, 104–115 (1984).
63. Dessein, A. *et al.* Immune evasion by Schistosoma mansoni: loss of susceptibility to antibody or complement-dependent eosinophil attack by schistosomula cultured in medium free of macromolecules. *Parasitology* **82**, 357–374 (1981).

64. Tchoubrieva, E. B., Ong, P. C., Pike, R. N., Brindley, P. J. & Kalinna, B. H. Vector-based RNA interference of cathepsin B1 in Schistosoma mansoni. *Cellular and Molecular Life Sciences* **67**, 3739–3748 (2010).
65. Wilson, R. A. Interferon gamma is a key cytokine in lung phase immunity to schistosomes but what is its precise role? *Brazilian Journal of Medical and Biological Research* **31**, 157–161 (1998).
66. Jankovic, D. *et al.* Optimal vaccination against Schistosoma mansoni requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. *Journal of immunology (Baltimore, Md. : 1950)* **162**, 345–51 (1999).
67. Gaucher, D. *et al.* Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. *Journal of Experimental Medicine* **205**, 3119–3131 (2008).
68. Akondy, R. S. *et al.* The Yellow Fever Virus Vaccine Induces a Broad and Polyfunctional Human Memory CD8+ T Cell Response. *The Journal of Immunology* **183**, 7919–7930 (2009).
69. Kannanganat, S., Ibegbu, C., Chennareddi, L., Robinson, H. L. & Amara, R. R. Multiple-Cytokine-Producing Antiviral CD4 T Cells Are Functionally Superior to Single-Cytokine-Producing Cells. *Journal of Virology* **81**, 8468–8476 (2007).
70. L'Huillier, A. G. *et al.* T-cell responses following Natural Influenza Infection or Vaccination in Solid Organ Transplant Recipients. *Scientific Reports* **10**, 1–9 (2020).
71. Savic, M. *et al.* Distinct T and NK cell populations may serve as immune correlates of protection against symptomatic pandemic influenza A(H1N1) virus infection during pregnancy. *PLOS ONE* **12**, e0188055 (2017).
72. Brown, D. M., Lee, S., Garcia-Hernandez, M. de la L. & Swain, S. L. Multifunctional CD4 Cells Expressing Gamma Interferon and Perforin Mediate Protection against Lethal Influenza Virus Infection. *Journal of Virology* **86**, 6792–6803 (2012).
73. Precopio, M. L. *et al.* Immunization with vaccinia virus induces polyfunctional and phenotypically distinctive CD8+ T cell responses. *Journal of Experimental Medicine* **204**, 1405–1416 (2007).
74. Darrah, P. A. *et al.* Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. *Nature Medicine* **13**, 843–850 (2007).

75. Kelton, J. G., Arnold, D. M. & Nazy, I. Lessons from vaccine-induced immune thrombotic thrombocytopenia. *Nature Reviews Immunology* **21**, 753–755 (2021).

76. Wilson, R. A., Li, X. H. & Castro-Borges, W. Do schistosome vaccine trials in mice have an intrinsic flaw that generates spurious protection data? *Parasites and Vectors* **9**, 1–16 (2016).

77. Perera, D. J. & Ndao, M. Promising Technologies in the Field of Helminth Vaccines. *Frontiers in Immunology* **12**, 3220 (2021).

3.10 Figures and legends

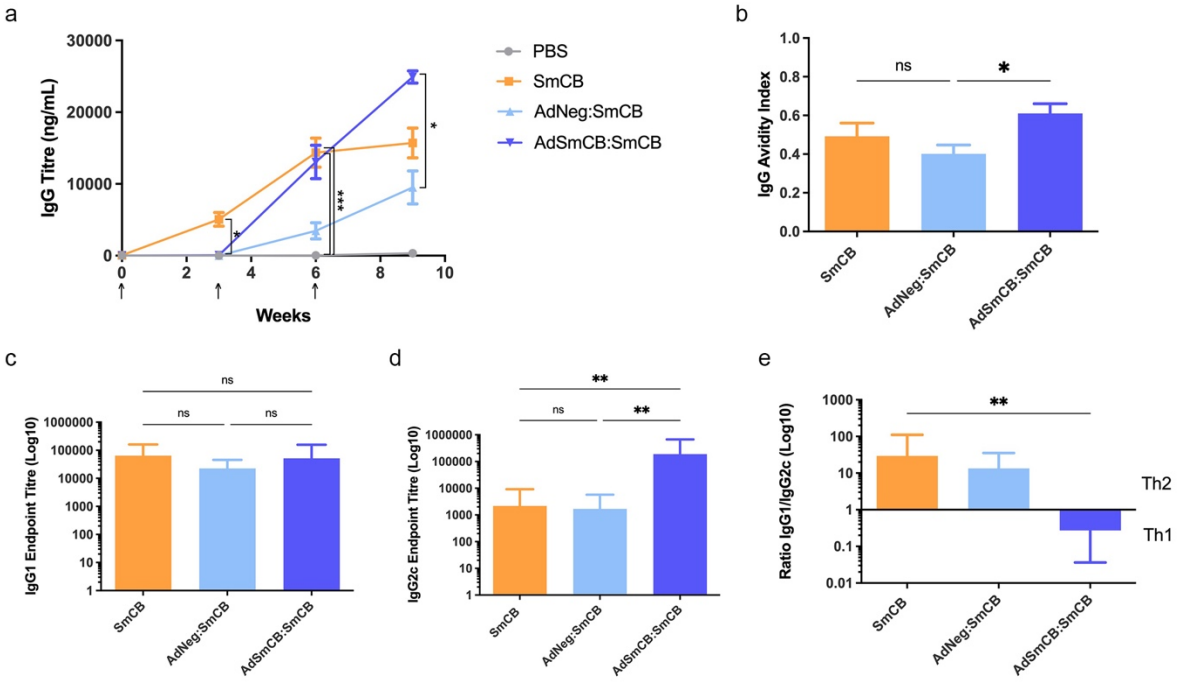


Figure 3.1 Humoral response to vaccination

(a) SmCB-specific IgG titres measured by ELISA. Immunizations are denoted by arrows. (b-e) Further analysis of the IgG antibody response at time of challenge (week 9). (b) Avidity of antigen-specific IgG reported as the avidity index. SmCB binding (c) IgG1 and (d) IgG2c measured by endpoint titre ELISA. (e) IgG immune skewing represented by the ratio of IgG1/IgG2c. All data are presented as the mean ± SEM of two independent experiments. (n=8). *p<0.05, **p<0.01, ***p<0.001 ((a,d-e) analysed using Kruskal-Wallis test (b-c) analysed using one-way ANOVA).

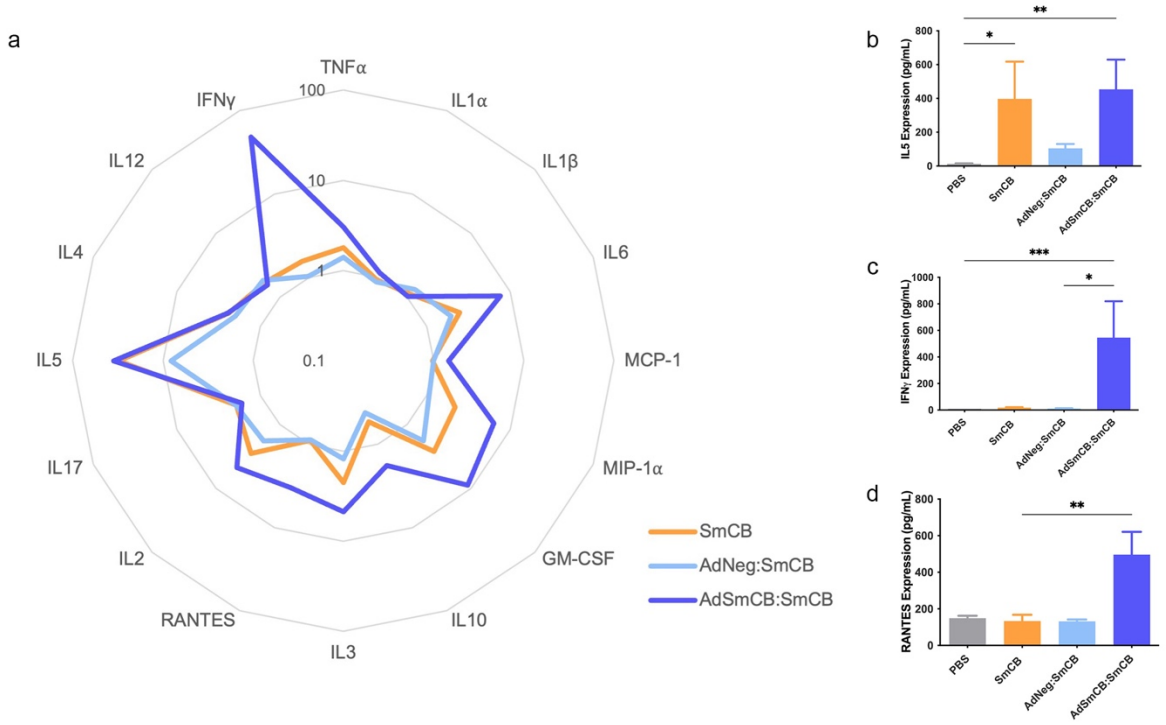


Figure 3.2 Cell-mediated memory responses to SmCB

Mean levels of cytokines and chemokines from restimulated splenocytes shown in the radar plot. Data are calculated as the fold change above the PBS control along the axis in log scale. Bar graphs depicting expression levels of cytokines (b) IL5, (c) IFNγ, and chemokine (d) RANTES (CCL5). All data are presented as the mean ± SEM of two independent experiments. (n=8). *p<0.05, **p<0.01, ***p<0.001 (Kruskal-Wallis test).

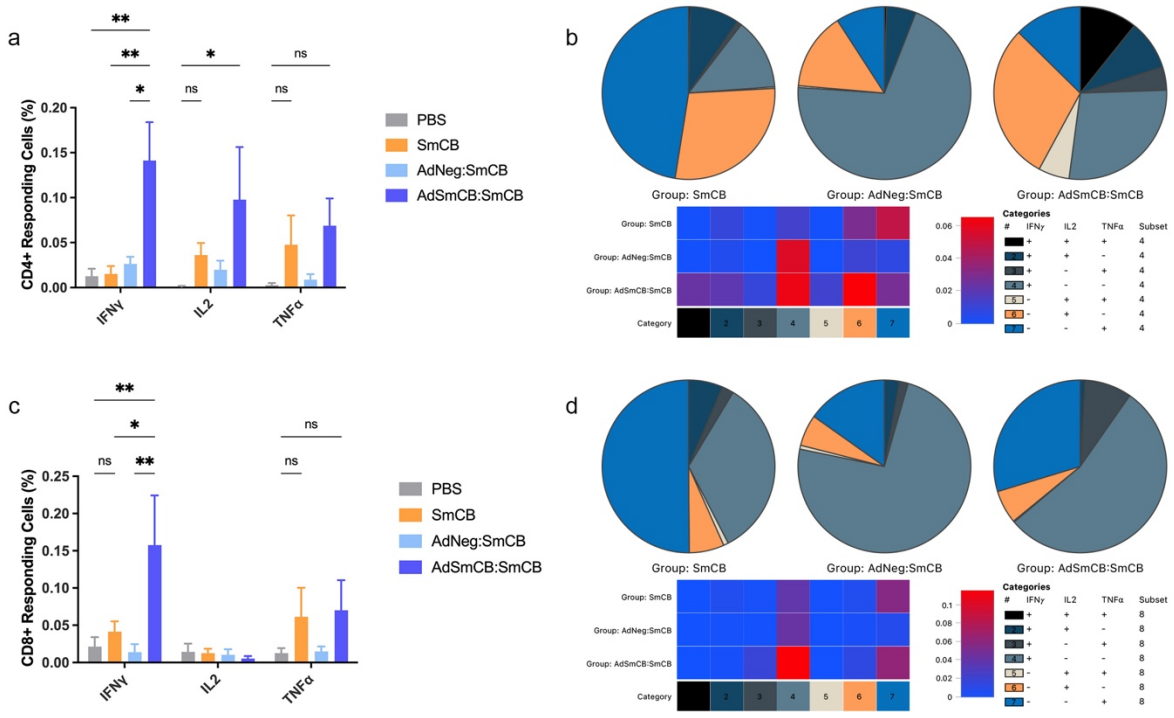


Figure 3.3 Responding T cell signature

Frequencies of (a) CD4+ and (c) CD8+ T cells expressing IFNγ, IL2, and TNFα shown as % of the parent population. Polyfunctional signatures of both (b) CD4+ and (d) CD8+ T cells shown in representative pie charts. Heat maps were included, for each subset of T cell, to describe the relative amounts of each polyfunctional profile. Both heat maps show the percentage of CD4+ or CD8+ T cell in each category on a continuum from 0% (blue) to increasing % (red). Numbered and colour coded categories within each pie chart/heat map represent various T cell profiles of cytokine expression and are explained in the included legends. All data are presented as the mean ± SEM of net values (stimulated cells – unstimulated cells) from two independent experiments. (n=8). *p<0.05, **p<0.01 (two-way ANOVA).

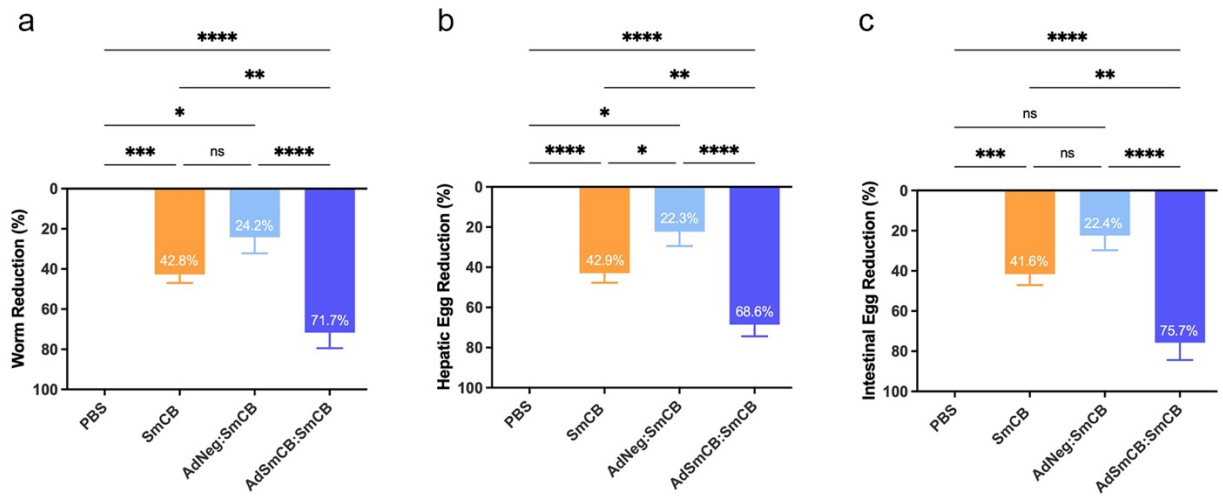


Figure 3.4 Parasite burden reduction

Reduction from the PBS control of (a) adult worms, (b) hepatic eggs, and (c) intestinal eggs at week 16. All data are presented as the mean ± SEM of two independent experiments. (n=8). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (one-way ANOVA).

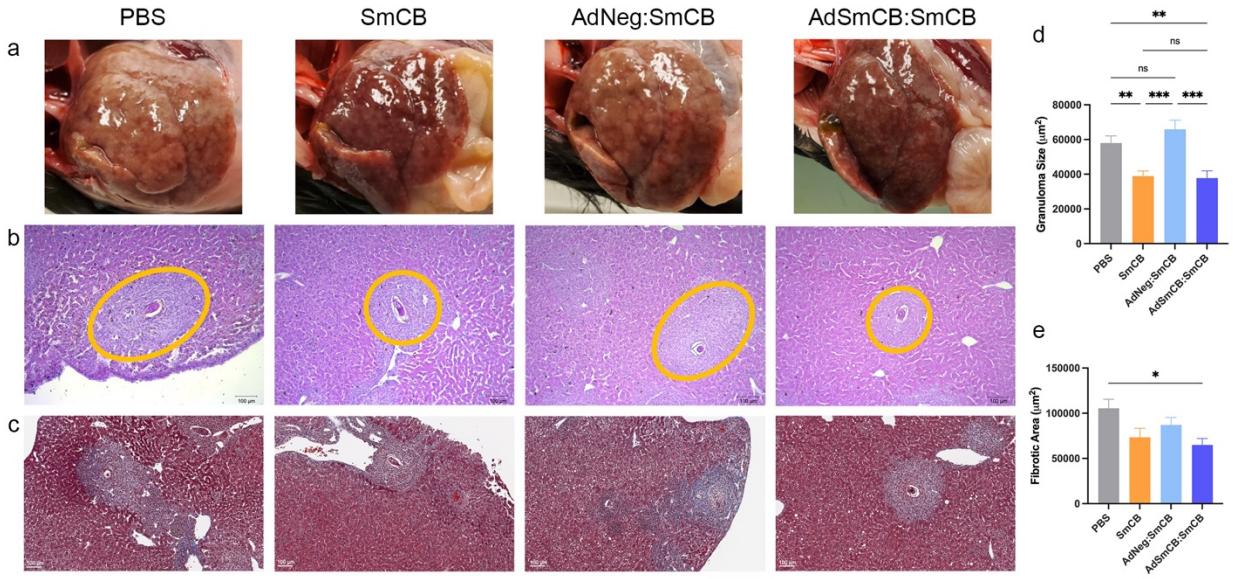
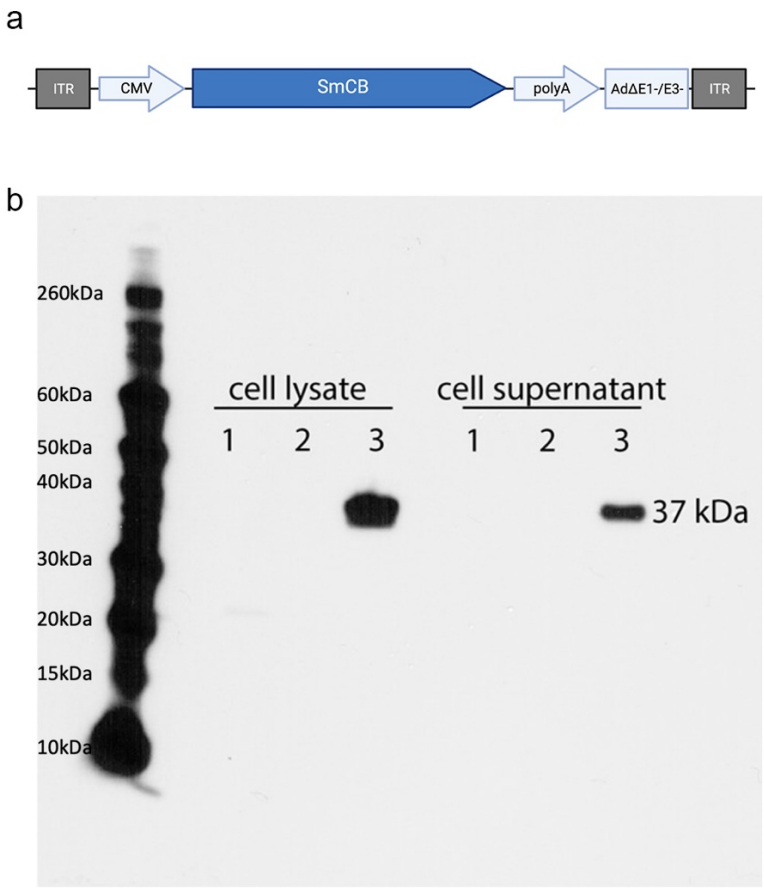


Figure 3.5 Pathological outcomes

(a) Whole livers imaged post-infection showing visual pathology. Liver portions were subsequently prepared for histology and stained using haematoxylin and eosin or Masson's trichrome. Representative images show qualitative (b) granuloma sizes (outlined in orange) and

(c) egg-induced fibrosis (collagen stained in blue) for each group. Scale bars represent 100 µm. (n=8). Quantitative (d) granuloma sizes (n=24-32) and (e) fibrotic areas (n=25-37) delimited and measured using ZenBlue and QuPath software, respectively. All data are presented as the mean ± SEM of two independent experiments. *p<0.05, **p<0.01, ***p<0.001 (Kruskal-Wallis test).

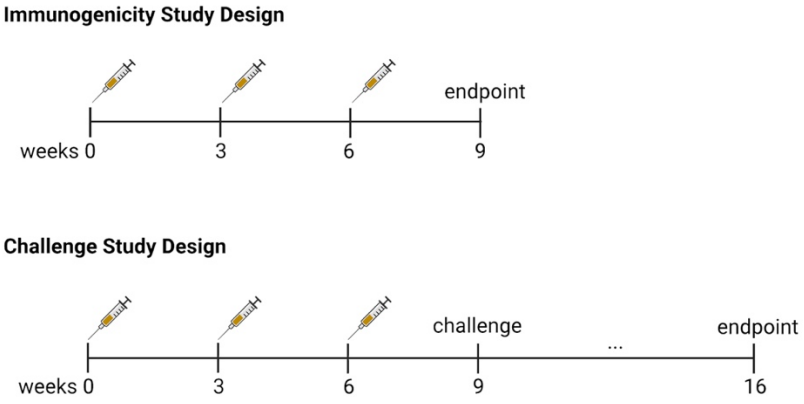
3.11 Supplemental data



Supplemental Figure 3.1 AdSmCB western blot

A graphical depiction of our SmCB expression cassette can be seen in (a). A western blot directed against the histidine tag of our protein was run to determine the expression and secretion of SmCB by our recombinant adenovirus (b). The presence of SmCB was analyzed in the cell lysates and culture medium. Mock-infected cells were used as a control. Lane 1: Control, Lane 2: empty adenovirus (AdNeg), Lane 3: recombinant adenovirus (AdSmCB).

a

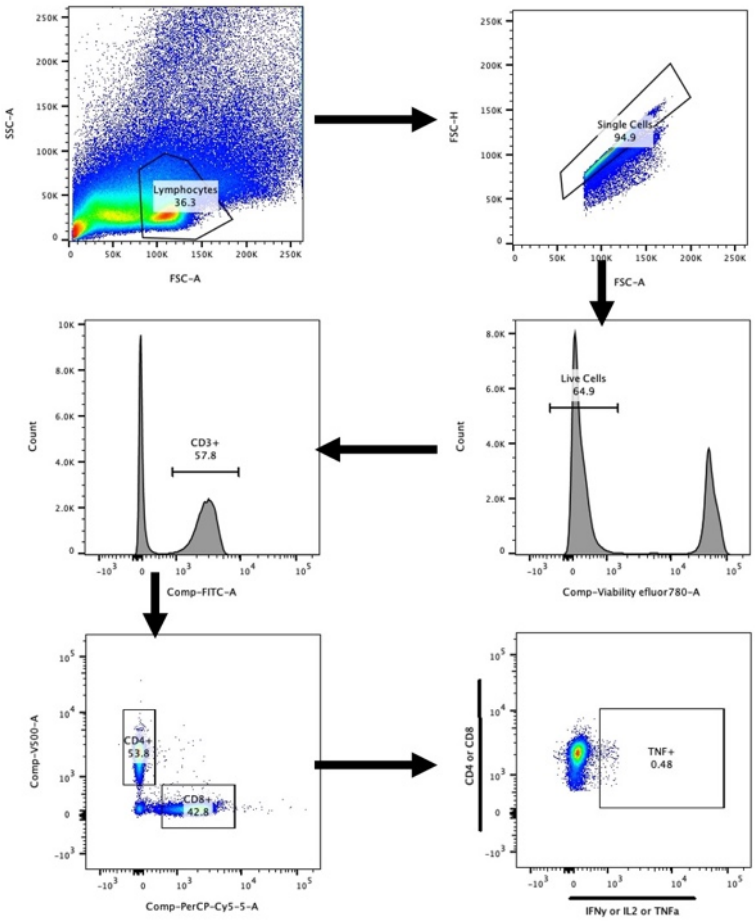


b

Group	Week 0	Week 3	Week 6
PBS	PBS	PBS	PBS
SmCB	rSmCB	rSmCB	rSmCB
AdNeg:SmCB	AdNeg 10^5 IU	rSmCB	rSmCB
AdSmCB:SmCB	AdSmCB 10^5 IU	rSmCB	rSmCB
AdNeg	AdNeg 10^5 IU	-	-

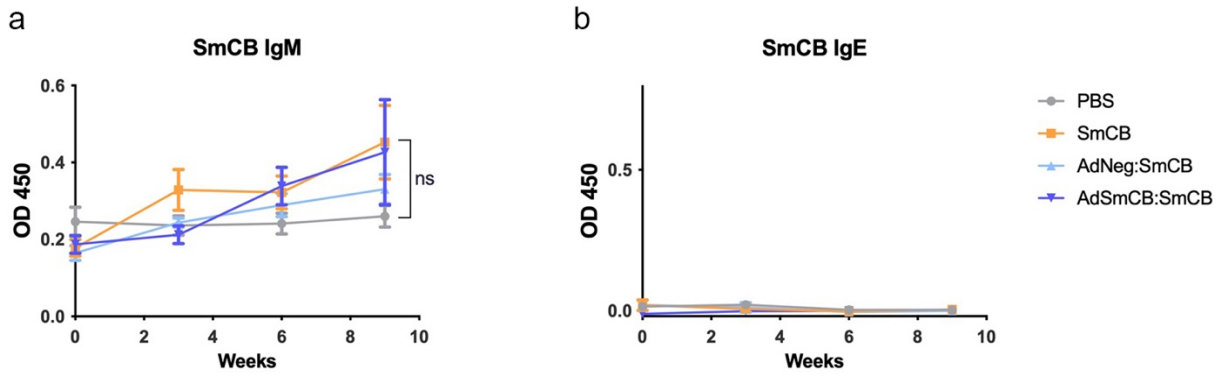
Supplemental Figure 3.2 Study design

Our immunization and challenge schedules can be seen in (a). Immunogenicity study (*n*=8) from two independent experiments. Challenge study (*n*=8) from two independent experiments. Experimental groups are shown in the table in (b). IU=infectious units, rSmCB=recombinant SmCB. AdNeg group was only included in the challenge study.



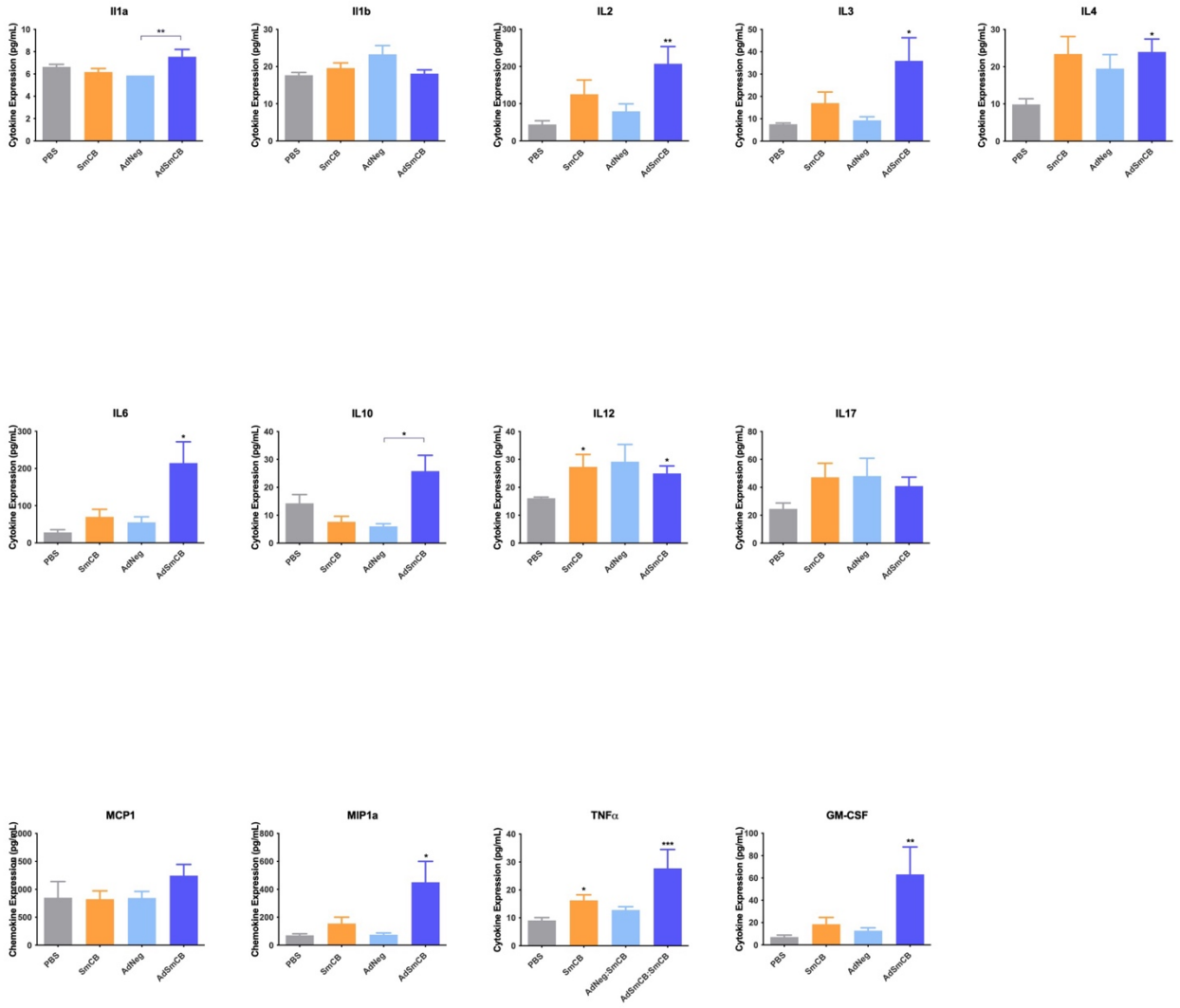
Supplemental Figure 3.3 Flow cytometry gating strategy

Here we show our gating strategy for flow cytometric analysis of stimulated splenocytes. IFNy=IFNγ, TNFa and TNF^{+}=TNFα.



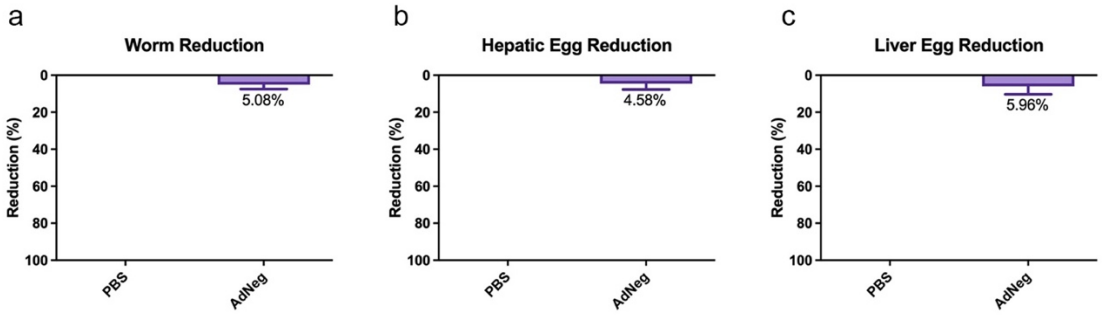
Supplemental Figure 3.4 SmCB IgM and IgE

Humoral responses were determined throughout the immunization schedule. No mice had detectable levels of antigen-specific IgM (a) or IgE (b) at baseline. Mice developed antigen-specific IgM in response to immunization with vaccines SmCB, AdNeg:SmCB, and AdSmCB:SmCB, however it was not significantly more than the PBS control. No animals developed antigen-specific IgE in response to vaccination. IgA was also assessed and was found not to be elicited in response to antigen. Data shows mean ± SEM from two independent experiments. (*n*=8). (Kruskal-Wallis test)



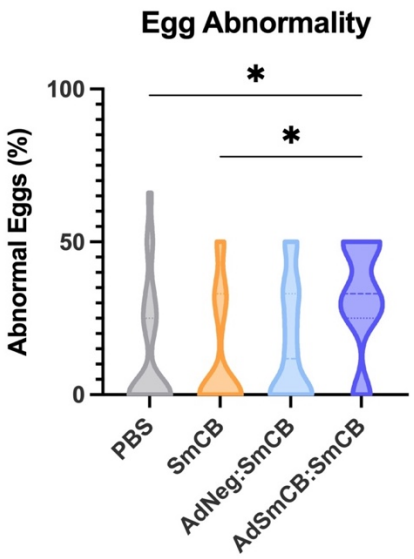
Supplemental Figure 3.5 Memory cytokine and chemokine responses

Splenocyte supernatants were run in singlet on a multiplex ELISA to detect the presence of 16 cytokines and chemokines: IL1α, IL1β, IL2, IL3, IL4, IL5, IL6, IL10, IL12, IL17, MCP-1, IFNγ, TNFα, MIP1α, RANTES, and GMCSF. Data for IL5, IFNγ, and RANTES are included in the manuscript. Data are represented by the mean ± SEM from two independent experiments. Significance is calculated against the PBS control unless otherwise denoted. (n=8). *p<0.05, **p<0.01, ***p<0.001 (Kruskal-Wallis test).



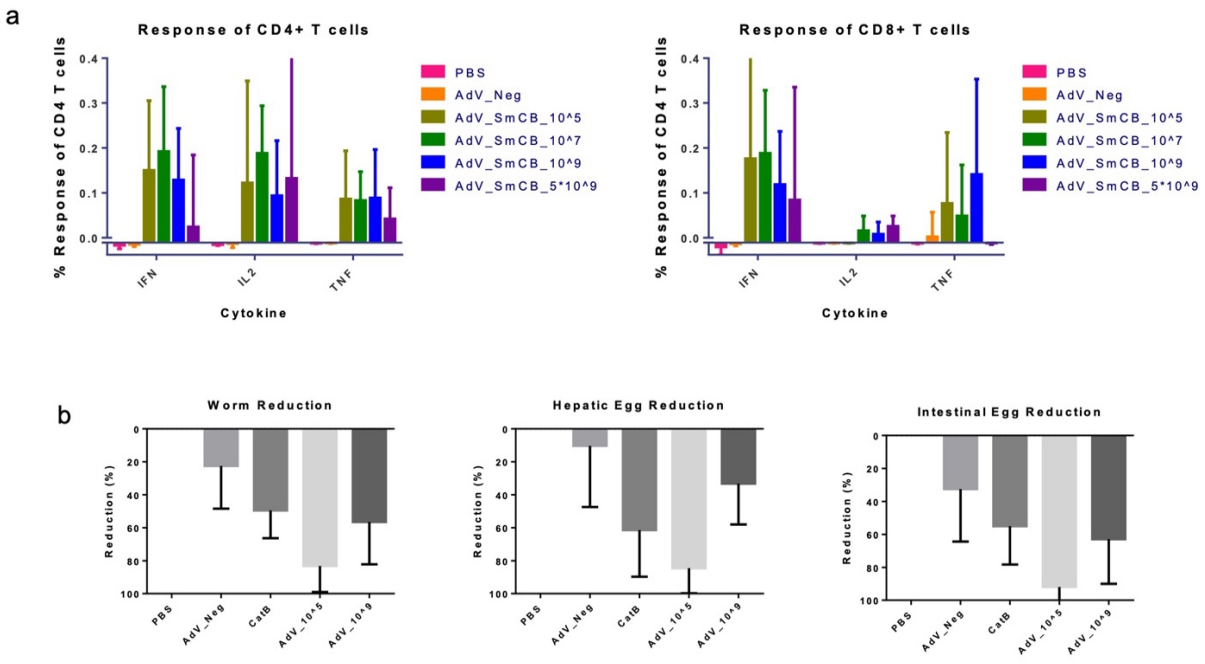
Supplemental Figure 3.6 Parasite burden reduction in control animals

A cohort of mice were included in challenge to determine any non-specific protection from the empty adenovirus vector. Mice were immunized with either PBS or 10^5 IU of AdNeg (empty vector) and were subsequently challenged with 150 cercaria. Adult worm burden (a), hepatic egg burden (b), and intestinal egg burden (c) reductions are shown. The resulting protection was negligible. Data are displayed as mean ± SEM, from two independent experiments (*n*=8).



Supplemental Figure 3.7 Egg abnormality

Egg abnormality was determined by assessing the percentage of visually abnormal eggs in histological preparations of livers from each group. Only the group AdSmCB:SmCB was able to significantly increase the observed amount of abnormal eggs compared to both PBS and SmCB groups. Data are displayed as mean ± SEM, from two independent experiments. (*n*=8). *$p<0.05$ (Kruskal-Wallis test)



Supplemental Figure 3.8 Cell mediated immunity dose response pilot study

CMI Dose Response (a). Pilot study comparing four doses of AdSmCB to determine cellular mediated immunity. Six- to eight-week-old female C57BL/6 mice were immunized for parasite burden reduction. Group 1: control: mice were injected with phosphate-buffered saline (PBS) (Wisent Bioproducts, St. Bruno, QC). Group 2: AdV_Neg: mice were immunized with 10^9 infectious units of empty adenovirus vector and boosted twice with 20 µg SmCB. Groups 3-6: mice were immunized with 10^5, 10^7, 10^9, or 5*10^9 infectious units of recombinant adenovirus vector and boosted twice with 20 µg SmCB. Each mouse was immunized at weeks 0, 3, and 6 intramuscularly in the thigh with 50 µL of vaccine. Mice were sacrificed at week 9 and splenocytes were harvested and restimulated with SmCB before being analyzed by flow cytometry as described in our paper. Data are shown as mean ± SEM. (*n*=5). Pilot challenge study comparing two doses of AdSmCB (b). Six- to eight-week-old female C57BL/6 mice were immunized for parasite burden reduction. Group 1: control: mice were injected with phosphate-buffered saline (PBS) (Wisent Bioproducts, St. Bruno, QC). Group 2: AdV_Neg: mice were immunized with 10^9 infectious units of empty adenovirus vector and boosted twice with 20 µg SmCB. Group 3: mice were immunized three times with 20 µg SmCB. Group 4: mice were immunized with 10^5 infectious units of recombinant adenovirus vector and boosted twice with 20 µg SmCB. Group 5: mice were immunized with 10^9 infectious units of recombinant adenovirus vector and boosted

twice with 20 μg SmCB. Each mouse was immunized at weeks 0, 3, and 6 intramuscularly in the thigh with 50 μL of vaccine. Mice were infected at week 9, and at week 16 parasite burden was assessed. Data are shown as mean ± SEM. (n=5).

Chapter 4

Enhancing an Adenovirus-based Schistosomiasis Vaccine Using Boosting Immunizations of Recombinant *Schistosoma mansoni* Cathepsin B Dispersed into AddaVax™

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4.1 Preface

Our previous vaccine efforts resulted in significant parasite burden protection and robust immunogenicity in murine models. However, there are limitations of using a mouse model to determine schistosomiasis vaccine efficacy. One of these proposed limitations is due to the physiological nature of murine lungs; in short term challenge models, non-specifically activated T cells and cytokines are at their maximum as parasites pass through pulmonary blood vessels, shunting them into the alveoli. To mitigate this risk, we combined our vaccine strategies from Chapters 2 and 3 to increase specific immune responses which we believe provided the highest protection from infection and *Schistosoma mansoni* pathology. Animals were also challenged on a

delayed schedule with a high infectious dose of parasite to determine if vaccine efficacy could persist to a period when non-specific immune activation in the lungs has subsided. In this chapter we also comprehensively assessed immune effectors from before and after challenge to identify correlates of immunity from intestinal schistosomiasis which should be sought after in future vaccine efforts.

4.2 Abstract

Schistosomiasis causes significant morbidity to over 250 million individuals worldwide. Intestinal schistosomiasis causes chronic liver and intestinal pathologies due to egg deposition by blood-dwelling worms which can lead to death if left untreated. The first line drug for schistosomiasis is praziquantel (PZQ); however, it does not protect from re-infection, or reverse existing tissue damage, and many patients in need are not able to access treatment. *Schistosoma mansoni* cathepsin B (SmCB) is a gut peptidase, necessary for parasite maturation, which has been targeted for vaccine development to remedy some of the limitations of PZQ. We have developed a heterologous, intramuscular, prime and boost vaccine formulation consisting of a priming immunization of a human adenovirus serotype 5 expressing SmCB followed by two boosting doses of recombinant SmCB adjuvanted with AddaVax (AdSmCB:SmCB/AddaVax). Our vaccine strategy resulted in significant humoral responses and production of antigen specific IgG1, IgG2c, and IgA. Systemic memory responses were also induced including, CD8+ T cell, CD19+ B cell, and CD49b+ cell proliferation and the production of several cytokines and chemokines including IFNγ and IL-5. Vaccination with AdSmCB:SmCB/AddaVax resulted in extended protection from challenge with parasite burden reductions of 60.7%, 50.4%, and 51.1% in adult worms, hepatic eggs, and intestinal eggs, respectively, with a specific targeting of adult female worms six weeks after immunization. Overall, we found that both T_H1 and T_H2 arms of immunity were activated by vaccination and may work in concert to protect from intestinal schistosomiasis in our mouse model. With nearly one billion at risk of infection, the development of a vaccine for schistosomiasis is an important public health control measure which should be advanced.

4.3 Introduction

Approximately one seventh of the world's population is at a risk of being infected with schistosomiasis[1]. This neglected tropical disease is caused by a parasite which is most prevalent in tropical and sub-tropical regions and can cause devastating morbidity to its host for upwards of 30 years. *Schistosoma mansoni* is the most widespread species which leads to schistosomiasis and results in intestinal disease. Pathology is largely attributable to eggs which become trapped in host tissues causing granuloma formation, fibrosis, and cirrhosis [2].

Schistosoma spp. adult worms can be effectively cleared with drug therapy, using the FDA approved drug praziquantel (PZQ)[3]. Unfortunately, mass drug administration (MDA) targeting at risk areas is not optimal and PZQ does not protect from re-infection or resolve pre-existing schistosomiasis pathology. Efforts to develop an effective vaccine against this helminth have been ongoing since the early 1950s; however, no vaccine has yet been approved for human use. Several *Schistosoma* vaccines in clinical trials target tegument proteins of the parasite's exterior[4–10]. To impede the growth of juvenile worms, before they mature into adult egg-laying females, our group has formulated a vaccine which targets *S. mansoni* cathepsin B (SmCB). This vaccine antigen has demonstrated promising efficacy in prophylactically protecting from *S. mansoni* infection in both mouse and hamster models[11–15].

Previously, we exploited the human adenovirus serotype 5 (hAdV5) as a vector to express SmCB (AdSmCB)[16]. To circumvent the limitation of neutralizing antibodies, we deployed a heterologous prime-and-boost vaccination strategy, following AdSmCB-prime with recombinant protein boosts. While SmCB alone contains inbuilt adjuvanticity, our objective was to augment and direct immune responses toward specific protective correlates; to enhance the effectiveness of boosting immunizations we combined SmCB with an adjuvant. To balance T_H1 skewed immunity delivered by our viral vector, we opted to utilize the squalene-based oil-in-water emulsion AddaVax™ (AddaVax), which promotes both T_H1 and T_H2 effectors.

Here, we assess the immune landscape created by AdSmCB when boosted by SmCB combined with AddaVax™ (AdSmCB:SmCB/AddaVax) and determine its efficacy in a mouse model of schistosomiasis.

4.4 Methods

4.4.1 Animal ethics

All animal procedures were performed in accordance with the Institutional Animal Care and Use Guidelines approved by the Animal Care and Use Committee at McGill University (Animal Use Protocol 8190). Mouse housing, husbandry, and environmental enrichment can be found within the McGill standard operating procedures (SOP) #502, #508, and #509. Animals were monitored for adverse events for 3 days post-vaccination and weekly until the end of each experiment. Humane intervention points were monitored according to McGill SOP #410. All animals were humanely sacrificed at endpoint by anaesthesia with isoflurane before euthanasia by carbon dioxide asphyxiation, followed by pneumothorax and blood collection by cardiac puncture.

4.4.2 Cell lines and reagents

Cell lines were obtained from commercial sources, passed quality control procedures, and were certified and validated by the manufacturer. All reagents were validated by the manufacturer or has been cited previously in the literature. When available, RRID tags have been listed in the text.

4.4.3 Author checklists

This manuscript has been developed with both the ARRIVE and MDAR author checklists.

4.4.4 Generation of AdSmCB vector

The AdSmCB construct was developed as previously described[16]. A second human adenovirus serotype 5 (ΔE1-, ΔE3-; 1st generation), lacking a gene cassette, was used as a negative control.

4.4.5 Protein expression and purification

Recombinant SmCB was prepared and purified as previously described[13]. Briefly, yeast cells modified using the PichiaPink™ system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) were grown and induced using methanol. Recombinant protein was purified by Ni-NTA chromatography (Ni-NTA Superflow by QIAGEN, Venlo, Limburg, Netherlands) and dialyzed into PBS.

4.4.6 Animals and immunization protocol

Six- to eight-week-old female C57BL/6 mice were purchased from Charles River Laboratories (RRID:IMSR_CRL:027) (Senneville, QC, Canada). Five groups of mice (n = 8) were immunized for humoral, and cell-mediated immunity assessment. Another four groups of mice (n = 10) were immunized and subsequently infected for parasite burden assessment. Each mouse was immunized at weeks 0, 3, and 6 by intramuscular injection in the thigh in a total volume of 50µL. Group 1 (PBS): mice were injected with PBS (Wisent Bioproducts, St. Bruno, QC, Canada). Group 2 (AdNeg:AddaVax): mice were immunized with $5x10^5$ infectious units (IU) of an empty adenovirus containing no gene cassette, followed by two boosts of AddaVax alone ((AddaVax) prepared according to manufacturer's guidelines). Group 3 (AdSmCB): mice were immunized with $5x10^5$ IU of AdSmCB with no boosting immunizations. Group 4 (AdSmCB:AddaVax): mice were immunized with $5x10^5$ IU of AdSmCB, followed by two boosts of AddaVax. Group 5 (AdSmCB:SmCB/AddaVax): mice were immunized with $5x10^5$ IU of AdSmCB, followed by two boosts of 20 µg of SmCB formulated in AddaVax.

Mice were bled from the saphenous vein at weeks 0, 3, and 6. Mice immunized for humoral and cell-mediated immunity assessment were euthanised three weeks after the final vaccination and blood and spleens were collected. Group 3 was excluded from the challenge study. Animals in the challenge study were challenged six weeks after the final vaccination and sacrificed at week 19 to determine parasite burden reduction. Spleens were also collected for multiplex ELISA.

4.4.7 Challenge with *S. mansoni*

Mice were infected with *S. mansoni* as previously described. Briefly, infectious cercariae were shed from *Biomphalaria glabrata* snails at week 12 and mice were blinded and challenged with 200 cercariae/mouse via tail exposure for one hour. Seven weeks post infection, animals were euthanized to determine correlates of immunity and to assess parasite burden. Before euthanasia faecal pellets were collected from infected animals. Images of mouse livers were taken during dissection using an iPhone 14 Pro camera (Apple, Cupertino, California, USA). Blood was collected by cardiac puncture and serum was isolated and stored at -20°C until use. Adult worms were perfused from the hepatic portal system and counted manually. Livers and intestines were weighed and digested overnight at 37°C in 4% potassium hydroxide. The following day, eggs present in these tissues were counted by microscopy and adjusted per gram of tissue. Burden reductions were calculated as previously described[16].

4.4.8 SmCB-specific IgG, IgG1, IgG2c, IgA, IgM, IgE quantification, and IgG avidity assays

Briefly, high binding 96-well plates (Greiner Bio-One, Frickenhausen, Germany) were coated with SmCB (0.5 μg/mL) in 100 mM bicarbonate/carbonate buffer (pH 9.6) along with various standard curves (IgG, IgG1, IgG2c, IgA: serially diluted from 2000 ng/mL to 1.953 ng/mL) overnight at 4°C. Then, plates were blocked with 2% bovine serum albumin (BSA; Sigma Aldrich, St. Louis, MO, USA) in PBS-T (blocking buffer) for 1 hour at 37°C before samples diluted in blocking buffer were added in duplicate. When running serum for total SmCB-IgG, an additional set of serum samples was run to determine IgG avidity. Plates were incubated for 1 hour at 37°C then washed with PBS (pH 7.4). For IgG avidity assessment, the additional set of samples received 10M urea, while blocking buffer was added to the first set and the standard curve. Plates were covered and incubated for 15 minutes at room temperature protected from light, washed 4 times, and then blocked again with blocking buffer for 1 hour at 37°C. Next, plates were washed with PBS and anti-mouse IgG-HRP (Sigma Aldrich) was diluted 1:20,000 in blocking buffer and applied for 30 minutes at 37°C. For other immunoglobulins, the same protocol was followed without the additional avidity steps and the appropriate HRP-conjugated antibody was applied.

Both IgG1- and IgG2c-HRP were diluted 1:20,000 in blocking buffer and applied for 30 minutes at 37°C. For IgA, HRP-conjugated anti-mouse IgA (Sigma Aldrich) was diluted 1:2,000 in blocking buffer and applied for 1 hour at 37°C. Plates were washed a final time with PBS and 3,3',5,5'-Tetramethyl benzidine (TMB) substrate (Sigma Aldrich) was added to each well. The reaction was stopped after 15 minutes using H_2SO_4 (0.5M; Fisher Scientific, Waltham, MA, USA) and the optical density (OD) was measured at 450 nm with an EL800 microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Concentrations of SmCB-specific antibodies were calculated by extrapolation from respective standard curves and multiplied by the dilution factor. IgG avidity indices were calculated by dividing the IgG titre in the urea conditions by the IgG titre in the non-treated condition. IgM and IgE were reported as OD values.

4.4.9 Quantification of cell proliferation by flow cytometry

Three weeks after the last immunization, mice were sacrificed, and spleens were harvested. Splenocytes were seeded at 10^6 cells in 200 µL/well in U-bottom 96-well plates (BD Falcon). Duplicate samples were stimulated with or without rSmCB in RPMI-1640 with 10% fetal bovine serum, 1 mM penicillin/streptomycin, 10 mM HEPES, 1X MEM non-essential amino acids, 1 mM sodium pyruvate, 1 mM L-glutamine (Wisent Bioproducts), and 0.05 mM 2-mercaptoethanol (Sigma Aldrich) (fancy RPMI, fRPMI) at 37 °C for 18 hours with 5% CO2. Incubated cells were then processed for flow cytometry as described elsewhere with some adjustments (45). Splenocytes were washed twice with 200 µL of cold PBS (pH 7.40; Wisent) and centrifuged at 400xg at 4°C for 7 minutes. Fixable viability dye eFluor 780 (1:375 dilution in PBS) (Affymetrix eBioscience, Waltham, MA) was applied and incubated for 15 min at 4°C protected from light. Cells were washed again with 1% BSA in PBS (PBS-BSA). Fc block (1:50 dilution in PBS; BD Science) was added and incubated at 4°C for 10 minutes. No washing step was required prior to extracellular staining. The extracellular cocktail consists of the following antibodies: CD3-AF700 (Clone 17A2, BioLegend®), CD4-V500 (RM4-5, BD Bioscience), CD8-BV650 (Clone 53-6.7, BioLegend®), CD19-PE-CF594 (Clone 1D3, BD Bioscience), and CD49b-BV605 (Clone HMa2, BD Bioscience). 50 µL of the extracellular cocktail or single stain was applied to corresponding wells for 25 minutes at 4°C. Cells were then washed and fixed with Foxp3 Transcription Factor Fixation solutions (eBioscience™) at 4°C in the dark, overnight. The next day, the plates were

washed with Foxp3 Transcription Factor Permeabilization wash (eBioscience™) and then stained with Ki67 eFluorTM 450 (Clone SolA15, eBioscience™) diluted 1:50 in Permeabilization wash for 25 minutes at 4°C. After staining, cells were washed and resuspended in PBS-BSA and analyzed on BD LSRFortessa X-20 (BD Science) using FlowJo TM software (version 10.0.8r1) (Treestar, Ashland, OR). Gating strategy shown in Supplemental Figure 4.1.

4.4.10 Cytokine production by multiplex ELISA

Splenocytes were incubated at 1 000 000 cells in 200 µL with SmCB in fRPMI (2.5 µg/mL recombinant protein). After 72 hours at 37°C + 5% CO_2, plates were centrifuged, and supernatant collected and stored at -80°C until analysis. Cell supernatants from animals pre-infection were assessed for the presence of 16 cytokines and chemokines (IL1α, IL1β, IL2, IL3, IL4, IL5, IL6, IL10, IL12p70, IL17, IFNγ, TNFα, MCP-1 (CCL2), MIP-1α (CCL3), GM-CSF (CSF2), and RANTES (CCL5)) using Q-plex Mouse Cytokine – Screen (16-plex) multiplex ELISA following the manufacturer's guidelines (Quansys Biosciences, Logan, UT, USA). Samples were run in singlet.

4.4.11 Statistical analysis

Experimental units are defined as individual animals. Sample sizes were empirically estimated based on previous data considering the anticipated variation of the results and statistical power needed, while also minimizing the number of animals used. C57BL/6 mice were randomly attributed to treatment groups. To minimise potential confounders, mice were matched for age and sex. Blinding: For all challenge experiments, staff performing infections and sample harvesting were blinded to the different groups and were only unblinded after data analysis. Inclusion/Exclusion: No animals were excluded from the analysis; however, outlying data were identified (ROUT, Q=1%) and when present they were removed. Statistical analysis was performed using GraphPad Prism 9 software (La Jolla, CA, USA) and statistical details of experiments can be found in figure legends. Data were assessed for normality using Shapiro-Wilk tests. Non-parametric data were analysed by Mann-Whitney tests and Kruskal-Wallis tests with Dunn's multiple comparisons. When appropriate, Mann-Whitney tests, and one-way and two-way

ANOVAs with Tukey's multiple comparisons were employed. P values <0.05 were considered significant.

4.5 Results

4.5.1 AdSmCB:SmCB/AddaVax induces robust antibody responses

Our three-dose vaccination schedule resulted in robust antigen specific antibody responses (Figure 4.1A). SmCB-specific IgG was produced as early as 3 weeks after the first AdSmCB immunization (1 444 ng/mL). However, boosting immunizations with adjuvanted protein were necessary to significantly increase antibody production (125 761 ng/mL at week 6; 144 267 ng/mL at week 9). Both PBS and AdNeg:AddaVax groups stayed seronegative for SmCB specific antibodies throughout the immunization schedule. When the avidity index of produced antibodies was assessed, we found that the avidity of antibodies produced by AdSmCB:SmCB/AddaVax was significantly higher (0.44%) than those produced by AdSmCB alone (0.07%) , or AdSmCB boosted by adjuvant (0.04%) (Figure 4.1B). We then determined the contributions of IgG subtypes 1 and 2c to the total IgG produced against SmCB. Although AdSmCB:SmCB/AddaVax produced significantly larger amounts of both IgG1 (Figure 4.1C) and IgG2c (Figure 4.1E), when the ratios of these subtypes were determined there were no significant differences between groups (Figure 4.1D). Interestingly we found that AdSmCB:SmCB/AddaVax was even capable of inducing serum SmCB-specific serum IgA (114 ng/mL) (Figure 4.1F); there were no significant differences in the amounts of antigen IgM (Figure 4.1G) or IgE (Figure 4.1H) produced between groups.

4.5.2 Vaccination results in significant antigen specific proliferation of CD8+ and CD19+ cells and cytokine expression

Three weeks after the final immunization we restimulated splenocytes from vaccinated animals to determine systemic, cell-mediated, memory responses. Initially, we used flow cytometry to determine cell proliferation from various lymphocytes. Only the group vaccinated with AdSmCB alone had an increased frequency of Ki67+ CD4+ T cells, although this difference was not statistically significant (Figure 4.2A). After stimulation, both CD8+ (Figure 4.2B) and

CD19+ (Figure 4.2C) from AdSmCB:SmCB/AddaVax animals had a significantly greater frequency of Ki67+ cells, 0.64% and 1.17%, respectively, compared to all other experimental groups. Additionally, the AdSmCB:SmCB/AddaVax group demonstrated an increased frequency of Ki67+ CD49b+ cells (10.28%) which was greater than those observed in PBS, AdNeg:AddaVax, and AdSmCB:AddaVax groups, although it was not significantly higher than that of the AdSmCB group (4.56%) (Figure 4.2D).

To create a broad visualization of what these proliferating cells may be producing in response to SmCB, we ran supernatants from stimulated cells on a multiplex ELISA. The immunological landscape of 16 cytokines and chemokines produced for each experimental group can be seen in Figure 4.2E depicted as the log of the fold change above the PBS control, demonstrating the immunogenicity of all groups which received AdSmCB and the increased immunogenicity when animals were boosted with adjuvanted protein. Of interest, AdSmCB:SmCB/AddaVax delivered increased expression of IL-5 (1590 pg/mL) (Figure 4.2F) compared to PBS (17.01 pg/mL) and AdSmCB (11.93 pg/mL) immunized mice, and IFNγ (4263 pg/mL) (Figure 4.2G) compared to the PBS (159.1 pg/mL), AdNeg:AddaVax (145.0 pg/mL), and AdSmCB (597.6 pg/mL) groups. Animals immunized with AdSmCB followed by AddaVax alone also demonstrated increased expression of IFNγ (1421 pg/mL) compared to the PBS and AdNeg:AddaVax groups.

4.5.3 Humoral response at challenge resembles the humoral response at week 9

Mice were challenged six weeks after the final immunization at week 12. We again assessed antibody responses to determine the humoral profile at infection. We found that SmCB IgG titres continued to rise in our AdSmCB:SmCB/AddaVax vaccinated animals (216 210 ng/mL) (Figure 4.3A); IgG avidity stayed consistent (0.41%) (Figure 4.3B). IgG subtype titres were also similar to those of week 9, IgG1 (Figure 4.3C) and IgG2c (Figure 4.3E) both significantly raised compared to the AdSmCB:AddaVax group with no significant difference in the ratios of those isotypes between groups (Figure 4.3D). SmCB-specific IgA produced by AdSmCB:SmCB/AddaVax persisted through to week 12 (123.6 ng/mL) (Figure 4.3F) and SmCB-IgM was not significantly higher than the PBS control in any animal groups (Figure 4.3G). Lastly, we observed an increase in SmCB-specific IgE in our AdSmCB:AddaVax group (0.03 OD 450) which was statistically

significant above the PBS control group (0.008 OD 450), but not the AdNeg:AddaVax control group (0.017 OD 450); we do not believe this increase above the background level is physiologically relevant.

4.5.4 AdSmCB:SmCB/AddaVax significantly reduces parasite burden compared to controls

Six weeks after the final immunization, mice were challenged with 175 cercariae to determine protective efficacy of our vaccine formulation. Seven weeks after challenge, animals were sacrificed, and parasite burden was assessed. PBS mice resulted in approximately 49.5 adult worms per mouse, and AdNeg:AddaVax resulted in 66 adult worms per mouse (Figure 4.4A). Mice had approximately 9 423 eggs per gram of liver tissue and 15 992 eggs per gram of intestinal tissue in the PBS group, and 13 234 eggs per gram of liver tissue and 12 911 eggs per gram of intestinal tissue in the AdNeg:AddaVax group (Figure 4.4B; Figure 4.4C). Since there were no statistical differences between the parasite burdens in control animals, vaccine induced parasite burden reduction was calculated against the AdNeg:AddaVax control.

Statistical differences in reduction were only seen when comparing our vaccine formulation (AdSmCB:SmCB/AddaVax) against both other groups. AdSmCB:SmCB/AddaVax was found to reduce parasite burden by 60.7%, 66.4%, and 54.6% in adult worms, female worms, and male worms, respectively (Figure 4.4D; Figure 4.4E; Figure 4.4F). When we assessed the ratio of adult worms found in animals, we found a higher male to female worm ratio (approximately 1.25) which was significantly higher than that found in the other groups (approximately 0.94) (Figure 4.4G). Parasitic egg burden was also calculated in both the liver and intestine, and we found that AdSmCB:SmCB/AddaVax reduced hepatic eggs by 50.4% and intestinal eggs by 51.1% (Figure 4.4H; Figure 4.4I).

4.5.5 Antibody responses persist during challenge and are disparate from control responses

To understand how the humoral response is altered during infection we determined antigen specific antibody levels at week 19. While there was a trend of increased SmCB-specific IgG in the AdSmCB:SmCB/AddaVax group, this increase was not statistically significant, and due to

challenge all animals had begun producing antibodies specific to SmCB (Figure 4.5A). However, when we determined the avidity of those antibodies, it was significantly greater in animals which were immunized with AdSmCB:SmCB/AddaVax (0.52%) (Figure 4.5B) compared to all other groups (0.28%-0.34%). Again, we determined the contributions of IgG1 and IgG2c and found that all mice produced a large amount of SmCB IgG1; the only difference between groups was that AdSmCB:SmCB/AddaVax produced less IgG1 than the AdNeg:AddaVax control (Figure 4.5C). Differently, AdSmCB:SmCB/AddaVax produced a significantly greater amount of IgG2c than both controls, PBS and AdNeg:AddaVax, but not the group AdSmCB:AddaVax (Figure 4.5E). When we combined both readouts to determine the IgG1/IgG2c ratios for each group we found that control animals (PBS and AdNeg:AddaVax) had a higher ratio (228 and 768, respectively) than those which received AdSmCB, and this difference was significant in animals from the AdSmCB:SmCB/AddaVax group (0.54) (Figure 4.5D).

SmCB specific production of serum IgA developed in all animals due to challenge, though the titres in the AdSmCB:SmCB/AddaVax group was significantly higher than all other groups (146 ng/mL) (Figure 4.5F). Antigen specific IgM and IgE was also produced in response to infection. Levels of SmCB specific IgM were found to be higher in control animals (3.1 OD 450 for both PBS and AdNeg:AddaVax) with a significant reduction in the AdSmCB:SmCB/AddaVax group (1.78 OD 450) (Figure 4.5G), and SmCB-IgE was produced in all groups; however, the AdNeg:AddaVax group had the highest titres (0.11 OD 450) with significant reductions in the AdSmCB:AddaVax (0.035 OD 450) and AdSmCB:SmCB/AddaVax groups (0.038 OD 450) (Figure 4.5H).

Lastly, we assessed antibody responses against parasite antigen preparations of soluble egg antigen (SEA) and soluble worm antigen preparation (SWAP). While there were no significant differences in SEA IgG between groups (Figure 4.6I), AdSmCB:SmCB/AddaVax produced a significantly larger amount of anti-SWAP IgG (42 519 ng/mL) than all other animal groups (24 545 ng/mL–26 581 ng/mL) (Figure 4.6J).

4.6 Discussion

Despite the prevalence of helminthic infections worldwide and their burden on global public health, there has yet to be a helminth vaccine approved for human use[17]. In fact, there are

billions of individuals at risk of helminth infection and almost a billion at risk for schistosomiasis specifically. Several vaccine strategies have been employed against schistosomiasis, most of them targeting external surface proteins of the parasite[18–20]. Conversely, we targeted a gut derived essential peptidase, SmCB, to starve the larval stage and adult worms as they begin feeding on blood macromolecules.

Schistosoma mansoni cathepsin B continues to prove itself to be an ideal vaccine target for intestinal schistosomiasis. We have previously reported the efficacy of SmCB in various vaccination platforms including adjuvanted protein[13–15], and vectored by bacterial[21] and viral vectors[16], resulting in varying protective capacities. Here, we describe a heterologous prime and boost vaccine formulation consisting of a human adenovirus serotype 5 (hAdV5) expressing SmCB followed by two boosting immunizations of recombinant SmCB admixed with AddaVax. We found that this vaccine strategy is capable of eliciting strong humoral, and cell mediated immunity providing significant protection from parasite infection.

Strong induction of both antigen specific IgG1 and IgG2c is a promising feature of our vaccine as IgG1 has been shown to kill larval stages of parasite *in vitro* and IgG2c could mediate effector functions through activated eosinophils[22]. In fact, the importance of the antibody response for protection from schistosomiasis was seen in passive transfer experiments[23,24]; the highest protection being mediated through IgG1 and IgG2 antibodies[25]. These IgG antibodies displayed high avidity demonstrating their affinity maturation over the course of our three-dose immunization schedule. Of interest, while not typical of intramuscular immunization, our adenovirus vaccine formulation elicited anti-SmCB IgA. While the protective capacity of IgA antibodies is not well studied, some work has proposed they may participate in protection from schistosomiasis[26]. Serum IgA is capable of inducing effector functions of both eosinophils and neutrophils[27]. Further, serum IgA-complexes have been shown to enhance phagocytosis and pro-inflammatory cytokine production[28] which may act on lung-stage schistosomula which are susceptible to immune cell destruction[29,30]. Of note, our vaccine did not elicit antigen specific IgE responses during immunization. Although human studies demonstrate the importance of IgE for schistosomiasis protection and helminth antigens commonly stimulate IgE production[31,32], vaccine-induced IgE may result in subsequent allergy-type hypersensitivity responses upon natural exposure[33].

To determine vaccine-induced cell mediated responses we first used flow cytometry to assess which systemic immune cells began expressing Ki67 upon exposure to SmCB. As might be expected alongside robust antibody production was an increased frequency of proliferating CD3- CD19+ B cells from the AdSmCB:SmCB/AddaVax group. We also found an increased frequency of proliferating CD8+ T cells and CD49b+ cells. Our group has previously demonstrated that natural killer (NK) cells from animals vaccinated with SmCB, adjuvanted with Montanide ISA 720 VG, played a key role in larval killing *in vitro*[34]. To capture this cell population, we used the same NK cell marker for mature, circulating NK cells, CD49b+ [35]; although, we acknowledge that NK cells could also be represented by other markers such as NK1.1[36]. Both CD8+ T cells and NK cells can express IFNγ, which we found significantly expressed in the supernatants of restimulated splenocytes from AdSmCB:SmCB/AddaVax animals. While T_H2 immunity is considered a key of helminth protection, radiation attenuated cercaria vaccination models showcase the importance of IFNγ[37] in protection from schistosomiasis. Our vaccine formulation also induced significant memory induction of IL-5 which we have also seen in our previous vaccine efforts[14,16,38], as well as other cytokines and chemokines. IL-5 is a potent activator and regulator of eosinophils. Not only are eosinophils important in the context of schistosomiasis pathology and granuloma formation[39], but we have also previously proposed the importance of eosinophils to target *S. mansoni* as it passes through circulation in the lungs. One mouse model of schistosomiasis shows that in natural infection eosinophils have no effect on worm burden or egg deposition using eosinophil ablated mice[40]. However, a history of literature has been published showing their ability to kill schistosomula *in vitro*[41–44], and their association with resistance to schistosome re-infection in humans[45,46].

Together, these immune responses led to significant protection from challenge with *S. mansoni*. Although the mouse model is not the ideal model for schistosomiasis, it continues to be to most widely used in the field due to efficiency and accessibility. To circumvent some of the concerns raised by Wilson et al.[47], we delayed our challenge infection to six weeks post final vaccination in comparison to the shorter schedules reported of about two weeks. Even in our delayed infection model, AdSmCB:SmCB/AddaVax reduced parasite burden by 60.7%, 50.4%, and 51.1% in adult worms, hepatic and intestinal eggs, respectively, compared to the AdNeg:AddaVax controls. This parasite burden decrease surpasses the 40% threshold of significant protection set by the World Health Organization[48]. Although we were unable to deliver

sterilizing immunity, mathematical modelling has been used to support the view that even partially protective schistosomiasis vaccines would contribute to reducing infection and interrupting parasite transmission[49]. Interestingly, our vaccine seems to target adult female worms, which lay pathology-causing eggs, giving a reduction of 66.4% compared to the AdNeg:AddaVax control group. This was exemplified in the resulting worm male:female sex ratio which was higher in vaccinated animals compared to controls. This sex-specific killing could stem from the observation that female schistosomes had higher SmCB activity in their extracts than male schistosomes[50].

Finally, we assessed humoral responses after challenge to determine how systemic antibody responses may change during infection. While all groups developed significant levels of anti-SmCB IgG, those from vaccinated animals were significantly more avid than those from other groups. We also found that primary expansion of IgG was the result of IgG1 antibodies, and animals in the AdSmCB:SmCB/AddaVax group had significantly more IgG2c and a more mixed T_H1/T_H2 humoral profile. While SmCB-IgA was induced by all groups it was higher in AdSmCB:SmCB/AddaVax vaccinated animals, and all other groups produced more SmCB-IgM than vaccinated animals inferring enhanced antibody isotype class-switching in this group. Only animals in the AdNeg:AddaVax group had significantly higher amounts of SmCB-IgE compared to both groups which received AdSmCB, which we hypothesize could be a direct result of adult worm burden as it is higher but not significantly different than the titres in the PBS control group. We also looked at antibody responses to parasite antigens and while we saw no changes in SEA-IgG in any of our animal groups, AdSmCB:SmCB/AddaVax animals had higher IgG titres specific to SWAP. As adult *S. haematobium* worms die in response to PZQ treatment, they release cryptic antigens which result in antibody development which is associated with protection from re-infection[51]; granted this is an IgE-mediated mechanism. It is reasonable to hypothesize that vaccine-induced killing of *S. mansoni* worms may also induce antibody production to a myriad of *Schistosoma* proteins found in SWAP; these antibodies may aid in protection from re-infection in subsequent parasite exposures.

The primary limitation of adenovirus vectored vaccines is their circulation in the human population leading to neutralizing antibodies which will render a vaccine ineffective[52]. This is an especially important consideration for schistosomiasis vaccine developments as in regions of Africa and Brazil where schistosomiasis is endemic, neutralizing antibodies to hAdV5 are prevalent[53–55]. This can be overcome using adenovirus serotypes with low prevalence in humans[56].

Alternatively, targeting vaccination of children between the ages of six months and two years of age may also ameliorate this problem since they are not only at high risk for schistosomiasis, but this age window is when Ad5 seroprevalence was reported to be at its lowest[57]. Post-COVID-19, adenoviral vectors have also been criticized for causing vaccine induced thrombocytopenia (VIT). During the SARS-CoV-2 pandemic vaccine roll-out it was reported that the Oxford/AstraZeneca (Chimpanzee adenovirus) and the Johnson & Johnson (HAd26) led to a rare blood clotting disorder in some cases[58,59]. Of note, our vaccine formulation delivers a very low dose of adenovirus ($5x10^5$ infectious units (IU)) which may prevent vaccine-induced adverse events such as this. While it is unclear if the adenoviral vector itself is responsible for causing VIT, or the Oxford/AstraZeneca and Johnson & Johnson formulations contain contaminants, our study serves as a proof-of-concept, demonstrating that a viral vectored vaccine may be a promising platform for schistosomiasis vaccine development; as such, other viral vectors may be explored.

With nearly one billion people at risk for schistosomiasis and no new drug treatments available, an effective prophylactic vaccine is necessary to prevent infection and stop transmission. Our vaccine formulation, AdSmCB:SmCB/AddaVax, provides significant protection from intestinal schistosomiasis in a mouse model and harnesses both T_H1 and T_H2 arms of immunity. This work opens the door to future studies of altering vaccine route administration to increase vaccine efficacy and provides insight to specific mechanisms which may be correlates of schistosomiasis immunity. The realization of an effective schistosomiasis vaccine would not only protect those at risk of infection but also travellers to endemic regions until schistosomiasis can be completely eradicated.

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4.8 References

1. Schistosomiasis (Bilharzia). *World Health Organization* https://www.who.int/health-topics/schistosomiasis#tab=tab_1 (2023).
2. Colley, D. G., Bustinduy, A. L., Secor, W. E. & King, C. H. Human schistosomiasis. *Lancet* **383**, 2253 (2014).
3. Cioli, D. & Pica-Mattoccia, L. Praziquantel. *Parasitol Res* **90** (Suppl 1), S3-S9 (2003).
4. Santini-Oliveira, M. *et al.* Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine* **34**, 586–594 (2016).
5. Study of Safety and Immune Response of the Sm14 Vaccine in Adults of Endemic Regions. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03041766?cond=schistosomiasis+vaccine&draw=2&rank=9.
6. Anti-Schistosomiasis Sm14-vaccine in Senegal. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT05658614?cond=schistosomiasis+vaccine&draw=2&rank=7.
7. Anti-Schistosomiasis Vaccine: Sm14 Phase 2b-Sn in School Children. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03799510?cond=schistosomiasis+vaccine&draw=2&rank=1.
8. Sm-TSP-2 Schistosomiasis Vaccine in Healthy Ugandan Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03910972?cond=schistosomiasis+vaccine&draw=2&rank=2.
9. A Study to Evaluate the Safety, Tolerability, and Immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) Candidate Vaccine in Healthy Adults in Burkina Faso and Madagascar. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT05762393?cond=schistosomiasis+vaccine&draw=2&rank=5.
10. Safety, Tolerability, and Immunogenicity Study of Sm-p80 + GLA-SE (SchistoShield(R)) Vaccine in Healthy Adults. *ClinicalTrials.gov*

https://classic.clinicaltrials.gov/ct2/show/NCT05292391?cond=schistosomiasis+vaccine&draw=2&rank=8.

11. El Ridi, R. *et al.* Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. *PLoS One* **9**, (2014).
12. Tallima, H. *et al.* Protective immune responses against Schistosoma mansoni infection by immunization with functionally active gut-derived cysteine peptidases alone and in combination with glyceraldehyde 3-phosphate dehydrogenase. *PLoS Negl Trop Dis* **11**, (2017).
13. Perera, D. J. *et al.* Adjuvanted Schistosoma mansoni-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model. *Front Immunol* **11**, (2020).
14. Ricciardi, A., Visitsunthorn, K., Dalton, J. P. & Ndao, M. A vaccine consisting of Schistosoma mansoni cathepsin B formulated in Montanide ISA 720 VG induces high level protection against murine schistosomiasis. *BMC Infect Dis* **16**, (2016).
15. Ricciardi, A., Dalton, J. P. & Ndao, M. Evaluation of the immune response and protective efficacy of Schistosoma mansoni Cathepsin B in mice using CpG dinucleotides as adjuvant. *Vaccine* **33**, 346–353 (2015).
16. Perera, D. J. *et al.* A low dose adenovirus vectored vaccine expressing Schistosoma mansoni Cathepsin B protects from intestinal schistosomiasis in mice. *EBioMedicine* **80**, (2022).
17. Perera, D. J. & Ndao, M. Promising Technologies in the Field of Helminth Vaccines. *Front Immunol* **12**, (2021).
18. Santini-Oliveira, M. *et al.* Development of the Sm14/GLA-SE Schistosomiasis Vaccine Candidate: An Open, Non-Placebo-Controlled, Standardized-Dose Immunization Phase Ib Clinical Trial Targeting Healthy Young Women. *Vaccines (Basel)* **10**, (2022).
19. Keitel, W. A. *et al.* A phase 1 study of the safety, reactogenicity, and immunogenicity of a Schistosoma mansoni vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. *Vaccine* **37**, 6500–6509 (2019).
20. Zhang, W. *et al.* Sm-p80-based schistosomiasis vaccine: double-blind preclinical trial in baboons demonstrates comprehensive prophylactic and parasite transmission-blocking efficacy. *Ann N Y Acad Sci* **1425**, 38–51 (2018).

21. Hassan, A. S. *et al.* Salmonella Typhimurium expressing chromosomally integrated Schistosoma mansoni Cathepsin B protects against schistosomiasis in mice. *NPJ Vaccines* **8**, (2023).
22. Khalife, J. *et al.* Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of Schistosoma mansoni. *J Immunol* **142**, 4422–7 (1989).
23. Mangold, B. L. & Dean, D. A. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against Schistosoma mansoni in mice. *J Immunol* **136**, 2644–8 (1986).
24. Mangold, B. L. & Dean, D. A. The role of IgG antibodies from irradiated cercaria-immunized rabbits in the passive transfer of immunity to Schistosoma mansoni-infected mice. *Am J Trop Med Hyg* **47**, 821–9 (1992).
25. Sher, A., Smithers, S. R., MacKenzie, P. & Broomfield, K. Schistosoma mansoni: Immunoglobulins involved in passive immunization of laboratory mice. *Exp Parasitol* **41**, 160–166 (1977).
26. Grzych, J. M. *et al.* IgA antibodies to a protective antigen in human Schistosomiasis mansoni. *J Immunol* **150**, 527–35 (1993).
27. Motegi, Y. & Kita, H. Interaction with Secretory Component Stimulates Effector Functions of Human Eosinophils But Not of Neutrophils. *The Journal of Immunology* **161**, 4340–4346 (1998).
28. Hansen, I. S., Hoepel, W., Zaat, S. A. J., Baeten, D. L. P. & den Dunnen, J. Serum IgA Immune Complexes Promote Proinflammatory Cytokine Production by Human Macrophages, Monocytes, and Kupffer Cells through FcαRI-TLR Cross-Talk. *J Immunol* **199**, 4124–4131 (2017).
29. David, J. R., Butterworth, A. E. & Vadas, M. A. Mechanism of the interaction mediating killing of Schistosoma mansoni by human eosinophils. *Am J Trop Med Hyg* **29**, (1980).
30. Capron, M. & Capron, A. Schistosomes and eosinophils. *Trans R Soc Trop Med Hyg* **74**, 44–50 (1980).
31. Fitzsimmons, C. M., Falcone, F. H. & Dunne, D. W. Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity. *Front Immunol* **5**, (2014).

32. Negrão-Corrêa, D. *et al.* Association of Schistosoma mansoni-specific IgG and IgE antibody production and clinical schistosomiasis status in a rural area of Minas Gerais, Brazil. *PLoS One* **9**, (2014).
33. Diemert, D. J. *et al.* Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: implications for the development of vaccines against helminths. *J Allergy Clin Immunol* **130**, (2012).
34. Ricciardi, A., Zelt, N. H., Visitsunthorn, K., Dalton, J. P. & Ndao, M. Immune mechanisms involved in schistosoma mansoni-Cathepsin B vaccine induced protection in mice. *Front Immunol* **9**, 398845 (2018).
35. Huntington, N. D., Vosshenrich, C. A. J. & Di Santo, J. P. Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nature Reviews Immunology* **7**, 703–714 (2007).
36. Goh, W. & Huntington, N. D. Regulation of Murine Natural Killer Cell Development. *Front Immunol* **8**, 130 (2017).
37. Jankovic, D. *et al.* Optimal vaccination against Schistosoma mansoni requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. *J Immunol* **162**, 345–51 (1999).
38. Perera, D. J. *et al.* Adjuvanted Schistosoma mansoni-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax^{TM} Provides Protection in a Pre-Clinical Schistosomiasis Model. *Front Immunol* **11**, 2990 (2020).
39. de Oliveira, V. G. *et al.* Eosinophils participate in modulation of liver immune response and tissue damage induced by Schistosoma mansoni infection in mice. *Cytokine* **149**, (2022).
40. Swartz, J. M. *et al.* Schistosoma mansoni infection in eosinophil lineage-ablated mice. *Blood* **108**, 2420–2427 (2006).
41. Hagan, P., Moore, P. J., Adjukiewicz, A. B., Greenwood, B. M. & Wilkins, H. A. In-vitro antibody-dependent killing of schistosomula of Schistosoma haematobium by human eosinophils. *Parasite Immunol* **7**, 617–24 (1985).
42. Glauert, A. M., Butterworth, A. E., Sturrock, R. F. & Houba, V. The mechansim of antibody-dependent, eosinophil-mediated damage to schistosomula of Schistosoma

mansoni in vitro: a study by phase-contrast and electron microscopy. *J Cell Sci* **34**, 173–92 (1978).

43. David, J. R. & Butterworth, A. E. Immunity ot Schistosoma mansoni: antibody-dependent eosinophil-mediated damage to schistosomula. *Fed Proc* **36**, 2176–80 (1977).
44. Butterworth, A. E. *et al.* Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* **256**, 727–729 (1975).
45. Sturrock, R. F. *et al.* Observations on possible immunity to reinfection among Kenyan schoolchildren after treatment for Schistosoma mansoni. *Trans R Soc Trop Med Hyg* **77**, 363–371 (1983).
46. Ganley-Leal, L. M. *et al.* Correlation between eosinophils and protection against reinfection with Schistosoma mansoni and the effect of human immunodeficiency virus type 1 coinfection in humans. *Infect Immun* **74**, 2169–2176 (2006).
47. Wilson, R. A., Li, X. H. & Castro-Borges, W. Do schistosome vaccine trials in mice have an intrinsic flaw that generates spurious protection data? *Parasit Vectors* **9**, (2016).
48. Bergquist, V. R. & Colley, D. G. Schistosomiasis vaccine:research to development. *Parasitol Today* **14**, 99–104 (1998).
49. Alsallaq, R. A., Gurarie, D., Ndeffo Mbah, M., Galvani, A. & King, C. Quantitative assessment of the impact of partially protective anti-schistosomiasis vaccines. *PLoS Negl Trop Dis* **11**, (2017).
50. Dalton, J. P., Clough, K. A., Jones, M. K. & Brindley, P. J. Characterization of the cathepsin-like cysteine proteinases of Schistosoma mansoni. *Infect Immun* **64**, 1328 (1996).
51. Hagan, P., Blumenthal, U. J., Dunn, D., Simpson, A. J. G. & Wilkins, H. A. Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. *Nature* **349**, 243–245 (1991).
52. Sakurai, F., Tachibana, M. & Mizuguchi, H. Adenovirus vector-based vaccine for infectious diseases. *Drug Metab Pharmacokinet* **42**, 100432 (2022).
53. Sumida, S. M. *et al.* Neutralizing Antibodies to Adenovirus Serotype 5 Vaccine Vectors Are Directed Primarily against the Adenovirus Hexon Protein. *The Journal of Immunology* **174**, 7179–7185 (2005).
54. Ersching, J. *et al.* Neutralizing antibodies to human and simian adenoviruses in humans and New-World monkeys. *Virology* **407**, 1–6 (2010).

55. Nwanegbo, E. *et al.* Prevalence of Neutralizing Antibodies to Adenoviral Serotypes 5 and 35 in the Adult Populations of The Gambia, South Africa, and the United States. *Clin Diagn Lab Immunol* **11**, 351 (2004).
56. Mendonça, S. A., Lorincz, R., Boucher, P. & Curiel, D. T. Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. *npj Vaccines* **6**, 1–14 (2021).
57. Thorner, A. R. *et al.* Age dependence of adenovirus-specific neutralizing antibody titers in individuals from sub-Saharan Africa. *J Clin Microbiol* **44**, 3781–3783 (2006).
58. Muir, K.-L., Kallam, A., Koepsell, S. A. & Gundabolu, K. Thrombotic Thrombocytopenia after Ad26.COV2.S Vaccination. *N Engl J Med* **384**, 1964–1965 (2021).
59. Greinacher, A. *et al.* Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* **384**, 2092–2101 (2021).

4.9 Figures and legends

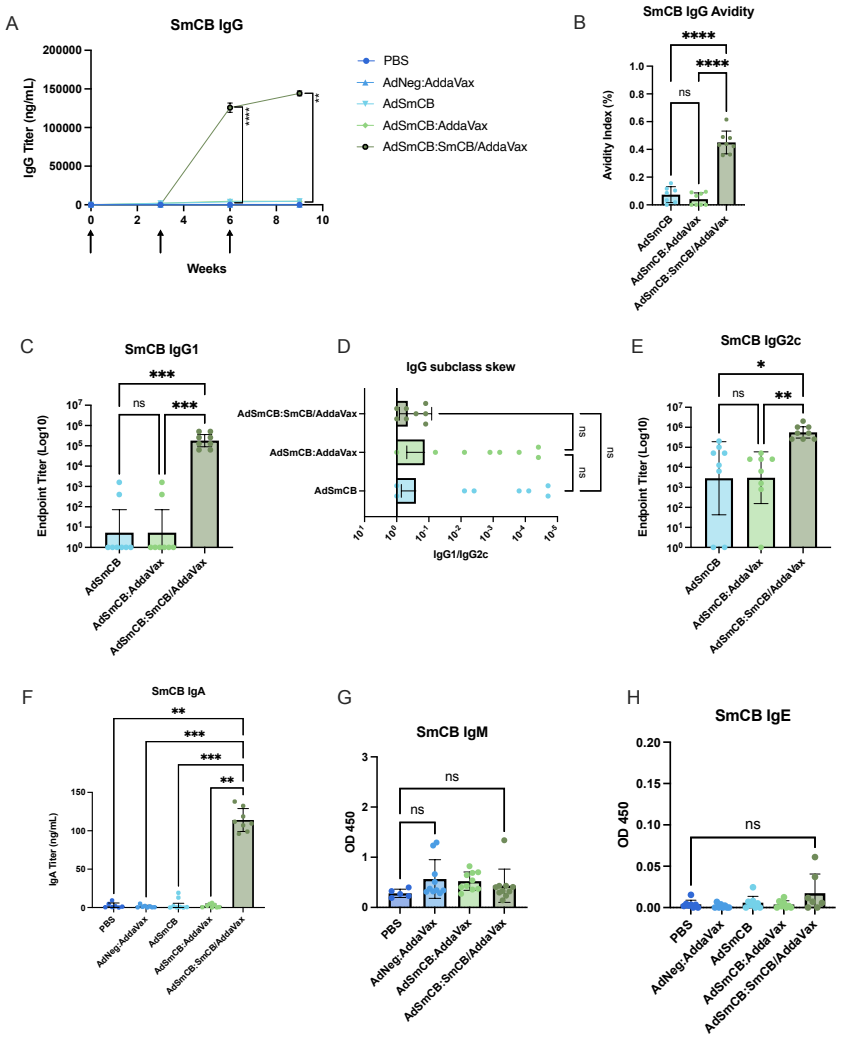


Figure 4.1 AdSmCB:SmCB/AddaVax induces robust antibody responses

(A) SmCB IgG titres measured by ELISA. Immunizations are indicated along the x-axis using arrows. (B-H) Other humoral responses were determined at week 9 by ELISA. (B) SmCB IgG avidity reported as the avidity index. SmCB (C) IgG1 and (E) IgG2c were measured by endpoint titre ELISA, and the ratio is given in (D). (F) SmCB-IgA measured by ELISA, and (G) SmCB-IgM and (H) IgE reported as OD 450. All data are represented as the mean ± SEM of two independent experiments. N=8-10. Ns=not significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. For (A week 6), (B), (G): one way ANOVA with Tukey's multiple comparisons. For (A week 9), (C)-(F), (H): Kruskal-Wallis test with Dunn's multiple comparisons.

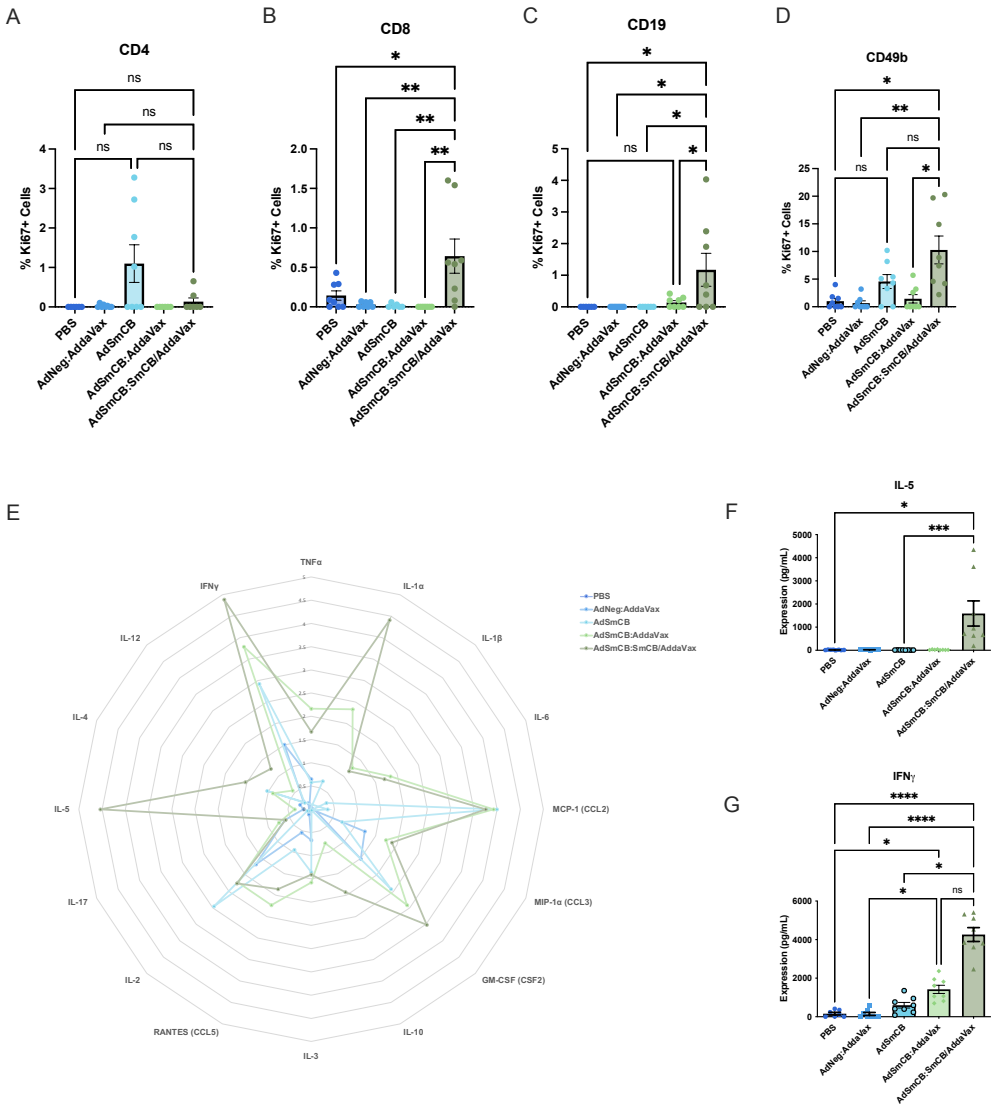


Figure 4.2 Vaccination results in significant antigen specific proliferation of CD8+ and CD19+ cells and cytokine expression

Splenocytes from vaccinated animals were restimulated for 18 hours with SmCB and assessed for proliferation using flow cytometry. Frequency of (A) CD3+CD4+, (B) CD3+CD8+, (C) CD3-CD19+, (D) CD49b+ cells expressing Ki67. Splenocytes were restimulated for 72 hours with SmCB to determine cytokine and chemokine responses. (E) Mean levels of cytokines and chemokines shown in the radar plot. Data are calculated as the fold change above the PBS converted into the natural log. Bar graphs depicting expression levels of cytokines (F) IL-5, (G) IFNγ. Ns=not significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. For (A), (D), (F), (G): Kruskal-Wallis test with Dunn's multiple comparisons. For (B), (C): one way ANOVA with Tukey's multiple comparisons.

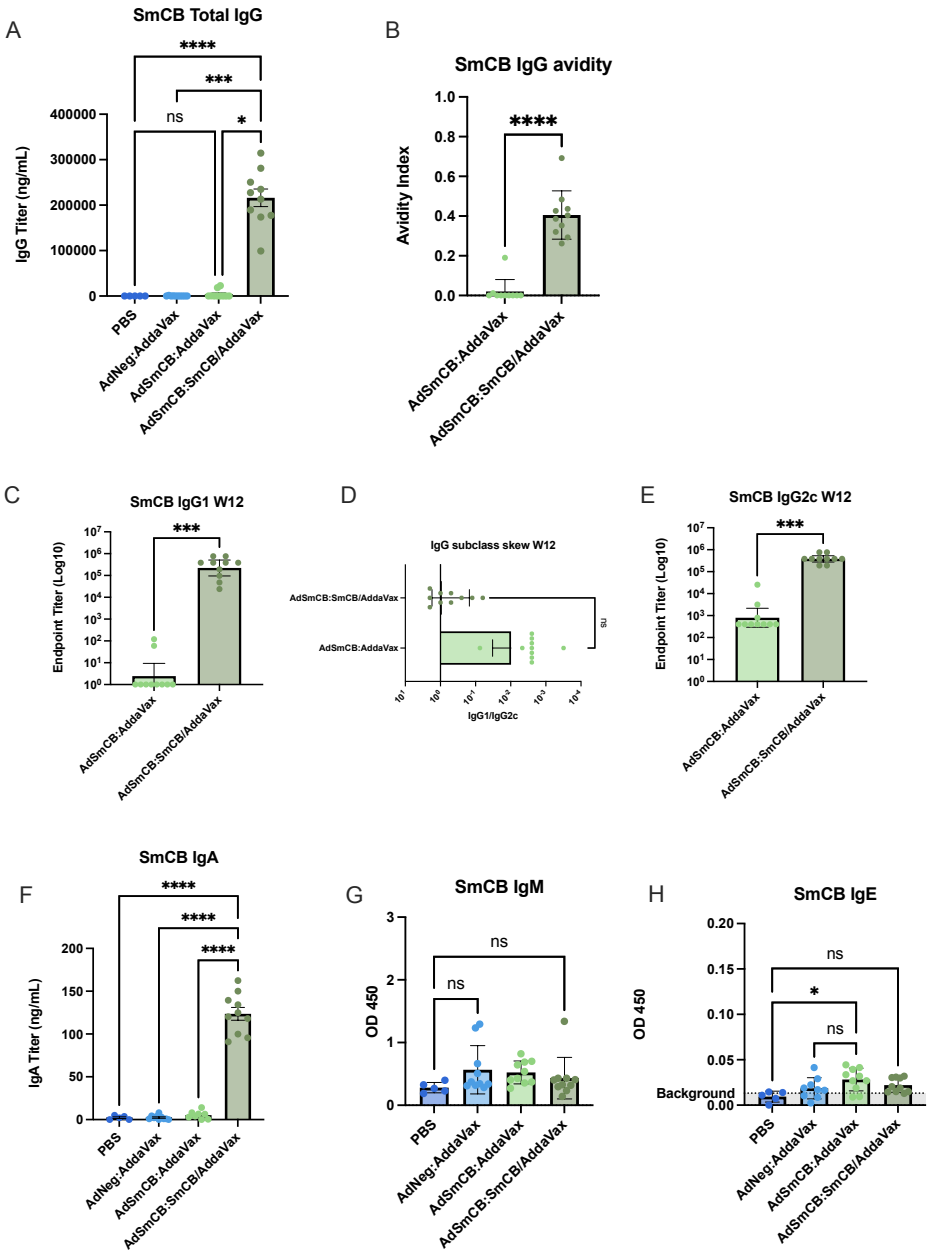


Figure 4.3 Humoral response at challenge resembles the humoral response at week 9

(A) SmCB IgG titres measured by ELISA at week 12. (B) SmCB IgG avidity reported as the avidity index. SmCB (C) IgG1 and (E) IgG2c were measured by endpoint titre ELISA, and the ratio is given in (D). (F) SmCB-IgA measured by ELISA, and (G) SmCB-IgM and (H) IgE reported as OD 450. All data are represented as the mean ± SEM of two independent experiments. PBS N=5, other groups n=10. Ns=not significant; *p<0.05, ***p<0.001, ****p<0.0001. For (A), (C)-(E), (H): Kruskal-Wallis test with Dunn's multiple comparisons. For (B): Mann-Whitney test. For (F), (G): one way ANOVA with Tukey's multiple comparisons.

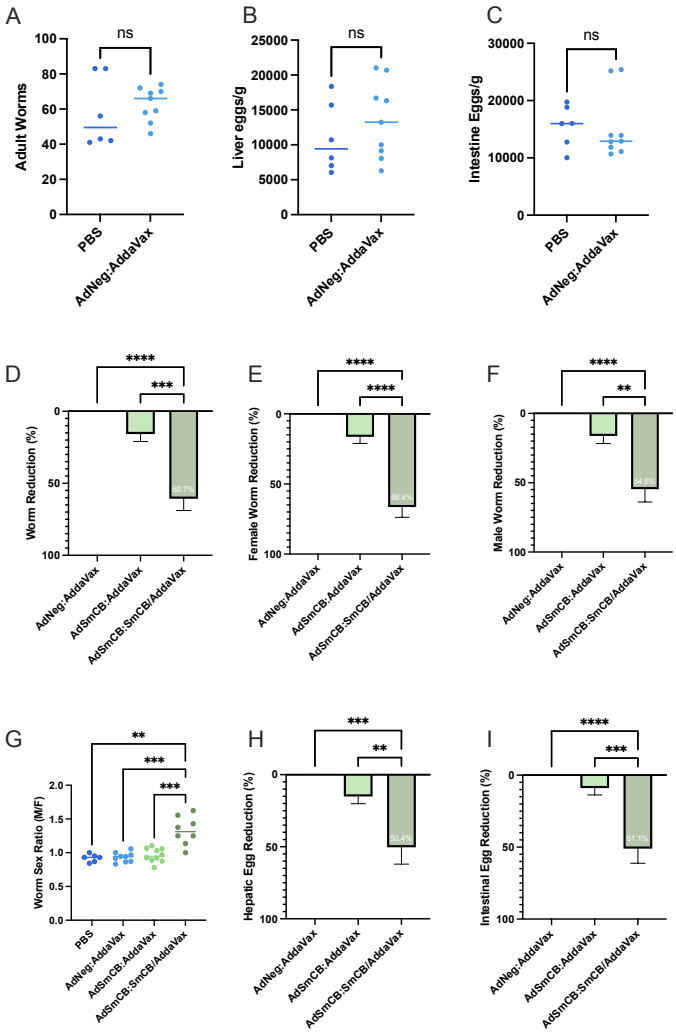


Figure 4.4 AdSmCB:SmCB/AddaVax significantly reduces parasite burden compared to controls

Parasite burdens in control groups showing (A) adult worms, (B) liver eggs per gram of tissue, (C) intestinal eggs per gram of tissue. Resulting parasite burden reduction calculated against the AdNeg:AddaVax group in (D) adult worms, (E) female worms, and (F) male worms. (G) Adult worm sex ratio given as male:female. (H) Hepatic egg reduction and (I) liver egg reduction compared to the AdNeg:AddaVax control. All data are represented as the mean ± SEM of two independent experiments. PBS N=6, other groups n=8-10. Ns=not significant; **p<0.01, ***p<0.001, ****p<0.0001. For (A), (C): Mann-Whitney test. For (B): unpaired t-test. For (D)-(I): one way ANOVA with Tukey's multiple comparisons.

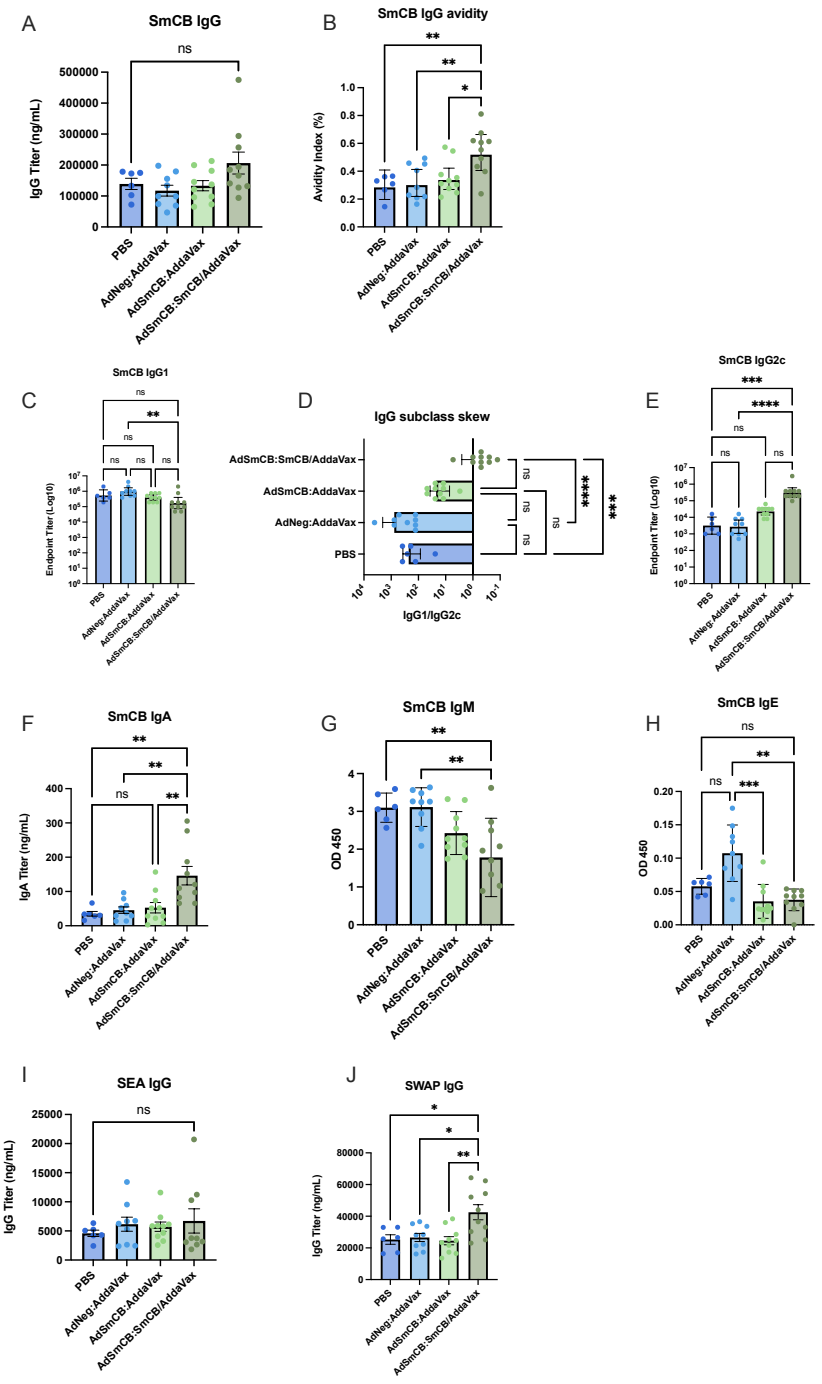
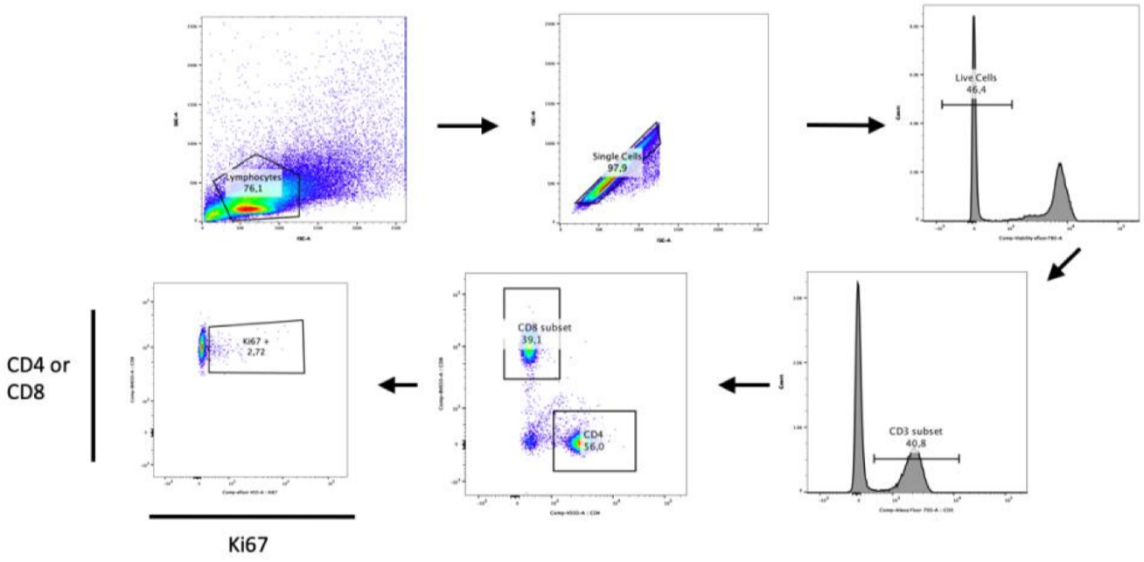


Figure 4.5 Antibody responses persist during challenge and are disparate from control responses

SmCB IgG titres measured by ELISA at week 19. (B) SmCB IgG avidity reported as the avidity index. SmCB (C) IgG1 and (E) IgG2c were measured by endpoint titre ELISA, and the ratio is given in (D). (F) SmCB-IgA measured by ELISA, and (G) SmCB-IgM and (H) IgE reported as

OD 450. (I) SEA-IgG and (J) SWAP-IgG measured by ELISA. All data are represented as the mean ± SEM of two independent experiments. PBS N=6, other groups n=9-10. Ns=not significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. For (A), (C)-(E), (H), (I): Kruskal-Wallis test with Dunn's multiple comparisons. For (B), (F), (G), (J): one way ANOVA with Tukey's multiple comparisons.

4.10 Supplemental data



Supplemental Figure 4.1 Flow cytometry gating strategy

Figure showing gating for proliferating CD4+ and CD8+ T cells. CD19 cells were similarly gated but on the CD3- population. CD49b+ cells were gated directly from the live cell population.

Chapter 5

General Discussion

Despite current schistosomiasis control measures, infection with *Schistosoma* spp. continues to affect a large proportion of the Global South. In fact, over 250 million people are infected each year, resulting in the attribution of disability adjusted life years to schistosomiasis ranging from 1.6 million[606] to as many as 70 million[607,608]. While MDA, WASH programs, and education help lower infection rates, developing a vaccine could significantly aid in interrupting schistosomiasis. Our group has developed several vaccines which target the cysteine peptidase cathepsin B from *Schistosoma mansoni*. This enzyme is necessary for parasite maturation and, when suppressed, leads to growth retardation in adult worms[609]. Being the most abundant peptidase secreted by *S. mansoni* our goal was to hinder this helminth's ability to acquire nutrients, thereby causing parasite starvation and death. Nonetheless, an important consideration in the field of vaccinology revolves around understanding the immune profile that will be triggered. It is important to harness appropriate correlates of immunity, specific to each infectious disease, to optimize efficacy. SmCB is a typically T_H2 skewing antigen[610] and T_H2 responses are well known to protect from helminthic infections[611]. Yet, it is becoming clear that T_H1 immunity can provide, and may be necessary for, long term protection from infection[110]. Vaccinology is in a constant state of development, with new platforms arising, to limit potential side effects while increasing protective responses and targeting specific arms of host immunity. The central goal of this work was to assess the protective capacity of SmCB when combined with various vaccination platforms to identify immune mechanisms that promote increased protection from schistosomiasis. Here, we show that while each vaccine formulation creates a unique immunological landscape that can protect from infection, both T_H1 and T_H2 arms of immunity tend to be activated and associated with significant protection.

5.1 Main Findings

It has been shown that SmCB confers protection from *S. mansoni* infection as a recombinant protein alone[610], when adjuvanted[501,612], and when expressed by vaccine

vectors[613,614]. These vaccination techniques elicited various antigen-specific immune responses and imparted protection between 50-80%; all, surpassing the WHO threshold, demonstrating a significant reduction of parasite burden[615].

Dr. Alessandra Ricciardi showed that when combined with CpG dinucleotides[612], or Montanide ISA 720 VG[501], the protective efficacy of SmCB could be increased from 50% towards 60%; shifting T_H2 towards T_H1 responses and T_H1/T_H2 responses, respectively. Using our well-established mouse model, and an arsenal of adjuvants introduced to us by the National Research Council of Canada (NRCC), we aimed to assess if a different adjuvant may be better suited to augment SmCB protective responses. We tested a variety of adjuvants and adjuvant combinations, finally focusing on sulfated lactosyl archaeol archaeosomes (SLA), developed at the NRCC, and AddaVax™ (AddaVax). We found that SmCB and SLA resulted in a parasite burden reduction of 56.6% across adult worms, and hepatic and intestinal eggs (PBR) compared to the PBS control. Conversely, when combined with AddaVax, SmCB gave a PBR of 83%. We also found that these vaccines created different immunological landscapes when systemic lymphocytes were restimulated with SmCB. SLA and SmCB resulted in primarily T_H1 and pro-inflammatory responses, whereas AddaVax and SmCB resulted in T_H2 and anti-inflammatory responses; both vaccines generated robust antigen specific humoral immunity and CD8+ T cell IFNγ production. This work was published in *Frontiers in Immunology* in 2020 and has been thoroughly discussed in Chapter 2.

We then sought to determine if vectoring SmCB using a virus may enhance protective efficacy. Using a viral vector for vaccine development creates a cost-effective immunization strategy which can be easily expanded for worldwide manufacturing and provides flexibility with administration routes. Initially, we developed a first-generation human adenovirus serotype 5 (Ad) expressing SmCB (AdSmCB) and administered it as a heterologous prime and boost vaccine with two boosting immunizations of recombinant SmCB (AdSmCB:SmCB). This vaccine strategy was able to maintain T_H2 responses typical of SmCB, while promoting type 1 immunity and CD4+ T cell polyfunctionality. Importantly, AdSmCB:SmCB resulted in a PBR of 72% with significant reductions in host pathology, measured by liver granuloma sizes and fibrosis around parasitic eggs. This work was published in *eBioMedicine* in 2022 and has been detailed in Chapter 3.

Finally, since the vaccine formulation described in Chapter 3 was unadjuvanted, we hypothesized that combining boosting immunizations with adjuvant might increase the protective

efficacy of this formulation further. Based on the data generated in Chapter 2, we decided to combine SmCB with AddaVax. This vaccine consisted of a priming immunization of AdSmCB followed by two boosts of SmCB and AddaVax (AdSmCB:SmCB/AddaVax). Here, we found that this vaccine formulation was highly immunogenic and combined many attributes of both vaccines discussed in Chapter 2 and Chapter 3. Highly avid antigen specific antibodies were produced and maintained throughout infection. These were accompanied by proliferation of CD8+ T cells, CD19+ B cells, and CD49+ NK cells. Both CD8+ T cells and NK cells have been shown to kill schistosomula *in vitro*[398] and are effective contributors of IFNγ which was also seen in animals immunized with AdSmCB:SmCB/AddaVax. This vaccine resulted in a PBR of 54% when animals were challenged with a high infectious dose of *S. mansoni* and six weeks post final immunization, compared to typical studies which challenge animals two weeks after the final dose[616]. Not only does a delayed challenge mitigate the limitations of the mouse model of *S. mansoni* infection, but it also demonstrates longer lasting protection from infection. Interestingly, we found that our vaccine targeted female worms and enhanced anti-SWAP IgG in challenged animals which may reduce egg deposition over time and protect from *S. mansoni* re-infection, ultimately aiding the interruption of schistosomiasis transmission. This work is being prepared for submission and has been discussed in Chapter 4.

5.2 Future perspectives

5.2.1 Protection from reinfection and therapeutic benefits

The work contained in this thesis describe vaccine efforts which protect from schistosomiasis prophylactically. However, the reality of schistosomiasis also includes the individuals already infected and those who are infected and receive treatment[617]. This means that an effective vaccine should provide therapeutic benefit; at the very least it should not create adverse events for infected individuals, and it should also stimulate enhanced protection for subsequent exposure following treatment. One aspect of helminth vaccines we did evaluate was whether our formulation was likely to elicit IgE hypersensitivity responses. As mentioned in Chapters 2-4, we found no evidence that this might be a danger.

It would be beneficial to determine if this vaccine can provide any therapeutic benefit within the context of a pre-existing schistosomiasis infection and further examine vaccine safety. Additionally, vaccine administration in the field would likely be conducted simultaneously with PZQ treatment. This vaccination schedule would also be an interesting avenue with direct translatability to the field. PZQ-killing of *S. haematobium* worms results in IgE production against internal worm antigens providing protection from re-infection[379]. The timing of vaccination should be carefully determined to work in concert with PZQ-acquired immunity and to avoid antigenic competition which has been demonstrated when multiple antigens are administered together[618].

5.2.2 Cross-protection

In a schistosomiasis vaccine meeting of several experts in the field, the utility of a schistosomiasis vaccine was discussed[498]. Of the proposed preferred product characteristics, one was that an effective schistosomiasis vaccine will ideally protect from several species of *Schistosoma*. This is especially important in regions where endemicity overlap[619,620]. The peptidase cathepsin B is expressed by all three major schistosome species: *S. mansoni*, *S. haematobium*, *S. japonicum,* as well as *S. mekongi*[621,622]. Interestingly, these cathepsins share homology with each other, and cysteine peptidases from other co-endemic parasites (Figure 5.1). We hypothesize that antibody responses developed against our vaccine formulation may cross-react and provide protection against other *Schistosoma* spp. and possibly even *Fasciola* spp. Cathepsin L from *F. hepatica* was able to provide protection from *S. mansoni* due to the similarity of their peptidases[495]. For this reason, it is reasonable to believe that *S. mansoni* cathepsin B might also be able to confer protection against *Fasciola* spp.

S. haematobium possesses an unnamed protein with a high homology to SmCB (82.65%). In fact, when *S. mansoni* cathepsin B was probed by serum from *S. haematobium* infected patients, IgG titres could be detected (Figure 5.2) demonstrating that these antigens share common epitopes and that protection might be shared between species.

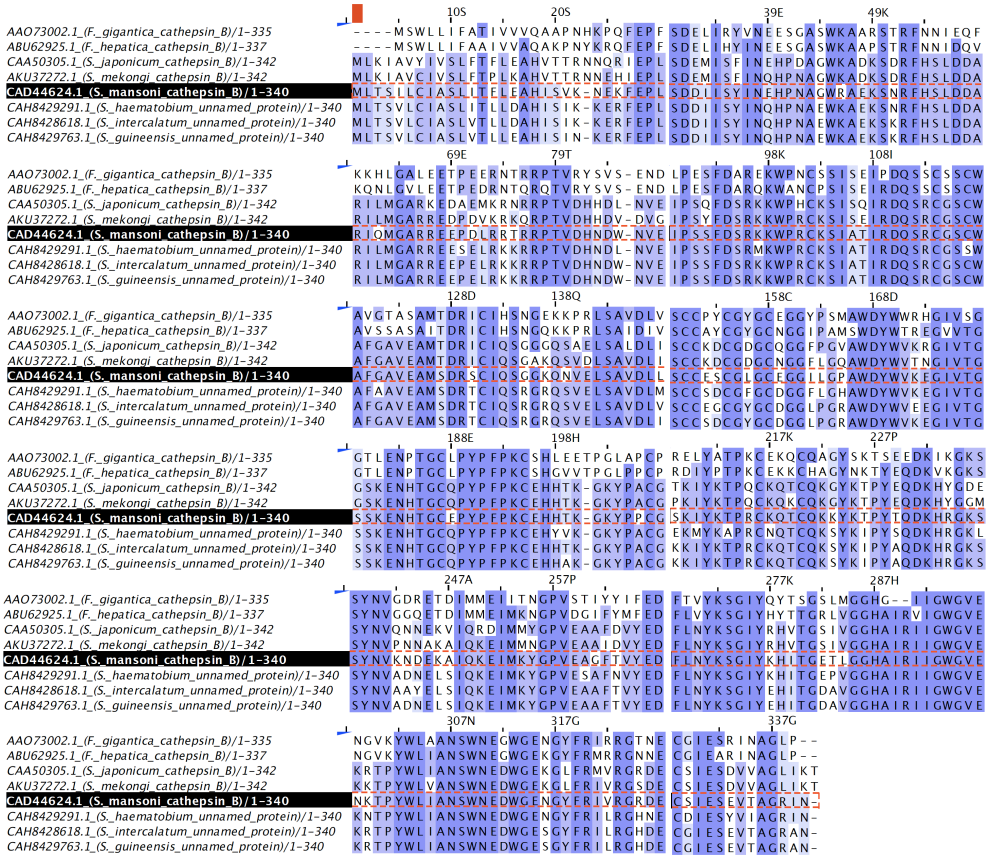


Figure 5.1 Sequence homology of various cysteine peptidases from related and co-endemic helminths

Multiple sequence homology was conducted using Clustal Omega and visualized using Jalview software. The following includes the percent identity of each peptidase compared to *S. mansoni* cathepsin B: *Fasciola gigantica* cathepsin B (50.15%), *F. hepatica* cathepsin B (50.75%), *S. japonicum* cathepsin B (75.88%), *S. mekongi* cathepsin B (76.18%), *S. haematobium* unnamed protein (82.65%), *S. intercalatum* unnamed protein (85.29%), and *S. guineensis* unnamed protein (85%).

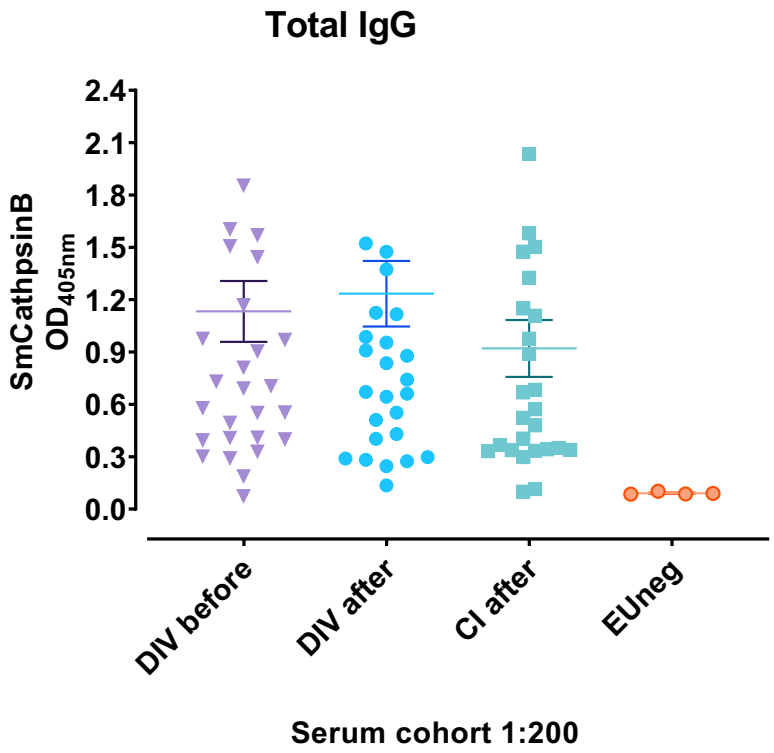


Figure 5.2 Serum IgG from *S. haematobium* infected patients binds SmCB

SmCB was probed with three different groups of serum samples: individuals who were resistant to infection and were found to be *Schistosoma* negative 18 months after receiving PZQ treatment (DIV after), serum from the same individuals before treatment (DIV before), and chronically infected individuals who became re-infected after PZQ treatment (CI). Sera from Europeans who had never been to an endemic region were used as a control (EUneg). ELISAs were conducted in a manner similar to that described in Chapters 2-4 using 1:5000 Anti-Human IgG (Fc)-HRP-conjugated secondary antibody and the resulting OD was read at 405 nm. *Contribution of authors: Dilhan Perera provided recombinant SmCB protein to Dr. Bemnet Tedla (James Cook University, Australia) who conducted the ELISA and generated this figure.*

5.2.3 Varying dosage and immunization route

There are infinite variables involved in vaccine formulation including, but not limited to: antigen dosage, adjuvant composition, vector dosage, immunization schedule and time between

boosting doses, number of immunizations, and route of administration. All these factors can affect vaccine efficacy and can be considered to enhance vaccine responses.

Vaccines can contain various quantities of antigen, and this can affect the immune response elicited. Although direct research into this idea is limited, it has been proposed that there may be an inverse relationship between antigen dose and delayed type hypersensitivity[623]. Further, it has been hypothesized that more antigen is necessary for T_H2 responses compared to T_H1 responses[624]; lower antigen doses also led to more favourable T cell responses[625–627]. Although we administered 20 μg of SmCB per immunization, this amount could be optimized to enhance protective immunity. Along the same vein, adjuvant dosages and various combinations too, can be reconsidered.

Typically, helminth vaccines consist of 3-dose vaccine regimens[110] to stimulate long lasting and significant protection. However, if a vaccine was efficacious in fewer doses this would be more clinically feasible as the vaccine would be more cost-effective and it would be easier to ensure the completion of vaccination schedules (less visits to or from the healthcare provider).

HAdV5 poses a limitation of neutralizing antibodies which can render vaccines ineffective if present before vaccination. Neutralizing antibodies can also negate subsequent immunizations in multiple dose vaccine formulations. In these instances, different adenovirus serotypes can be used and/or combined; they can also be carefully chosen to best serve target populations. In addition, adenoviral vectors easily allow for changing the route of vaccine administration. For example, hAdV5 vectored vaccines have been administered orally, subcutaneously, intranasally, and intramuscularly; some of these less conventional routes of administration have even circumvented the problem of pre-existing adenovirus immunity[628–633]. While our studies have administered AdSmCB intramuscularly, intranasal administration is of particular interest. Early in the schistosome lifecycle, the larval parasite spends approximately one week in the lungs. Intranasal vaccination may stimulate mucosal immunity in the lungs to target schistosomula and eliminate infection before the parasite can mature into egg laying adults. In fact, preliminary studies showed that AdSmCB was highly immunogenic when administered intranasally (AdSmCB (IN)), resulting in antigen specific lung IgA and high titres of antigen specific IgG which persisted up to 7 months post vaccination (Figure 5.3). Interestingly, AdSmCB (IN) alone did not elicit significant amounts of antigen specific cytokine or chemokine release from restimulated splenocytes, showing that cell mediated immunity might be localized to the lungs; when AdSmCB

(IN) was administered simultaneously with a single dose of recombinant SmCB intramuscularly, restimulated splenocytes from those animals expressed elevated levels of IL-3, IL-5, and IL-6 (Figure 5.4). A single dose of AdSmCB (IN) was able to confer reasonable protection from *S. mansoni* challenge (52% PBR; low level infection) and when administered with an intramuscular dose of SmCB, protection was slightly lower (46% PBR; low level infection) (Figure 5.5). A single dose intranasal vaccine is effectively a single dose needle-free vaccine. In developing countries, where schistosomiasis is endemic, it is not uncommon for needles to be reused without sterilization; unsafe injection practices result in millions of cases of viral hepatitis B and C, and HIV infections[634,635]. It is also known that vaccine hesitancy is common in Africa[636]. Development of a needle free vaccine for schistosomiasis could not only increase vaccine compliance but also reduce patient exposure to additional infectious agents by unsterile needles.

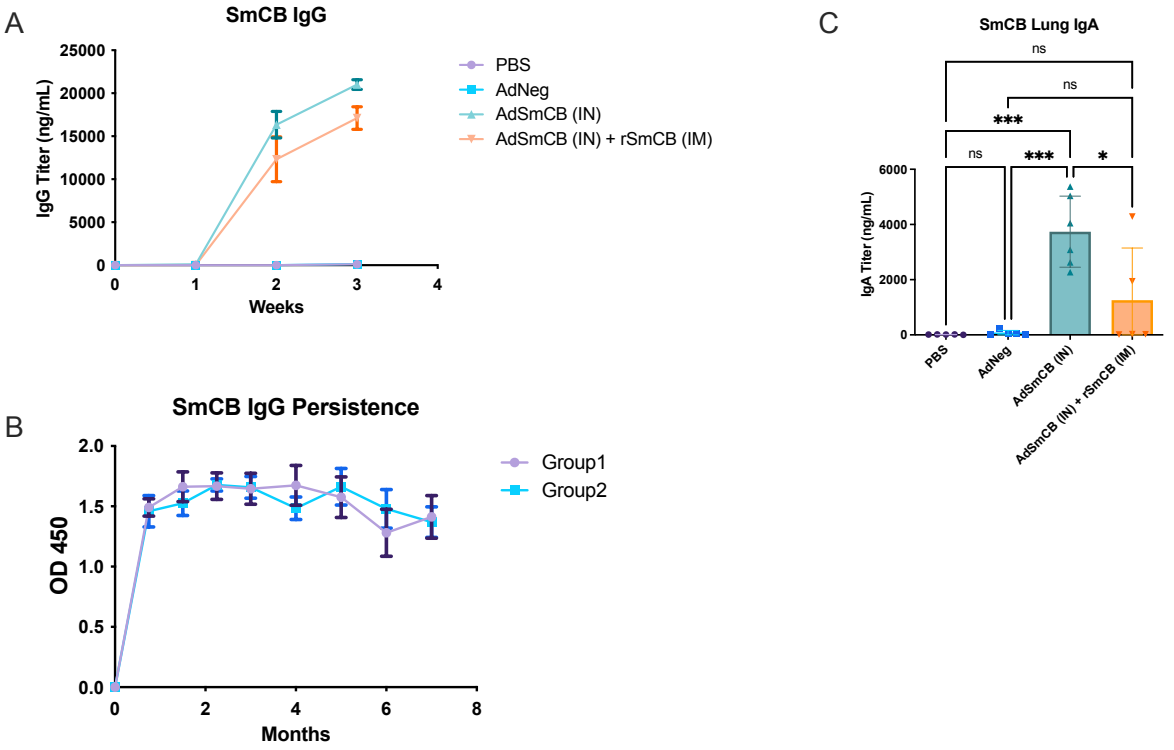


Figure 5.3 AdSmCB (IN) produces persistent humoral responses

C57BL/6 female mice were immunized intranasally with 10^9 infectious units of AdSmCB. (A) Serum SmCB-IgG was assessed by ELISA up until three weeks after immunization. n=4-5. (B) Some animals were followed to month seven after immunization to determine SmCB-IgG

persistence in the serum. n=4. (C) At week 3 after immunization, some animals were sacrificed to determine lung IgA. After perfusion with PBS, lungs were homogenized, and SmCB-IgA was assessed by ELISA. n=5. Statistical analysis: one way ANOVA, ns=not significant; *p<0.05, ***p<0.001. Data is shown as mean ± SEM. ELISAs were conducted in a manner similar to that described in Chapters 2-4.

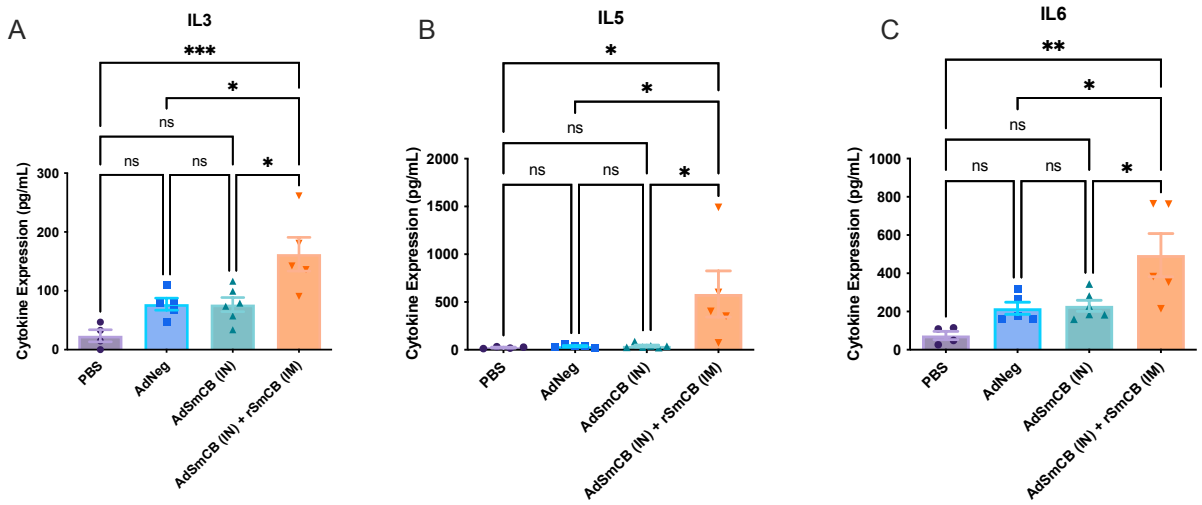


Figure 5.4 AdSmCB (IN) results in cytokine memory responses when combined with concurrent intramuscular administration of recombinant SmCB

Three weeks after immunization with AdSmCB (IN) animals were sacrificed. Splenocytes were isolated and restimulated for 72 hours with recombinant SmCB. Supernatants were assessed for cytokine and chemokine production by multiplex ELISA as described in Chapters 2-4. This figure shows the supernatant expression of (A) IL-3, (B) IL-5, and (C) IL-6. n=5. Statistical analysis: one way ANOVA, ns=not significant; *p<0.05; **p<0.01; ***p<0.001. Data is shown as mean ± SEM.

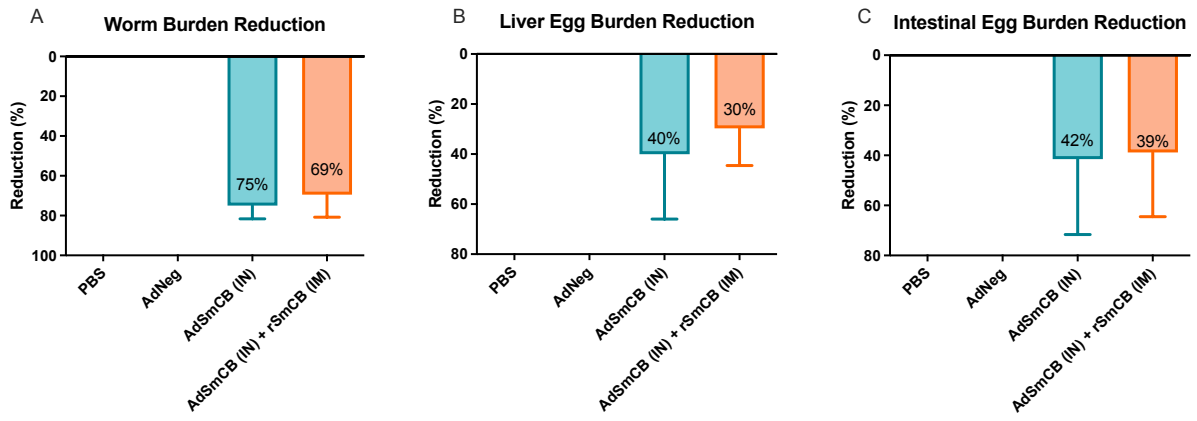


Figure 5.5 AdSmCB (IN) provides protection from *S. mansoni*

Three weeks post immunization with AdSmCB (IN) or multimodal vaccine (simultaneous administration of AdSmCB(IN) with an intramuscular dose of recombinant SmCB), animals were challenged with a low infectious level of *Schistosoma*. Seven weeks after challenge, animals were sacrificed to determine protective efficacy of vaccination. PBS and AdNeg immunized animals had adult worm burdens of approximately 20 worms. Parasite burden reductions were calculated as described in Chapters 2-4. (A) Worm burden reduction. (B) Liver egg burden reduction. (C) Intestinal egg burden reduction. All parasite burden reduction measures were calculated against the PBS control. n=3. Data is shown as mean ± SEM.

5.2.4 Multi-antigenic vaccines

While vaccines targeting a single antigen are commonly efficacious against viral and bacterial pathogens, *Schistosoma* spp. are large, complex, eukaryotic pathogens with several life stages within the host. SmCB is expressed by cercariae, schistosomula, and adult worms[637] making it an ideal target for vaccine development. In adult worms, SmCB is secreted into the gut and has been found in somatic extracts and within gastrodermal cells[638]. However, other schistosome antigens have been exploited for their protective abilities.

Specifically, *S. mansoni* tetraspanin-2 (SmTSP2) and the large subunit of calpain (Sm-p80). SmTSP2 is a transmembrane protein expressed on every life cycle stage of *S. mansoni* and is thought to be involved in tegument turnover[639]. It has been used numerous times in pre-clinical vaccine efforts[522,640] and is currently in phase I/II clinical trials[578,580,641]. In murine models, SmTSP2 has a PBR of 30-54% depending on the study[522,640]. Sm-p80, is also a protein expressed

on the exterior of the parasite and plays a role in surface membrane biogenesis and renewal[642]. In baboons the highest PBR achieved was 82%[497].

HAdV5 has a relatively large carrying capacity and is poised to contain several antigens within the same virus. By vaccinating using SmCB and an exterior membrane protein of *Schistosoma* we can harness the immune system to starve the worm and target its external surface collaboratively. This may involve creating bi- and tri-cistronic adenoviruses containing a combination of SmCB and SmTSP-2/Sm-p80. To begin this work, we have already created a recombinant adenovirus carrying SmTSP2 (AdSmTSP2) as a proof of concept (Figure 5.6). Our next steps will be to administer AdSmCB and AdSmTSP2 together, to clone a bi-cistronic adenovirus containing SmCB and SmTSP2, and to clone recombinant adenoviruses containing Sm-p80.

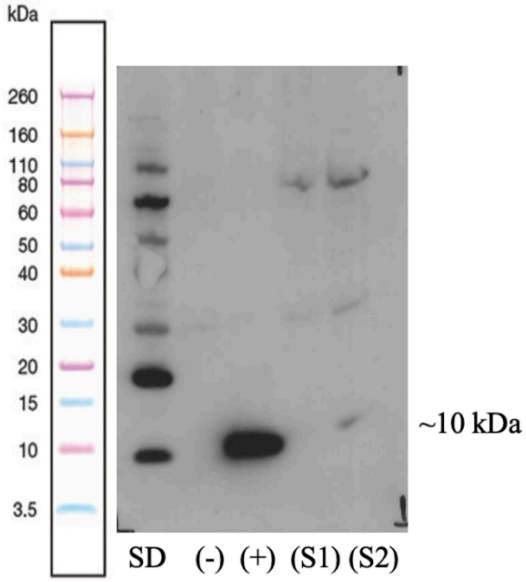


Figure 5.6 AdSmTSP2 expresses TSP2 *in vitro*

A western blot was conducted on two HEK293 cell lysate samples (S1;S2) infected with AdSmTSP2. Blot was incubated with 1:5000 rabbit anti-SmTSP2 followed by 1:20 000 anti-rabbit IgG-HRP. After substrate incubation for 5 minutes, the blot was exposed to film for 15 seconds and developed. Negative control is non-transfected HEK293 cells (-); positive control is purified recombinant SmTSP2 protein (+); protein ladder (SD). SmTSP2 is shown in the (+) and (S2) lanes at around 10 kDa. *Contribution of authors: Dilhan Perera ordered the SmTSP2 gene cassette and*

oversaw the production of AdSmTSP2. Cloning of this virus and generation of this figure was completed by Francesca Battelli (McGill University, Canada).

5.2.5 Novel vaccination methods

5.2.5.1 *Leishmania major* as a vaccine vector

In addition to hAdV5 there are many novel vaccine platforms in pre-clinical research. One of which is the attenuated *Leishmania major* as a vector to express antigen. *L. major* is the causative agent of the parasitic infection leishmaniasis and results in the cutaneous form of the disease. By using CRISPR gene editing, the *L. major centrin* gene has been removed ($LmCen^{-/-}$) and this attenuated protozoan is antibiotic resistant marker free and is unable to cause disease in immunocompromised mice[643]. Currently, $LmCen^{-/-}$ is used as a leishmanization agent to confer protection from both cutaneous and visceral leishmaniasis[643,644].

In collaboration with Dr. Greg Matlashewski, we have developed a $LmCen^{-/-}$ harboring a plasmid containing SmCB (LmΔCen+SmCB). These parasites express SmCB *in vitro* (Figure 5.7) and can be used as a vectored vaccine for schistosomiasis as well as serve its original purpose as a vaccine for leishmaniasis. *Schistosoma* spp. and *Leishmania* spp. are co-endemic to many regions inside of Africa, South America, the Middle East, and Asia[25,645]. LmΔCen+SmCB, if effective, could protect hundreds of millions of individuals from both parasitic infections. This vectored vaccine will be tested for efficacy against *L. major* (cutaneous disease), *L. donovani* (visceral disease), and *S. mansoni*.

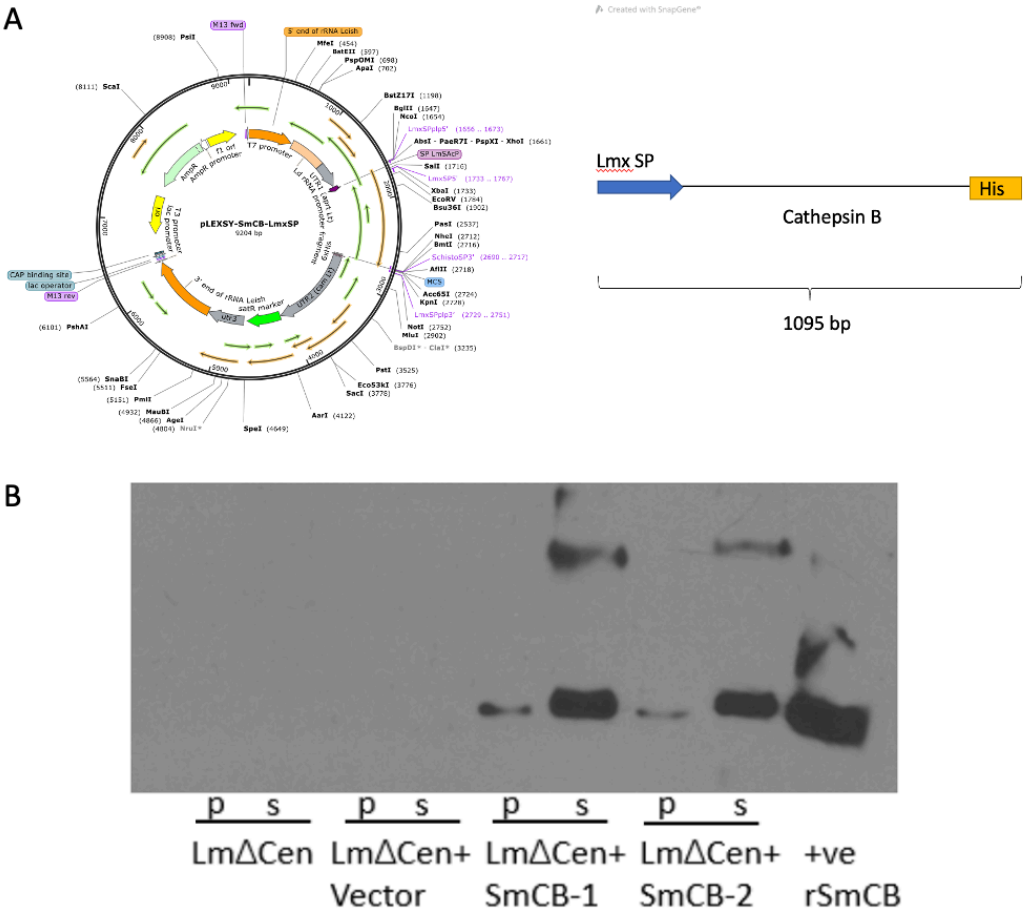


Figure 5.7 LmΔCen+SmCB expresses SmCB *in vitro*

(A) Plasmid map of pLEXSY with SmCB gene construct expressed using *L. major* signal peptide. SmCB was cloned with a 6X his tag for confirmation of protein expression. (B) Western blot against the his tag, conducted in a similar manner to that described in Chapter 2. Parasite internal and membrane proteins were assessed (p) alongside cell culture supernatants (s). LmΔCen, and LmΔCen containing an empty plasmid (LmΔCen+Vector) were included as controls. Two clones of LmΔCen+SmCB were tested (-1;-2) with a positive control of recombinant SmCB (+ve rSmCB). *Contribution of authors: Dilhan Perera provided recombinant SmCB and SmCB containing DNA to Kayla Paulini (McGill University, Canada) who created LmΔCen+SmCB clones and generated this figure.*

5.2.5.2 Newcastle disease virus as a vaccine vector

Newcastle disease virus (NDV) is an enveloped RNA virus and has been used as a vaccine vector[646,647]. NDV is typically an avian virus limiting the problem of neutralizing antibodies, and due to host-range restriction, it is highly attenuated in all non-avian species including humans[646]. The safety and immunogenicity of these vaccines have been extensively studied in non-human primates and have been shown not to cause disease[648]. Further, this vector has been reported to be restricted to the respiratory tract making it a promising vector for intranasal administration[649].

Recently, NDV has been exploited as a vector to deliver the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[557]. When mice were immunized intramuscularly, they were afforded sterilizing immunity from subsequent SARS-CoV-2 infection with no viral titres or viral antigen found in their lungs. In collaboration with Dr. Weina Sun we have developed a recombinant NDV expressing SmCB (NDV-SmCB) (Figure 5.8). This vaccine vector could be used on its own, intramuscularly, intranasally, or in combination with AdSmCB as a heterologous vaccine.

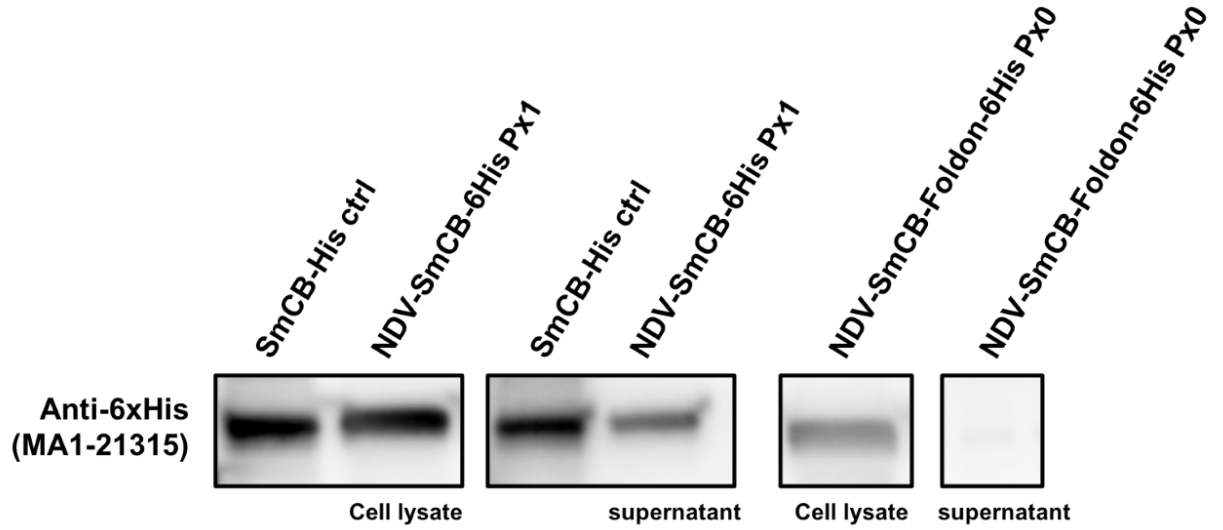


Figure 5.8 NDV-SmCB expresses SmCB *in vitro*

SmCB was cloned into NDV, and western blots directed against the 6X his tag were conducted on infected DF-1s and VERO E6 cells and supernatants. Western blot was conducted in a similar manner to that described in Chapter 2. Recombinant SmCB was used as a control (SmCB-His ctrl). *Contribution of authors: Dilhan Perera provided recombinant SmCB and SmCB containing DNA*

to Dr. Stefan Slamanig (Icahn School of Medicine at Mount Sinai, USA) who created NDV-SmCB clones and generated this figure.

5.2.5.3 *Schistosoma* antigen mRNA vaccines

Finally, the COVID-19 pandemic accelerated mRNA vaccine strategies to the forefront of vaccinology, introducing them to the population at a global scale. These vaccines are safe, easy, and cost-effective to manufacture, and have shown efficacy in SARS-CoV-2 protection in adults and children[650,651]. Similar mRNA vaccines have been developed against other parasitic infections including toxoplasmosis[652,653], malaria[654], and leishmaniasis[655]. In fact, this platform is also capable of expressing multiple antigens and one toxoplasmosis RNA vaccine also confers protection against H1N1 influenza and Ebola virus[652]. A large limitation of mRNA vaccines, however, is the necessity of the cold chain which may not be feasible for tropical regions endemic for schistosomiasis. Fortunately, lyophilization of these vaccines can increase their stability as seen with a rabies vaccine that did not lose potency over several months when stored at oscillating temperatures between 4°C and 56°C[656].

5.3 A short note on one health

To eliminate schistosomiasis, control measures must be multifaceted and look towards the development of an efficacious vaccine. According to the one health philosophy, these measures must consider not only humans, but the role animals play in transmission as well. For instance, *S. japonicum* is zoonotic with bovines playing a large role in human infection. Additionally, the proximity of humans and animals increase the risk of *Schistosoma* hybridization seen in both *S. haematobium* and *S. mansoni*[30,657].

While this thesis focused on the preparation of a vaccine for human use, vaccines can be adapted to preventing *Schistosoma* infection in animal reservoirs. Vaccination of cattle against schistosomiasis resulted in a significant decrease in human *S. japonicum* infection in the Philippines[658]. The best outcome of this study was when vaccination was combined with molluscicide deployment in transmission hotspots. The benefit of animal vaccination is the reduction of schistosomiasis in animal reservoirs that release parasite into the environment, and

consequently, the protection of humans from infection. Moreover, animal vaccine development generally has less rigorous regulatory requirements[659].

5.4 Concluding remarks

For thousands of years, *Schistosoma* spp. have lived alongside humans, causing growth and cognitive deficits in children[660] and significant chronic morbidity and mortality around the world[661]. Current parasite control measures including MDA, snail control, and WASH programs have not been enough to eradicate the disease in all the regions it is endemic. It is estimated that in 2021, over 251.4 million people required PZQ MDA, with less than half of them reported to have been treated[23]. Although effective for individuals, periodic large-scale population treatment alone is not sufficient to completely eradicate schistosomiasis. This is because PZQ does not prevent against reinfection and in regions where transmission is high, infection prevalence returns to baseline after within 18-24 months of treatment[252].

A valuable addition to schistosomiasis control efforts would be the development and deployment of a vaccine. In fact, when 50 experts in the field ranked vaccine development priority based on feasibility and need, schistosomiasis was the highest-ranking parasite on the list, at #7[603]. The work collected in this thesis demonstrate the promise of a S. *mansoni* vaccine targeting SmCB. We have shown significant protection from parasitic infection and pathology in a murine model and described several aspects of resistance which may play significant roles in mediating immunity. Specifically, we demonstrated that significant parasite burden reduction coincides with the induction of both T_H1 and T_H2 arms of immunity.

While there is plenty of research yet to be completed, we believe that this work characterizes several vaccine formulations which can be progressed towards clinical trials. Moreover, we discussed the importance of harnessing multiple arms of immunity to direct parasite killing from many angles. We propose that by combining and exploring novel vaccine strategies, we can enhance vaccine efficacy. In turn, this may pave the way for the development of an effective schistosomiasis vaccine, aiding the eradication of this helminth definitively.

5.5 References

1. Mawa, P. A., Kincaid-Smith, J., Tukahebwa, E. M., Webster, J. P. & Wilson, S. Schistosomiasis Morbidity Hotspots: Roles of the Human Host, the Parasite and Their Interface in the Development of Severe Morbidity. *Front Immunol* **12**, (2021).
2. Schistosomiasis (Bilharzia). *World Health Organization* https://www.who.int/health-topics/schistosomiasis#tab=tab_1 (2023).
3. Schistosomiasis (Bilharzia). *World Health Organization* https://www.who.int/health-topics/schistosomiasis#tab=tab_3 (2023).
4. Molehin, A. J., McManus, D. P. & You, H. Vaccines for Human Schistosomiasis: Recent Progress, New Developments and Future Prospects. *Int J Mol Sci* **23**, (2022).
5. Bilharz, T. *Ein Beitrag Zur Helminthographia Humana: Aus Brieflichen Mittheilungen Des Dr. Bilharz in Cairo, Nebst Bemerkungen Von Prof. C. Th. V. Siebold in Breslau*. (W. Engelmann, 1852).
6. Anastasiou, E., Lorentz, K. O., Stein, G. J. & Mitchell, P. D. Prehistoric schistosomiasis parasite found in the Middle East. *Lancet Infect Dis* **14**, 553–4 (2014).
7. Leake, C. D. *The old Egyptian medical papyri*. (Lawrence, Kan., University of Kansas Press, 1952).
8. Di Bella, S., Riccardi, N., Giacobbe, D. R. & Luzzati, R. History of schistosomiasis (bilharziasis) in humans: from Egyptian medical papyri to molecular biology on mummies. *Pathog Glob Health* **112**, 268–273 (2018).
9. Zakaria, H. Historical Study of Schistosoma haematobium and its Intermediate Host, Bulinus truncatus, in Central Iraq. *J Fac Med Baghdad* **1**, 2–10 (1959).
10. Ruffer, M. A. Note on the presence of 'Bilharzia haematobia' in Egyptian mummies of the twentieth dynasty [1250-1000 B.C.]. *Br Med J* **1**, 16 (1910).
11. Miller, R. L. *et al.* Palaeoepidemiology of Schistosoma infection in mummies. *BMJ* **304**, 555–6 (1992).
12. Matheson, C. D., David, R., Spigelman, M. & Donoghue, H. D. Molecular confirmation of Schistosoma and family relationship in two ancient Egyptian mummies. in (Verlag Dr. Friedrich Pfeil, 2014).
13. Adamson, P. B. Schistosomiasis in antiquity. *Med Hist* **20**, 176–188 (1976).

14. Nelson, G. S., Teesdale, C. & Highton, R. B. The Role of Animals as Reservoirs of Bilharziasis in Africa. 127–156 (2009) doi:10.1002/9780470719312.CH7.
15. Zhou, Y., Chen, Y. & Jiang, Q. History of Human Schistosomiasis (bilharziasis) in China: From Discovery to Elimination. *Acta Parasitol* **66**, 760–769 (2021).
16. Kajihara, N. & Hirayama, K. The War against a Regional Disease in Japan A History of the Eradication of Schistosomiasis japonica. *Trop Med Health* **39**, 3 (2011).
17. Logan, O. T. A Case of Dysentery in Hunan Province, caused by the Trematode, Schistosoma japonicum. *Chin Med J (Engl)* **19**, 243–245 (1905).
18. Ross, A. G. P. *et al.* Road to the elimination of schistosomiasis from Asia: the journey is far from over. *Microbes Infect* **15**, 858–865 (2013).
19. Araújo, A. & Ferreira, L. F. Paleoparasitology of schistosomiasis. *Mem Inst Oswaldo Cruz* **92**, 717 (1997).
20. Wiscovitch-Russo, R. *et al.* Pre-Columbian zoonotic enteric parasites: An insight into Puerto Rican indigenous culture diets and life styles. *PLoS One* **15**, e0227810 (2020).
21. Bouchet, F., Harter, S., Paicheler, J. C., Aráujo, A. & Ferreira, L. F. First recovery of Schistosoma mansoni eggs from a latrine in Europe (15-16th centuries). *J Parasitol* **88**, 404–405 (2002).
22. Brumpt, E. *Précis de parasitologie*. (Masson et Cie, 1936).
23. Schistosomiasis. *World Health Organization* https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (2023).
24. Schistosomiasis - Biology. *Centers for Disease Control and Prevention* https://www.cdc.gov/parasites/schistosomiasis/biology.html (2023).
25. Schistosomiasis - Epidemiology & Risk Factors. *Centers for Disease Control and Prevention* https://www.cdc.gov/parasites/schistosomiasis/epi.html (2023).
26. DPDx - Schistosomiasis Infection. *Centers for Disease Control and Prevention* https://www.cdc.gov/dpdx/schistosomiasis/index.html (2023).
27. Angora, E. K. *et al.* Population genetic structure of Schistosoma haematobium and Schistosoma haematobium × Schistosoma bovis hybrids among school-aged children in Côte d'Ivoire. *Parasite* **29**, (2022).
28. Onyekwere, A. M. *et al.* Population Genetic Structure and Hybridization of Schistosoma haematobium in Nigeria. *Pathogens* **11**, (2022).

29. Moné, H. *et al.* Introgressive hybridizations of Schistosoma haematobium by Schistosoma bovis at the origin of the first case report of schistosomiasis in Corsica (France, Europe). *Parasitol Res* **114**, 4127–4133 (2015).
30. Kincaid-Smith, J. *et al.* Morphological and genomic characterisation of the Schistosoma hybrid infecting humans in Europe reveals admixture between Schistosoma haematobium and Schistosoma bovis. *PLoS Negl Trop Dis* **15**, (2021).
31. Chaine, J. & Malek EA. Urinary schistosomiasis in the Sahelian region of the Senegal River Basin. *Trop Geogr Med* **35**, 249–256 (1983).
32. Picquet, M. *et al.* Royal Society of Tropical Medicine and Hygiene meeting at Manson House, London, 18 May 1995. The epidemiology of human schistosomiasis in the Senegal river basin. *Trans R Soc Trop Med Hyg* **90**, 340–346 (1996).
33. Talla, I. *et al.* Outbreak of intestinal schistosomiasis in the Senegal River Basin. *Ann Soc Belg Med Trop* **70**, 173–180 (1990).
34. Talla, I., Kongs, A. & Verlé, P. Preliminary study of the prevalence of human schistosomiasis in Richard-Toll (the Senegal river basin). *Trans R Soc Trop Med Hyg* **86**, 182 (1992).
35. Warren, K. S., Mahmoud, A. A. F., Cummings, P., Murphy, D. J. & Houser, H. B. Schistosomiasis mansoni in Yemeni in California: duration of infection, presence of disease, therapeutic management. *Am J Trop Med Hyg* **23**, 902–909 (1974).
36. Winsberg, G. R., Moriearty, P., Yogore, M. & Lewert, R. Schistosomiasis in Chicago: a study of the Westown Puerto Rican population. *Am J Epidemiol* **100**, 324–332 (1974).
37. Miller, J. M. *et al.* Malaria, intestinal parasites, and schistosomiasis among Barawan Somali refugees resettling to the United States: a strategy to reduce morbidity and decrease. *Am J Trop Med Hyg* **62**, 958 (2000).
38. Posey, D. L. *et al.* High prevalence and presumptive treatment of schistosomiasis and strongyloidiasis among African refugees. *Clinical Infectious Diseases* **45**, 1310–1315 (2007).
39. Costiniuk, C. T., Cooper, C. L., Doucette, S. & Kovacs, C. M. Parasitic Disease Screening among HIV Patients from Endemic Countries in a Toronto Clinic. *Canadian Journal of Infectious Diseases and Medical Microbiology* **23**, 23–27 (2012).

40. Brant, S. V. & Loker, E. S. Schistosomes in the Southwest United States and their potential for causing cercarial dermatitis or “swimmer’s itch”. *J Helminthol* **83**, 191 (2009).
41. Cook, A. K. Schistosomiasis in the United States. *Vet Clin North Am Small Anim Pract* **52**, 1283–1303 (2022).
42. Horák, P. *et al.* Avian schistosomes and outbreaks of cercarial dermatitis. *Clin Microbiol Rev* **28**, 165–190 (2015).
43. Gordy, M. A., Cobb, T. P. & Hanington, P. C. Swimmer’s itch in Canada: a look at the past and a survey of the present to plan for the future. *Environ Health* **17**, (2018).
44. Lévesque, B., Giovenazzo, P., Guerrier, P., Laverdière, D. & Prud’homme, H. Investigation of an outbreak of cercarial dermatitis. *Epidemiol Infect* **129**, 379–386 (2002).
45. Al-Jubury, A., Bygum, A., Susannatracz, E., Koch, C. N. & Buchmann, K. Cercarial Dermatitis at Public Bathing Sites (Region Zealand, Denmark): A Case Series and Literature Review. *Case Rep Dermatol* **13**, 360–365 (2021).
46. Juhász, A., Majoros, G. & Cech, G. Threat of cercarial dermatitis in Hungary: A first report of Trichobilharzia franki from the mallard (Anas platyrhynchos) and European ear snail (Radix auricularia) using molecular methods. *Int J Parasitol Parasites Wildl* **18**, 92–100 (2022).
47. Davis, N. E., Blair, D. & Brant, S. V. Diversity of Trichobilharzia in New Zealand with a new species and a redescription, and their likely contribution to cercarial dermatitis. *Parasitology* **149**, 380–395 (2022).
48. Leiper, R. T. Observations on the Mode of Spread and Prevention of Vesical and Intestinal Bilharziosis in Egypt, with additions to August, 1916. *Proc R Soc Med* **9(Gen Rep)**, 145-172 (1916).
49. Chabasse, D., Bertrand, G., Leroux, J. P., Gauthey, N. & Hocquet, P. [Developmental bilharziasis caused by Schistosoma mansoni discovered 37 years after infestation]. *Bull Soc Pathol Exot Filiales* **78**, 643–7 (1985).
50. Carson, J. P. *et al.* A comparative proteomics analysis of the egg secretions of three major schistosome species. *Mol Biochem Parasitol* **240**, 111322 (2020).
51. Ishii, A., Tsuji, M., Tada, I. History of Katayama disease: schistosomiasis japonica in Katayama district, Hiroshima, Japan. *Parasitol Int* **52**, 313-319 (2003).

52. Gryseels, B., Polman, K., Clerinx, J. & Kestens, L. Human schistosomiasis. *The Lancet* **368**, 1106–1118 (2006).
53. Ross, A. G. P. *et al.* Schistosomiasis in the People's Republic of China: Prospects and challenges for the 21st century. *Clin Microbiol Rev* **14**, 270–295 (2001).
54. Eloi-Santos, S. M. *et al.* Idiotypic sensitization in utero of children born to mothers with schistosomiasis or Chagas' disease. *J Clin Invest* **84**, (1989).
55. Schwartz, C. & Fallon, P. G. Schistosoma "Eggs-iting" the host: Granuloma formation and egg excretion. *Front Immunol* **9**, 2492 (2018).
56. Cheever, A. A quantitative post-mortem study of Schistosomiasis mansoni in man. *American Journal of Tropical Medicine and Hygiene* **17**, (1968).
57. Chen, M. & Mott, K. Progress in assement of morbidity due to Schistosoma Japonicum Infection. *Trop Dis Bull* (1988).
58. Qin, X. *et al.* The clinical features of chronic intestinal schistosomiasis-related intestinal lesions. *BMC Gastroenterol* **21**, 1–11 (2021).
59. Chen, M. & Mott, K. Progress in assessment of morbidity due to Schistosoma intercalatum infection: a review of recent literature. *Trop Dis Bull* (1989).
60. Lambertucci, J. R. Schistosoma mansoni: pathological and clinical aspects. *Human schistosomiasis.* 195–235 (1993).
61. Gryseels, B. & Polderman, A. M. Morbidity, due to schistosomiasis mansoni, and its control in Subsaharan Africa. *Parasitology Today* **7**, 244–248 (1991).
62. Dessein, A. J. *et al.* Severe Hepatic Fibrosis in Schistosoma mansoni Infection Is Controlled by a Major Locus That Is Closely Linked to the Interferon-γ Receptor Gene. *The American Journal of Human Genetics* **65**, 709–721 (1999).
63. Homeida, M. *et al.* Morbidity Associated with Schistosoma Mansoni Infection as Determined by Ultrasound: A Study in Gezira, Sudan. *Am J Trop Med Hyg* **39**, 196–201 (1988).
64. Chen, M. G. Schistosoma Japonicum and S. japonicum-like infections: epidemiology, clinical and parasitological findings. *Human sehistosomiasi. Willingham, Oxon, CAB International* (1998).

65. Vale, N. *et al.* Comparison of findings using ultrasonography and cystoscopy in urogenital schistosomiasis in a public health centre in rural Angola. *South African Medical Journal* **105**, 312–315 (2015).
66. King, C. H. *et al.* Urinary tract morbidity in schistosomiasis haematobia: associations with age and intensity of infection in an endemic area of Coast Province, Kenya. *Am J Trop Med Hyg* **39**, 361–368 (1988).
67. Von Lichtenberg, F. Schistosomiasis as a worldwide problem: pathology. *J Toxicol Environ Health* **1**, 175–184 (1975).
68. Ursini, T. *et al.* Assessing the prevalence of Female Genital Schistosomiasis and comparing the acceptability and performance of health worker-collected and self-collected cervical-vaginal swabs using PCR testing among women in North-Western Tanzania: The ShWAB study. *PLoS Negl Trop Dis* **17**, e0011465 (2023).
69. Hotez, P. J., Engels, D., Gyapong, M., Ducker, C. & Malecela, M. N. Female Genital Schistosomiasis. *N Engl J Med* **381**, 2493–2495 (2019).
70. Christinet, V., Lazdins-Helds, J. K., Stothard, J. R. & Reinhard-Rupp, J. Female genital schistosomiasis (FGS): from case reports to a call for concerted action against this neglected gynaecological disease. *Int J Parasitol* **46**, 395–404 (2016).
71. Jourdan, P. M., Holmen, S. D., Gundersen, S. G., Roald, B. & Kjetland, E. F. HIV target cells in Schistosoma haematobium-infected female genital mucosa. *Am J Trop Med Hyg* **85**, 1060 (2011).
72. Crump, J. A., Murdoch, D. R., Chambers, S. T., Aickin, D. R. & Hunter, L. A. Female genital schistosomiasis. *J Travel Med* **7**, 30 (2000).
73. Kjetland, E. F. *et al.* Association between genital schistosomiasis and HIV in rural Zimbabwean women. *AIDS* **20**, 593–600 (2006).
74. Kayuni, S. *et al.* A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa. *Parasite Epidemiol Control* **4**, e00077 (2019).
75. Leutscher, P. D. C. *et al.* Coexistence of urogenital schistosomiasis and sexually transmitted infection in women and men living in an area where Schistosoma haematobium is endemic. *Clinical Infectious Diseases* **47**, 775–782 (2008).

76. Vilana, R., Corachan, M., Gascon, J., Valls, E. & Bru, C. Schistosomiasis of the male genital tract: transrectal sonographic findings. *J Urol* **158**, 1491–1493 (1997).
77. Leandro, D. M. *et al.* Clinical and histopathological profile of female genital schistosomiasis. *Research, Society and Development* **10**, e47410716652–e47410716652 (2021).
78. Brindley, P. J., Costa, J. M. C. Da & Sripa, B. Why Does Infection with Some Helminths Cause Cancer? *Trends Cancer* **1**, 174–182 (2015).
79. Mostafa, M. H., Sheweita, S. A. & O'Connor, P. J. Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev* **12**, 97–111 (1999).
80. Botelho, M. C., Alves, H. & Richter, J. Halting Schistosoma haematobium - Associated Bladder Cancer. *International Journal of Cancer Management* **10**, 9430 (2017).
81. Honeycutt, J., Hammam, O., Fu, C. L. & Hsieh, M. H. Controversies and challenges in research on urogenital schistosomiasis-associated bladder cancer. *Trends Parasitol* **30**, 324–332 (2014).
82. Pittella, J. E. H. Neuroschistosomiasis. *Brain Pathology* **7**, 649–662 (1997).
83. Carod-Artal, F. J. Neuroschistosomiasis. *Expert Rev Anti Infect Ther* **8**, 1307–1318 (2010).
84. Ross, A. G. *et al.* Neuroschistosomiasis. *J Neurol* **259**, 22–32 (2012).
85. Jauréguiberry, S. *et al.* Acute neuroschistosomiasis: two cases associated with cerebral vasculitis. *Am J Trop Med Hyg* **76**, 964–6 (2007).
86. Jauréguiberry, S. & Caumes, E. Neurological involvement during Katayama syndrome. *Lancet Infect Dis* **8**, 9–10 (2008).
87. Sibomana, J. P. *et al.* Schistosomiasis Pulmonary Arterial Hypertension. *Front Immunol* **11**, 608883 (2020).
88. Vella, A. T., Hulsebosch, M. D. & Pearce, E. J. Schistosoma mansoni eggs induce antigen-responsive CD44-hi T helper 2 cells and IL-4-secreting CD44-lo cells. Potential for T helper 2 subset differentiation is evident at the precursor level. *J Immunol* **149**, 1714–22 (1992).
89. Chiaramonte, M. G., Donaldson, D. D., Cheever, A. W. & Wynn, T. A. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2–dominated inflammatory response. *J Clin Invest* **104**, 777–785 (1999).

90. Tang, J. *et al.* Involvement of IL-13 and tissue transglutaminase in liver granuloma and fibrosis after Schistosoma japonicum infection. *Mediators Inflamm* **2014**, (2014).
91. Wilson, M. S. *et al.* Immunopathology of schistosomiasis. *Immunol Cell Biol* **85**, 148–154 (2007).
92. Chiaramonte, M. G. *et al.* Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. *J Exp Med* **197**, 687–701 (2003).
93. Barreto, A. V. M. S. *et al.* Evaluation of serum levels of IL-9 and IL-17 in human Schistosoma mansoni infection and their relationship with periportal fibrosis. *Immunobiology* **221**, 1351–1354 (2016).
94. Chen, D. *et al.* Characteristics of IL-17 induction by Schistosoma japonicum infection in C57BL/6 mouse liver. *Immunology* **139**, 523 (2013).
95. Nady, S., Shata, M. T. M., Mohey, M. A. & El-Shorbagy, A. Protective role of IL-22 against Schistosoma mansoni soluble egg antigen-induced granuloma in Vitro. *Parasite Immunol* **39**, (2017).
96. Sertorio, M. *et al.* IL-22 and IL-22 binding protein (IL-22BP) regulate fibrosis and cirrhosis in hepatitis C virus and schistosome infections. *Hepatology* **61**, 1321–1331 (2015).
97. Dunne, D. W., Jones, F. M. & Doenhoff, M. J. The purification, characterization, serological activity and hepatotoxic properties of two cationic glycoproteins (alpha 1 and omega 1) from Schistosoma mansoni eggs. *Parasitology* **103 Pt 2**, 225–236 (1991).
98. Lu, L. *et al.* Loss of natural resistance to schistosome in T cell deficient rat. *PLoS Negl Trop Dis* **14**, 1–21 (2020).
99. Jankovic, D. *et al.* Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J Immunol* **163**, 337–42 (1999).
100. Fallon, P. G., Richardson, E. J., McKenzie, G. J. & McKenzie, A. N. J. Schistosome Infection of Transgenic Mice Defines Distinct and Contrasting Pathogenic Roles for IL-4 and IL-13: IL-13 Is a Profibrotic Agent. *The Journal of Immunology* **164**, 2585–2591 (2000).

101. Hesse, M. *et al.* Differential Regulation of Nitric Oxide Synthase-2 and Arginase-1 by Type 1/Type 2 Cytokines In Vivo: Granulomatous Pathology Is Shaped by the Pattern of l-Arginine Metabolism. *The Journal of Immunology* **167**, 6533–6544 (2001).
102. Herbert, D. R. *et al.* Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* **20**, 623–635 (2004).
103. Schwartz, C., Oeser, K., Prazeres da Costa, C., Layland, L. E. & Voehringer, D. T cell-derived IL-4/IL-13 protects mice against fatal Schistosoma mansoni infection independently of basophils. *J Immunol* **193**, 3590–3599 (2014).
104. Hoffmann, K. F., Cheever, A. W. & Wynn, T. A. IL-10 and the Dangers of Immune Polarization: Excessive Type 1 and Type 2 Cytokine Responses Induce Distinct Forms of Lethal Immunopathology in Murine Schistosomiasis. *The Journal of Immunology* **164**, 6406–6416 (2000).
105. Baumgart, M., Tompkins, F., Leng, J. & Hesse, M. Naturally occurring CD4+Foxp3+ regulatory T cells are an essential, IL-10-independent part of the immunoregulatory network in Schistosoma mansoni egg-induced inflammation. *J Immunol* **176**, 5374–5387 (2006).
106. Singh, K. P., Gerard, H. C., Hudson, A. P., Reddy, T. R. & Boros, D. L. Retroviral Foxp3 gene transfer ameliorates liver granuloma pathology in Schistosoma mansoni infected mice. *Immunology* **114**, 410–417 (2005).
107. Hesse, M. *et al.* The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *J Immunol* **172**, 3157–3166 (2004).
108. McKee, A. S. & Pearce, E. J. CD25+CD4+ cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *J Immunol* **173**, 1224–1231 (2004).
109. Abruzzi, A. & Fried, B. Coinfection of Schistosoma (Trematoda) with Bacteria, Protozoa and Helminths. *Adv Parasitol* **77**, 1–85 (2011).
110. Perera, D. J. & Ndao, M. Promising Technologies in the Field of Helminth Vaccines. *Front Immunol* **12**, 711650 (2021).
111. Meurs, L. *et al.* Epidemiology of mixed Schistosoma mansoni and Schistosoma haematobium infections in northern Senegal. *Int J Parasitol* **42**, 305–311 (2012).

112. Cunin, P., Tchuem Tchuenté, L. A., Poste, B., Djibrilla, K. & Martin, P. M. V. Interactions between Schistosoma haematobium and Schistosoma mansoni in humans in north Cameroon. *Tropical Medicine & International Health* **8**, 1110–1117 (2003).
113. Cunin, P., Griffet, A., Poste, B., Djibrilla, K. & Martin, P. M. V. Epidemic Schistosoma mansoni in a known S. haematobium area. *Trans R Soc Trop Med Hyg* **94**, 657–660 (2000).
114. Ugbomoiko, U. S., Dalumo, V., Danladi, Y. K., Heukelbach, J. & Ofoezie, I. E. Concurrent urinary and intestinal schistosomiasis and intestinal helminthic infections in schoolchildren in Ilobu, South-western Nigeria. *Acta Trop* **123**, 16–21 (2012).
115. Anyan, W. K. *et al.* Concurrent Schistosoma mansoni and Schistosoma haematobium infections in a peri-urban community along the Weija dam in Ghana: A wake up call for effective National Control Programme. *Acta Trop* **199**, (2019).
116. Gouvras, A. N. *et al.* The impact of single versus mixed Schistosoma haematobium and S. mansoni infections on morbidity profiles amongst school-children in Taveta, Kenya. *Acta Trop* **128**, 309–317 (2013).
117. Knowles, S. C. L. *et al.* Epidemiological Interactions between Urogenital and Intestinal Human Schistosomiasis in the Context of Praziquantel Treatment across Three West African Countries. *PLoS Negl Trop Dis* **9**, (2015).
118. Koukounari, A. *et al.* The impact of single versus mixed schistosome species infections on liver, spleen and bladder morbidity within Malian children pre- and post-praziquantel treatment. *BMC Infect Dis* **10**, (2010).
119. Meurs, L. *et al.* Cytokine Responses to Schistosoma mansoni and Schistosoma haematobium in Relation to Infection in a Co-endemic Focus in Northern Senegal. *PLoS Negl Trop Dis* **8**, e3080 (2014).
120. Meurs, L. *et al.* Bladder Morbidity and Hepatic Fibrosis in Mixed Schistosoma haematobium and S. mansoni Infections: A Population-Wide Study in Northern Senegal. *PLoS Negl Trop Dis* **6**, e1829 (2012).
121. Fall, C. B. *et al.* Hybridized zoonotic Schistosoma infections result in hybridized morbidity profiles: A clinical morbidity study amongst co-infected human populations of Senegal. *Microorganisms* **9**, 1776 (2021).

122. Garba, A. *et al.* Efficacy and safety of two closely spaced doses of praziquantel against Schistosoma haematobium and S. mansoni and re-infection patterns in school-aged children in Niger. *Acta Trop* **128**, 334–344 (2013).
123. Panzner, U. & Boissier, J. Natural Intra- and Interclade Human Hybrid Schistosomes in Africa with Considerations on Prevention through Vaccination. *Microorganisms* **9**, 1465 (2021).
124. Leger, E. & Webster, J. P. Hybridizations within the Genus Schistosoma: implications for evolution, epidemiology and control. *Parasitology* **144**, 65–80 (2017).
125. Soil-transmitted helminth infections. *World Health Organization* https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections (2023).
126. Yajima, A., Gabrielli, A. F., Montresor, A. & Engels, D. Moderate and high endemicity of schistosomiasis is a predictor of the endemicity of soil-transmitted helminthiasis - Systematic review. *Trans R Soc Trop Med Hyg* **105**, 68 (2011).
127. Geiger, S. M. Immuno-epidemiology of Schistosoma mansoni infections in endemic populations co-infected with soil-transmitted helminths: Present knowledge, challenges, and the need for further studies. *Acta Trop* **108**, 118–123 (2008).
128. Bazzone, L. E. *et al.* Coinfection with the intestinal nematode Heligmosomoides polygyrus markedly reduces hepatic egg-induced immunopathology and proinflammatory cytokines in mouse models of severe schistosomiasis. *Infect Immun* **76**, 5164–5172 (2008).
129. Bickle, Q. D., Solum, J. & Helmby, H. Chronic intestinal nematode infection exacerbates experimental Schistosoma mansoni infection. *Infect Immun* **76**, 5802–5809 (2008).
130. Le, L. *et al.* Chronic whipworm infection exacerbates Schistosoma mansoni egg-induced hepatopathology in non-human primates. *Parasit Vectors* **13**, (2020).
131. McSorley, H. J. & Maizels, R. M. Helminth infections and host immune regulation. *Clin Microbiol Rev* **25**, 585–608 (2012).
132. De Moira, A. P. *et al.* Effects of treatment on IgE responses against parasite allergen-like proteins and immunit to reinfection in childhood schistosome and hookworm coinfections. *Infect Immun* **81**, 23–32 (2013).

133. De Moira, A. P. *et al.* Suppression of basophil histamine release and other IgE-dependent responses in childhood Schistosoma mansoni/hookworm coinfection. *Journal of Infectious Diseases* **210**, 1198–1206 (2014).
134. Sumbele, I. U. N. *et al.* Polyparasitism with Schistosoma haematobium, Plasmodium and soil-transmitted helminths in school-aged children in Muyuka-Cameroon following implementation of control measures: a cross sectional study. *Infect Dis Poverty* **10**, (2021).
135. McDowell, D., Hurt, L., Kabatereine, N. B., Stothard, J. R. & Lello, J. Infection History and Current Coinfection With Schistosoma mansoni Decreases Plasmodium Species Intensities in Preschool Children in Uganda. *J Infect Dis* **225**, 2181–2186 (2022).
136. Hailu, T., Yimer, M., Mulu, W. & Abera, B. Synergetic Effects of Plasmodium, Hookworm, and Schistosoma mansoni Infections on Hemoglobin Level among Febrile School Age Children in Jawe Worda, Northwest Ethiopia. *J Parasitol Res* **2018**, (2018).
137. Orish, V. N. *et al.* Prevalence of Polyparasitic Infection Among Primary School Children in the Volta Region of Ghana. *Open Forum Infect Dis* **6**, (2019).
138. Anchang-Kimbi, J. K., Elad, D. M., Sotoing, G. T. & Achidi, E. A. Coinfection with Schistosoma haematobium and Plasmodium falciparum and Anaemia Severity among Pregnant Women in Munyenge, Mount Cameroon Area: A Cross-Sectional Study. *J Parasitol Res* **2017**, (2017).
139. Tuasha, N., Hailemeskel, E., Erko, B. & Petros, B. Comorbidity of intestinal helminthiases among malaria outpatients of Wondo Genet health centers, southern Ethiopia: implications for integrated control. *BMC Infect Dis* **19**, 659 (2019).
140. Nyarko, R., Torpey, K. & Ankomah, A. Schistosoma haematobium, Plasmodium falciparum infection and anaemia in children in Accra, Ghana. *Trop Dis Travel Med Vaccines* **4**, 1–6 (2018).
141. Morenikeji, O. A., Eleng, I. E., Atanda, O. S. & Oyeyemi, O. T. Renal related disorders in concomitant Schistosoma haematobium-Plasmodium falciparum infection among children in a rural community of Nigeria. *J Infect Public Health* **9**, 136–142 (2016).
142. Phillips, M. A. *et al.* Malaria. *Nature Reviews Disease Primers* **3**, 1–24 (2017).
143. Doherty, J. F., Grant, A. D. & Bryceson, A. D. Fever as the presenting complaint of travellers returning from the tropics. *QJM* **88**, 277–81 (1995).

144. Nji, C. P., Nguedia Assob, J. C. & Kihla Akoachere, J. F. T. Predictors of Urinary Tract Infections in Children and Antibiotic Susceptibility Pattern in the Buea Health District, South West Region, Cameroon. *Biomed Res Int* **2020**, (2020).
145. Yirenya-Tawiah, D. R. *et al.* Female Genital Schistosomiasis, Genital Tract Infections and HIV Co-infection in the Volta Basin of Ghana. *Int J Trop Dis Health* **3**, 94–103 (2014).
146. Eyong, M. E., Ikepeme, E. E. & Ekanem, E. E. Relationship between Schistosoma haematobium infection and urinary tract infection among children in South Eastern, Nigeria. *Niger Postgrad Med J* **15**, 89–93 (2008).
147. Adeyeba, O. & Ojeaga, S. Urinary schistosomiasis and concomitant urinary tract pathogens among school children in metropoitan Ibadan, Nigeria. *African Journal of Biomedical Research* **5**, 103–107 (2010).
148. Nmorsi, O. P. G., Kwandu, U. N. C. D. & Ebiaguanye, L. M. Schistosoma haematobium and urinary tract pathogens co-infections in a rural community of Edo State, Nigeria. *J Commun Dis* **39**, 85–90 (2007).
149. J.Amoo, K., Amoo, O., Oke, A. & Adegboyega, T. Prevalence of Urinary Tract Infection (UTI) and Concomitant Urinary Schistosomiasis among Primary School Children in Remo North Local Government, Ogun State, Nigeria. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)* **16**, 68–73 (2017).
150. Ayoade, F., Moro, D. D. & Ebene, O. L. Prevalence and Antimicrobial Susceptibility Pattern of Asymptomatic Urinary Tract Infections of Bacterial and Parasitic Origins among University Students in Redemption Camp, Ogun State, Nigeria. *Open J Med Microbiol* **03**, 219–226 (2013).
151. Kone, K. J., Onifade, A. K. & Dada, E. O. Risk factors affecting the occurrence of urinary schistosomiasis and urinary tract infections in some communities of Ondo State, Nigeria. *J Water Health* **21**, 27–34 (2023).
152. Soyannwo, M. A. *et al.* Studies on the prevalence of renal disease and hypertension in relation to schistosomiasis. III. Proteinuria, haematuria, pyuria and bacteriuria in the rural community of Nigeria. *Niger Med J* **8**, 451–64 (1978).
153. Sobh, M. A. *et al.* Impact of schistosomiasis on patient and graft outcome after kidney transplantation. *Nephrol Dial Transplant* **7**, 858–64 (1992).

154. Ossai, O. P. *et al.* Bacteriuria and urinary schistosomiasis in primary school children in rural communities in Enugu State, Nigeria, 2012. *Pan Afr Med J* **18**, 15 (2014).
155. Chidinma U. U. *et al*. Epidemiological studies on urinary schistosomiasis and bacterial co-infection in some rural communities of Abia State, Nigeria. *World Journal of Biology Pharmacy and Health Sciences* **10**, 065–072 (2022).
156. Seynabou, L. *et al.* Profile of bacterial and parasitic urinary infections in Saint Louis Senegal between 2000 and 2010. *Afr J Microbiol Res* **10**, 1061–1065 (2016).
157. Anosike, J. C. *et al.* Endemicity of urinary schistosomiasis in north central zone of Abia state, Nigeria. *Int. J. Environ. Hlth. Human. Develop.* **1**, 6–15 (2001).
158. Nwachukwu, I. O. *et al.* Urinary Schistosomiasis and Concomitant Bacteriuria among School Age Children in Some Parts of Owerri, Imo State. *International Journal of Research In Advance Engineering* **3**, 107–115 (2018).
159. Mahmoud, K. M. *et al.* Impact of schistosomiasis on patient and graft outcome after renal transplantation: 10 years' follow-up. *Nephrol Dial Transplant* **16**, 2214–2221 (2001).
160. Wishahi, M. M., El-Sherbeni, E. & El-Baz, H. Granulomatous cystitis: a reversible lesion in children. *Eur Urol* **10**, 368–369 (1984).
161. el Hawey, A., Massoud, A., Badr el Din, N., Waheeb, A. & Abd-el Hamid, S. Bacterial flora in hepatic encephalopathy in bilharzial and non-bilharzial patients. *J Egypt Soc Parasitol* **19**, 797–804 (1989).
162. Dada, E. O. & Alagha, B. A. Urinary Schistosomiasis and Asymptomatic Bacteriuria among Individuals of Ipogun, Nigeria: Detection of Predominant Microorganisms and Antibiotic Susceptibility Profile. *Journal of Medical and Health Studies* **2**, 70–80 (2021).
163. Nimorsi, O. P., Egwunyenga, A. O. & Bajomo, D. O. Survey of urinary schistosomiasis and trichomoniasis in a rural community in Edo State, Nigeria. *J Commun Dis* **33**, 96–101 (2001).
164. Gadoth, A. *et al.* Urogenital Schistosomiasis and Sexually Transmitted Coinfections among Pregnant Women in a Schistosome-Endemic Region of the Democratic Republic of Congo. *Am J Trop Med Hyg* **101**, 828 (2019).
165. Hennequin, C., Bourée, P. & Halfon, P. [Salmonella paratyphi A urinary infection in Schistosoma mansoni bilharziasis]. *Rev Med Interne* **12**, 141–2 (1991).

166. Uwaezuoke, J., Anosike, J., Udujih, O. & Onyeka, P. Epidemiological and Bacteriological Studies On Vesical Schistosomiasis in Ikwo Area, Ebonyi State, Nigeria. *Journal of Applied Sciences and Environmental Management* **12**, 75–80 (2010).
167. Uneke, C. J., Ugwuoke-Adibuah, S., Nwakpu, K. O. & Ngwu, B. A. An Assessment of Schistosoma haematobium infection and urinary tract bacterial infection amongst school children in rural eastern Nigeria. *The Internet Journal of Laboratory Medicine* **4**, (2009).
168. Hsieh, Y.-J., Fu, C.-L. & Hsieh, M. 1150 Interleukin-4 signaling promotes bacteriuria during urinary tract co-infection. *J Urol* **189**, e470 (2013).
169. Hsieh, Y. J., Fu, C. L. & Hsieh, M. H. Helminth-induced interleukin-4 abrogates invariant natural killer T cell activation-associated clearance of bacterial infection. *Infect Immun* **82**, 2087–2097 (2014).
170. Mbanefo, E. C. *et al.* IPSE, a urogenital parasite-derived immunomodulatory molecule, suppresses bladder pathogenesis and anti-microbial peptide gene expression in bacterial urinary tract infection. *Parasit Vectors* **13**, 1–11 (2020).
171. About HIV/AIDS. *Centers for Disease Control and Prevention* https://www.cdc.gov/hiv/basics/whatishiv.html (2023).
172. Furch, B. D., Koethe, J. R., Kayamba, V., Heimburger, D. C. & Kelly, P. Interactions of Schistosoma and HIV in Sub-Saharan Africa: A Systematic Review. *Am J Trop Med Hyg* **102**, 711–718 (2020).
173. Downs, J. A. *et al.* Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. *PLoS Negl Trop Dis* **11**, e0005968 (2017).
174. Secor, W. E. The effects of schistosomiasis on HIV/AIDS infection, progression and transmission. *Curr Opin HIV AIDS* **7**, 254–259 (2012).
175. Midzi, N., Mduluza, T., Mudenge, B., Foldager, L. & Leutscher, P. D. C. Decrease in Seminal HIV-1 RNA Load After Praziquantel Treatment of Urogenital Schistosomiasis Coinfection in HIV-Positive Men—An Observational Study. *Open Forum Infect Dis* **4**, (2017).
176. Yegorov, S. *et al.* Schistosoma mansoni treatment reduces HIV entry into cervical CD4+ T cells and induces IFN-I pathways. *Nat Commun* **10**, (2019).

177. Patel, P. *et al.* Association of schistosomiasis and HIV infections: A systematic review and meta-analysis. *International Journal of Infectious Diseases* **102**, 544–553 (2021).
178. Kayuni, S. A. *et al.* Prospective pilot study on the relationship between seminal HIV-1 shedding and genital schistosomiasis in men receiving antiretroviral therapy along Lake Malawi. *Scientific Reports* **13**, 1–9 (2023).
179. Plesniarski, A., Siddik, A. B. & Su, R. C. The Microbiome as a Key Regulator of Female Genital Tract Barrier Function. *Front Cell Infect Microbiol* **11**, 790627 (2021).
180. Brenchley, J. M. & Douek, D. C. HIV infection and the gastrointestinal immune system. *Mucosal Immunol* **1**, 23–30 (2008).
181. Yang, Y. *et al.* Immune Dysfunction and Coinfection with Human Immunodeficiency Virus and Schistosoma japonicum in Yi People. *J Immunol Res* **2018**, (2018).
182. Mazigo, H. D. *et al.* Periportal fibrosis, liver and spleen sizes among S. mansoni mono or co-infected individuals with human immunodeficiency virus-1 in fishing villages along Lake Victoria shores, North-Western, Tanzania. *Parasit Vectors* **8**, 1–13 (2015).
183. Ocama, P. *et al.* The burden, pattern and factors that contribute to periportal fibrosis in HIV-infected patients in an S. mansoni endemic rural Uganda. *Afr Health Sci* **17**, 301–307 (2017).
184. Marti, A. I. *et al.* Increased hepatotoxicity among HIV-infected adults co-infected with Schistosoma mansoni in Tanzania: A cross-sectional study. *PLoS Negl Trop Dis* **11**, (2017).
185. Colombe, S. *et al.* Decreased Sensitivity of Schistosoma sp. Egg Microscopy in Women and HIV-Infected Individuals. *Am J Trop Med Hyg* **98**, 1159–1164 (2018).
186. Sanya, R. E. *et al.* Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. *Trop Med Int Health* **20**, 1201–1208 (2015).
187. Omar, H. H. Impact of chronic schistosomiasis and HBV/HCV co-infection on the liver: current perspectives. *Hepat Med* **11**, 131–136 (2019).
188. Mohd Hanafiah, K., Groeger, J., Flaxman, A. D. & Wiersma, S. T. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. *Hepatology* **57**, 1333–1342 (2013).
189. Omar, H. H., Taha, S. A., Hassan, W. H. & Omar, H. H. Impact of schistosomiasis on increase incidence of occult hepatitis B in chronic hepatitis C patients in Egypt. *J Infect Public Health* **10**, 761–765 (2017).

190. Khatami, A. *et al.* Two rivals or colleagues in the liver? Hepatit B virus and Schistosoma mansoni co-infections: A systematic review and meta-analysis. *Microb Pathog* **154**, (2021).
191. Omar, H. H. Impact of chronic schistosomiasis and HBV/HCV co-infection on the liver: current perspectives. *Hepat Med* **11**, 131–136 (2019).
192. Zhang, Y. *et al.* Prevalence and co-infection of schistosomiasis/hepatitis B among rural populations in endemic areas in Hubei, China. *Trans R Soc Trop Med Hyg* **114**, 155–161 (2020).
193. Abruzzi, A., Friedx, B. & Alikhan, S. B. Coinfection of Schistosoma Species with Hepatitis B or Hepatitis C Viruses. *Adv Parasitol* **91**, 111–231 (2016).
194. Pottie, K. *et al.* Evidence-based clinical guidelines for immigrants and refugees. *CMAJ* **183**, (2011).
195. Mutapi, F. Changing policy and practice in the control of pediatric schistosomiasis. *Pediatrics* **135**, 536–544 (2015).
196. Wang, L., Utzinger, J. & Zhou, X. N. Schistosomiasis control: experiences and lessons from China. *Lancet* **372**, 1793–1795 (2008).
197. Glinz, D. *et al.* Comparing diagnostic accuracy of Kato-Katz, Koga Agar Plate, Ether-Concentration, and FLOTAC for Schistosoma mansoni and Soil-transmitted helminths. *PLoS Negl Trop Dis* **4**, (2010).
198. Barda, B. D. *et al.* Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. *PLoS Negl Trop Dis* **7**, (2013).
199. World Health Organization. Schistosomiasis: progress report 2001-2011, strategic plan 2012-2020. (2013).
200. Knopp, S., Becker, S. L., Ingram, K. J., Keiser, J. & Utzinger, J. Diagnosis and treatment of schistosomiasis in children in the era of intensified control. *Expert Rev Anti Infect Ther* **11**, 1237–1258 (2013).
201. Zhang, Y. Y. *et al.* Evaluation of Kato-Katz examination method in three areas with low-level endemicity of schistosomiasis japonica in China: A Bayesian modeling approach. *Acta Trop* **112**, 16–22 (2009).
202. Koukounari, A. *et al.* A Latent Markov Modelling Approach to the Evaluation of Circulating Cathodic Antigen Strips for Schistosomiasis Diagnosis Pre- and Post-Praziquantel Treatment in Uganda. *PLoS Comput Biol* **9**, (2013).

203. Haggag, A. A. *et al.* Thirty-Day Daily Comparisons of Kato-Katz and CCA Assays of 45 Egyptian Children in Areas with Very Low Prevalence of Schistosoma mansoni. *Am J Trop Med Hyg* **100**, 578–583 (2019).
204. Barda, B. *et al.* Mini-FLOTAC and Kato-Katz: Helminth eggs watching on the shore of lake Victoria. *Parasit Vectors* **6**, (2013).
205. Dazo, B. C. & Biles, J. E. Two new field techniques for detection and counting of Schistosoma haematobium eggs in urine samples, with an evaluation of both methods. *Bull World Health Organ* **51**, 399–408 (1974).
206. Bogoch, I. I., Andrews, J. R., Dadzie Ephraim, R. K. & Utzinger, J. Simple questionnaire and urine reagent strips compared to microscopy for the diagnosis of Schistosoma haematobium in a community in northern Ghana. *Trop Med Int Health* **17**, 1217–1221 (2012).
207. Emukah, E. *et al.* Urine heme dipsticks are useful in monitoring the impact of praziquantel treatment on Schistosoma haematobium in sentinel communities of Delta State, Nigeria. *Acta Trop* **122**, 126–131 (2012).
208. Stete, K. *et al.* Dynamics of Schistosoma haematobium egg output and associated infection parameters following treatment with praziquantel in school-aged children. *Parasit Vectors* **5**, (2012).
209. Sarhan, R. M., Aminou, H. A. K., Saad, G. A. R. & Ahmed, O. A. Comparative analysis of the diagnostic performance of adult, cercarial and egg antigens assessed by ELISA, in the diagnosis of chronic human Schistosoma mansoni infection. *Parasitol Res* **113**, 3467–3476 (2014).
210. Xu, X. *et al.* Serodiagnosis of Schistosoma japonicum infection: Genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. *Lancet Infect Dis* **14**, 489–497 (2014).
211. Duus, L. M. *et al.* The Schistosoma-specific antibody response after treatment in non-immune travellers. *Scand J Infect Dis* **41**, 285–290 (2009).
212. Stothard, J. R. *et al.* Schistosoma mansoni Infections in Young Children: When Are Schistosome Antigens in Urine, Eggs in Stool and Antibodies to Eggs First Detectable? *PLoS Negl Trop Dis* **5**, e938 (2011).

213. Bierman, W. F. W., Wetsteyn, J. C. F. M. & Van Gool, T. Presentation and Diagnosis of Imported Schistosomiasis: Relevance of Eosinophilia, Microscopy for Ova, and Serology. *J Travel Med* **12**, 9–13 (2005).
214. Ahmed, M. M., Hussein, H. M. & el-Hady, H. M. Evaluation of cercarien hullen reaction (CHR) as a diagnostic test in chronic schistosomiasis and as a parameter for reinfection in acute cases. *J Egypt Soc Parasitol* **23**, 365–71 (1993).
215. Alarcón de Noya, B. *et al.* Detection of schistosomiasis cases in low-transmission areas based on coprologic and serologic criteria The Venezuelan experience. *Acta Trop* **103**, 41–49 (2007).
216. Van Gool, T. *et al.* Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with Schistosoma mansoni adult worm antigens and an enzyme-linked immunosorbent assay with S. mansoni egg antigens. *J Clin Microbiol* **40**, 3432–3437 (2002).
217. Oyeyemi, O. T., Corsini, C. A., Gonçalves, G., de Castro Borges, W. & Grenfell, R. F. Q. Evaluation of schistosomula crude antigen (SCA) as a diagnostic tool for Schistosoma mansoni in low endemic human population. *Scientific Reports* **11**, 1–8 (2021).
218. Deelder, A. M. *et al.* Quantitative diagnosis of Schistosoma infections by measurement of circulating antigens in serum and urine. *Trop Geogr Med* **46**, 233–8 (1994).
219. Hamilton, J. V., Doenhoff, M. J. & Klinkert, M. Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. *Parasitology* **117 Suppl**, S41 (1998).
220. Corstjens, P. L. A. M. *et al.* Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* **141**, 1841–1855 (2014).
221. van Grootveld, R. *et al.* Improved diagnosis of active Schistosoma infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. *European Journal of Clinical Microbiology and Infectious Diseases* **37**, 1709–1716 (2018).
222. van Dam, G. J. *et al.* An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of Schistosoma japonicum in a low-endemic area, People's Republic of China. *Acta Trop* **141**, 190–197 (2015).

223. Van Dam, G. J. *et al.* Evaluation of banked urine samples for the detection of circulating anodic and cathodic antigens in Schistosoma mekongi and S. japonicum infections: A proof-of-concept study. *Acta Trop* **141**, 198–203 (2015).
224. Shane, H. L. *et al.* Evaluation of Urine CCA Assays for Detection of Schistosoma mansoni Infection in Western Kenya. *PLoS Negl Trop Dis* **5**, e951 (2011).
225. Colley, D. G. *et al.* A Five-Country Evaluation of a Point-of-Care Circulating Cathodic Antigen Urine Assay for the Prevalence of Schistosoma mansoni. *Am J Trop Med Hyg* **88**, 426 (2013).
226. Assare, R. K. *et al.* Accuracy of Two Circulating Antigen Tests for the Diagnosis and Surveillance of Schistosoma mansoni Infection in Low-Endemicity Settings of Côte d'Ivoire. *Am J Trop Med Hyg* **105**, 677–683 (2021).
227. Lodh, N., Naples, J. M., Bosompem, K. M., Quartey, J. & Shiff, C. J. Detection of Parasite-Specific DNA in Urine Sediment Obtained by Filtration Differentiates between Single and Mixed Infections of Schistosoma mansoni and S. haematobium from Endemic Areas in Ghana. *PLoS One* **9**, e91144 (2014).
228. Kjetland, E. F. *et al.* Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women. *American Journal of Tropical Medicine and Hygiene* **81**, 1050 (2009).
229. Huyse, T. *et al.* Bidirectional Introgressive Hybridization between a Cattle and Human Schistosome Species. *PLoS Pathogens* **5**, e1000571 (2009).
230. Kato-Hayashi, N. *et al.* Use of cell-free circulating schistosome DNA in serum, urine, semen, and saliva to monitor a case of refractory imported schistosomiasis hematobia. *J Clin Microbiol* **51**, 3435–3438 (2013).
231. Wichmann, D. *et al.* Diagnosing Schistosomiasis by Detection of Cell-Free Parasite DNA in Human Plasma. *PLoS Negl Trop Dis* **3**, e422 (2009).
232. Cnops, L., Soentjens, P., Clerinx, J. & van Esbroeck, M. A Schistosoma haematobium-Specific Real-Time PCR for Diagnosis of Urogenital Schistosomiasis in Serum Samples of International Travelers and Migrants. *PLoS Negl Trop Dis* **7**, e2413 (2013).
233. van Lieshout, L. & Roestenberg, M. Clinical consequences of new diagnostic tools for intestinal parasites. *Clinical Microbiology and Infection* **21**, 520–528 (2015).

234. Allam, A. F. *et al.* Performance of loop-mediated isothermal amplification (LAMP) for detection of Schistosoma mansoni infection compared with Kato-Katz and real-time PCR. *J Helminthol* **96**, (2022).
235. Gomes, E. C. de S., Barbosa Júnior, W. L. & Melo, F. L. de. Evaluation of SmITS1-LAMP performance to diagnosis schistosomiasis in human stool samples from an endemic area in Brazil. *Exp Parasitol* **242**, (2022).
236. Fernández-Soto, P. *et al.* Detection of Schistosoma mansoni-derived DNA in human urine samples by loop-mediated isothermal amplification (LAMP). *PLoS One* **14**, (2019).
237. Xu, J. *et al.* DNA detection of Schistosoma japonicum: diagnostic validity of a LAMP assay for low-intensity infection and effects of chemotherapy in humans. *PLoS Negl Trop Dis* **9**, (2015).
238. Doenhoff, M. J., Cioli, D. & Utzinger, J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr Opin Infect Dis* **21**, 659–667 (2008).
239. Zwang, J. & Olliaro, P. L. Clinical efficacy and tolerability of praziquantel for intestinal and urinary schistosomiasis-a meta-analysis of comparative and non-comparative clinical trials. *PLoS Negl Trop Dis* **8**, (2014).
240. Simarro, P. P., Sima, F. O., Mir, M. & Ndong, P. Effect of repeated targeted mass treatment with praziquantel on the prevalence, intensity of infection and morbidity due to Schistosoma intercalatum in an urban community in equatorial Guinea. *Trop Med Parasitol* **42**, 167–171 (1991).
241. Nash, T. E., Hofstetter, M., Cheever, A. W. & Ottesen, E. A. Treatment of Schistosoma mekongi with praziquantel: a double-blind study. *Am J Trop Med Hyg* **31**, 977–982 (1982).
242. Control of Neglected Tropical Diseases. *World Health Organization* https://www.who.int/teams/control-of-neglected-tropical-diseases/schistosomiasis/control-and-elimination-programmes (2023).
243. Lloyd, A. E., Honey, B. L., John, B. M. & Condren, M. Treatment Options and Considerations for Intestinal Helminthic Infections. *J Pharm Technol* **30**, 130 (2014).
244. Bustinduy, A. L. *et al.* Population Pharmacokinetics and Pharmacodynamics of Praziquantel in Ugandan Children with Intestinal Schistosomiasis: Higher Dosages Are Required for Maximal Efficacy. *mBio* **7**, (2016).
245. Ross, A. G. P. *et al.* Schistosomiasis. *N Engl J Med* **346**, 1212–1220 (2002).

246. Colley, D. G., Bustinduy, A. L., Secor, W. E. & King, C. H. Human schistosomiasis. *The Lancet* **383**, 2253–2264 (2014).
247. Caffrey, C. R. Schistosomiasis and its treatment. *Future Med Chem* **7**, 675–676 (2015).
248. Andrews, P., Thomas, H., Pohlke, R. & Seubert, Jr. Praziquantel. *Med Res Rev* **3**, 147–200 (1983).
249. Kovač, J., Vargas, M. & Keiser, J. In vitro and in vivo activity of R-and S-praziquantel enantiomers and the main human metabolite trans-4-hydroxy-praziquantel against Schistosoma haematobium. doi:10.1186/s13071-017-2293-3.
250. Waechtler, A. *et al.* Praziquantel – 50 Years of Research. *ChemMedChem* **18**, e202300154 (2023).
251. Ross, A. G. P., Olveda, R. M. & Li, Y. An audacious goal: the elimination of schistosomiasis in our lifetime through mass drug administration. *Lancet* **385**, 2220–2221 (2015).
252. Gray, D. J. *et al.* Schistosomiasis elimination: lessons from the past guide the future. *Lancet Infect Dis* **10**, 733–736 (2010).
253. Gryseels, B. *et al.* Are poor responses to praziquantel for the treatment of Schistosoma mansoni infections in Senegal due to resistance? An overview of the evidence. *Trop Med Int Health* **6**, 864–873 (2001).
254. Ismail, M. *et al.* Characterization of isolates of Schistosoma mansoni from Egyptian villagers that tolerate high doses of praziquantel. *Am J Trop Med Hyg* **55**, 214–218 (1996).
255. Stelma, F. F. *et al.* Efficacy and side effects of praziquantel in an epidemic focus of Schistosoma mansoni. *Am J Trop Med Hyg* **53**, 167–170 (1995).
256. Fong, G. C. & Cheung, R. T. Caution with praziquantel in neurocysticercosis. *Stroke* **28**, 1648–9 (1997).
257. Lawn, S. D., Lucas, S. B. & Chiodini, P. L. Case report: Schistosoma mansoni infection: failure of standard treatment with praziquantel in a returned traveller. *Trans R Soc Trop Med Hyg* **97**, 100–101 (2003).
258. Melman, S. D. *et al.* Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. *PLoS Negl Trop Dis* **3**, (2009).
259. Danso-Appiah, A. & De Vlas, S. J. Interpreting low praziquantel cure rates of Schistosoma mansoni infections in Senegal. *Trends Parasitol* **18**, 125–129 (2002).

260. Ismail, M. M., Taha, S. A., Farghaly, A. M. & el-Azony, A. S. Laboratory induced resistance to praziquantel in experimental schistosomiasis. *J Egypt Soc Parasitol* **24**, 685–95 (1994).
261. Lotfy, W. M., Hishmat, M. G., El Nashar, A. S. & Abu El Einin, H. M. Evaluation of a method for induction of praziquantel resistance in Schistosoma mansoni. *Pharm Biol* **53**, 1214–1219 (2015).
262. Fallon, P. G. & Doenhoff, M. J. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specific. *Am J Trop Med Hyg* **51**, 83–88 (1994).
263. Hong-Jun, L. I. *et al.* Studies on resistance of Schistosoma to praziquantel XIV Experimental comparison of susceptibility to praziquantel between PZQ? resistant isolates and PZQ? susceptible isolates of Schistosoma japonicum in stages of adult worms, miracidia and cercariae. *Chinese Journal of Schistosomiasis Control* **23**, 611 (2011).
264. Liang, Y.-S. *et al.* Studies on resistance of Schistosoma to praziquantel XIII resistance of Schistosoma japonicum to praziquantel is experimentally induced in laboratory. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* **23**, 605–610 (2011).
265. Liang, Y. J. *et al.* New insight into the antifibrotic effects of praziquantel on mice in infection with Schistosoma japonicum. *PLoS One* **6**, (2011).
266. Couto, F. F. B. *et al.* Schistosoma mansoni: a method for inducing resistance to praziquantel using infected Biomphalaria glabrata snails. *Mem Inst Oswaldo Cruz* **106**, 153–157 (2011).
267. Mitchell, K. M. *et al.* Predicted impact of mass drug administration on the development of protective immunity against Schistosoma haematobium. *PLoS Negl Trop Dis* **8**, (2014).
268. Gurarie, D. *et al.* Modelling control of Schistosoma haematobium infection: predictions of the long-term impact of mass drug administration in Africa. *Parasit Vectors* **8**, (2015).
269. Amer, E. I., Abou-El-Naga, I. F., Boulos, L. M., Ramadan, H. S. & Younis, S. S. Praziquantel-encapsulated niosomes against Schistosoma mansoni with reduced sensitivity to praziquantel. *Biomedica* **42**, (2022).
270. Chevalier, F. D. *et al.* Independent origins of loss-of-function mutations conferring oxamniquine resistance in a Brazilian schistosome population. *Int J Parasitol* **46**, 417–424 (2016).

271. Abay, S. M., Tilahun, M., Fikrie, N. & Habtewold, A. Plasmodium falciparum and Schistosoma mansoni coinfection and the side benefit of artemether-lumefantrine in malaria patients. *J Infect Dev Ctries* **7**, 468–474 (2013).
272. Mbanzulu, K. M. *et al.* The impact of artesunate-amodiaquine on schistosoma mansoni infection among children infected by plasmodium in rural area of lemfu, kongo central, democratic Republic of the Congo. *Biomed Res Int* **2018**, (2018).
273. Roucher, C. *et al.* Evaluation of Artesunate-mefloquine as a Novel Alternative Treatment for Schistosomiasis in African Children (SchistoSAM): protocol of a proof-of-concept, open-label, two-arm, individually-randomised controlled trial. *BMJ Open* **11**, (2021).
274. Akilimali, A. *et al.* Self-medication and Anti-malarial Drug Resistance in the Democratic Republic of the Congo (DRC): A silent threat. *Trop Med Health* **50**, 1–5 (2022).
275. Pereira, L. H., Coelho, P. M., Costa, J. O. & de Mello, R. T. Activity of 9-acridanone-hydrazone drugs detected at the pre-postural phase, in the experimental schistosomiasis mansoni. *Mem Inst Oswaldo Cruz* **90**, 425–428 (1995).
276. Sayed, A. A. *et al.* Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nature Medicine* **14**, 407–412 (2008).
277. Guglielmo, S. *et al.* New praziquantel derivatives containing NO-donor furoxans and related furazans as active agents against Schistosoma mansoni. *Eur J Med Chem* **84**, 135–145 (2014).
278. Li, G. *et al.* Synthesis of oxadiazole-2-oxide derivatives as potential drug candidates for schistosomiasis targeting SjTGR. *Parasit Vectors* **14**, (2021).
279. Li, G. *et al.* Impact of the coronavirus disease 2019 lockdown on Schistosoma host Oncomelania hupensis density in Wuhan. *Acta Trop* **226**, (2022).
280. Kura, K., Ayabina, D., Toor, J., Hollingsworth, T. D. & Anderson, R. M. Disruptions to schistosomiasis programmes due to COVID-19: an analysis of potential impact and mitigation strategies. *Trans R Soc Trop Med Hyg* **115**, 236–244 (2021).
281. Tanaka, H. & Tsuji, M. From discovery to eradication of schistosomiasis in Japan: 1847–1996. *Int J Parasitol* **27**, 1465–1480 (1997).
282. Coelho, P. M. Z. & Caldeira, R. L. Critical analysis of molluscicide application in schistosomiasis control programs in Brazil. *Infect Dis Poverty* **5**, 1–6 (2016).

283. Dai, J. R. *et al.* A novel molluscicidal formulation of niclosamide. *Parasitol Res* **103**, 405–412 (2008).
284. Incani, R. N. The Venezuelan experience in the control of Schistosomiasis mansoni. *Mem Inst Oswaldo Cruz* **82**, 89–93 (1987).
285. Laamrani, H. *et al.* Evaluation of environmental methods to control snails in an irrigation system in Central Morocco. *Tropical Medicine & International Health* **5**, 545–552 (2000).
286. Steinmann, P., Keiser, J., Bos, R., Tanner, M. & Utzinger, J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* **6**, 411–425 (2006).
287. Sokolow, S. H. *et al.* Nearly 400 million people are at higher risk of schistosomiasis because dams block the migration of snail-eating river prawns. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**, (2017).
288. Howarth, F. G. Environmental Impacts of Classical Biological Control. *Annu Rev Entomol* **36**, 485–509 (1991).
289. Pointier, J. P., David, P. & Jarne, P. The biological control of the snail hosts of schistosomes: The role of competitor snails and biological invasions. *Biomphalaria Snails and Larval Trematodes* 215–238 (2011) doi:10.1007/978-1-4419-7028-2_9/COVER.
290. Sokolow, S. H. *et al.* Global Assessment of Schistosomiasis Control Over the Past Century Shows Targeting the Snail Intermediate Host Works Best. *PLoS Negl Trop Dis* **10**, e0004794 (2016).
291. Grimes, J. E. T. *et al.* The relationship between water, sanitation and schistosomiasis: a systematic review and meta-analysis. *PLoS Negl Trop Dis* **8**, (2014).
292. Tayo, M. A., Pugh, R. N. H. & Bradley, A. K. Malumfashi Endemic Diseases Research Project, XI. Water-contact activities in the schistosomiasis study area. *Ann Trop Med Parasitol* **74**, 347–354 (1980).
293. Rollinson, D. *et al.* Time to set the agenda for schistosomiasis elimination. *Acta Trop* **128**, 423–440 (2013).
294. Jones, M. K. *et al.* Correlative and Dynamic Imaging of the Hatching Biology of Schistosoma japonicum from Eggs Prepared by High Pressure Freezing. *PLoS Negl Trop Dis* **2**, e334 (2008).

295. Standen, O. D. The effects of temperature, light and salinity upon the hatching of the ova of Schistosoma mansoni. *Trans R Soc Trop Med Hyg* **45**, 241–325 (1951).
296. Yi-zheng Xu & Dresden, M. H. Leucine aminopeptidase and hatching of Schistosoma mansoni eggs. *Journal of Parasitology* **72**, 507–511 (1986).
297. Fogarty, C. E. *et al.* Identification of Schistosoma mansoni miracidia attractant candidates in infected Biomphalaria glabrata using behaviour-guided comparative proteomics. *Front Immunol* **13**, 954282 (2022).
298. Wang, T. *et al.* A Biomphalaria glabrata peptide that stimulates significant behaviour modifications in aquatic free-living Schistosoma mansoni miracidia. *PLoS Negl Trop Dis* **13**, (2019).
299. Lamberton, P. H. L., Norton, A. J., Webster, J. P. & Wilson, K. Propagule Behavior and Parasite Transmission. *Encyclopedia of Animal Behavior* 646–652 (2019) doi:10.1016/B978-0-12-809633-8.01187-0.
300. Xia, M. Y. & Jourdane, J. Penetration and migration routes of Schistosoma japonicum miracidia in the snail Oncomelania hupensis. *Parasitology* **103 Pt 1**, 77–83 (1991).
301. Nacif-Pimenta, R. *et al.* Schistosoma mansoni in Susceptible and Resistant Snail Strains Biomphalaria tenagophila: In Vivo Tissue Response and In Vitro Hemocyte Interactions. *PLoS One* **7**, e45637 (2012).
302. Krupa, P. L., Lewis, L. M. & Vecchio, P. Del. Schistosoma haematobium in Bulinus guernei: Electron microscopy of hemocyte-sporocyst interactions. *J Invertebr Pathol* **30**, 35–45 (1977).
303. De Moraes Bezerra, F. S. *et al.* Effect of gamma radiation on the activity of hemocytes and on the course of Schistosoma mansoni infection in resistant Biomphalaria tenagophila snails. *Mem Inst Oswaldo Cruz* **98**, 73–75 (2003).
304. Le Clec'h, W., Chevalier, F. D., Jutzeler, K. & Anderson, T. J. C. No evidence for schistosome parasite fitness trade-offs in the intermediate and definitive host. *Parasites & Vectors* **16**, 1–13 (2023).
305. Graefe, G., Hohorst, W. & Dräger, H. Forked Tail of the Cercaria of Schistosoma mansoni—a Rowing Device. *Nature* **215**, 207–208 (1967).

306. Stirewalt, M. A. & Hackey, J. R. Penetration of Host Skin by Cercariae of Schistosoma mansoni. I. Observed Entry into Skin of Mouse, Hamster, Rat, Monkey and Man. *J Parasitol* **42**, 565 (1956).
307. Hockley, D. J. & McLaren, D. J. Schistosoma mansoni: Changes in the outer membrane of the tegument during development from cercaria to adult worm. *Int J Parasitol* **3**, 13–20 (1973).
308. Ramaswamy, K., Kumar, P. & He, Y.-X. A role for parasite-induced PGE2 in IL-10-mediated host immunoregulation by skin stage schistosomula of Schistosoma mansoni. *J Immunol* **165**, 4567–4574 (2000).
309. He, Y. X., Chen, L. & Ramaswamy, K. Schistosoma mansoni, S. haematobium, and S. japonicum: Early events associated with penetration and migration of schistosomula through human skin. *Exp Parasitol* **102**, 99–108 (2002).
310. Hogg, K. G., Kumkate, S., Anderson, S. & Mountford, A. P. Interleukin-12 p40 secretion by cutaneous CD11c+ and F4/80+ cells is a major feature of the innate immune response in mice that develop Th1-mediated protective immunity to Schistosoma mansoni. *Infect Immun* **71**, 3563–3571 (2003).
311. Angeli, V. *et al.* Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J Exp Med* **193**, 1135–1147 (2001).
312. Chen, L., Rao, K. V. N., He, Y. X. & Ramaswamy, K. Skin-stage schistosomula of Schistosoma mansoni produce an apoptosis-inducing factor that can cause apoptosis of T cells. *J Biol Chem* **277**, 34329–34335 (2002).
313. Sher, A., James, S. L., Simpson, A. J., Lazdins, J. K. & Meltzer, M. S. Macrophages as effector cells of protective immunity in murine schistosomiasis. III. Loss of susceptibility to macrophage-mediated killing during maturation of S. mansoni schistosomula from the skin to the lung stage. *J Immunol* **128**, 1876–1879 (1982).
314. Wilson, R. A., Draskau, T., Miller, P. & Lawson, J. R. Schistosoma mansoni: the activity and development of the schistosomulum during migration from the skin to the hepatic portal system. *Parasitology* **77**, 57–73 (1978).
315. Goldring, O. L., Clegg, J. A., Smithers, S. R. & Terry, R. J. Acquisition of human blood group antigens by Schistosoma mansoni. *Clin Exp Immunol* **26**, 181–7 (1976).

316. Sher, A., Sacks, D. L., Simpson, A. J. G. & Singer, A. Dichotomy in the tissue origin of schistosome acquired class I and class II major histocompatibility complex antigens. *J Exp Med* **159**, 952–957 (1984).
317. Mclaren, D. J. & Terry, R. J. The protective role of acquired host antigens during schistosome maturation. *Parasite Immunol* **4**, 129–148 (1982).
318. Ramalho-Pinto, F. J., Carvalho, E. M. & Horta, M. F. Mechanisms of evasion of Schistosoma mansoni schistosomula to the lethal activity of complement. *Mem Inst Oswaldo Cruz* **87**, 111–116 (1992).
319. Redpath, S. A., van der Werf, N., MacDonald, A. S., Maizels, R. M. & Taylora, M. D. Schistosoma mansoni Larvae Do Not Expand or Activate Foxp3+ Regulatory T Cells during Their Migratory Phase. *Infect Immun* **83**, 3881–3889 (2015).
320. Dunne, D. W. & Cooke, A. A worm's eye view of the immune system: consequences for evolution of human autoimmune disease. *Nature Reviews Immunology* **5**, 420–426 (2005).
321. Egesa, M. *et al.* Schistosoma mansoni schistosomula antigens induce Th1/Pro-inflammatory cytokine responses. *Parasite Immunol* **40**, e12592 (2018).
322. Yang, J. *et al.* Molecular cloning and functional characterization of Schistosoma japonicum enolase which is highly expressed at the schistosomulum stage. *Parasitol Res* **107**, 667–677 (2010).
323. Figueiredo, B. C., Da'dara, A. A., Oliveira, S. C. & Skelly, P. J. Schistosomes Enhance Plasminogen Activation: The Role of Tegumental Enolase. *PLoS Pathog* **11**, (2015).
324. Tarleton, R. L. & Kemp, W. M. Demonstration of IgG-Fc and C3 receptors on adult Schistosoma mansoni. *J Immunol* **126**, 379–84 (1981).
325. Castro-Borges, W., Dowle, A., Curwen, R. S., Thomas-Oates, J. & Wilson, R. A. Enzymatic shaving of the tegument surface of live schistosomes for proteomic analysis: a rational approach to select vaccine candidates. *PLoS Negl Trop Dis* **5**, (2011).
326. Loukas, A., Jones, M. K., King, L. T., Brindley, P. J. & McManus, D. P. Receptor for Fc on the surfaces of schistosomes. *Infect Immun* **69**, 3646–3651 (2001).
327. Torpier, G., Capron, A. & Ouaissi, M. A. Receptor for IgG(Fc) and human beta2-microglobulin on S. mansoni schistosomula. *Nature* **278**, 447–449 (1979).

328. Dagenais, M., Gerlach, J. Q., Geary, T. G. & Long, T. Sugar Coating: Utilisation of Host Serum Sialoglycoproteins by Schistosoma mansoni as a Potential Immune Evasion Mechanism. *Pathogens* **11**, (2022).
329. Leung, M. K. *et al.* Schistosoma mansoni: The presence and potential use of opiate-like substances. *Exp Parasitol* **81**, 208–215 (1995).
330. Salzet, M., Capron, A. & Stefano, G. B. Molecular crosstalk in host-parasite relationships: schistosome- and leech-host interactions. *Parasitol Today* **16**, 536–540 (2000).
331. Duvaux-Miret, O., Stefano, G. B., Smith, E. M., Dissous, C. & Capron, A. Immunosuppression in the definitive and intermediate hosts of the human parasite Schistosoma mansoni by release of immunoactive neuropeptides. *Proc Natl Acad Sci U S A* **89**, 778–781 (1992).
332. Liu, Q., Li, F. C., Elsheikha, H. M., Sun, M. M. & Zhu, X. Q. Identification of host proteins interacting with Toxoplasma gondii GRA15 (TgGRA15) by yeast two-hybrid system. *Parasit Vectors* **10**, 1–7 (2017).
333. Morais, S. B. *et al.* Schistosoma mansoni SmKI-1 serine protease inhibitor binds to elastase and impairs neutrophil function and inflammation. *PLoS Pathog* **14**, (2018).
334. L. S. Alves, C. *et al.* Immunomodulatory properties of Schistosoma mansoni proteins Sm200 and SmKI-1 in vitro and in a murine model of allergy to the mite Blomia tropicalis. *Mol Immunol* **124**, 91–99 (2020).
335. Sotillo, J. *et al.* Extracellular vesicles secreted by Schistosoma mansoni contain protein vaccine candidates. *Int J Parasitol* **46**, 1–5 (2016).
336. Samoil, V. *et al.* Vesicle-based secretion in schistosomes: Analysis of protein and microRNA (miRNA) content of exosome-like vesicles derived from Schistosoma mansoni. *Sci Rep* **8**, (2018).
337. Pérez-Sánchez, R., Ramajo-Hernández, A., Ramajo-Martín, V. & Oleaga, A. Proteomic analysis of the tegument and excretory-secretory products of adult Schistosoma bovis worms. *Proteomics* **6 Suppl 1**, (2006).
338. Hall, S. L. *et al.* Insights into blood feeding by schistosomes from a proteomic analysis of worm vomitus. *Mol Biochem Parasitol* **179**, 18–29 (2011).
339. Chen, L., He, B., Hou, W. & He, L. Cysteine protease inhibitor of Schistosoma japonicum - A parasite-derived negative immunoregulatory factor. *Parasitol Res* **116**, 901–908 (2017).

340. Cao, X. *et al.* iTRAQ-based comparative proteomic analysis of excretory-secretory proteins of schistosomula and adult worms of Schistosoma japonicum. *J Proteomics* **138**, 30–39 (2016).
341. Mastorakos, G., Karoutsou, E. I. & Mizamtsidi, M. Corticotropin Releasing Hormone And The Immune/Inflammatory Response. *European Journal of Endocrinology, Supplement* **155**, (2015).
342. Karl, S. *et al.* The iron distribution and magnetic properties of schistosome eggshells: implications for improved diagnostics. *PLoS Negl Trop Dis* **7**, e2219 (2013).
343. Woolley, P. G. & Huffman, O. V. The ova of Schistosoma japonicum and the absence of spines. *Parasitology* **4**, 131–132 (1911).
344. Leiper, R. T. Note on the presence of a lateral spine in the eggs of Schistosoma japonicum. *Trans R Soc Trop Med Hyg* **4**, 132–136 (1911).
345. Southgate, V. R. & Bray, R. A. Medical helminthology. in *Manson's Tropical Diseases* 1633–1698 (Elsevier, 2009).
346. Ashton, P. D., Harrop, R., Shah, B. & Wilson, R. A. The schistosome egg: development and secretions. *Parasitology* **122**, 329–338 (2001).
347. Grzych, J. M. *et al.* Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J Immunol* **146**, 1322–7 (1991).
348. Vella, A. T. & Pearce, E. J. CD4+ Th2 response induced by Schistosoma mansoni eggs develops rapidly, through an early, transient, Th0-like stage. *J Immunol* **148**, 2283–90 (1992).
349. Abdulla, M. H., Lim, K. C., McKerrow, J. H. & Caffrey, C. R. Proteomic identification of IPSE/alpha-1 as a major hepatotoxin secreted by Schistosoma mansoni eggs. *PLoS Negl Trop Dis* **5**, (2011).
350. Meyer, N. H. *et al.* A Crystallin Fold in the Interleukin-4-inducing Principle of Schistosoma mansoni Eggs (IPSE/α-1) Mediates IgE Binding for Antigen-independent Basophil Activation. *J Biol Chem* **290**, 22111–22126 (2015).
351. Schramm, G. *et al.* Cutting edge: IPSE/alpha-1, a glycoprotein from Schistosoma mansoni eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *J Immunol* **178**, 6023–6027 (2007).

352. Everts, B. *et al.* Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *J Exp Med* **206**, 1673–1680 (2009).
353. Zaccone, P. *et al.* Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. *Eur J Immunol* **39**, 1098–1107 (2009).
354. Dunne, D. W. *et al.* Identification and partial purification of an antigen (ω1 from Schistosoma mansoni eggs which is putatively hepatotoxic in T-cell deprived mice. *Trans R Soc Trop Med Hyg* **75**, 54–71 (1981).
355. Hagen, J. *et al.* Omega-1 knockdown in Schistosoma mansoni eggs by lentivirus transduction reduces granuloma size in vivo. *Nat Commun* **5**, (2014).
356. Smith, P. *et al.* Schistosoma mansoni secretes a chemokine binding protein with antiinflammatory activity. *J Exp Med* **202**, 1319–1325 (2005).
357. Cheever, E. A., Macedonia, J. G., Mosimann, J. E. & Cheever, A. W. Kinetics of Egg Production and Egg Excretion by Schistosoma mansoni and S. japonicum in Mice Infected with a Single Pair of Worms. *Am J Trop Med Hyg* **50**, 281–295 (1994).
358. Zheng, B. *et al.* T Lymphocyte-Mediated Liver Immunopathology of Schistosomiasis. *Front Immunol* **11**, (2020).
359. Lundy, S. K. & Lukacs, N. W. Chronic schistosome infection leads to modulation of granuloma formation and systemic immune suppression. *Front Immunol* **4**, (2013).
360. Wolde, M. *et al.* Human monocytes/macrophage inflammatory cytokine changes following in vivo and in vitro Schistosoma mansoni infection. *J Inflamm Res* 35–43 (2020).
361. Kamdem, S. D., Moyou-Somo, R., Brombacher, F. & Nono, J. K. Host Regulators of Liver Fibrosis During Human Schistosomiasis. *Front Immunol* **9**, (2018).
362. Dewals, B. G. *et al.* IL-4Ralpha-independent expression of mannose receptor and Ym1 by macrophages depends on their IL-10 responsiveness. *PLoS Negl Trop Dis* **4**, (2010).
363. Layland, L. E. *et al.* Pronounced Phenotype in Activated Regulatory T Cells during a Chronic Helminth Infection. *The Journal of Immunology* **184**, 713–724 (2010).
364. Taylor, J. J., Mohrs, M. & Pearce, E. J. Regulatory T Cell Responses Develop in Parallel to Th Responses and Control the Magnitude and Phenotype of the Th Effector Populatio. *The Journal of Immunology* **176**, 5839–5847 (2006).

365. Lundy, S. K. & Boros, D. L. Fas ligand-expressing B-1a lymphocytes mediate CD4(+)-T-cell apoptosis during schistosomal infection: induction by interleukin 4 (IL-4) and IL-10. *Infect Immun* **70**, 812–819 (2002).
366. Lundy, S. K., Lerman, S. P. & Boros, D. L. Soluble egg antigen-stimulated T helper lymphocyte apoptosis and evidence for cell death mediated by FasL(+) T and B cells during murine Schistosoma mansoni infection. *Infect Immun* **69**, 271–280 (2001).
367. Boctor, F. N. & Peter, J. B. IgG subclasses in human chronic schistosomiasis: over-production of schistosome-specific and non-specific IgG4. *Clin Exp Immunol* **82**, 574–578 (2008).
368. Van De Veen, W. *et al.* IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol* **131**, 1204–1212 (2013).
369. Oliveira, R. R. *et al.* Factors associated with resistance to Schistosoma mansoni infection in an endemic area of Bahia, Brazil. *Am J Trop Med Hyg* **86**, 296–305 (2012).
370. Kamal, S. M. & El Sayed Khalifa, K. Immune modulation by helminthic infections: worms and viral infections. *Parasite Immunol* **28**, 483–496 (2006).
371. Fitzsimmons, C. M. *et al.* Progressive cross-reactivity in IgE responses: an explanation for the slow development of human immunity to schistosomiasis? *Infect Immun* **80**, 4264–4270 (2012).
372. Mutapi, F., Billingsley, P. F. & Secor, W. E. Infection and treatment immunizations for successful parasite vaccines. *Trends Parasitol* **29**, 135–141 (2013).
373. Wilson, S. *et al.* Rapidly boosted Plasma IL-5 induced by treatment of human Schistosomiasis haematobium is dependent on antigen dose, IgE and eosinophils. *PLoS Negl Trop Dis* **7**, (2013).
374. Mutapi, F. *et al.* Chemotherapy accelerates the development of acquired immune responses to Schistosoma haematobium infection. *J Infect Dis* **178**, 289–293 (1998).
375. Osakunor, D. N. M., Woolhouse, M. E. J. & Mutapi, F. Paediatric schistosomiasis: What we know and what we need to know. *PLoS Negl Trop Dis* **12**, (2018).
376. Mitchell, K. M., Mutapi, F., Savill, N. J. & Woolhouse, M. E. J. Explaining observed infection and antibody age-profiles in populations with urogenital schistosomiasis. *PLoS Comput Biol* **7**, (2011).

377. Dunne, D. W. *et al.* Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* **22**, 1483–1494 (1992).
378. Dunne, D. W., Butterworth, A. E., Fulford, A. J., Ouma, J. H. & Sturrock, R. F. Human IgE responses to Schistosoma mansoni and resistance to reinfection. *Mem Inst Oswaldo Cruz* **87 Suppl 4**, 99–103 (1992).
379. Hagan, P., Blumenthal, U. J., Dunn, D., Simpson, A. J. G. & Wilkins, H. A. Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. *Nature* **349**, 243–245 (1991).
380. Ganley-Leal, L. M. *et al.* Correlation between eosinophils and protection against reinfection with Schistosoma mansoni and the effect of human immunodeficiency virus type 1 coinfection in humans. *Infect Immun* **74**, 2169–2176 (2006).
381. Negrão-Corrêa, D. *et al.* Association of Schistosoma mansoni-specific IgG and IgE antibody production and clinical schistosomiasis status in a rural area of Minas Gerais, Brazil. *PLoS One* **9**, (2014).
382. Mwinzi, P. N. M. *et al.* Circulating CD23+ B cell subset correlates with the development of resistance to Schistosoma mansoni reinfection in occupationally exposed adults who have undergone multiple treatments. *J Infect Dis* **199**, 272–279 (2009).
383. Grzych, J. M. *et al.* IgA antibodies to a protective antigen in human Schistosomiasis mansoni. *The Journal of Immunology* **150**, 527–535 (1993).
384. Dessein, A. J. *et al.* Human resistance to Schistosoma mansoni is associated with IgG reactivity to a 37-kDa larval surface antigen. *The Journal of Immunology* **140**, 2727–2736 (1988).
385. Auriault, C. *et al.* Antibody response of Schistosoma mansoni-infected human subjects to the recombinant P28 glutathione-S-transferase and to synthetic peptides. *J Clin Microbiol* **28**, 1918–1924 (1990).
386. Colley, D. G. & Secor, W. E. Immunology of human schistosomiasis. *Parasite Immunol* **36**, 347–357 (2014).
387. Wilson, M. S., Cheever, A. W., White, S. D., Thompson, R. W. & Wynn, T. A. IL-10 blocks the development of resistance to re-infection with Schistosoma mansoni. *PLoS Pathog* **7**, (2011).

388. Nono, J. K. *et al.* Schistosomiasis Burden and Its Association With Lower Measles Vaccine Responses in School Children From Rural Cameroon. *Front Immunol* **9**, (2018).
389. Chan, B. C. L., Lam, C. W. K., Tam, L. S. & Wong, C. K. IL33: Roles in allergic inflammation and therapeutic perspectives. *Front Immunol* **10**, 394368 (2019).
390. Cortes-Selva, D. & Fairfax, K. Schistosome and intestinal helminth modulation of macrophage immunometabolism. *Immunology* **162**, 123–134 (2021).
391. Schwartz, C. *et al.* ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control. *Journal of Experimental Medicine* **214**, 2507–2521 (2017).
392. Viana, I. R. C. *et al.* Comparison of antibody isotype responses to Schistosoma mansoni antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunol* **17**, 297–304 (1995).
393. Viana, I. R. C. *et al.* Interferon-γ production by peripheral blood mononuclear cells from residents of an area endemic for Schistosoma mansoni. *Trans R Soc Trop Med Hyg* **88**, 466–470 (1994).
394. Bahia-Oliveira, L. M. G. *et al.* Differential cellular reactivity to adult worm antigens of patients with different clinical forms of schistosomiasis mansoni. *Trans R Soc Trop Med Hyg* **86**, 57–61 (1992).
395. Correa-Oliveira, R. *et al.* The human immune response to defined immunogens of Schistosoma mansoni: elevated antibody levels to paramyosin in stool-negative individuals from two endemic areas in Brazil. *Trans R Soc Trop Med Hyg* **83**, 798–804 (1989).
396. Caldas, I. R. *et al.* Susceptibility and resistance to Schistosoma mansoni reinfection: parallel cellular and isotypic immunologic assessment. *Am J Trop Med Hyg* **62**, 57–64 (2000).
397. Brito, C. F. A., Caldas, I. R., Coura Filho, P., Correa-Oliveira, R. & Oliveira, S. C. CD4+ T Cells of Schistosomiasis Naturally Resistant Individuals Living in an Endemic Area Produce Interferon-γ and Tumour Necrosis Factor-α in Response to The Recombinant 14kda Schistosoma mansoni Fatty Acid-Binding Protein. *Scand J Immunol* **51**, 595–601 (2000).
398. Ricciardi, A., Zelt, N. H., Visitsunthorn, K., Dalton, J. P. & Ndao, M. Immune mechanisms involved in Schistosoma mansoni-Cathepsin B vaccine induced protection in mice. *Front Immunol* **9**, 1710 (2018).

399. Wynn, T. A. *et al.* Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with Schistosoma mansoni. *The Journal of Immunology* **153**, 5200–5209 (1994).
400. Pearce, E. J. & James, S. L. Post lung-stage schistosomula of Schistosoma mansoni exhibit transient susceptibility to macrophage-mediated cytotoxicity in vitro that may relate to late phase killing in vivo. *Parasite Immunol* **8**, 513–527 (1986).
401. Smithers, S. R. & Terry, R. J. Resistance to experimental infection with Schistosoma mansoni in rhesus monkeys induced by the transfer of adult worms. *Trans R Soc Trop Med Hyg* **61**, 517–533 (1967).
402. Buck, J. C., De Leo, G. A. & Sokolow, S. H. Concomitant Immunity and Worm Senescence May Drive Schistosomiasis Epidemiological Patterns: An Eco-Evolutionary Perspective. *Front Immunol* **11**, 490788 (2020).
403. Brunet, L. R., Kopf, M. A. & Pearce, E. J. Schistosoma mansoni: IL-4 is necessary for concomitant immunity in mice. *J Parasitol* **85**, 734–6 (1999).
404. Salim, A. M. & Al-Humiany, A. R. Concomitant immunity to Schistosoma mansoni in mice. *Turkiye Parazitol Derg* **37**, 19–22 (2013).
405. He, L. *et al.* Schistosoma japonicum: establishment of a mouse model that demonstrates concomitant immunity. *Chin Med J (Engl)* **117**, 947–9 (2004).
406. Sher, A., Smithers, S. R. & Mackenzie, P. Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice. *Parasitology* **70 Part 3**, 347–357 (1975).
407. Dean, D. A., Minard, P., Stirewalt, M. A., Vannier, W. E. & Murrell, K. D. Resistance of mice to secondary infection with Schistosoma mansoni. I. Comparison of bisexual and unisexual initial infections. *Am J Trop Med Hyg* **27**, 951–956 (1978).
408. Dumont, M. *et al.* Influence of pattern of exposure, parasite genetic diversity and sex on the degree of protection against reinfection with Schistosoma mansoni. *Parasitol Res* **101**, 247–252 (2007).
409. Harrison, R. A., Bickle, Q. & Doenhoff, M. J. Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. Evidence that the mechanisms which mediate resistance during early patent infections may lack immunological specificity. *Parasitology* **84**, 93–110 (1982).

410. Dissous, C., Grzych, J. M. & Capron, A. Schistosoma mansoni surface antigen defined by a rat monoclonal IgG2a. *J Immunol* **129**, 2232–4 (1982).
411. Dissous, C. & Capron, A. Schistosoma mansoni: antigenic community between schistosomula surface and adult worm incubation products as a support for concomitant immunity. *FEBS Lett* **162**, 355–359 (1983).
412. James, S. L., Sher, A., Lazdins, J. K. & Meltzer, M. S. Macrophages as effector cells of protective immunity in murine schistosomiasis. II. Killing of newly transformed schistosomula in vitro by macrophages activated as a consequence of Schistosoma mansoni infection. *J Immunol* **128**, 1535–40 (1982).
413. Chensue, S. W., Otterness, I. G., Higashi, G. I., Forsch, C. S. & Kunkel, S. L. Monokine production by hypersensitivity (Schistosoma mansoni egg) and foreign body (Sephadex bead)-type granuloma macrophages. Evidence for sequential production of IL-1 and tumor necrosis factor. *J Immunol* **142**, 1281–6 (1989).
414. Hagan, P., Garside, P. & Kusel, J. R. Is tumour necrosis factor alpha the molecular basis of concomitant immunity in schistosomiasis? *Parasite Immunol* **15**, 553–7 (1993).
415. James, S. L., Glaven, J., Goldenberg, S., Meltzer, M. S. & Pearce, E. Tumour necrosis factor (TNF) as a mediator of macrophage helminthotoxic activity. *Parasite Immunol* **12**, 1–13 (1990).
416. Zeng, X. J. *et al.* Effect of integrated control intervention on soil-transmitted helminth infections in Jiangxi province in southeast China. *Acta Trop* **194**, 148–154 (2019).
417. Rojanapanus, S. *et al.* How Thailand eliminated lymphatic filariasis as a public health problem. *Infect Dis Poverty* **8**, 1–15 (2019).
418. Bah, Y. M. *et al.* Achievements and challenges of lymphatic filariasis elimination in sierra leone. *PLoS Negl Trop Dis* **14**, 1–16 (2020).
419. Chiamah, O., Ubachukwu, P., Anorue, C. & Ebi, S. Urinary schistosomiasis in Ebonyi State, Nigeria from 2006 to 2017. *Journal of Vector Borne Diseases* **56**, 87–91 Preprint at https://doi.org/10.4103/0972-9062.263721 (2019).
420. Weatherhead, J. E., Hotez, P. J. & Mejia, R. The Global State of Helminth Control and Elimination in Children. *Pediatric Clinics of North America* **64**, 867–877 Preprint at https://doi.org/10.1016/j.pcl.2017.03.005 (2017).

421. Geerts, S. & Gryseels, B. Anthelmintic resistance in human helminths: A review. *Tropical Medicine and International Health* **6**, 915–921 Preprint at https://doi.org/10.1046/j.1365-3156.2001.00774.x (2001).
422. Vale, N. *et al.* Praziquantel for schistosomiasis: Single-drug metabolism revisited, mode of action, and resistance. *Antimicrobial Agents and Chemotherapy* **61**, Preprint at https://doi.org/10.1128/AAC.02582-16 (2017).
423. Biswas, G., Sankara, D. P., Agua-Agum, J. & Maiga, A. Dracunculiasis (guinea worm disease): Eradication without a drug or a vaccine. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**, Preprint at https://doi.org/10.1098/rstb.2012.0146 (2013).
424. Dracunculiasis eradication: global surveillance summary, 2020. *World Health Organization* https://www.who.int/publications/i/item/who-wer9621-173-194 (2021).
425. Rauch, S., Jasny, E., Schmidt, K. E. & Petsch, B. New vaccine technologies to combat outbreak situations. *Frontiers in Immunology* **9**, 1963 Preprint at https://doi.org/10.3389/fimmu.2018.01963 (2018).
426. Cox, F. E. G. History of human parasitology. *Clinical Microbiology Reviews* **15**, 595–612 Preprint at https://doi.org/10.1128/CMR.15.4.595-612.2002 (2002).
427. Zarowiecki, M. & Berriman, M. What helminth genomes have taught us about parasite evolution. *Parasitology* **142**, S85–S97 Preprint at https://doi.org/10.1017/S0031182014001449 (2015).
428. Ravindran, B., Satapathy, A. K., Sahoo, P. K. & Mohanty, M. C. Protective immunity in human lymphatic filariasis: Problems and prospects. in *Medical Microbiology and Immunology* **192**, 41–46 (Springer Verlag, 2003).
429. Kwarteng, A. & Ahuno, S. T. Immunity in Filarial Infections: Lessons from Animal Models and Human Studies. *Wiley Online Library* **85**, 251–257 (2017).
430. Dixon, J. B. Echinococcosis. *Comp Immunol Microbiol Infect Dis* **20**, 87–94 (1997).
431. Ploeger, H. W. & Eysker, M. Protection against and establishment of Dictyocaulus viviparus following primary infection at different dose levels. *Vet Parasitol* **106**, 213–223 (2002).
432. Taylor, S. M. *et al.* Induction of protective immunity to Dictyocaulus viviparus in calves while under treatment with endectocides. *Vet Parasitol* **88**, (2000).

433. Morris, C. P., Evans, H., Larsen, S. E. & Mitre, E. A Comprehensive, Model-Based Review of Vaccine and Repeat Infection Trials for Filariasis. *Am Soc Microbiol* (2013) doi:10.1128/CMR.00002-13.
434. Haque, A., Chassoux, D., Capron, A. & Ogilvie, B. M. Dipetalonema viteae infection in hamsters: Enhancement and suppression of microfilaraemia. *Parasitology* **76**, 77–84 (1978).
435. Rajakumar, S. *et al.* Concomitant immunity in a rodent model of filariasis: The infection of Meriones unguiculatus with Acanthocheilonema viteae. *Journal of Parasitology* **92**, 41–45 (2006).
436. Claerebout, E. & Geldhof, P. Helminth Vaccines in Ruminants: From Development to Application. *Veterinary Clinics of North America - Food Animal Practice* **36**, 159–171 Preprint at https://doi.org/10.1016/j.cvfa.2019.10.001 (2020).
437. Gause, W. C., Rothlin, C. & Loke, P. Heterogeneity in the initiation, development and function of type 2 immunity. *Nature Reviews Immunology* **20**, 603–614 Preprint at https://doi.org/10.1038/s41577-020-0301-x (2020).
438. Turner, J. D. *et al.* Allergen-specific IgE and IgG4 are markers of resistance and susceptibility in a human intestinal nematode infection. *Microbes Infect* **7**, 990–996 (2005).
439. Medhat, A. *et al.* Increased interleukin-4 and interleukin-5 production in response to Schistosoma haematobium adult worm antigens correlates with lack of reinfection after treatment. *Journal of Infectious Diseases* **178**, 512–519 (1998).
440. Yasuda, K. & Kuroda, E. Role of eosinophils in protective immunity against secondary nematode infections. *Immunological Medicine* **42**, 148–155 Preprint at https://doi.org/10.1080/25785826.2019.1697135 (2019).
441. Herbert, D. R., Douglas, B. & Zullo, K. Group 2 innate lymphoid cells (ILC2): Type 2 immunity and helminth immunity. *Int J Mol Sci* **20**, (2019).
442. Fitzsimmons, C. M., Falcone, F. H. & Dunne, D. W. Helminth allergens, parasite-specific IgE, and its protective role in human immunity. *Frontiers in Immunology* **5**, Preprint at https://doi.org/10.3389/fimmu.2014.00061 (2014).
443. Turner, J. *et al.* A comparison of cellular and humoral immune responses to trichuroid derived antigens in human trichuriasis. *Parasite Immunol* **24**, 83–93 (2002).

444. Muhsin, M. *et al.* IL-6 is required for protective immune responses against early filarial infection. *Int J Parasitol* **48**, 925–935 (2018).
445. Faulkner, H. *et al.* Age- and infection intensity-dependent cytokine and antibody production in human trichuriasis: The importance of IgE. *Journal of Infectious Diseases* **185**, 665–672 (2002).
446. Cooper, P. J. & Figuieredo, C. A. Immunology of Ascaris and Immunomodulation. in *Ascaris: The Neglected Parasite* 3–19 (Elsevier Inc., 2013). doi:10.1016/B978-0-12-396978-1.00001-X.
447. Babu, S. & Nutman, T. B. Immunology of lymphatic filariasis. *Parasite Immunology* **36**, 338–346 Preprint at https://doi.org/10.1111/pim.12081 (2014).
448. Yong, L. *et al.* B1 cells protect against Schistosoma japonicum–induced liver inflammation and fibrosis by controlling monocyte infiltration. *PLoS Negl Trop Dis* **13**, e0007474 (2019).
449. Maizels, R. M. & McSorley, H. J. Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology* **138**, 666–675 (2016).
450. Zakeri, A., Hansen, E. P., Andersen, S. D., Williams, A. R. & Nejsum, P. Immunomodulation by helminths: Intracellular pathways and extracellular vesicles. *Frontiers in Immunology* **9**, 2349 Preprint at https://doi.org/10.3389/fimmu.2018.02349 (2018).
451. Soloviova, K., Fox, E. C., Dalton, J. P., Caffrey, C. R. & Davies, S. J. A secreted schistosome cathepsin B1 cysteine protease and acute schistosome infection induce a transient T helper 17 response. *PLoS Negl Trop Dis* **13**, (2019).
452. Zhang, S. The role of transforming growth factor β in T helper 17 differentiation. *Immunology* **155**, 24–35 Preprint at https://doi.org/10.1111/imm.12938 (2018).
453. Wong, M. M., Fredericks, H. J. & Ramachandran, C. P. Studies on Immunization against Brugia malayi Infection in the Rhesus Monkey. *Bull Org Mond Sante* **40**, (1969).
454. Schrempf-Eppstein, B., Kern, A., Textor, G. & Lucius, R. Acanthocheilonema viteae: Vaccination with irradiated L3 induces resistance in three species of rodents (Meriones unguiculatus, Mastomys coucha, Mesocricetus auratus). *Tropical Medicine and International Health* **2**, 104–110 (1997).

455. Babayan, S. A. *et al.* Vaccination against filarial nematodes with irradiated larvae provides long-term protection against the third larval stage but not against subsequent life cycle stages. *Int J Parasitol* **36**, 903–914 (2006).
456. Torrero, M. N. *et al.* Basophils help establish protective immunity induced by irradiated larval vaccination for filariasis. *Vaccine* **31**, 3675–3682 (2013).
457. Hafeez, M. & Rao, B. V. Studies on amphistomiasis in Andhra Pradesh (India) VI. Immunization of lambs and kids with gamma irradiated metacerereariae of Cercariae indicae XXVI. *J Helminthol* **55**, 29–32 (1981).
458. Urban, J. F. & Tromba, F. G. Development of immune responsiveness to Ascaris suum antigens in pigs vaccinated with ultraviolet-attenuated eggs. *Vet Immunol Immunopathol* **3**, 399–409 (1982).
459. Tromba, F. G. Immunization of pigs against experimental Ascaris suum infection by feeding ultraviolet-attenuated eggs. *Journal of Parasitology* **64**, 651–656 (1978).
460. Jian, X. & *et al*. Protective immunity elicited by ultraviolet-irradiated third-stag. *Southeast Asian Journal of Tropical Medicine and Public Health* **37**, (2006).
461. Conder, G. A. & Williams, J. F. Immunization with infective larvae of Strongyloides ratti (Nematoda) exposed to microwave radiation. *Journal of Parasitology* **69**, 83–87 (1983).
462. Boag, P. R., Parsons, J. C., Presidente, P. J. A., Spithill, T. W. & Sexton, J. L. Characterisation of humoral immune responses in dogs vaccinated with irradiated Ancylostoma caninum. *Vet Immunol Immunopathol* **92**, 87–94 (2003).
463. Fujiwara, R. T. *et al.* Vaccination with irradiated Ancylostoma caninum third stage larvae induces a Th2 protective response in dogs. *Vaccine* **24**, 501–509 (2006).
464. Creaney, J. *et al.* Fasciola hepatica: Irradiation-induced alterations in carbohydrate and cathepsin-B protease expression in newly excysted juvenile liver fluke. *Exp Parasitol* **83**, 202–215 (1996).
465. Creaney, J. *et al.* Attempted immunisation of sheep against Fasciola hepatica using γ-irradiated metacercariae. *Int J Parasitol* **25**, 853–856 (1995).
466. Nansen, P. Resistance in cattle to Fasciola hepatica induced by γ ray attenuated larvae: results from a controlled field trial. *Res Vet Sci* **19**, 278–283 (1975).

467. Younis, S. A., Yagi, A. I., Haroun, E. M., Gameel, A. A. & Taylor, M. G. Immunization of zebu calves against Fasciola gigantica, using irradiated metacercariae. *J Helminthol* **60**, 123–134 (1986).
468. Hafez, E. N., Hafez, M. N. & Amin, M. M. Effect of vaccination with irradiated Toxocara canis larvae or thyme oil treatment on testicular histochemical and immunohistochemical changes of rats. *Trop Biomed* **36**, 430–442 (2019).
469. Hafez, E. N., Awadallah, F. M., Ibrahim, S. A., Amin, M. M. & El-Nawasera, N. Z. Assessment of vaccination with gamma radiation-attenuated infective Toxocara canis eggs on murine toxocariasis. *Trop Biomed* **37**, 89–102 (2020).
470. Nakayama, H. *et al.* Immunization of laboratory animals with ultraviolet-attenuated larvae against homologous challenge infection with Trichinella britovi. *Southeast Asian Journal of Tropical Medicine and Public Health* **29**, 563–566 (1998).
471. Ali, S. M., El-Zawawy, L. A., El-Said, D. & Gaafar, M. R. Immunization against trichinellosis using microwaved larvae of Trichinela spiralis. *J Egypt Soc Parasitol* **37**, 121–133 (2007).
472. Lange, A. M., Yutanawiboonchai, W., Lok, J. B., Trpis, M. & Abraham, D. Induction of protective immunity against larval Onchocerca volvulus in a mouse model. *American Journal of Tropical Medicine and Hygiene* **49**, 783–788 (1993).
473. Taylor, M. J. *et al.* Protective immunity against Onchocerca volvulus and O. lienalis infective larvae in mice. *Tropical Medicine and Parasitology* **45**, 17–23 (1994).
474. Prince, A. M. *et al.* Onchocerca volvulus: Immunization of chimpanzees with X-irradiated third-stage (L3) larvae. *Exp Parasitol* **74**, 239–250 (1992).
475. Fu, S. Q. *et al.* Resistance to reinfection in rats induced by irradiated metacercariae of Clonorchis sinensis. *Mem Inst Oswaldo Cruz* **100**, 549–554 (2005).
476. Movsesijan, M., Sokolić, A. & Mladenović, Z. Studies on the immunological potentiality of irradiated Echinococcus granulosus forms: immunization experiments in dogs. *Br Vet J* **124**, 425–428 (1968).
477. Smithers, S. R. Immunizing effect of irradiated cercariæ of Schistosoma mansoni in rhesus monkeys. *Nature* **194**, 1146–1147 (1962).

478. Stek, M. Fr., Minard, P., Dean, D. A., Hall J. E. Immunization of baboons with Schistosoma mansoni cercariae attenuated by gamma irradiation. *Science* **212**, 1518-20 (1981) doi:10.1126/science.7233238.
479. Kariuki, T. M. *et al.* Parameters of the attenuated schistosome vaccine evaluated in the olive baboon. *Infect Immun* **72**, 5526–5529 (2004).
480. Webbe, G., Sturrock, R. F., James, E. R. & James, C. Schistosoma haematobium in the baboon (Papio anubis): Effect of vaccination with irradiated larvae on the subsequent infection with percutaneously applied cercariae. *Trans R Soc Trop Med Hyg* **76**, 354–361 (1982).
481. Agnew, A. M., Murare, H. M. & Doenhoff, M. J. Specific cross-protection between Schistosoma bovis and S. haematobium induced by highly irradiated infections in mice. *Paiasitr Immunology* **11**, (1989).
482. Dean, D. A., Mangold, B. L., Harrison, R. A. & Ricciardone, M. D. Homologous and heterologous protective immunity to Egyptian strains of Schistosoma mansoni and S. haematobium induced by ultraviolet-irradiated cercariae. *Parasite Immunol* **18**, 403–410 (1996).
483. Harrison, R. A. *et al.* Immunization of baboons with attenuated schistosomula of schistosoma haematobium: Levels of protection induced by immunization with larvae irradiated with 20 and 60 krad. *Trans R Soc Trop Med Hyg* **84**, 89–99 (1990).
484. Hsü, H. F., Hsü, S. Y. L. & Osborne, J. W. Immunization against Schistosoma japonicum in rhesus monkeys produced by irradiated cercariæ. *Nature* **194**, 98–99 (1962).
485. Lin, D. *et al.* Multiple vaccinations with UV- attenuated cercariae in pig enhance protective immunity against Schistosoma japonicum infection as compared to single vaccination. *Parasit Vectors* **4**, (2011).
486. Smythies, L. E., Coulson, P. S. & Wilson, R. A. Monoclonal antibody to IFN-gamma modifies pulmonary inflammatory responses and abrogates immunity to Schistosoma mansoni in mice vaccinated with attenuated cercariae. *J Immunol* **149**, 3654–8 (1992).
487. Wilson, R. A., Coulson, P. S., Betts, C., Dowling, M. A. & Smythies, L. E. Impaired immunity and altered pulmonary responses in mice with a disrupted interferon-gamma receptor gene exposed to the irradiated Schistosoma mansoni vaccine. *Immunology* **87**, 275–82 (1996).

488. Jankovic, D. *et al.* Optimal vaccination against Schistosoma mansoni requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. *J Immunol* **162**, 345–51 (1999).
489. Hewitson, J. P., Hamblin, P. A. & Mountford, A. P. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunol* **27**, 271–280 (2005).
490. Vaccine Types. *National Institute of Allergy and Infectious Diseases* https://www.niaid.nih.gov/research/vaccine-types (2021).
491. Kalita, P., Lyngdoh, D. L., Padhi, A. K., Shukla, H. & Tripathi, T. Development of multi-epitope driven subunit vaccine against Fasciola gigantica using immunoinformatics approach. *Int J Biol Macromol* **138**, 224–233 (2019).
492. Sanches, R. C. O. *et al.* Immunoinformatics Design of Multi-Epitope Peptide-Based Vaccine Against Schistosoma mansoni Using Transmembrane Proteins as a Target. *Front Immunol* **12**, 621706 (2021).
493. Zawawi, A. *et al.* In silico design of a T-cell epitope vaccine candidate for parasitic helminth infection. *PLoS Pathog* **16**, (2020).
494. Jain, S. *et al.* The parasite-derived rOv-ASP-1 is an effective antigen-sparing CD4+ T cell-dependent adjuvant for the trivalent inactivated influenza vaccine, and functions in the absence of MyD88 pathway. *Vaccine* **36**, 3650–3665 (2018).
495. El Ridi, R. *et al.* Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. *PLoS One* **9**, (2014).
496. Adjuvants and Vaccines | Vaccine Safety. *Centers for Disease Control and Prevention* https://www.cdc.gov/vaccinesafety/concerns/adjuvants.html (2021).
497. Zhang, W. *et al.* Sm-p80-based schistosomiasis vaccine: double-blind preclinical trial in baboons demonstrates comprehensive prophylactic and parasite transmission-blocking efficacy. *Ann N Y Acad Sci* **1425**, 38–51 (2018).
498. Mo, A. X. & Colley, D. G. Workshop report: Schistosomiasis vaccine clinical development and product characteristics. *Vaccine* **34**, 995–1001 (2016).
499. Chauhan, N., Khatri, V., Banerjee, P. & Kalyanasundaram, R. Evaluating the Vaccine Potential of a Tetravalent Fusion Protein (rBmHAXT) Vaccine Antigen Against Lymphatic Filariasis in a Mouse Model. *Front Immunol* **9**, 372719 (2018).

500. Khatri, V. *et al.* Prospects of developing a prophylactic vaccine against human lymphatic filariasis – evaluation of protection in non-human primates. *Int J Parasitol* **48**, 773–783 (2018).
501. Ricciardi, A., Visitsunthorn, K., Dalton, J. P. & Ndao, M. A vaccine consisting of Schistosoma mansoni cathepsin B formulated in Montanide ISA 720 VG induces high level protection against murine schistosomiasis. *BMC Infect Dis* **16**, (2016).
502. Wei, J. *et al.* Yeast-expressed recombinant As16 protects mice against Ascaris suum infection through induction of a Th2-skewed immune response. *PLoS Negl Trop Dis* **11**, e0005769 (2017).
503. Golden, O. *et al.* Protection of cattle against a natural infection of Fasciola hepatica by vaccination with recombinant cathepsin L1 (rFhCL1). *Vaccine* **28**, 5551–5557 (2010).
504. Pérez-Caballero, R. *et al.* Pathological, immunological and parasitological study of sheep vaccinated with the recombinant protein 14-3-3z and experimentally infected with Fasciola hepatica. *Vet Immunol Immunopathol* **202**, 115–121 (2018).
505. Rivera, F. & Espino, A. M. Adjuvant-enhanced antibody and cellular responses to inclusion bodies expressing FhSAP2 correlates with protection of mice to Fasciola hepatica. *Exp Parasitol* **160**, 31–38 (2016).
506. Zafra, R. *et al.* Efficacy of a multivalent vaccine against Fasciola hepatica infection in sheep. *Vet Res* **52**, 13 (2021).
507. Wu, H. W. *et al.* Vaccination with recombinant paramyosin in Montanide ISA206 protects against Schistosoma japonicum infection in water buffalo. *Vaccine* **35**, 3409–3415 (2017).
508. Yang, J. *et al.* Partially protective immunity induced by the 14-3-3 protein from Trichinella spiralis. *Vet Parasitol* **231**, 63–68 (2016).
509. Xu, J. *et al.* Influence of adjuvant formulation on inducing immune response in mice immunized with a recombinant serpin from *Trichinella spiralis*. *Parasite Immunol* **39**, e12437 (2017).
510. Zhan, B. *et al.* Ligand binding properties of two Brugia malayi fatty acid and retinol (FAR) binding proteins and their vaccine efficacies against challenge infection in gerbils. *PLoS Negl Trop Dis* **12**, e0006772 (2018).

511. Martínez-Fernández, A. R. *et al.* Vaccination of mice and sheep with Fh12 FABP from Fasciola hepatica using the new adjuvant/immunomodulator system ADAD. *Vet Parasitol* **126**, 287–298 (2004).
512. Hernández-Goenaga, J. *et al.* Peptides Derived of Kunitz-Type Serine Protease Inhibitor as Potential Vaccine Against Experimental Schistosomiasis. *Front Immunol* **10**, 469865 (2019).
513. Vicente, B. *et al.* A Fasciola hepatica-derived fatty acid binding protein induces protection against schistosomiasis caused by Schistosoma bovis using the adjuvant adaptation (ADAD) vaccination system. *Exp Parasitol* **145**, 145–151 (2014).
514. Li, L. & Petrovsky, N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Review of Vaccines* **15**, 313–329 Preprint at https://doi.org/10.1586/14760584.2016.1124762 (2016).
515. Yang Lee, L. Y., Izzard, L. & Hurt, A. C. A review of DNA vaccines against influenza. *Frontiers in Immunology* **9**, Preprint at https://doi.org/10.3389/fimmu.2018.01568 (2018).
516. Porter, K. R. & Raviprakash, K. DNA Vaccine Delivery and Improved Immunogenicity. *Current issues in molecular biology* **22**, 129–138 Preprint at https://doi.org/10.21775/cimb.022.129 (2017).
517. Suschak, J. J., Williams, J. A. & Schmaljohn, C. S. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity. *Human Vaccines and Immunotherapeutics* **13**, 2837–2848 Preprint at https://doi.org/10.1080/21645515.2017.1330236 (2017).
518. Li, L. & Petrovsky, N. Molecular Adjuvants for DNA Vaccines. *Current issues in molecular biology* **22**, 17–40 Preprint at https://doi.org/10.21775/cimb.022.017 (2017).
519. Liu, H. F., Li, W., Lu, M. B. & Yu, L. J. Pharmacokinetics and risk evaluation of DNA vaccine against Schistosoma japonicum. *Parasitol Res* **112**, 59–67 (2013).
520. Kutzler, M. A. & Weiner, D. B. DNA vaccines: Ready for prime time? *Nature Reviews Genetics* **9**, 776–788 Preprint at https://doi.org/10.1038/nrg2432 (2008).
521. Tang, F., Xu, L., Yan, R., Song, X. & Li, X. A DNA vaccine co-expressing Trichinella spiralis MIF and MCD-1 with murine ubiquitin induces partial protective immunity in mice. *J Helminthol* **87**, 24–33 (2013).

522. Gonçalves De Assis, N. R. *et al.* DNA Vaccine Encoding the Chimeric Form of Schistosoma mansoni Sm-TSP2 and Sm29 Confers Partial Protection against Challenge Infection. *PLoS One* **10**, (2015).
523. Yang, Y. *et al.* Protective immune response induced by co-immunization with the Trichinella spiralis recombinant Ts87 protein and a Ts87 DNA vaccine. *Vet Parasitol* **194**, 207–210 (2013).
524. Wang, J. *et al.* Vaccination with DNA encoding ES 43-kDa /45-kDa antigens significantly reduces Trichinella spiralis infection in mice. *Res Vet Sci* **120**, 4–10 (2018).
525. Gupta, J., Misra, S. & Misra-Bhattacharya, S. Immunization with Brugia malayi myosin as heterologous DNA prime protein boost induces protective immunity against B. Malayi infection in Mastomys coucha. *PLoS One* **11**, (2016).
526. Joardar, N., Mondal, C. & Sinha Babu, S. P. A review on the interactions between dendritic cells, filarial parasite and parasite-derived molecules in regulating the host immune responses. *Scandinavian Journal of Immunology* **93**, Preprint at https://doi.org/10.1111/sji.13001 (2021).
527. Sharma, A., Sharma, P., Vishwakarma, A. L. & Srivastava, M. Functional impairment of murine dendritic cell subsets following infection with infective larval stage 3 of Brugia malayi. *Infect Immun* **85**, (2017).
528. Zhang, W. *et al.* Fifteen Years of Sm-p80-Based Vaccine Trials in Nonhuman Primates: Antibodies From Vaccinated Baboons Confer Protection in vivo and in vitro From Schistosoma mansoni and Identification of Putative Correlative Markers of Protection. *Front Immunol* **11**, (2020).
529. Torben, W. *et al.* Role of antibody dependent cell mediated cytotoxicity (ADCC) in Sm-p80-mediated protection against Schistosoma mansoni. *Vaccine* **30**, 6753–8 (2012).
530. Versteeg, L., Almutairi, M. M., Hotez, P. J. & Pollet, J. Enlisting the mRNA vaccine platform to combat parasitic infections. *Vaccines* **7**, Preprint at https://doi.org/10.3390/vaccines7040122 (2019).
531. Oliver, S. E. *et al.* The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Pfizer-BioNTech COVID-19 Vaccine — United States, December 2020. *Morbidity and Mortality Weekly Report* **69**, 1922 (2020).

532. FDA & CBER. *Vaccines and Related Biological Products Advisory Committee December 17, 2020 Meeting Briefing Document - FDA*. (2020).
533. Maruggi, G., Zhang, C., Li, J., Ulmer, J. B. & Yu, D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. *Molecular Therapy* **27**, 757–772 Preprint at https://doi.org/10.1016/j.ymthe.2019.01.020 (2019).
534. Jackson, L. A. *et al.* An mRNA Vaccine against SARS-CoV-2 — Preliminary Report. *New England Journal of Medicine* **383**, 1920–1931 (2020).
535. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine* **383**, 2603–2615 (2020).
536. Cafri, G. *et al.* mRNA vaccine–induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. *Journal of Clinical Investigation* **130**, 5976–5988 (2020).
537. Maruyama, J. *et al.* Adenoviral vector-based vaccine is fully protective against lethal Lassa fever challenge in Hartley guinea pigs. *Vaccine* **37**, 6824–6831 (2019).
538. Norton, T. D. *et al.* Lentiviral Vector-Based Dendritic Cell Vaccine Suppresses HIV Replication in Humanized Mice. *Molecular Therapy* **27**, 960–973 (2019).
539. Yusuf, Y. *et al.* A viral-vectored multi-stage malaria vaccine regimen with protective and transmission-blocking efficacies. *Front Immunol* **10**, (2019).
540. Rothel, J. S. *et al.* Sequential nucleic acid and recombinant adenovirus vaccination induces host-protective immune responses against Taenia ovis infection in sheep. *Parasite Immunol* **19**, 221–227 (1997).
541. Smaill, F. *et al.* A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. *Sci Transl Med* **5**, (2013).
542. Liu, F. *et al.* Development of recombinant goatpox virus expressing Echinococcus granulosus EG95 vaccine antigen. *J Virol Methods* **261**, 28–33 (2018).
543. Liu, F. *et al.* Development of reverse genetics system for small ruminant morbillivirus: Rescuing recombinant virus to express Echinococcus granulosus EG95 antigen. *Virus Res* **261**, 50–55 (2019).
544. Marsland, B. J., Tisdall, D. J., Heath, D. D. & Mercer, A. A. Construction of a recombinant orf virus that expresses an Echinococcus granulosus vaccine antigen from a novel genomic insertion site. *Arch Virol* **148**, 555–562 (2003).

545. Tan, J. L., Ueda, N., Heath, D., Mercer, A. A. & Fleming, S. B. Development of orf virus as a bifunctional recombinant vaccine: Surface display of Echinococcus granulosus antigen EG95 by fusion to membrane structural proteins. *Vaccine* **30**, 398–406 (2012).
546. Dutton, S. *et al.* Delivery of Echinococcus granulosus antigen EG95 to mice and sheep using recombinant vaccinia virus. *Parasite Immunol* **34**, 312–317 (2012).
547. Hota-Mitchell, S., Clarke, M. W., Podesta, R. B. & Dekaban, G. A. Recombinant vaccinia viruses and gene gun vectors expressing the large subunit of Schistosoma mansoni calpain used in a murine immunization- challenge model. *Vaccine* **17**, 1338–1354 (1999).
548. Wei, F. *et al.* Development and immunogenicity of a recombinant pseudorabies virus expressing Sj26GST and SjFABP from Schistosoma japonicum. *Vaccine* **28**, 5161–5166 (2010).
549. Dong, B., Zarlenga, D. S. & Ren, X. An overview of live attenuated recombinant pseudorabies viruses for use as novel vaccines. *Journal of Immunology Research* **2014**, Preprint at https://doi.org/10.1155/2014/824630 (2014).
550. Kelly, B. J., Fraefel, C., Cunningham, A. L. & Diefenbach, R. J. Functional roles of the tegument proteins of herpes simplex virus type 1. *Virus Research* **145**, 173–186 Preprint at https://doi.org/10.1016/j.virusres.2009.07.007 (2009).
551. Dai, Y. *et al.* Construction and evaluation of replication-defective recombinant optimized triosephosphate isomerase adenoviral vaccination in Schistosoma japonicum challenged mice. *Vaccine* **32**, 771–778 (2014).
552. Dai, Y. *et al.* Enhancement of Protective Efficacy through Adenoviral Vectored Vaccine Priming and Protein Boosting Strategy Encoding Triosephosphate Isomerase (SjTPI) against Schistosoma japonicum in Mice. *PLoS One* **10**, e0120792 (2015).
553. Logunov, D. Y. *et al.* Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *The Lancet* **396**, 887–897 (2020).
554. Folegatti, P. M. *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *The Lancet* **396**, 467–478 (2020).

555. Milligan, I. D. *et al.* Safety and immunogenicity of novel adenovirus type 26-and modified vaccinia Ankara-vectored Ebola vaccines: A randomized clinical trial. *Journal of the American Medical Association* **315**, 1610–1623 (2016).
556. Humphreys, I. R. & Sebastian, S. Novel viral vectors in infectious diseases. *Immunology* **153**, 1–9 Preprint at https://doi.org/10.1111/imm.12829 (2018).
557. Sun, W. *et al.* Newcastle disease virus (NDV) expressing the spike protein of SARS-CoV-2 as a live virus vaccine candidate. *EBioMedicine* **62**, 103132 (2020).
558. Diemert, D. J. *et al.* Generalized urticaria induced by the Na-ASP-2 hookworm vaccine: Implications for the development of vaccines against helminths. *Journal of Allergy and Clinical Immunology* **130**, 169-176.e6 (2012).
559. Riveau, G. *et al.* Safety and efficacy of the rSh28GST urinary schistosomiasis vaccine: A phase 3 randomized, controlled trial in Senegalese children. *PLoS Negl Trop Dis* **12**, e0006968 (2018).
560. Diemert, D. J. *et al.* Safety and immunogenicity of the Na-GST-1 hookworm vaccine in Brazilian and American adults. *PLoS Negl Trop Dis* **11**, e0005574 (2017).
561. Safety and Immunogenicity Study of Na-GST-1 With or Without CpG. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT02143518?term=helminth+vaccine&draw=3 .
562. Safety and Immunogenicity of a Human Hookworm Candidate Vaccine With Different Doses of a Novel Adjuvant. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT01385189?term=helminth+vaccine&draw=3 .
563. Safety and Immunogenicity of the Na-APR-1 Hookworm Vaccine in Healthy Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT01717950?term=helminth+vaccine&draw=2 &rank=10.
564. Safety and Immunogenicity of Co-Administered Hookworm Vaccine Candidates Na-GST-1 and Na-APR-1 in Gabonese Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT02126462?term=helminth+vaccine&draw=2 .

565. Adegnika, A. A. *et al.* Safety and immunogenicity of co-administered hookworm vaccine candidates Na-GST-1 and Na-APR-1 in Gabonese adults: a randomised, controlled, double-blind, phase 1 dose-escalation trial. *Lancet Infect Dis* **21**, 275–285 (2021).
566. Immunisation, Treatment and Controlled Human Hookworm Infection. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03702530.
567. Phase 1 Trial of Na-ASP-2 Hookworm Vaccine in Previously Infected Brazilian Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT00473967?term=vaccine&cond=hookworm&draw=2&rank=1.
568. Bethony, J. M. *et al.* Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 Hookworm Vaccine in unexposed adults. *Vaccine* **26**, 2408–2417 (2008).
569. Study of Na-ASP-2 Human Hookworm Vaccine in Healthy Adults Without Evidence of Hookworm Infection. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT00120081?term=vaccine&cond=hookworm&draw=2&rank=3.
570. Efficacy of Bilhvax in Association With Praziquantel for Prevention of Clinical Recurrences of Schistosoma Haematobium. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT00870649?term=vaccine&cond=schistosomiasis&draw=2.
571. Riveau, G. *et al.* Safety and Immunogenicity of rSh28GST Antigen in Humans: Phase 1 Randomized Clinical Study of a Vaccine Candidate against Urinary Schistosomiasis. *PLoS Negl Trop Dis* **6**, e1704 (2012).
572. Clinical Trial of Bilhvax, a Vaccine Candidate Against Schistosomiasis. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT01512277?term=vaccine&cond=schistosomiasis&draw=2.
573. Tendler, M., Almeida, M. S., Vilar, M. M., Pinto, P. M. & Limaverde-Sousa, G. Current Status of the Sm14/GLA-SE Schistosomiasis Vaccine: Overcoming Barriers and Paradigms towards the First Anti-Parasitic Human(itarian) Vaccine. *Trop Med Infect Dis* **3**, (2018) doi:10.20944/PREPRINTS201810.0334.V1.
574. Study of Safety and Immune Response of the Sm14 Vaccine in Adults of Endemic Regions. *ClinicalTrials.gov*

https://classic.clinicaltrials.gov/ct2/show/NCT03041766?term=vaccine&cond=schistosomiasis&draw=2.

575. Anti-Schistosomiasis Vaccine: Sm14 Phase 2b-Sn in School Children. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03799510?term=vaccine&cond=schistosomiasis&draw=2.
576. Study to Evaluate the Safety of the Vaccine Prepared sm14 Against Schistosomiasis. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT01154049?term=vaccine&cond=schistosomiasis&draw=2&rank=1.
577. Santini-Oliveira, M. *et al.* Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine* **34**, 586–594 (2016).
578. Sm-TSP-2 Schistosomiasis Vaccine in Healthy Ugandan Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03910972?term=vaccine&cond=schistosomiasis&draw=2.
579. A Phase Ib Study of the Safety, Reactogenicity, and Immunogenicity of Sm-TSP-2/Alhydrogel)(R) With or Without AP 10-701 for Intestinal Schistosomiasis in Healthy Exposed Adults. *ClinicalTrials.gov* https://classic.clinicaltrials.gov/ct2/show/NCT03110757?term=vaccine&cond=schistosomiasis&draw=2.
580. Keitel, W. A. *et al.* A phase 1 study of the safety, reactogenicity, and immunogenicity of a Schistosoma mansoni vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. *Vaccine* **37**, 6500–6509 (2019).
581. Bruschi, F., Korenaga, M. & Watanabe, N. Eosinophils and Trichinella infection: toxic for the parasite and the host? *Trends Parasitol* **24**, 462–467 (2008).
582. Klion, A. D. & Nutman, T. B. The role of eosinophils in host defense against helminth parasites. *Journal of Allergy and Clinical Immunology* **113**, 30–37 (2004).
583. Huang, L. & Appleton, J. A. Eosinophils in helminth infection: defenders and dupes. *Trends Parasitol* **32**, 798 (2016).
584. Gentil K, Hoerauf A & Layland LE. Eosinophil-mediated responses toward helminths. *In: Lee JJ, Rosenberg HF (eds) Eosinophils in health and disease.* 303–312 (2013).

585. Ramalho-Pinto, F. J., McLaren, D. J. & Smithers, S. R. Complement-mediated killing of schistosomula of Schistosoma mansoni by rat eosinophils in vitro. *Journal of Experimental Medicine* **147**, 147–156 (1978).
586. Ondigo, B. N. *et al.* Functional Studies of T Regulatory Lymphocytes in Human Schistosomiasis in Western Kenya. *Am J Trop Med Hyg* **98**, 1770 (2018).
587. Zhou, S. *et al.* SjHSP60 induces CD4+CD25+Foxp3+ Tregs via TLR4-Mal-drived production of TGF-β in macrophages. *Immunol Cell Biol* **96**, 958–968 (2018).
588. Zhou, W. *et al.* Inhibition of Rho-Kinase Downregulates Th17 Cells and Ameliorates Hepatic Fibrosis by Schistosoma japonicum Infection. *Cells* **8**, 1262 (2019).
589. Vuitton, D. A. Echinococcosis and allergy. *Clinical Reviews in Allergy and Immunology* **26**, 93–104 Preprint at https://doi.org/10.1007/s12016-004-0004-2 (2004).
590. Babu, S., Ganley, L. M., Klei, T. R., Shultz, L. D. & Rajan, T. V. Role of gamma interferon and interleukin-4 in host defense against the human filarial parasite Brugia malayi. *Infect Immun* **68**, 3034–3035 (2000).
591. Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology* **18**, 134–147 (2017).
592. Bouchery, T. *et al.* Hookworms Evade Host Immunity by Secreting a Deoxyribonuclease to Degrade Neutrophil Extracellular Traps. *Cell Host Microbe* **27**, 277-289.e6 (2020).
593. Bonne-Année, S. *et al.* Extracellular traps are associated with human and mouse neutrophil and macrophage mediated killing of larval Strongyloides stercoralis. *Microbes Infect* **16**, 502–511 (2014).
594. Paust, S. & Andrian, U. H. von. Natural killer cell memory. *Nature Immunology* **12**, 500–508 (2011).
595. Min-Oo, G., Kamimura, Y., Hendricks, D. W., Nabekura, T. & Lanier, L. L. Natural killer cells: walking three paths down memory lane. *Trends Immunol* **34**, 251–258 (2013).
596. MacDonald, A. J. *et al.* rOv-ASP-1, a recombinant secreted protein of the helminth Onchocerca volvulus, is a potent adjuvant for inducing antibodies to ovalbumin, HIV-1 polypeptide and SARS-CoV peptide antigens. *Vaccine* **23**, 3446–3452 (2005).
597. Van Meulder, F. *et al.* Granule exocytosis of granulysin and granzyme B as a potential key mechanism in vaccine-induced immunity in cattle against the nematode ostertagia ostertagi. *Infect Immun* **81**, 1798–1809 (2013).

598. Villa-Mancera, A., Reynoso-Palomar, A., Utrera-Quintana, F. & Carreón-Luna, L. Cathepsin L1 mimotopes with adjuvant Quil A induces a Th1/Th2 immune response and confers significant protection against Fasciola hepatica infection in goats. *Parasitol Res* **113**, 243–250 (2014).
599. Sun, G. G. *et al.* Intranasal immunization with recombinant Trichinella spiralis serine protease elicits protective immunity in BALB/c mice. *Exp Parasitol* **201**, 1–10 (2019).
600. Perera, D. J. *et al.* Adjuvanted Schistosoma mansoni-Cathepsin B With Sulfated Lactosyl Archaeol Archaeosomes or AddaVax™ Provides Protection in a Pre-Clinical Schistosomiasis Model. *Front Immunol* **11**, (2020).
601. Gupta, J., Pathak, M., Misra, S. & Misra-Bhattacharya, S. CpG enhances the immunogenicity of heterologous DNA-prime/protein-boost vaccination with the heavy chain myosin of Brugia malayi in BALB/c mice. *Parasitol Res* **118**, 1943–1952 (2019).
602. Zhu, L. *et al.* Construction, purification, and evaluation of multivalent DNA vaccine against Schistosoma japonicum. *Parasitol Res* **108**, 115–121 (2011).
603. Cohen, J. UNFILLED VIALS: Scientifically feasible vaccines against major diseases are stalled for lack of funds. Science names 10 top candidates that need a boost. *Science (1979)* **351**, 16–18 (2016).
604. Alsallaq, R. A., Gurarie, D., Ndeffo Mbah, M., Galvani, A. & King, C. Quantitative assessment of the impact of partially protective anti-schistosomiasis vaccines. *PLoS Negl Trop Dis* **11**, (2017).
605. Williams, G. M. *et al.* Mathematical modelling of schistosomiasis japonica: Comparison of control strategies in the People's Republic of China. *Acta Trop* **82**, 253–262 (2002).
606. Montresor, A., Mwinzi, P., Mupfasoni, D. & Garba, A. Reduction in DALYs lost due to soil-transmitted helminthiases and schistosomiasis from 2000 to 2019 is parallel to the increase in coverage of the global control programmes. *PLoS Negl Trop Dis* **16**, (2022).
607. Ogongo, P., Nyakundi, R. K., Chege, G. K. & Ochola, L. The Road to Elimination: Current State of Schistosomiasis Research and Progress Towards the End Game. *Front Immunol* **13**, 846108 (2022).
608. Hotez, P. J. *et al.* The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases. *PLoS Negl Trop Dis* **8**, e2865 (2014).

609. Correnti, J. M., Brindley, P. J. & Pearce, E. J. Long-term suppression of cathepsin B levels by RNA interference retards schistosome growth. *Mol Biochem Parasitol* **143**, 209–215 (2005).
610. El Ridi, R., Tallima, H., Dalton, J. P. & Donnelly, S. Induction of protective immune responses against schistosomiasis using functionally active cysteine peptidases. *Front Genet* **5**, (2014).
611. Pearce, E. J. *et al.* Th2 response polarization during infection with the helminth parasite Schistosoma mansoni. *Immunol Rev* **201**, 117–126 (2004).
612. Ricciardi, A., Dalton, J. P. & Ndao, M. Evaluation of the immune response and protective efficacy of Schistosoma mansoni Cathepsin B in mice using CpG dinucleotides as adjuvant. *Vaccine* **33**, 346–353 (2015).
613. Hassan, A. S. *et al.* Salmonella Typhimurium expressing chromosomally integrated Schistosoma mansoni Cathepsin B protects against schistosomiasis in mice. *NPJ Vaccines* **8**, (2023).
614. Hassan, A. S., Perera, D. J., Ward, B. J. & Ndao, M. Therapeutic activity of a Salmonella-vectored Schistosoma mansoni vaccine in a mouse model of chronic infection. *Vaccine* **39**, 5580–5588 (2021).
615. Bergquist, V. R. & Colley, D. G. Schistosomiasis vaccine:research to development. *Parasitol Today* **14**, 99–104 (1998).
616. Wilson, R. A., Li, X. H. & Castro-Borges, W. Do schistosome vaccine trials in mice have an intrinsic flaw that generates spurious protection data? *Parasit Vectors* **9**, (2016).
617. Lo, N. C. *et al.* Review of 2022 WHO guidelines on the control and elimination of schistosomiasis. *Lancet Infect Dis* **22**, e327–e335 (2022).
618. Kallas, E. G. *et al.* Antigenic competition in CD4+ T cell responses in a randomized, multicenter, double-blind clinical HIV vaccine trial. *Sci Transl Med* **11**, (2019).
619. Ojo, J. A. *et al.* Prevalence of urogenital and intestinal schistosomiasis among school children in South-west Nigeria. *PLoS Negl Trop Dis* **15**, (2021).
620. Jones, I. J. *et al.* Schistosome infection in Senegal is associated with different spatial extents of risk and ecological drivers for Schistosoma haematobium and S. mansoni. *PLoS Negl Trop Dis* **15**, (2021).

621. Buathong, S., Leelayoova, S., Mungthin, M. & Tan-ariya, P. Role of Cathepsin B in Schistosoma japonicum Infection. *The Journal of Tropical Medicine and Parasitology* **37**, 43–53 (2014).
622. Sangfuang, M. *et al.* Schistosoma mekongi cathepsin B and its use in the development of an immunodiagnosis. *Acta Trop* **155**, 11–19 (2016).
623. Salvin, S. B. D. *et al.* Occurrence of delayed hypersensitivity during the development of arthus type hypersensitivity. *Journal of Experimental Medicine* **107**, 109–124 (1958).
624. Rudulier, C. D., McKinstry, K. K., Al-Yassin, G. A., Kroeger, D. R. & Bretscher, P. A. The Number of Responding CD4 T Cells and the Dose of Antigen Conjointly Determine the Th1/Th2 Phenotype by Modulating B7/CD28 Interactions. *The Journal of Immunology* **192**, 5140–5150 (2014).
625. Billeskov, R., Beikzadeh, B. & Berzofsky, J. A. The effect of antigen dose on T cell-targeting vaccine outcome. *Hum Vaccin Immunother* **15**, 407 (2019).
626. Luabeya, A. K. K. *et al.* First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. *Vaccine* **33**, 4130–4140 (2015).
627. Aagaard, C. *et al.* Protection and Polyfunctional T Cells Induced by Ag85B-TB10.4/IC31® against Mycobacterium tuberculosis Is Highly Dependent on the Antigen Dose. *PLoS One* **4**, e5930 (2009).
628. Appledorn, D. M., Aldhamen, Y. A., Godbehere, S., Seregin, S. S. & Amalfitano, A. Sublingual administration of an adenovirus serotype 5 (Ad5)-based vaccine confirms Toll-like receptor agonist activity in the oral cavity and elicits improved mucosal and systemic cell-mediated responses against HIV antigens despite preexisting Ad5 immunity. *Clin Vaccine Immunol* **18**, 150–160 (2011).
629. Zaric, M. *et al.* Skin immunisation activates an innate lymphoid cell-monocyte axis regulating CD8+ effector recruitment to mucosal tissues. *Nat Commun* **10**, (2019).
630. Jeyanathan, M. *et al.* Aerosol delivery, but not intramuscular injection, of adenovirus-vectored tuberculosis vaccine induces respiratory-mucosal immunity in humans. *JCI Insight* **7**, (2022).

631. Joyce, C. *et al.* Orally administered adenoviral-based vaccine induces respiratory mucosal memory and protection against RSV infection in cotton rats. *Vaccine* **36**, 4265–4277 (2018).
632. Maunder, H. E., Taylor, G., Leppard, K. N. & Easton, A. J. Intranasal immunisation with recombinant adenovirus vaccines protects against a lethal challenge with pneumonia virus of mice. *Vaccine* **33**, 6641–6649 (2015).
633. De Andrade Pereira, B., Bouillet, L. E. M., Dorigo, N. A., Fraefel, C. & Bruna-Romero, O. Adenovirus Specific Pre-Immunity Induced by Natural Route of Infection Does Not Impair Transduction by Adenoviral Vaccine Vectors in Mice. *PLoS One* **10**, e0145260 (2015).
634. Kane, M. Unsafe injections. *Bull World Health Organ* **76**, 99–100 (1998).
635. Gyawali, S., Rathore, D. S., Shankar, P. R. & Vikash Kumar, K. C. Strategies and challenges for safe injection practice in developing countries. *J Pharmacol Pharmacother* **4**, 8 (2013).
636. Ackah, B. B. B. *et al.* COVID-19 vaccine hesitancy in Africa: a scoping review. *Glob Health Res Policy* **7**, 1–20 (2022).
637. Kašný, M. *et al.* Chapter 4 Peptidases of Trematodes. *Adv Parasitol* **69**, 205–297 (2009).
638. Sajid, M. *et al.* Functional expression and characterization of Schistosoma mansoni cathepsin B and its trans-activation by an endogenous asparaginyl endopeptidase. *Mol Biochem Parasitol* **131**, 65–75 (2003).
639. Tran, M. H. *et al.* Suppression of mRNAs encoding tegument tetraspanins from Schistosoma mansoni results in impaired tegument turnover. *PLoS Pathog* **6**, 1–10 (2010).
640. Pearson, M. S. *et al.* Enhanced protective efficacy of a chimeric form of the schistosomiasis vaccine antigen Sm-TSP-2. *PLoS Negl Trop Dis* **6**, (2012).
641. Diemert, D. J. *et al.* A randomized, controlled Phase 1b trial of the Sm-TSP-2 Vaccine for intestinal schistosomiasis in healthy Brazilian adults living in an endemic area. *PLoS Negl Trop Dis* **17**, (2023).
642. Siddiqui, A. A. & Siddiqui, S. Z. Sm-p80-Based Schistosomiasis Vaccine: Preparation for Human Clinical Trials. *Trends Parasitol* **33**, 194–201 (2017).
643. Zhang, W. W. *et al.* A second generation leishmanization vaccine with a markerless attenuated Leishmania major strain using CRISPR gene editing. *Nat Commun* **11**, (2020).

644. Fiuza, J. A. *et al.* Intradermal Immunization of Leishmania donovani Centrin Knock-Out Parasites in Combination with Salivary Protein LJM19 from Sand Fly Vector Induces a Durable Protective Immune Response in Hamsters. *PLoS Negl Trop Dis* **10**, (2016).
645. Burza, S., Croft, S. L. & Boelaert, M. Leishmaniasis. *Lancet* **392**, 951–970 (2018).
646. Shirvani, E. & Samal, S. K. Newcastle Disease Virus as a Vaccine Vector for SARS-CoV-2. *Pathogens* **9**, 619 (2020).
647. Huang, Y., Wan, H. Q., Liu, H. Q., Wu, Y. T. & Liu, X. F. Genomic sequence of an isolate of Newcastle disease virus isolated from an outbreak in geese: a novel six nucleotide insertion in the non-coding region of the nucleoprotein gene. Brief Report. *Arch Virol* **149**, 1445–1457 (2004).
648. Bukreyev, A. *et al.* Recombinant newcastle disease virus expressing a foreign viral antigen is attenuated and highly immunogenic in primates. *J Virol* **79**, 13275–13284 (2005).
649. DiNapoli, J. M. *et al.* Newcastle disease virus, a host range-restricted virus, as a vaccine vector for intranasal immunization against emerging pathogens. *Proc Natl Acad Sci U S A* **104**, 9788–9793 (2007).
650. Kackos, C. M. *et al.* mRNA Vaccine Mitigates SARS-CoV-2 Infections and COVID-19. *Microbiol Spectr* **11**, (2023).
651. Hause, A. M. *et al.* Safety Monitoring of Bivalent COVID-19 mRNA Vaccine Booster Doses Among Children Aged 5-11 Years - United States, October 12-January 1, 2023. *MMWR Morb Mortal Wkly Rep* **72**, 39–43 (2023).
652. Chahal, J. S. *et al.* Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. *Proc Natl Acad Sci U S A* **113**, E4133–E4142 (2016).
653. Luo, F. *et al.* Induction of Protective Immunity against Toxoplasma gondii in Mice by Nucleoside Triphosphate Hydrolase-II (NTPase-II) Self-amplifying RNA Vaccine Encapsulated in Lipid Nanoparticle (LNP). *Front Microbiol* **8**, (2017).
654. Baeza Garcia, A. *et al.* Neutralization of the Plasmodium-encoded MIF ortholog confers protective immunity against malaria infection. *Nature Communications* **9**, 1–13 (2018).
655. Duthie, M. S. *et al.* Heterologous Immunization With Defined RNA and Subunit Vaccines Enhances T Cell Responses That Protect Against Leishmania donovani. *Front Immunol* **9**, (2018).

656. Stitz, L. *et al.* A thermostable messenger RNA based vaccine against rabies. *PLoS Negl Trop Dis* **11**, (2017).
657. Morgan, J. A. T. *et al.* First report of a natural hybrid between Schistosoma mansoni and S. rodhaini. *J Parasitol* **89**, 416–8 (2003).
658. Ross, A. G. *et al.* First bovine vaccine to prevent human schistosomiasis - a cluster randomised Phase 3 clinical trial. *International Journal of Infectious Diseases* **129**, 110–117 (2023).
659. Meeusen, E. N. T., Walker, J., Peters, A., Pastoret, P. P. & Jungersen, G. Current Status of Veterinary Vaccines. *Clin Microbiol Rev* **20**, 489 (2007).
660. Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I. & Kappo, A. P. Impact of human schistosomiasis in sub-Saharan Africa. *The Brazilian Journal of Infectious Diseases* **19**, 196–205 (2015).
661. Nigo, M. M. *et al.* Morbidity associated with Schistosoma mansoni infection in north-eastern Democratic Republic of the Congo. *PLoS Negl Trop Dis* **15**, e0009375 (2021).