T cell Expression of Bcl-6 Curtails an Anti-helminth Immunoregulatory Network in the Intestine

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Table of Contents

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Contributions

The main objectives of the thesis were planned and directed by the principal investigator, Dr. Irah King. Experiment planning was conducted by Dr. King and Gabriel Russell. Experiments and data analysis were performed by Gabriel Russell with assistance from Dr. Irah King and members of the King laboratory. Additional technical assistance was provided by Dr. Erwan Pernet and Dr. Annie Rochette. Experimental animal models were managed and overseen by Dr. Ghislaine Fontés. Funding for this MSc thesis was provided by a Canada Graduate Scholarship – Masters Award.

Abstract

CD4+ T cells consist of multiple subtypes that contribute to the control of infectious diseases by providing help to immune and non-immune target cells. T helper 2 (Th2) and T follicular helper (Tfh) cells are two CD4+ subsets that are critical for orchestrating host immunity against helminth infection – a global health problem affecting almost 2 billion people worldwide. However, the molecular mechanisms regulating these distinct effector cell lineages remain to be clearly defined. Here we describe an unexpected role for the Tfh cell-defining transcriptional repressor, B cell lymphoma (BCL)-6, in regulating Th2 effector function during experimental helminth infection. Specifically, we discovered that intrinsic deletion of Bcl-6 in CD4+ T cells led to an increase in Th2 cell-derived Interleukin (IL)-10 and accelerated parasite expulsion. Further investigation into the mechanisms driving host resistance revealed that, in combination with the classical type 2 cytokines IL-4 and IL-13, IL-10 imparts a variety of profound changes on the cellular networks activated during helminth infection, including an enhancement of the intestinal epithelial 'weep and sweep' response as well as amplification of alternatively activated, tissue-reparative macrophage differentiation. Collectively, our work demonstrates how lineage decision-making in T lymphocytes can lead to a cascade of intercellular signaling events that determine host resistance to infection.

Résumé

Les cellules lymphocytes T CD4+ sont constituées de plusieurs sous-types qui contribuent au contrôle des maladies infectieuses en aidant les cellules cibles immunitaires et non immunitaires. Les cellules T auxiliaires 2 (Th2) et T auxiliaires folliculaires (Tfh) sont deux sous-ensembles de cellules CD4+ qui sont essentiels pour orchestrer l'immunité de l'hôte contre les helminthes, un problème sanitaire mondial affectant près de 2 milliards de personnes à travers le monde. Cependant, les mécanismes moléculaires régulant ces lignées distinctes de cellules effectrices restent à être clairement définis. Nous décrivons ici un rôle inattendu du répresseur transcriptionnel caractéristique des cellules Tfh, Bcl-6, dans la régulation de la fonction effectrice des cellules Th2 lors de l'infection par les helminthes. Plus précisément, nous avons découvert que la suppression intrinsèque de Bcl-6 dans les cellules T CD4+ entraînait une augmentation de l'interleukine 10 (IL-10) dérivée des cellules Th2 et accélérait l'expulsion du parasite. Une étude plus approfondie sur les mécanismes à l'origine de la résistance de l'hôte a révélé que, en combinaison avec les cytokines classiques de type 2, IL-4 et IL-13, IL-10 induit une variété de profondes modifications sur les réseaux cellulaires activés au cours de l'infection par les helminthes, inlcuant une amélioration de la réponse intestinale épithéliale «weep and sweep», ainsi que l'amplification de la différentiation des macrophages activés alternativement et réparateurs des tissus. Collectivement, notre travail explique comment le processus décisionnel des lymphocytes T à engendrer une lignée cellulaire spécifique peut conduire à une cascade d'événements de signalisation intercellulaire qui détermine la résistance de l'hôte à l'infection.

Introduction

Intestinal helminths are parasitic worms that infect close to 2 billion people worldwide (Bethony et al., 2006; Hotez et al., 2008). While they seldom kill the host, helminths lead to a variety of detrimental co-morbidities including diarrhea, anemia, cognitive delays, and more. In addition to these direct effects, helminths are estimated to cause significant mortality through exacerbating infection by other microbes. Indeed, studies have observed that helminth co-infection with human immunodeficiency virus (H.I.V.) or malaria, for example, can significantly worsen the transmission and outcome of these lethal diseases (Borkow and Bentwich, 2006; Druilhe et al., 2005; Salgame et al., 2013). As such, due to both direct and indirect effects, as well as their ubiquitous nature, helminths pose an infectious global health challenge of unmatched scale.

While modern sanitation has largely eradicated helminths from many regions of the world, these parasites remain incredibly pervasive, infecting almost a quarter of all humans on earth (Chan, 1997). Two major factors contribute to the unparalleled success of these worms as pathogens. First, helminth infection is often chronic, meaning that host mechanisms of expulsion are generally ineffective against these pathogens (Maizels et al., 2004). Additionally, even if helminths are cleared by way of anthelmintic drugs, re-infection rates remain high in endemic regions of the world (Jia et al., 2012), indicating there exists poor natural immunity against these parasites. To develop better therapeutic strategies to combat helminth infection and re-infection, we must first better understand fundamental aspects of the immune response they evoke. An in-depth characterization of the immune mechanisms involved in the expulsion of these parasites

will lay the foundation for strategies that can either bolster or negate mechanisms that are beneficial or counterproductive, respectively.

In contrast to microbial pathogens such as bacteria and viruses, helminth parasites almost universally elicit a distinct immune response referred to as a type 2 response. Type 2 immunity is most fundamentally characterized by the production of a specific repertoire of cytokines including IL-4, IL-5, and IL-13. The 2 major sources of these defining cytokines during infection are T helper type 2 (Th2) cells as well as innate lymphoid type 2 cells (ILC2s), and as such, these cells are generally viewed as central orchestrators of type 2 immunity. In recent years, however, it has become increasingly well-appreciated that there exist a multitude of other critical immunological events that precede, as well as follow, the production of these cytokines by ILC2s and Th2 cells (Image 1). For example, the initiation of type 2 responses has been shown to involve signalling derived from intestinal epithelial cells (IECs) at the site of infection, that ultimately drives the production of IL-4/5/13. Through a specialized subset of chemo-sensory cells termed ‘tuft cells’, IECs can directly sense the parasite-associated metabolite succinate, and respond by secreting the cytokine IL-25, which promotes ILC2 activation and Th2 differentiation (Gerbe et al., 2016; Howitt et al., 2016; von Moltke et al., 2016; Wong et al., 2007). Likewise, the release of alarmins such as IL-33 or ATP by dying IECs during the tissue-invasive stage of infection can directly and indirectly trigger type 2 cytokine release by a variety of immune cell types (Ho et al., 2007; Kurowska-Stolarska et al., 2008; Schmitz et al., 2005; Shimokawa et al., 2017), while the secretion of factors such as thymic stromal lymphopoietin (TSLP) are critical for priming the adaptive Th2 response (Ziegler and Artis, 2010). In addition to IECs, recent advances have revealed similar roles for other non-hematopoietic cell types in the initiation of type 2 responses. Intestinal neurons, for example, have been demonstrated to play an upstream

role in the sensing of helminth infection, helping drive type 2 immunity through the secretion of ILC2-activating neuropeptides such as neurominidin U (NMU) (Cardoso et al., 2017; Klose et al., 2017; Wallrapp et al., 2017). In summation, the initiation of type 2 responses against helminths appears to involve intricate co-ordination between immune and non-immune cell types, culminating in the activation of ILC2s and Th2 cells.

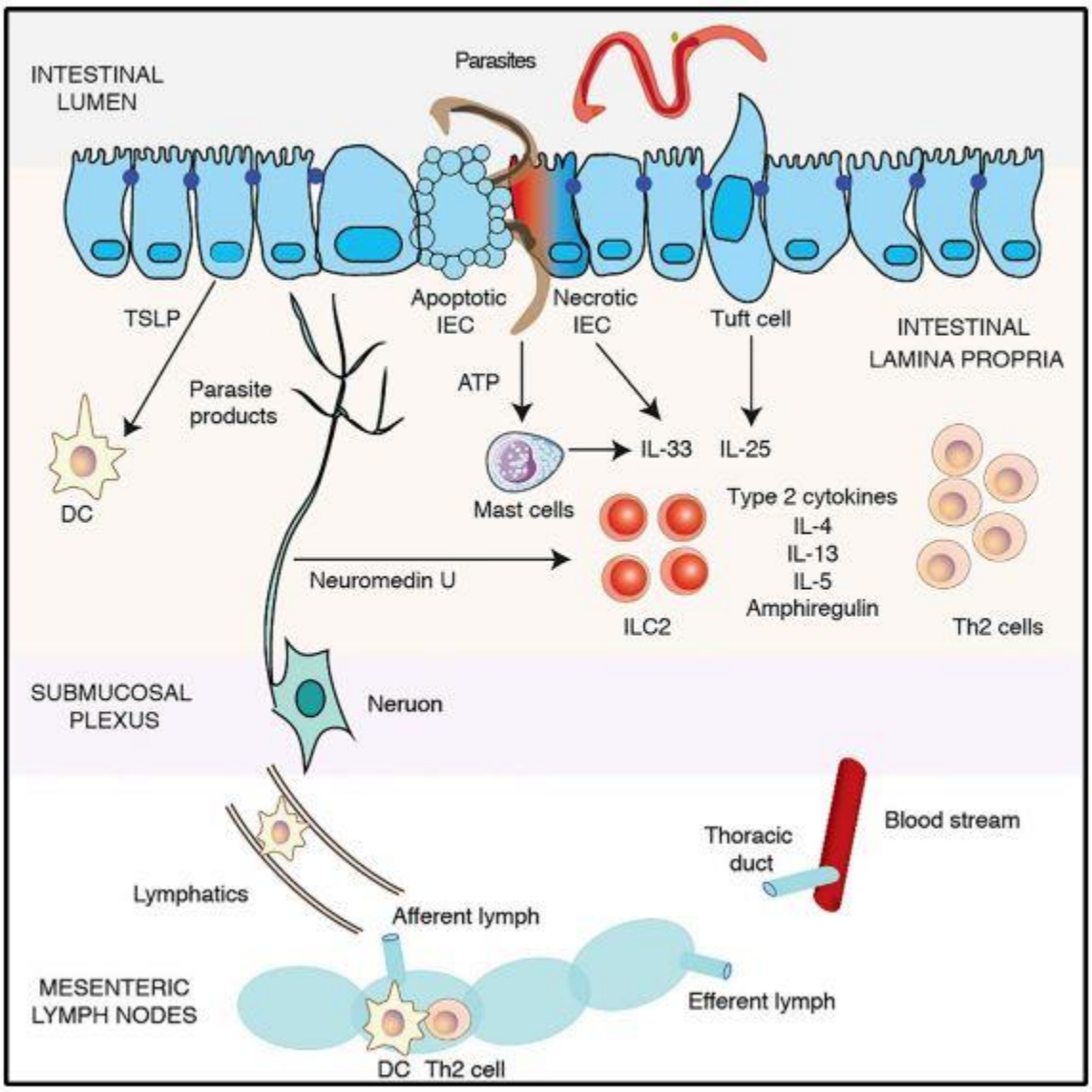


Image 1 – Overview of type 2 immunity against helminths. Type 2 immunity features cross-talk between hematopoietic and non-hematopoietic cell types, leading to the production of defining type 2 cytokines IL-4/5/13 by Th2 cells and ILC2s (Harris and Loke, 2017).

Once activated, Th2 cells and ILC2s release a variety of soluble mediators including canonical type 2 cytokines IL-4, IL-5, and IL-13. These factors exert a wide variety of profound effects on surrounding cellular networks during infection. Such changes include the recruitment and/or activation of type 2 granulocytes such as eosinophils and basophils, class-switching of B cells to produce IgE antibody, as well as changes to the intestinal epithelium and smooth muscle cells (Image 2) (Anthony et al., 2007). Despite this plethora of downstream consequences, studies utilizing animal models of helminth infection have found relatively few of these Th2-driven mechanisms to appreciably impact parasitic resistance. The murine roundworm *Heligmosomoides polygyrus bakeri* (*Hpb*), for example, has been shown to parasitize subjects despite robust IgE production and eosinophilia, hallmarks of type 2 immunity (Reynolds et al., 2012). Instead, helminth resistance appears to be impacted by only a select few Th2-driven mechanisms that include the intestinal 'weep and sweep' response, as well as the alternative activation of macrophages. Whether there exist additional Th2-driven mechanisms of helminth expulsion remains to be determined.

Overall, Th2 cells and ILC2s are central orchestrators of the type 2 immune response against helminths, but many important questions remain regarding the immunological events that precede, as well as follow, the production of IL-4/5/13 by these cells. For example, while succinate has been shown to trigger tuft cell release of IL-25, this process is not required for the expulsion of certain parasitic species (Nadjsombati et al., 2018), and type 2 responses can occur in settings where sources of this metabolite are generally absent (such as under germ-free conditions). In addition to such questions concerning upstream events in type 2 immunity, there remain even more critical knowledge gaps concerning the downstream mechanisms of Th2 cell

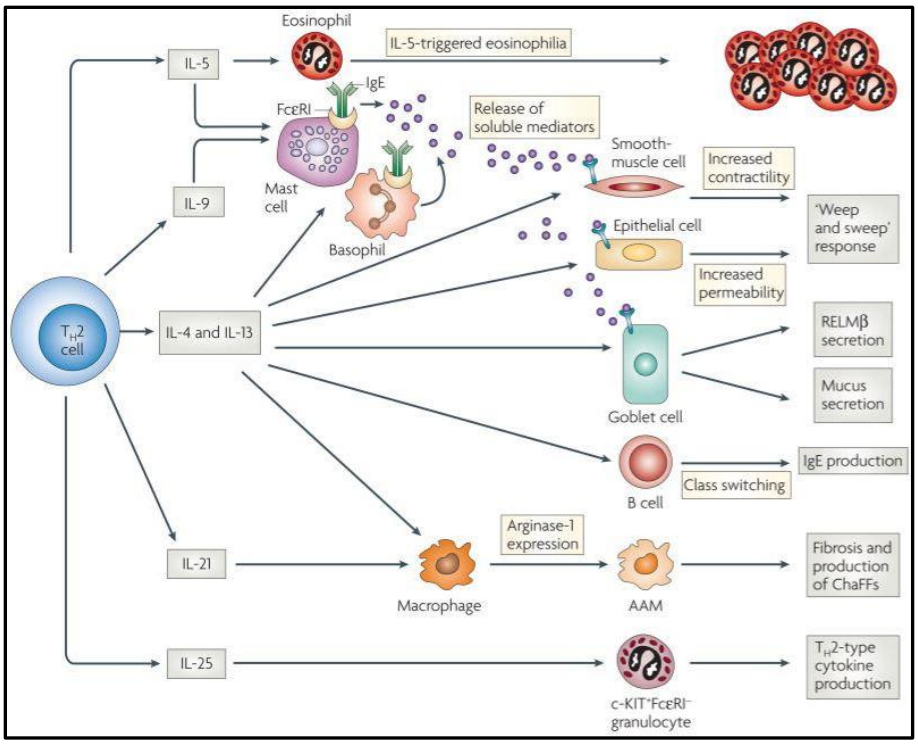


Image 2 – Downstream effects of Th2 activation. Activation of Th2 cells (as well as ILC2s) during intestinal helminth infection leads to the secretion of soluble factors including IL-4/5/13, that impart a plethora of changes on the surrounding environment, many of which are incompletely understood (Anthony *et al.*, 2007).

effector function. There exist relatively few known mechanisms of parasitic expulsion downstream of IL-4/5/13, and further, the signalling and regulators that controls these known mechanisms are poorly characterized. This lack of characterization regarding expulsion mechanisms represents a particularly important obstacle in the development of improved therapeutic and prophylactic strategies to combat helminth infection. The work in this thesis aims to better characterize and understand factors impacting mechanisms of helminth resistance. Building upon past work, these studies will help cement a foundation of knowledge that will ultimately aid in combatting parasitic infection, as well as other diseases associated with type 2 immunity.

Review of Relevant Literature

Immunity to Hpb infection

A proper characterization of anti-helminth immunity requires an understanding of cell types and mechanisms that can effectively promote parasitic expulsion. Studies utilizing the natural murine parasite *Heligmosomoides polygyrus bakeri (Hpb)* have shown CD4+ T cells to be a major immune cell type responsible for controlling parasite burden and fitness during infection. Antibody-mediated depletion of CD4+ T cells throughout the course of primary infection, for example, has been found to significantly abrogate host resistance to this pathogen (Urban et al., 1991). Similarly, studies examining the infection of severe combined immunodeficient (SCID) mice found these lymphocyte-lacking subjects to display elevated parasite burden and fitness (Urban et al., 1995). In addition to *Hpb* infection, a role for CD4+ T cells in driving primary clearance has been observed across a diversity of parasitic models, indicating a broad relevance for this immune cell type in protection against helminths (Katona et al., 1988; Koyama et al., 1995). Finally, in addition to driving primary resistance, CD4+ T cells have also been shown to be critical for the development of protective memory against *Hpb*, with depletion leading to severely compromised resistance to secondary challenge (Urban *et al.*, 1991). Thus, CD4+ T cells are universally important protectors against helminth infection and can contribute to multiple facets of parasitic resistance.

CD4+ T cells consist of multiple subsets that play unique roles during infection. The differentiation of each subset is driven by a particular cytokine environment, and similarly, effector cells of each lineage will produce distinct cytokine repertoires that define their

functional capabilities (Zhu et al., 2010). *Hpb* infection has been shown to induce high levels of type 2 cytokines including IL-4 and IL-13 (Svetić et al., 1993), which in turn are permissive to a robust expansion of Th2 cells, as well as Tfh cells (Meli et al., 2016). These two CD4+ T cell subsets contribute in unique ways to anti-*Hpb* immunity. In general, Th2 cells are considered to mediate resistance to primary *Hpb* infection, while Tfh cells are seen as essential to protection against secondary challenge. The role for Th2 cells in driving primary resistance has been demonstrated directly through adoptive transfer experiments (Minutti et al., 2017), as well as indirectly through studies revealing roles for Th2-derived cytokines IL-4 and IL-13 in driving worm expulsion (Herbert et al., 2009; Minutti *et al.*, 2017; Urban *et al.*, 1995). In contrast, the role for Tfh cells in controlling secondary resistance has only been indirectly implicated. Unlike primary infection, protection against secondary *Hpb* challenge has been shown to depend on the production of parasite-specific IgG1 antibody (McCoy et al., 2008), which mediates the entrapment of invading larvae, preventing their maturation into adult worms (Hewitson et al., 2015). Given their role in licensing antibody class-switching, Tfh cells are thus assumed to be essential for this process, though an explicit requirement for this cell type in both secondary and primary infection has never been directly assessed.

CD4+ T cell Differentiation and Function

CD4+ T cells exert their effector functions largely through the secretion of soluble mediators such as cytokines that provide help to both hematopoietic as well as non-hematopoietic cell types. Different CD4+ T cell subsets secrete unique cytokine repertoires that define their functional capabilities. On the molecular level, T cell cytokine profiles are controlled

by the expression of lineage-specific transcription factors that are activated by a combination of T cell receptor signals, co-stimulation, and polarizing cytokine responsiveness. For example, the differentiation of Th2 cells during immune responses such as that mounted against *Hpb* is governed by the lineage-defining transcription factor GATA-3 (Zheng and Flavell, 1997). GATA3 expression is induced by STAT6 signalling in response to the Th2-differentiating cytokine IL-4, and in turn this transcription factor drives the production of Th2-defining effector cytokines IL-4, IL-5, and IL-13 (though STAT6-independent Th2 differentiation has been observed) (Kaplan et al., 1996; Ouyang et al., 2000; Takeda et al., 1996). The differentiation of Tfh cells, the other major CD4+ T cell subtype induced during *Hpb* infection, is driven by high expression levels of the transcriptional repressor Bcl-6 (Johnston et al., 2009; Nurieva et al., 2009).

Additional regulatory elements outside of the subset-defining transcription factors have been found to play important roles in CD4+ T cell differentiation and function. For example, the Tfh-defining transcriptional repressor Bcl-6 has been demonstrated to exist in a mutually antagonistic relationship with another transcriptional repressor called B Lymphocyte-induced Maturation Protein-1 (BLIMP-1) (Johnston *et al.*, 2009). The balance between Bcl-6 and BLIMP-1 expression has been shown to determine the differentiation of CD4+ T cells into Tfh vs. non-Tfh effector subsets, with some studies suggesting that high BLIMP-1 levels can further promote expression of specific non-Tfh lineages (Cimmino et al., 2008; He et al., 2020). In addition to influencing differentiation, further studies have demonstrated that BLIMP-1 expression can influence the function of terminally differentiated CD4+ T cells, affecting cytokine production (Kallies et al., 2006; Martins et al., 2006). It remains unclear if Bcl-6 also influences function in such a manner, as non-Tfh CD4+ T cells downregulate and maintain very

low levels of Bcl-6 expression upon terminal differentiation (Crotty et al., 2010). In summation, CD4+ T cell differentiation is most fundamentally characterized by the expression of lineage-specific transcription factors, however additional layers of transcriptional regulators profoundly influence the processes of both differentiation and function.

Anti-helminth functions of Th2 cells – the 'weep and sweep'

Th2 cells are the major CD4+ T cell sub-type responsible for driving resistance during primary helminth infection, promoting parasite expulsion largely through the secretion of IL-4 and IL-13. The major mechanism of worm expulsion driven by these cytokines is the intestinal 'weep and sweep' response – a collection of profound changes to the gut epithelium that drive the clearance of lumen-dwelling adult parasites. There are many different facets of the intestinal 'weep and sweep' response. The most well-examined in the context of *Hpb* infection is the 'weep', which involves changes to a specialized subset of mucus-secreting IECs known as goblet cells. Type 2 cytokines induced during *Hpb* infection have been shown to alter the differentiation (Minutti *et al.*, 2017), as well as the function of intestinal goblet cells, in ways that promote parasite clearance. For example, IL-4 and IL-13 signalling drives goblet cells to synthesize increased levels of glycoproteins known as mucins (Campbell et al., 2019; Dabbagh et al., 1999; Hasnain et al., 2011). These factors, such as *Muc5ac*, have been found to directly inhibit worm fitness, and have been shown to facilitate the expulsion of helminths including *Hpb* (Hasnain *et al.*, 2011). Alongside mucins, helminth infection and associated type 2 cytokines will lead to the production of additional anti-parasitic molecules. Both IL-4 and IL-13, for example, have been shown to induce goblet cell expression of Resistin-like Molecule β (RELM-β) – that directly

inhibits feeding by multiple gastrointestinal nematodes including *Hpb* (Artis et al., 2004; Herbert *et al.,* 2009). Altogether, the intestinal 'weep' response comprises a diversity of events that alter both the differentiation and function of goblet cells. While these various changes often go hand in hand (ie: increased mucin secretion accompanying increased goblet cell differentiation), this may not always be the case. In one study of note, CD4+ T cell depletion during infection with *Nippostronglyus braziliensis* was found to compromise intestinal mucus output, while having no affect on total goblet cell levels (Khan et al., 1995). Thus, while often linked, the unique sub-components of the intestinal 'weep' response may be regulated by independent mechanisms.

In addition to changes in goblet cells and their associated factors, there exist additional less-examined alterations to the gut epithelium that occur in response to Th2-derived cytokines, that altogether comprise the 'sweep' portion of this Th2-driven response. An increased rate of epithelial cell proliferation and turnover in response to IL-13, for example, has been shown to impair the survival of the colon-dwelling whipworm model *Trichuris muris* (Cliffe et al., 2005). Additionally, elevated rates of smooth muscle contraction in response to IL-4 and IL-13 has been shown to occur during infection with multiple helminth species (Akiho et al., 2002; Zhao et al., 2003; Zhao et al., 2008), with one study suggesting these alterations may impact worm clearance (Zhao et al., 2008). Finally, type 2 cytokines have also been demonstrated to exert other effects on intestinal epithelial cell function such as increased permeability and electrolyte secretion (Shea-Donohue et al., 2001), exerting putative negative effects on the survival of luminal-dwelling parasites.

Anti-helminth functions of Th2 cells – AAMs

In addition to promoting the intestinal 'weep and sweep' response, Th2 cell-derived cytokines orchestrate a plethora of other events during a type 2 immune response. Another major Th2-induced mechanism of parasitic resistance is the induction of alternatively activated macrophages (AAMs) at the site of infection. AAMs are a phenotype of macrophage induced by type 2 cytokines and are denoted by the expression of markers such as CD206, arginase1, and PD-L2. Th2 cell-derived IL-4 and IL-13 has been shown to drive the alternative activation of macrophages *in vivo* (Bosurgi et al., 2017), and the accumulation of AAMs has been shown to occur during *Hpb* infection (Anthony et al., 2006).

AAMs play a variety of important roles during helminth infection, as well as other models of infection and immunity. For example, AAMs have been shown to promote tissue repair in response to intestinal damage (Anthony *et al.*, 2006; Mantovani et al., 2013). During *Hpb* infection, these cells have also been described as having an important niche role in mediating protection against secondary challenge (Anthony *et al.*, 2006). Indeed, in addition to depending on the production of IgG1 for larval entrapment, protection against secondary *Hpb* infection also requires arginase1-expressing AAMs (Anthony *et al.*, 2006), which have been suggested to promote parasitic clearance through Fc-receptor engagement (Esser-von Bieren et al., 2015).

While AAMs clearly promote resistance to secondary infection, the function of these cells during primary infection, a setting in which parasite-specific antibodies have yet to be generated, remains unclear. Macrophages have been shown to be capable of modulating intestinal epithelial turnover through the secretion of Wnt or epidermal growth factor (EGF)

family ligands (Minutti et al., 2019; Saha et al., 2016), suggesting a role in parasitic expulsion through modulation of the intestinal 'weep and sweep' response. One study even found muscularis-resident AAMs to facilitate smooth muscle contraction in response to IL-4 and IL-13 signalling during *Nippostrongylus braziliensis* infection (Zhao *et al.*, 2008). Despite promoting parasitic expulsion in certain contexts, however, AAMs have paradoxically been shown to exert immunosuppressive affects on T cell responses during helminth infection (Loke et al., 2000). Taken together, AAMs play diverse roles during helminth infection, all of which have important consequences for host immunity and tolerance.

IL-10 as a regulator of resistance mechanisms

The full array of factors that regulate mechanisms of parasitic resistance remain incompletely characterized, and it has not been fully elucidated what other cytokines can contribute to regulating these processes. IL-10 is an example of a cytokine that has gone relatively underexamined in the context of *Hpb* infection, and its potential effects on the 'weep and sweep' response and the alternative activation of macrophages. This is despite IL-10 having been shown to have a multitude of potential cellular sources, and even being initially described as a Th2 cytokine (Ouyang and O'Garra, 2019). The few studies that have examined IL-10 during *Hpb* infection have focused on protective roles for this cytokine during experimental colitis (Setiawan et al., 2007), while the effects of IL-10 on mechanisms of parasitic resistance have gone unexamined. Despite a lack of direct experimental assessment, IL-10 has generally been assumed to play an immunosuppressive role, promoting chronicity of infection. This assumption is based on the immunosuppressive role IL-10 has been observed to play in many

contexts of infection (Ouyang and O'Garra, 2019; Schopf et al., 2002), as well as one study observing that *Hpb* has evolved to secrete a T regulatory cell (Treg)-inducing Transforming growth factor β (TGF-β) mimic (where induced Tregs produce IL-10 in addition to TGF- β) (Johnston et al., 2017).

One recent publication examining the colonic whipworm *trichuris muris* found that mice lacking IL-10Rα displayed significantly worsened mortality during infection (Duque-Correa et al., 2019). Interestingly, this was attributed to decreased levels of colonic goblet cells. These data suggest that IL-10 may play a role driving the intestinal 'weep and sweep' response, and parasite expulsion in certain contexts, though this possibility has not been explored in other contexts of helminth infection, including *Hpb* infection. Further studies into whether IL-10 can impact goblet cell responses, and other measures of mucosal anti-parasitic defense, will provide a better understanding of possible pathways through which anti-helminth immunity can be amplified and bolstered.

Preliminary Findings

Parasite-specific IgG1 antibody production has been shown to be required for resistance against secondary *Hpb* infection (McCoy *et al.*, 2008). Presumably, Tfh cells (which license the class-switching of antibody isotypes) are required for this protection, however the importance of this cell type has never been explicitly shown. Previous work from our lab sought to validate the requirement for Tfh cells in protection against secondary *Hpb* challenge (Meli, 2019). To this end, resistance to *Hpb* infection was assessed utilizing 4get.KN2.Bcl6$^{fl/fl}$CD4-cre mice that

harbour an intrinsic deletion of the Tfh-defining transcriptional repressor Bcl-6 in all CD4+ T cells, and are bred on an IL-4 dual-reporter '4get.KN2' background (Mohrs et al., 2005). As expected, 4get.KN2.Bcl6$^{fl/fl}$CD4-cre subjects (henceforth referred to as simply Bcl6$^{fl/fl}$CD4-cre) were found to display significantly impaired resistance to secondary *Hpb* challenge, relative to 4get.KN2.Bcl6$^{fl/fl}$ littermate controls (henceforth referred to as simply Bcl6$^{fl/fl}$).

In addition to secondary resistance, the ability of these mice to control primary *Hpb* infection was also assessed. Given that class-switched antibody has been demonstrated to not impact worm burden during primary *Hpb* infection (McCoy *et al.*, 2008), it was expected that primary resistance would be unaltered by the intrinsic deletion of the transcriptional repressor Bcl-6. Unexpectedly, Bcl6$^{fl/fl}$CD4-cre mice displayed a significant acceleration of parasite expulsion relative to Bcl6$^{fl/fl}$ controls by 4 weeks post-infection. Resistance to primary *Hpb* infection has been demonstrated to be a Th2-driven process (Minutti *et al.*, 2017), however Bcl6$^{fl/fl}$CD4-cre mice have previously been shown to display unaltered rates of Th2 cell differentiation during *Hpb* infection (Meli et al., 2017). Additionally, it was found that the production of canonical type 2 cytokines IL-4, IL-5, and IL-13 by Th2 cells during *Hpb* infection was unchanged in Bcl6$^{fl/fl}$CD4-cre mice relative to Bcl6$^{fl/fl}$ controls, in both the draining lymph nodes as well as at the site of infection in the intestine.

Previously it has been demonstrated that elevated levels of the transcriptional repressor BLIMP-1 in Th2 cells results in elevated IL-10 production (Poholek et al., 2016). Since Bcl-6 exists in a mutually antagonistic relationship with BLIMP-1 (Johnston *et al.*, 2009), it was hypothesized that Th2 cells in Bcl6$^{fl/fl}$CD4-cre mice produce elevated IL-10 levels during *Hpb* infection. Indeed, it was found that Th2 cells, but not other CD4+ T cell subsets, isolated from the mesenteric lymph nodes and small intestine produced increased IL-10 levels during *Hpb*

infection compared to littermate controls. Mice harbouring a selective Bcl-6 deletion in B cells ($Bcl6^{fl/fl}$Mb1-cre) were not found to display elevated Th2 cell production of IL-10, indicating the source of elevated cytokine production was not a result of germinal centre loss or lymph node architectural defects. Additionally, bone marrow chimera experiments were employed to demonstrate that Bcl-6 acts in a T cell intrinsic manner to curb IL-10 production by Th2 cells. IL-10 signalling has not been shown previously to be capable of driving *Hpb* expulsion, however antibody-mediated blockade of the IL-10R was found to significantly abrogate increased resistance in $Bcl6^{fl/fl}$CD4-cre mice. These collective results formed the foundation for the studies comprising this thesis, seeking to better understand the consequences of Bcl-6 signalling, and IL-10-driven expulsion during *Hpb* infection.

Objective and Hypothesis

These preliminary findings indicate that Th2 cell-derived IL-10 acts to promote *Hpb* expulsion during primary infection (Image 3).

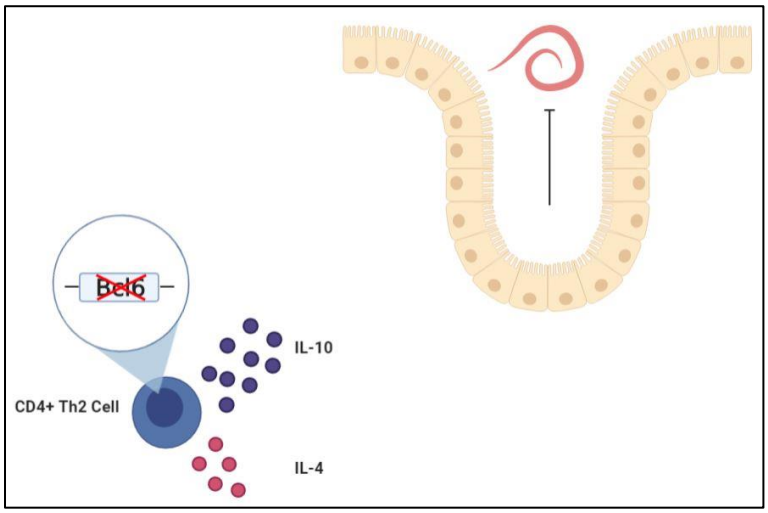


Image 3. Preliminary findings concerning Bcl6$^{fl/fl}$CD4-cre mice. Preliminary data from our laboratory has revealed the intrinsic deletion of Bcl-6 in CD4+ T cells to lead to a cell-intrinsic elevation of IL-10 production by Th2 cells during *Hpb* infection, ultimately promoting accelerated parasite clearance (Meli, 2019).

The objective of this thesis project is to assess the effect of IL-10 on canonical mechanisms of primary resistance to *Hpb*. To assess this objective, we have devised two major aims:

1. Assess the effects of Th2 cell-derived IL-10 on the intestinal 'weep and sweep' response in Bcl6$^{fl/fl}$CD4-cre mice.
2. Assess the effects of IL-10 on the alternative activation of macrophages.

We hypothesize that Th2-derived IL-10 in Bcl6$^{fl/fl}$CD4-cre mice will lead to an amplification of both the intestinal 'weep and sweep' response, as well as the alternative activation of macrophages, synergizing to promote IL-10R-dependent parasite expulsion.

Results

Bcl6$^{fl/fl}$CD4-cre mice exhibit an enhanced intestinal 'weep and sweep' response.

Our preliminary findings establish that Th2 cell-derived IL-10 augments resistance in Bcl6$^{fl/fl}$CD4-cre mice (Meli, 2019). We first sought to identify how worm expulsion is enhanced by the intrinsic deletion of Bcl-6 in CD4+ T cells. To this end, we assessed key readouts of the most well-established means of *Hpb* expulsion - the intestinal 'weep and sweep' response, which we hypothesized to be enhanced in Bcl6$^{fl/fl}$CD4-cre subjects. Periodic-Acid Schiff (P.A.S.; labels mucous) staining of intestinal tissue at two weeks post-*Hpb* infection revealed Bcl6$^{fl/fl}$CD4-cre mice to display significant goblet cell hyperplasia relative to Bcl6$^{fl/fl}$ controls (Figure 1A and B). Analysis of 5-bromo-2'-deoxyuridine (BrdU) incorporation to label proliferating cells further revealed greater intestinal epithelial cell proliferation in Bcl6$^{fl/fl}$CD4-cre mice relative to controls (Figure 1C). Similarly, IECs isolated from the duodenum of two-week infected Bcl6$^{fl/fl}$CD4-cre mice were found to display a modest, but significant elevation of Ki67 expression (Figure 1D and E). Overall, these data demonstrate that Bcl6$^{fl/fl}$CD4-cre mice exhibit an enhanced 'weep and sweep' response, likely mediating enhanced worm expulsion.

IL-10R signalling drives the enhanced intestinal 'weep and sweep' response in Bcl6$^{fl/fl}$CD4-cre mice.

We next sought to assess if the enhanced intestinal 'weep and sweep' response in Bcl6$^{fl/fl}$CD4-cre mice requires IL-10R signalling, as IL-10 has generally not been shown to be a driver of these mucosal defences during *Hpb* infection. Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ control mice

were subjected to αIL-10R antibody treatment throughout the course of infection, and 'weep and sweep' parameters were assessed. IL-10R blockade was found to significantly abrogate the increase in goblet cell levels observed in Bcl6$^{fl/fl}$CD4-cre mice (Figure 2A and B), and treatment appeared to also curb goblet cell formation to an extent in Bcl6$^{fl/fl}$ mice. A similar effect of IL-10R blockade was observed on BrdU incorporation in the intestinal epithelium (Figure 2C). Altogether, these findings demonstrate that IL-10R signalling enhances the intestinal 'weep and sweep' response in Bcl6$^{fl/fl}$CD4-cre mice.

Bcl6$^{fl/fl}$CD4-cre mice display elevated intestinal pSTAT3.

Having established these changes in the 'weep and sweep' response to be IL-10R-signalling dependent, we next sought to elucidate if they were a result of Th2-derived IL-10 acting directly on the intestinal epithelium. Activation of the IL-10R induces the phosphorylation of STAT3 (Rodig et al., 1998; Weber-Nordt et al., 1996), and this post-translational modification can be used as a proxy for IL-10R engagement on cells. We assessed phosphorylated-STAT3 (pSTAT3) levels in the intestine of two-week infected Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ control mice to assay for alterations induced by IL-10R signalling. pSTAT3 levels were found to be noticeably elevated throughout the intestine of Bcl6$^{fl/fl}$CD4-cre mice (Figure 3). This included epithelial cells comprising the lengths of intestinal villi, as well as non-epithelial cells located throughout the lamina propria. Bcl6$^{fl/fl}$ control mice displayed an absence of any pSTAT3 throughout the epithelial layer, and infrequent signal throughout the lamina propria. Overall, these results are highly suggestive of a direct effect of IL-10 on the intestinal epithelium in Bcl6$^{fl/fl}$CD4-cre mice, and further suggest that this cytokine impacts other cell types throughout the lamina propria.

IL-10 enhances IL-4-driven alternative activation of macrophages.

We next sought to better understand how Th2-derived IL-10 in Bcl6$^{fl/fl}$CD4-cre mice could be impacting immune cellular networks, apart from driving changes in the epithelium. In addition to the 'weep and sweep' response, Th2 cells promote the accumulation of AAMs during helminth infection (Anthony *et al.*, 2006). These cells have been shown to be critical for tissue repair, and can play varied roles in helminth infection, ranging from T cell suppression to driving expulsion (Anthony *et al.*, 2006; Loke *et al.*, 2000; Mantovani *et al.*, 2013). Previous work from our laboratory found Bcl6$^{fl/fl}$CD4-cre mice to display an increase in CD206+ AAM accumulation at both the mesenteric lymph nodes and small intestine during *Hpb* infection (Meli, 2019). Interestingly, this accumulation of AAMs was found to be IL-10R-signalling dependent, indicating that Th2-derived IL-10 is driving this process. Typically, alternative activation of macrophages has been shown to be driven by Th2-cell derived IL-4 or IL-13 (Anthony *et al.*, 2006; Bosurgi *et al.*, 2017), though it has been suggested that IL-10 can augment this process (Makita et al., 2015; Van de Velde et al., 2017). We sought to assess this possibility *in vitro*, and to this end, we cultured bone-marrow derived macrophages (BMDMs) from C57BL/6-WT mice, and measured expression of markers of alternative activation after 24 hours of stimulation with recombinant cytokine. While IL-10 alone did not induce expression levels of any markers of alternative activation, as expected, it was found to significantly enhance IL-4 driven expression of arginase1 (Figure 4A). Similarly, levels of active arginase enzyme were found to be significantly increased in cell lysates from BMDMs dual-stimulated with IL-4 and IL-10, relative to those stimulated with IL-4 alone (Figure 4B).

As a more physiologically relevant method of macrophage stimulation, we stimulated BMDMs with supernatants from naïve CD4+ T cells polarized under Th2 conditions *in vitro*.

Th2 cells from Bcl6$^{fl/fl}$CD4-cre mice expectedly displayed a striking increase in IL-10 secretion after 72 hours of stimulation, relative to Bcl6$^{fl/fl}$ controls, with negligible increases in IL-4 and IL-13 (Figure 4C). BMDMs stimulated with these supernatants were found to express significantly higher levels of arginase1 expression (Figure 4D), with mostly complete abrogation through IL10R blockade. We finally sought to assess if the striking changes in arginase1 expression observed *in vitro* would also be found under conditions of elevated IL-10 *in vivo*, in Bcl6$^{fl/fl}$CD4-cre mice. Due to difficulties observing differences in gene expression through qPCR of whole gut tissue, we adopted an *in situ* hybridization technique, RNAscope, to visualize arginase1 transcript at the site of infection. At 2 weeks post-*Hpb* infection, analysis of duodenal sections revealed Bcl6$^{fl/fl}$CD4-cre mice to display elevated arginase1 transcript levels relative to Bcl6$^{fl/fl}$ controls (Figure 3E). Interestingly, the majority of arginase1 transcript was found localized to large aggregates in the muscularis layer of the intestine, though some signal was observed within the lamina propria. Altogether, these findings demonstrate that IL-10 alone is sufficient to significantly augment IL-4 driven alternative macrophage activation *in vitro*, and that markers of alternative activation are elevated in Bcl6$^{fl/fl}$CD4-cre mice during *Hpb* infection.

IL-10 and IL-4 have additive effects on macrophage metabolism.

Upon stimulation with IL-4, macrophages have been shown to upregulate processes promoting fatty acid oxidation, ultimately fueling an increase in mitochondrial oxidative phosphorylation (Huang et al., 2014). This alteration of cellular metabolism is a key phenotypic change of AAMs, and studies have shown these modifications to even be essential to the polarization process (Vats et al., 2006). We sought to assess if synergistic alterations to cellular metabolism would accompany the changes in gene expression observed in macrophages dual-

stimulated with IL-4 and IL-10. Analysis of macrophage bioenergetics found IL-10 and IL-4 to impart a modest increase in overall levels of oxygen consumption (a readout of oxidative phosphorylation) relative to IL-4 alone (Figure 5A), while the rate of extracellular acidification (a readout of glycolysis) remained unaffected by combinatorial stimulus (Figure 5B). Analysis of metabolic sub-parameters found this trend to be upheld, where dual cytokine stimulation had a modest but, non-significant effect on different sub-components of macrophage metabolism (Figure 5C-F). In particular, spare respiratory capacity and maximal respiration appeared to be somewhat increased in IL4/10 stimulated macrophages (Figure 5E & F), however relative to IL-4 alone these increases did not reach statistical significance. Additionally, IL-10 stimulation itself appeared to induce minor metabolic changes to macrophages. Altogether, these *in vitro* findings reveal IL-10 and IL-4 to have additive, but not synergistic effects on AAM metabolism.

IL-10 and IL-4 synergize to induce an immunosuppressive AAM phenotype

We next sought to assess other functional impacts that IL-10 could be imparting on AAMs. While AAMs have been shown to drive expulsion in niche contexts of *Hpb* infection (Anthony *et al.*, 2006), these cells have paradoxically been shown to inhibit T cell responses during helminth infection, through the expression of T cell inhibitory ligands such as Programmed Death Ligand 2 (PDL2) (Loke *et al.*, 2000). Flow cytometric analysis of cytokine stimulated BMDMs revealed IL-4 and IL-10 to synergistically induce high expression levels of PDL2, relative to macrophages stimulated with IL-4 alone (Figure 6A & B). In line with the high expression of this T cell inhibitory marker, we found that, while macrophages stimulated with IL-4 alone exhibited a clear upregulation of antigen presentation molecule major histocompatibility complex II (MHC-II), dual-stimulation of macrophages with IL-4 and IL-10

led to a near complete abrogation of this expression (Figure 6C & D). Finally, we sought to assess if this phenotype resultant from IL-10 and type 2 cytokine synergy, namely the striking abrogation of MHC-II expression, would be observable *in vivo*. Immunofluorescence staining of duodenal tissue from 2-week *Hpb* infected Bcl6$^{fl/fl}$CD4-cre mice revealed a stark abrogation of MHC-II expression levels throughout the intestinal lamina propria and muscularis layer, relative to Bcl6$^{fl/fl}$ controls (Figure 6E). Overall, these data reveal that IL-10 selectively augments immunosuppressive AAM qualities, that are observable in Bcl6$^{fl/fl}$CD4-cre mice during *Hpb* infection.

CCR2+ Monocyte-derived macrophages curb expulsion in Bcl6$^{fl/fl}$CD4-cre mice.

Our observations that IL-4/10-stimulated macrophages express high PDL2 and low MHC-II levels led us to hypothesize that AAMs, in the context of Bcl6$^{fl/fl}$CD4-cre mice, suppress T cell responses and curtail expulsion. To formally test this hypothesis, we first established an adoptive transfer methodology where total CD4+ T cells from 2-week infected Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ mice were transferred into 1-week infected recipient mice, and infection was allowed to progress for another 3 weeks before worm burden was assessed (Figure 7A). Through this methodology, we first validated that increased *Hpb* expulsion is a CD4+ T cell-transferrable phenomenon, where C57BL/6-WT mice receiving Bcl6$^{fl/fl}$CD4-cre CD4+ T cells displayed accelerated clearance relative to C57BL/6-WT mice receiving Bcl6$^{fl/fl}$ control CD4+ T cells (Figure 7B). We then performed a variation of this protocol where CD4+ T cells were transferred into 1-week infected CCR2 +/- or -/- recipient mice, the latter of which lack the ability to recruit monocyte-derived macrophages during *Hpb* infection. The utilization of CCR2 -/- recipient mice was a decision informed by unpublished observations from our lab finding *Hpb* infection to

feature a robust infiltration of monocyte-derived macrophages to the gut. Additionally, our *in vitro* model of bone-marrow derived macrophages, which are differentiated *ex vivo* prior to stimulation, serve as a proxy for monocyte-derived macrophages which similarly differentiate at the site of infection, unlike tissue-resident macrophages. Through this model, it was found that CCR2 -/- mice receiving Bcl6$^{fl/fl}$CD4-cre CD4+ T cells displayed a rate of *Hpb* expulsion that was accelerated even compared to CCR2 +/- mice receiving Bcl6$^{fl/fl}$CD4-cre CD4+ T cells (Figure 7C). Collectively, these data demonstrate that, in line with our *in vitro* and *in vivo* findings indicating an immunosuppressive phenotype, monocyte-derived macrophages in Bcl6$^{fl/fl}$CD4-cre mice curtail *Hpb* expulsion, forming a negative feedback regulatory loop in the gut.

Figures

Figure 1

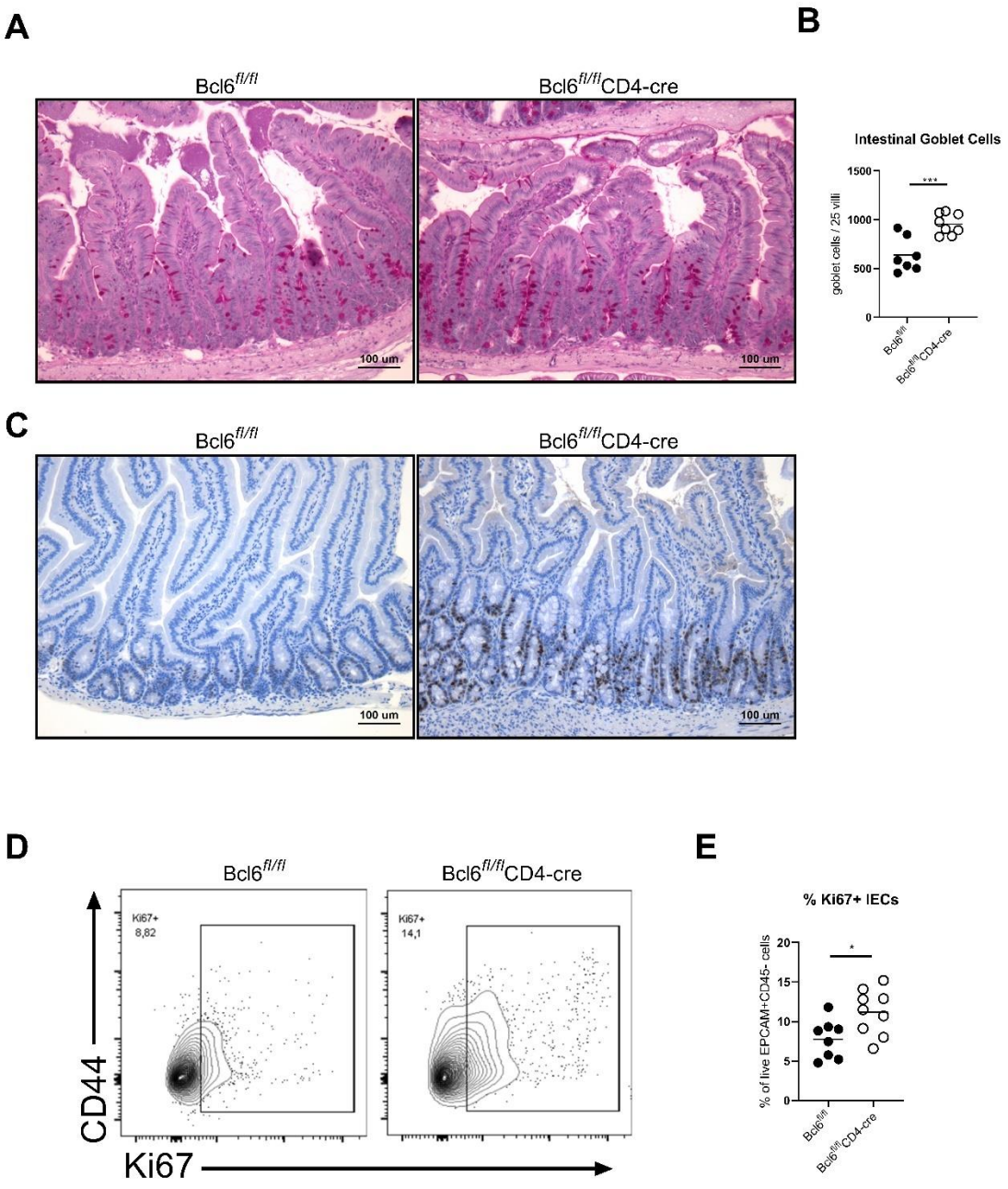
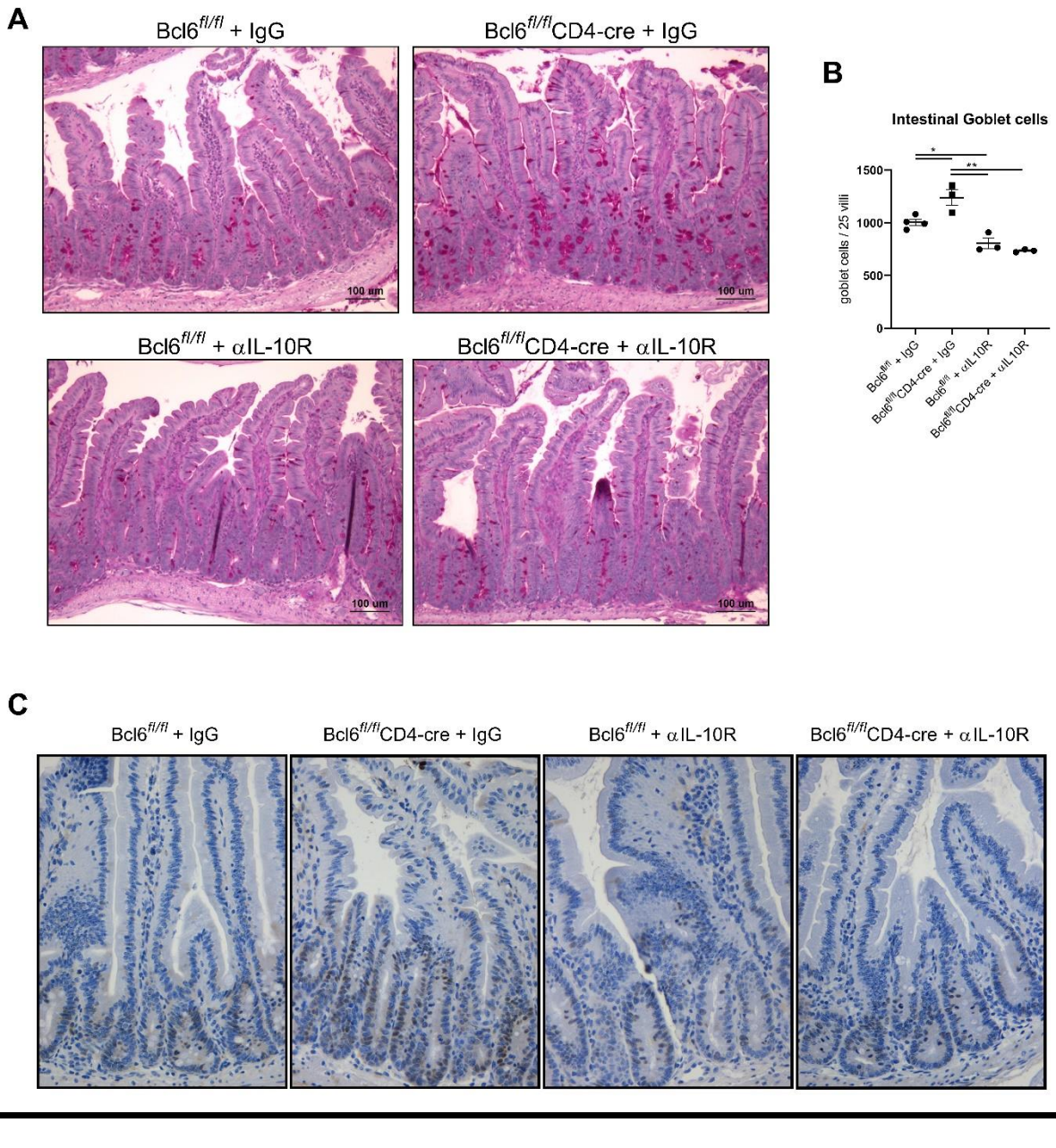


Figure 2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Bcl6fl/fl + αIL-10R
Bcl6fl/flCD4-cre + αIL-10R
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B
Intestinal Goblet cells
goblet cells / 25 villi
1500
1000
500
0
*
**
Bcl6fl/fl + IgG
Bcl6fl/flCD4-cre + IgG
Bcl6fl/fl + αIL10R
Bcl6fl/flCD4-cre + αIL10R
C
Bcl6fl/fl + IgG
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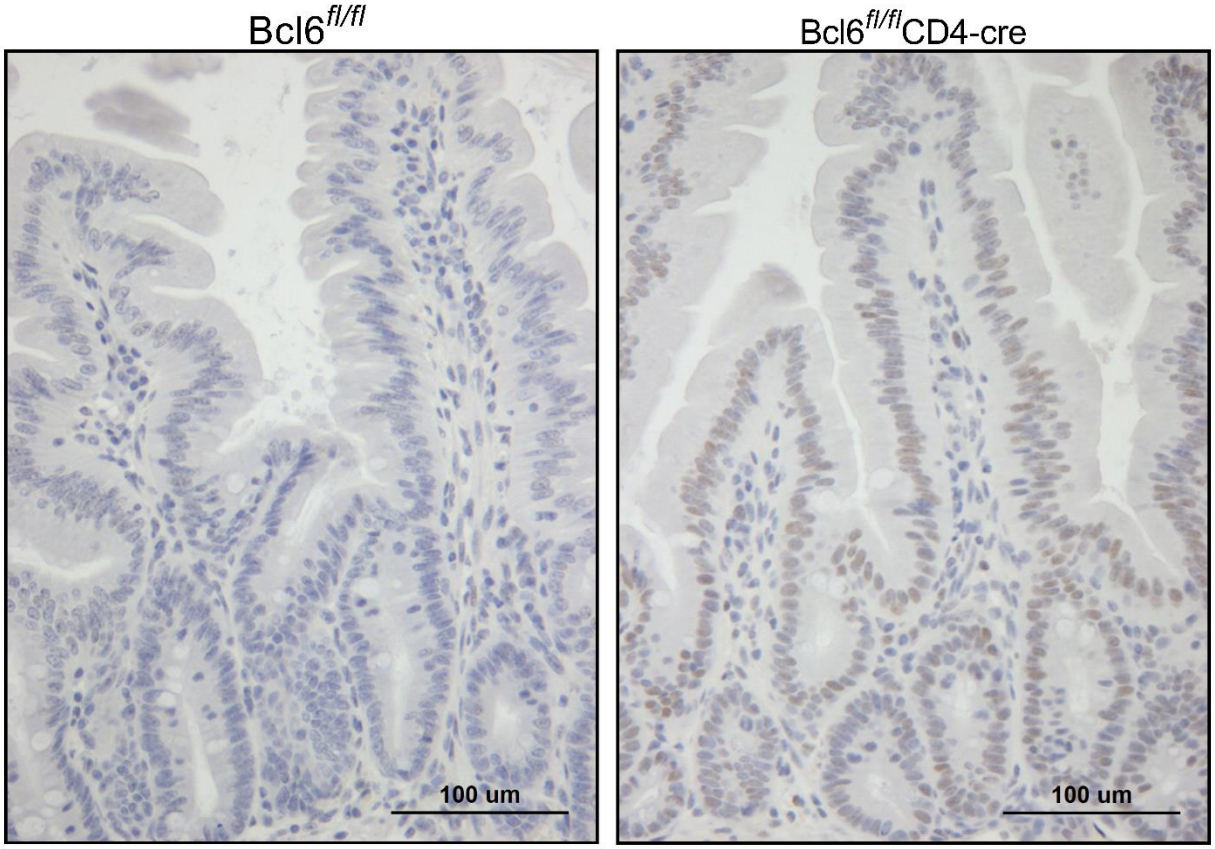
Bcl6fl/flCD4-cre + αIL-10R

Figure 3



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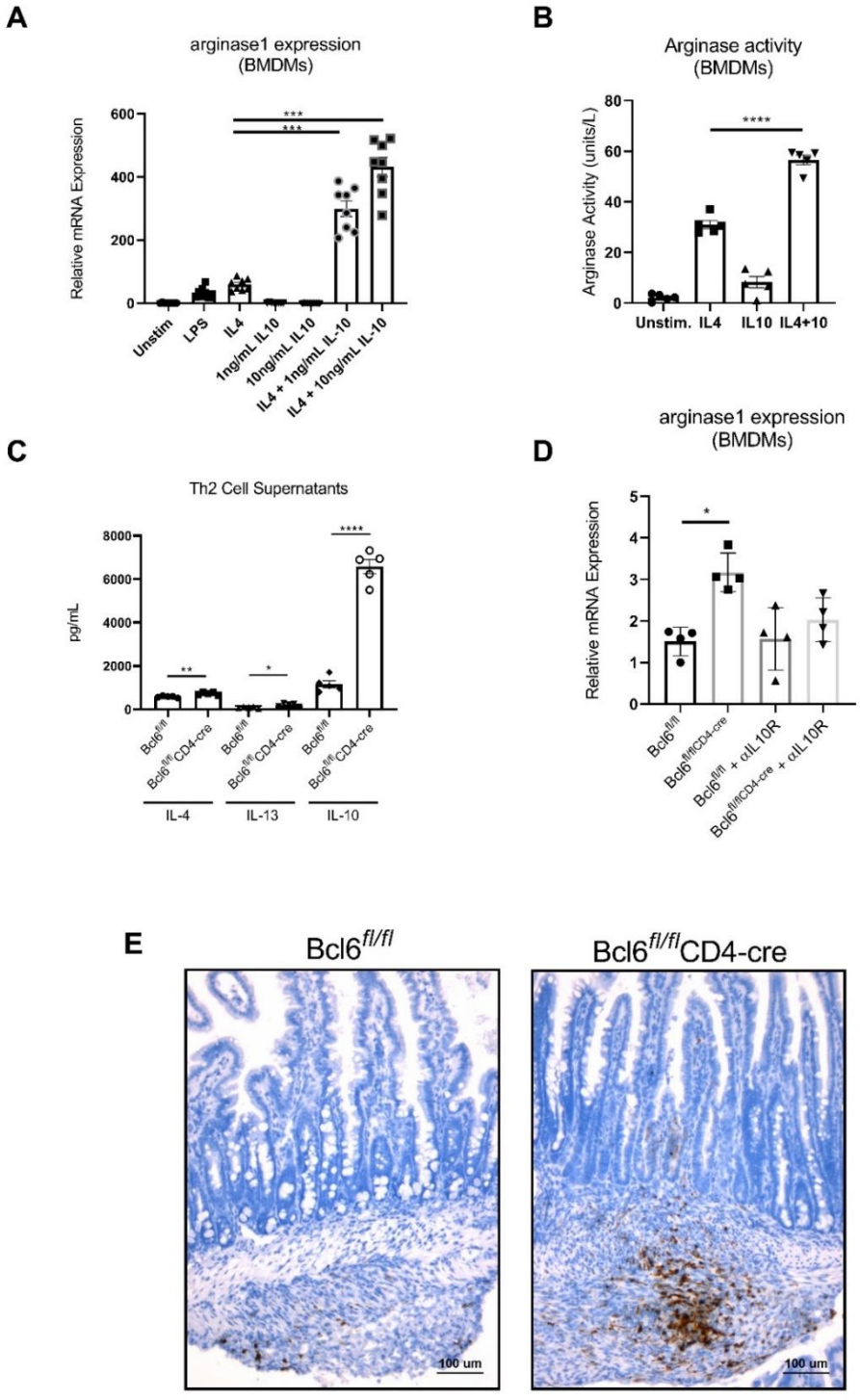
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arginase1 expression
(BMDMs)
Relative mRNA Expression
600
400
200
0
***
***
Unstim
LPS
IL4
1ng/mL IL10
10ng/mL IL10
IL4 + 1ng/mL IL-10
IL4 + 10ng/mL IL-10
B
Arginase activity
(BMDMs)
Arginase Activity (units/L)
80
60
40
20
0
****
Unstim.
IL4
IL10
IL4+10
C
Th2 Cell Supernatants
pg/mL
8000
6000
4000
2000
0
**
*
****
Bcl6fl/fl
Bcl6fl/flCD4-cre
IL-4
IL-13
IL-10
D
arginase1 expression
(BMDMs)
Relative mRNA Expression
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4
3
2
1
0
*
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Bcl6fl/fl + αIL10R
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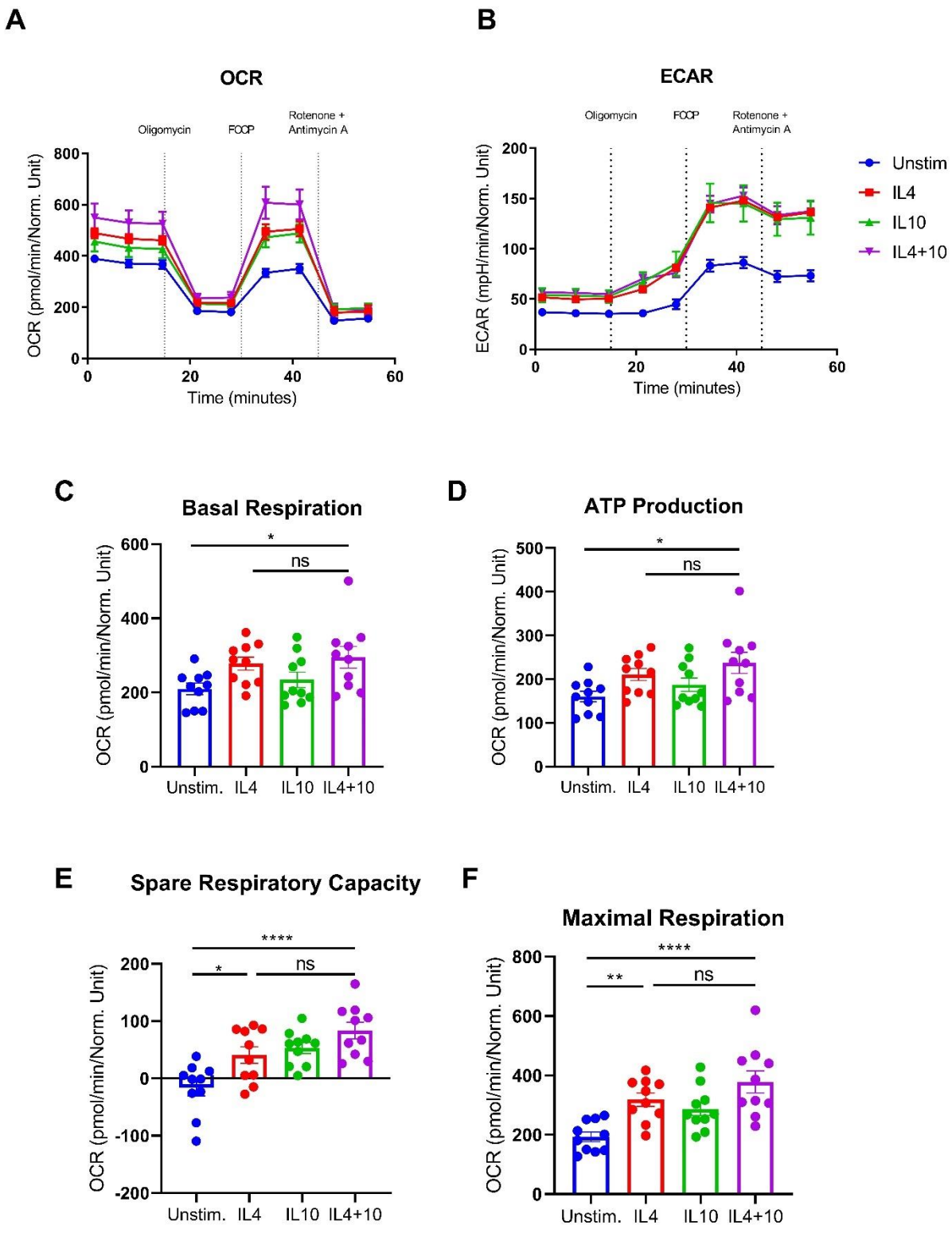
E
Bcl6fl/fl
Bcl6fl/flCD4-cre
100 um
100 um

Figure 5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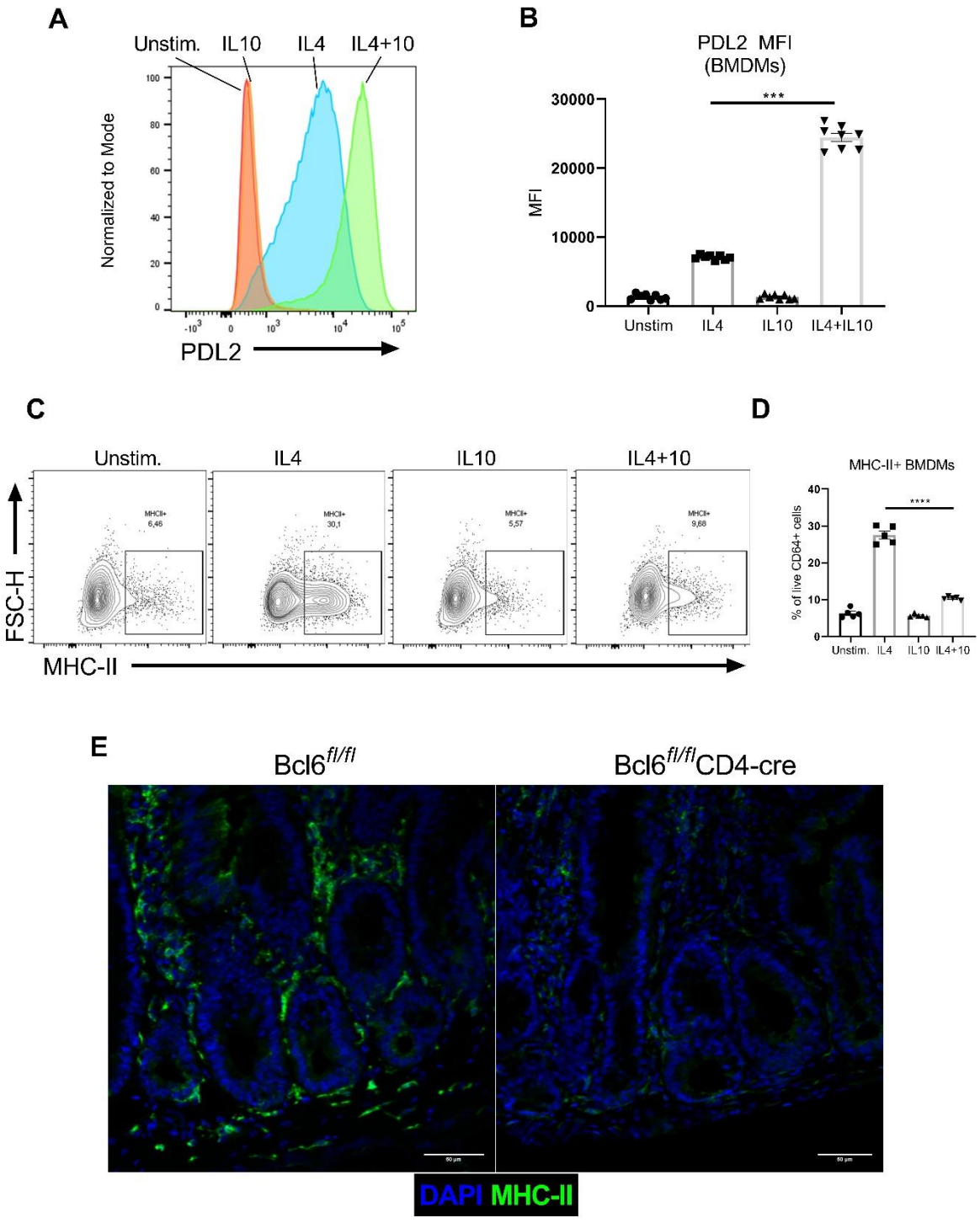
OCR
Oligomycin
FCCP
Rotenone + Antimycin A
OCR (pmol/min/Norm. Unit)
Time (minutes)
B
ECAR
ECAR (mpH/min/Norm. Unit)
Unstim
IL4
IL10
IL4+10
C
Basal Respiration
D
ATP Production
E
Spare Respiratory Capacity
F
Maximal Respiration
Unstim.
IL4
IL10
IL4+10
ns

Figure 6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Unstim.
IL10
IL4
IL4+10
Normalized to Mode
PDL2
B
PDL2 MFI
(BMDMs)
***
MFI
30000
20000
10000
0
Unstim
IL4
IL10
IL4+IL10
C
Unstim.
IL4
IL10
IL4+10
FSC-H
MHC-II
D
MHC-II+ BMDMs
****
% of live CD64+ cells
Unstim.
IL4
IL10
IL4+10
E
Bcl6fl/fl
Bcl6fl/flCD4-cre
DAPI MHC-II

Figure 7

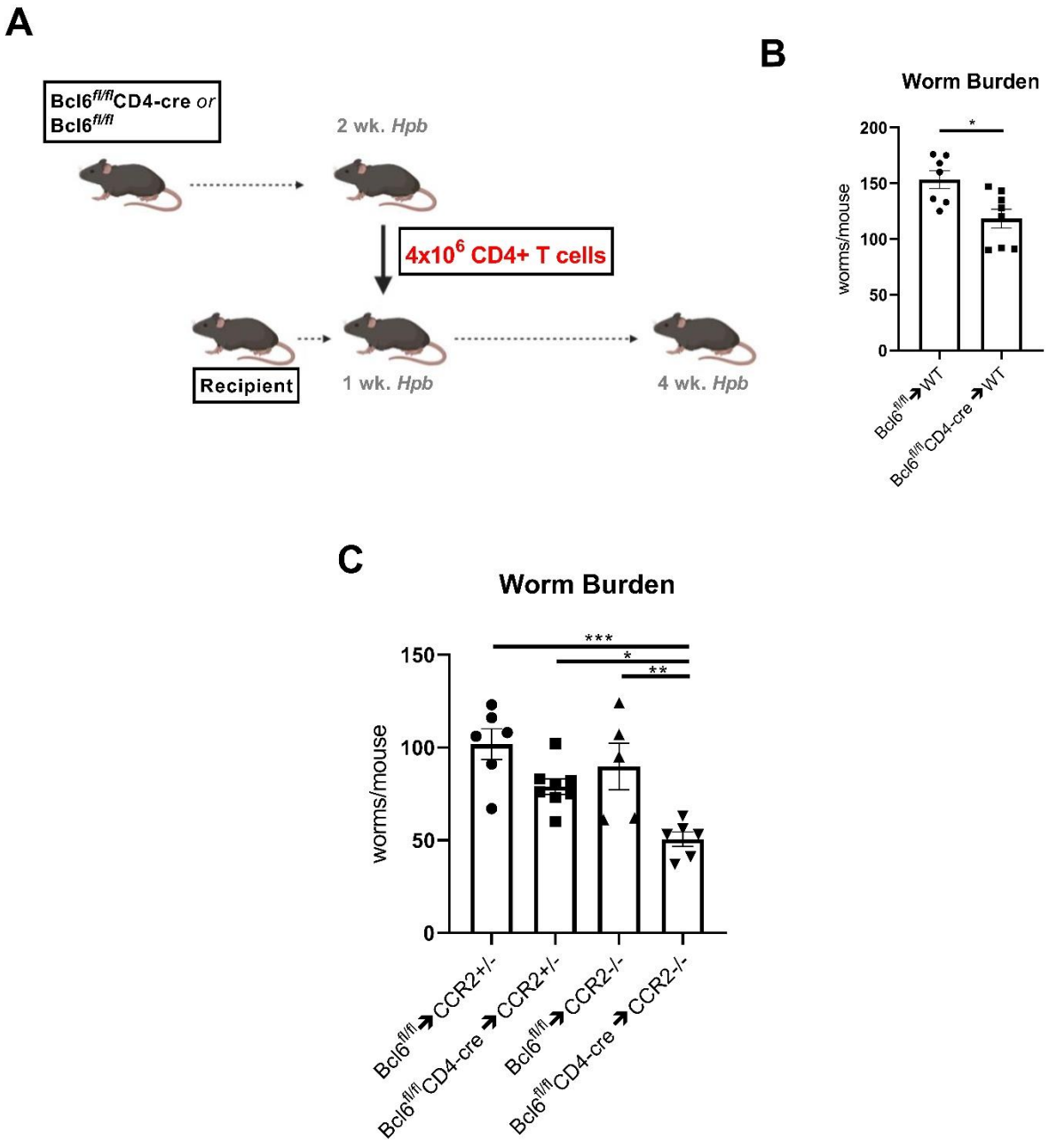
A
Bcl6fl/flCD4-cre or
Bcl6fl/fl
2 wk. Hpb
4x10^6 CD4+ T cells
Recipient
1 wk. Hpb
4 wk. Hpb
B
Worm Burden
worms/mouse
200
150
100
50
0
*
Bcl6fl/fl → WT
Bcl6fl/flCD4-cre → WT
C
Worm Burden
worms/mouse
150
100
50
0
***
*
**
Bcl6fl/fl → CCR2+/-
Bcl6fl/flCD4-cre → CCR2+/-
Bcl6fl/fl → CCR2-/-
Bcl6fl/flCD4-cre → CCR2-/-

Figure Legends

Figure 1. Bcl6$^{fl/fl}$CD4-cre mice exhibit an enhanced intestinal 'weep and sweep' response. *Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ mice were infected for 2 weeks with Hpb and duodenal tissue was harvested, cut, cleared and rolled. Duodenal tissue was fixed in 10% formalin for 24 hours, and paraffin-embedded sections were stained with Periodic Acid Schiff (P.A.S.) dye for goblet cells (shown in bright pink). (A) Representative images and (B) enumerations of goblet cells are shown. A minimum of 15 intestinal villi were manually counted using ImageJ, and the number of goblet cells per 25 villi is shown. In (C), mice were injected intraperitoneally with >3mg of BrdU 4 hours prior to sacrifice, and immunohistochemistry for BrdU was performed on FFPE sections. In (D) and (E), intestinal epithelial cells were isolated from the small intestine and flow cytometric analysis was performed. (D) Representative plots and (E) frequencies of live Ki67+CD45.2-EPCAM+ intestinal epithelial cells are shown. Data in (A-D) are representative of at least 2 independent experiments with (n=3-4) per group, while data in (E) is from 2 pooled experiments with (n=3-4) per group. An unpaired student's t test was performed for statistical analyses. * denotes $p < 0.05$. *** denotes $p < 0.001$*

Figure 2. IL-10R signalling drives the enhanced intestinal 'weep and sweep' response in Bcl6$^{fl/fl}$CD4-cre mice.

*Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ mice were infected for 2 weeks with Hpb and duodenal tissue was harvested, cut, cleared and rolled. Every 72 hours (starting at day 0), mice were treated intraperitoneally with αIL10-R antibody (clone 1B1.3A) or Rat IgG control. Duodenal tissue was fixed in 10% formalin for 24 hours, and paraffin-embedded sections were stained with Periodic Acid Schiff (P.A.S.) dye for goblet cells (shown in bright pink). (A) Representative images and (B) enumerations of goblet cells are shown. A minimum of 15 intestinal villi were manually counted using ImageJ, and the number of goblet cells per 25 villi is shown. In (C), mice were injected intraperitoneally with >3mg of BrdU 4 hours prior to sacrifice, and immunohistochemistry for BrdU was performed on FFPE sections. Data are representative of at least 2 independent experiments with (n=2-4) per group. A one-way ANOVA was performed for statistical analyses. * denotes $p < 0.05$. ** denotes $p < 0.01$*

Figure 3. Bcl6$^{fl/fl}$CD4-cre mice display elevated intestinal pSTAT3. *Bcl6$^{fl/fl}$CD4-cre or Bcl6$^{fl/fl}$ mice were infected for 2 weeks with Hpb before duodenal tissue was cleared, cut, rolled, and fixed in 10% formalin for 24 hours. 5um thick Paraffin-embedded sections were stained by immunohistochemistry for phosphorylated STAT3 (residue Try705), and representative images are shown. pSTAT3 is marked by brown DAB substrate signal. Images are representative of 2 independent experiments with (n=3-4) per group.*

Figure 4. IL-10 enhances IL-4-driven alternative activation of macrophages*. C57BL/6-WT bone-marrow derive macrophages (BMDMs) were stimulated with LPS (50ng/mL), IL-4*

*(10ng/mL), and/or the denoted concentrations of IL-10 for 24 hours. (A) mRNA expression levels of arginase1 normalized to HPRT are shown. (B) Stimulated BMDMs were lysed and an arginase activity assay was performed on lysates. (C) Naïve CD4+ T cells were isolated from the total lymph nodes of $Bcl6^{fl/fl}$CD4-cre or $Bcl6^{fl/fl}$ mice, and stimulated under Th2-polarizing conditions (IL-2, IL-4, and αIFN-γ) for 72-96 hours with αCD3/28 stimulation. After 72-96 hours of stimulation, supernatant IL-4, IL-13, and IL-10 levels were quantified by ELISA. (D) BMDMs were stimulated with 25% of their cell medium comprising of $Bcl6^{fl/fl}$CD4-cre or $Bcl6^{fl/fl}$ Th2 cell supernatants for 24 hours, with some cells receiving 10ug/mL αIL-10R antibody (clone 1B1.3A), and mRNA expression levels of arginase1 normalized to HPRT were assessed. (E) RNAscope for arginase1 mRNA was performed on formalin-fixed paraffin embedded tissue sections from 2-week infected $Bcl6^{fl/fl}$CD4-cre or $Bcl6^{fl/fl}$ mice. Data in (A) are from 2 pooled experiments each with (n=3-5), while all other data is representative of at least 2 independent experiments with a minimum of (n=4) per group. A one-way ANOVA was performed for statistical analyses. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$, **** denotes $p < 0.0001$, ns = not significant*

Figure 5. IL-10 and IL-4 have additive effects on macrophage metabolism**. C57BL/6-WT bone-marrow derive macrophages (BMDMs) were stimulated with IL-4 (10ng/mL) and/or IL-10 (10ng/mL) for 24 hours. After a 1 hour rest period, metabolic capacity was assessed through a Seahorse analyser. Mitochondrial function was assessed through stress tests involving the addition of the following mitochondrial inhibitors: oligomycin A (1.5 μM), carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) (2 μM), and antimycin A (2 μM). Data in (A-B) are representative of 2 independent experiments each with (n=5). Data in (C-F) are from 2 pooled experiments each with (n=5) per group. A one-way ANOVA was performed for statistical analyses. * denotes $p < 0.05$, ** denotes $p < 0.01$, * denotes $p < 0.0001$, ns = not significant*

Figure 6. IL-10 and IL-4 synergize to induce an immunosuppressive AAM phenotype.** C57BL/6-WT bone-marrow derive macrophages (BMDMs) were stimulated with IL-4 (10ng/mL) and/or IL-10 (10ng/mL) for 24 hours. Cells were detached and processed for flow cytometric analysis. Macrophage surface expression of (A & B) PDL2 and (C & D) MHC-II are shown. In (E), $Bcl6^{fl/fl}$CD4-cre or $Bcl6^{fl/fl}$ mice were infected with 200 L3 Hpb larvae, and at 2 weeks post-infection duodenal tissue was cut, cleared, rolled, and live-frozen in OCT. Immunofluorescence staining was performed for MHC-II (green) as well as DAPI (blue) and analyzed by confocal microscopy. Data is representative of 2 independent experiments with a (n>=3) per group. A one-way ANOVA was performed for statistical analyses. *** denotes $p < 0.001$, * denotes $p < 0.0001$,*

***Figure 7. CCR2+ Monocyte-derived macrophages curb expulsion in $Bcl6^{fl/fl}$CD4-cre mice.** (A) Schematic of adoptive transfer protocol is shown. $Bcl6^{fl/fl}$CD4-cre or $Bcl6^{fl/fl}$ mice were infected with 200 L3 Hpb larvae, and CD4+ T cells were isolated from the mesenteric lymph nodes at 2-weeks post-infection. $4x10^6$ cells were transferred intravenously into recipient mice, and 3 weeks post-transfer (4 weeks post-Hpb infection) intestinal worm burden was assessed. In (B), cells were transferred into C57BL/6-WT recipients. In (C) cells were transferred into either CCR2 +/-*

*or CCR2 -/- recipients. Data is pooled from 2 independent experiments with (n=2-5) per group. A one-way ANOVA was performed for statistical analyses. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$*

Discussion

These studies characterize a previously unidentified pathway of helminth expulsion resulting from the intrinsic deletion of Bcl-6 in CD4+ T cells. More broadly, our results reveal IL-10 exerts a variety of profound effects on immune mechanisms of helminth resistance. Our characterization of the intestinal 'weep and sweep' response in $Bcl6^{fl/fl}$CD4-cre mice reveals that IL-10R signalling can significantly augment multiple facets of the intestinal epithelium's response to parasitic infection. Indeed, blockade of IL-10R signalling was found to significantly abrogate both the intestinal goblet cell response, as well as the proliferation of intestinal epithelial cells during *Hpb* infection in $Bcl6^{fl/fl}$CD4-cre mice (Figure 2). Furthermore, our observation that IL-10R blockade leads to a modest abrogation of goblet cell frequency in $Bcl6^{fl/fl}$ control mice indicates that IL-10R signalling can play an important role in the maintenance of mucosal immunity in a wild-type context. It remains to be determined if IL-10R signalling also impacts other important sub-components of the intestinal 'weep and sweep' response, such as the expression of mucins or anti-parasitic factors such as RELM-β. Future investigation into this topic will be of great importance, as some studies have found that changes to mucus production, goblet cell differentiation, and mucin expression do not necessarily occur in parallel (Khan *et al.*, 1995). Thus, it may be possible that, while modestly promoting goblet cell differentiation, IL-10R signalling contributes in even greater magnitude to the expression of mucins or other goblet cell-associated factors in the context of wild-type *Hpb* infection.

Our observation of elevated pSTAT3 levels in the duodenum of $Bcl6^{fl/fl}$CD4-cre mice relative to $Bcl6^{fl/fl}$ controls is highly suggestive of a direct effect of IL-10 on the intestinal epithelium (Figure 3). While STAT3 is not exclusive to IL-10 signaling, this model of direct

signalling is supported by the observation that pSTAT3 was observed along the lengths of the villi, running parallel to the intra-epithelial regions where lymphocytes (such as Th2 cells) reside. One alternative possibility is that, in Bcl6$^{fl/fl}$CD4-cre mice, Th2-derived IL-10 acts on an intermediate cell type which in turn produces the bulk of IL-10 responsible for changes to the epithelium. Indeed, macrophages have been shown to be capable of secreting IL-10 in such a positive-feedback loop (Yao et al., 2019). Regardless of the precise cellular source, however, our findings pinpoint a role for direct IL-10 signalling to the intestinal epithelium that drives profound changes impacting parasitic resistance. These results indicate a possible therapeutic role for IL-10 in combatting intestinal helminth infection, though further studies will be required to assess if exogenous administration of IL-10 alone is sufficient to drive the phenotype observed.

In addition to the intestinal 'weep and sweep' response, this study reveals that IL-10 can impart significant changes on immune cellular networks during *Hpb* infection, leading to increased AAM polarization. Our *in vitro* phenotyping studies found IL-10 to synergize with IL-4, inducing changes to markers of alternative activation. One such striking change was the profound upregulation in arginase1 mRNA and protein expression that was found in dual-stimulated BMDMs (Figure 4A & B). These results are in line with previous observations examining arginase1 mRNA levels in IL-4/10-stimulated peritoneal macrophages (Van de Velde *et al.*, 2017), and expand upon these studies, demonstrating an increase in functional protein expression, in addition to transcriptional changes. In addition to serving as a marker of alternative activation (Corraliza et al., 1995), arginase1 is a rate-limiting enzyme in metabolic pathways of macrophage polyamine biosynthesis (Kepka-Lenhart et al., 2000), and these molecules have been shown to perform variety of important biological functions. One previous

publication demonstrated direct larvicidal activity for polyamines against *Hpb* (Esser-von Bieren et al., 2013). Additionally, these molecules have been found to have direct effects on the polarization of macrophages themselves (Latour et al., 2020). Future studies examining the production and potential effects of polyamine family members in IL-4/10-stimulated macrophages may give further insight into this unique polarization phenotype.

Our RNAscope analysis confirmed elevated arginase1 transcript levels to be observable *in vivo* in Bcl6$^{fl/fl}$CD4-cre mice (Figure 4E). Of note, most arginase1 transcript was found localized to distinct foci in the muscularis layer of the duodenum. We speculate these to be macrophages remaining from the tissue-invasive stage of *Hpb* that features the formation of a macrophage-dense granuloma in the underlying smooth muscle of the intestine (Gentile et al., 2019; Reynolds *et al.*, 2012). Similar to arginase1, changes to MHC-II expression were observed in BMDMs dual-stimulated with IL-4/10 *in vitro* (Figure 6C & D). *In vivo* analysis of MHC-II expression, however, found expression changes to affect macrophages in both the lamina propria as well as the underlying muscularis (Figure 6E). These data indicate that the effects of Th2-derived IL-10 are likely not restricted to any one region of the intestine, and as such, further studies will be required to fully elucidate the reason for such strikingly localized arginase1 expression *in vivo*.

Contrary to our initial hypothesis, our adoptive transfer studies found that in the context of Bcl6$^{fl/fl}$CD4-cre mice, monocyte-derived macrophages play an inhibitory role, curbing the rate of *Hpb* expulsion (Figure 7C). Our *in vitro* phenotyping provides a rationale basis for the underlying mechanism, suggesting that high levels of the T cell inhibitory ligand PDL2 combined with low levels of MHC-II surface expression are the causes of this immunosuppressive activity (Figure 6). As such, while imparting direct effects on the intestinal

epithelium that drive worm expulsion, IL-10 also appears to synergize with type 2 cytokines to induce a phenotype of AAM that serve a negative feedback role in Bcl6$^{fl/fl}$CD4-cre mice (Image 4). It is important to note that intestinal macrophages are highly heterogenous, and comprise multiple subpopulations each with unique function and origin (Bain and Schridde, 2018). As such, it remains to be determined if CCR2- tissue-resident macrophages also adopt a similar immunosuppressive phenotype in response to elevated IL-10, or if this a unique response of this monocyte-derived population.

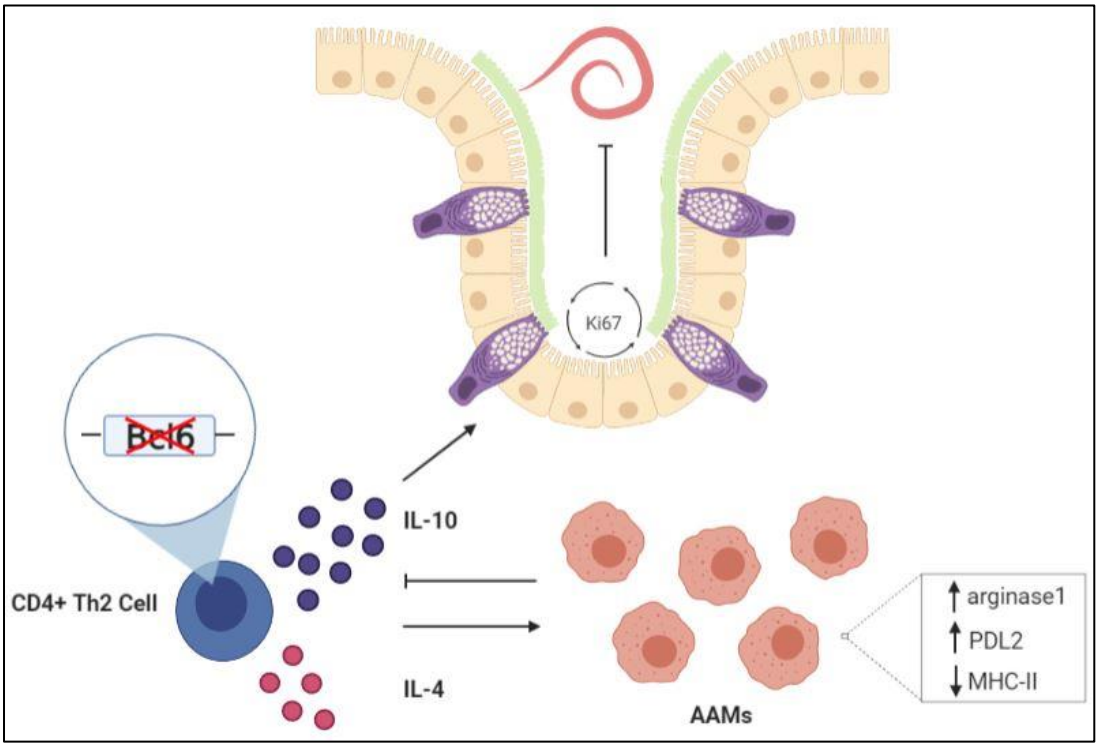

Image 4. Working Model. Our findings reveal that in Bcl6$^{fl/fl}$CD4-cre mice, Th2-derived IL-10 exerts opposing effects on two mechanisms of helminth resistance – directly amplifying the intestinal 'weep and sweep' response, while inducing an immunosuppressive phenotype of macrophage that acts to curb the rate of expulsion through direct negative feedback on T cells.

In conclusion, our work has defined how amplification of IL-10 signals promotes anti-helminth immunity, while simultaneously supporting an immunoregulatory environment. In

addition to detailing an unexpected role for Bcl-6 in regulating the function of terminally differentiated Th2 cells, we have shown that IL-10 synergizes with type 2 cytokines to profoundly impact multiple facets of anti-helminth immunity. These changes contrast and balance each other, as IL-10 directly augments epithelial mechanisms of expulsion, while collaterally inducing an immunosuppressive AAM phenotype that negatively regulates T cell activity. Overall, these findings shed light on how transcriptional regulation of T cell cytokine production can have important consequences on multiple cell types during the course of a complex intestinal infection.

Materials and Methods

Animals and Infections

All experiments were approved by the McGill University Animal Care Committee, and mice were used at 6-16 weeks of age. 4get/KN2, KN2/KN2, CD4-cre, Bcl6$^{fl/fl}$, and CCR2+/- mice on a C57BL/6 background were bred and kept under specific pathogen-free conditions at McGill University. Through all experiments, a sex-balanced mixture of male and female mice were used among groups, and no sex-based differences were observed.

Infectious L3 *Hpb* larvae were generated by fecal-culture methods as previously described (Gentile *et al.*, 2019) and checked for viability prior to infection. For some experiments, mice were administered 250ug of anti-IL10Rα/CD210 (1B1.3A) or rat IgG (BioXcell) i.p. every 72 hours post *Hpb* infection.

Intestinal Epithelial Cell Extraction

For intestinal epithelial cell extraction and analysis, roughly 2cm of small intestinal tissue was taken, cut open with scissors and cleared of worms and debris. Tissue was washed manually in cold HBSS buffer (containing 10% FBS and 15mM HEPES). Tissue was then placed in 15mLs of HBSS+EDTA buffer (5mM EDTA, 10% FBS and 15mM HEPES) and incubated at 37°C, 250 rpm, for 20 minutes. After shaking, the tissue was filtered through a 100μm filter and the flow-through was collected and spun down, while the tissue was placed into 15mL of fresh

HBSS+EDTA buffer to repeat the shake a second time. Intestinal epithelial cells were washed with EDTA-free HBSS buffer prior to cell counting and staining.

Flow Cytometry

Data were acquired on a FACS Canto II or LSR Fortessa (BD Biosciences) and analyzed using FlowJo software. Antibodies used include: Ki67 (SolA15), EPCAM (G8.8), CD45.2 (104), CD44 (IM7), MHCII (M5/144.15.2), CD64 (X54-5/7.1), PDL2 (TY25). Fixable viability dye (Thermofisher) was used to exclude dead cells.

Adoptive Transfer of CD4+ T cells

Total CD4+ T cells were isolated from the mesenteric lymph nodes of 4get.KN2.Bcl6$^{fl/fl}$CD4-cre or 4get.KN2.Bcl6$^{fl/fl}$ control mice on day 14 of *Hpb* infection. CD4+ T cells were isolated using the EasySep™ Mouse Naive CD4+ T Cell Isolation Kit as per the manufacturer's protocol (STEMCELL), with an omission of memory T cell depletion step. Flow cytometric analysis confirmed CD4+ T cell purity to be >97% by this method. Cells were resuspended in PBS, and $4x10^6$ cells were intravenously administered to 7-day infected C57BL/6-WT or CCR2+/+ or +/- recipients. After 21 days post-transfer (28 days post-infection), mice were sacrificed, and worm burden was assessed.

Fecundity

At least 0.2g of feces was collected and vortexed in a 0.75-1mL saturated NaCl solution. Suspensions were incubated at room temperature for 24 hours, followed by at least 6 days at 4°C. Then, the top 2 layers (clear and milky) were extracted and centrifuged at 4000rpm for 5 minutes, supernatants were discarded, and egg pellets were re-suspended in ddH_2O for counting. Eggs were counted among 10ul strips.

RNA extraction and reverse-transcription

PCR mRNA was extracted using TRIzol (Sigma), and cDNA samples were prepared as previously described (Meli, 2019). Quantitative real-time RT-PCR was performed using SYBR green detection using the Biorad CFX96TM Real-Time System and software. Fold expression was calculated using the ΔΔCT method and Hprt as reference genes. The below primers were generated using Primer3 software.

Hprt: forward (5'- AGGACCTCTCGAAGTGTTGG-3'), reverse (5'-AACTTGCGCTCATCTTAGGC-3')

Arginase1: forward (5' – TGATGGAAGAGACCTTCAGC – 3'), reverse (5' – CACCTCCTCTGCTGTCTTCC – 3')

Histology

All tissues were fixed with 10% formalin phosphate for at least 24 hours. After 24 hours, fixed tissue was briefly washed twice in PBS, and transferred to 70% ethanol. Paraffin embedding and sectioning of tissue was performed by the Histology Core at the RI-MUHC. Select tissue sections were further stained with Periodic Acid Schiff (P.A.S).

BrdU

For 5-bromo-2'-deoxyuridine (BrdU) administration, a minimum of 3mg of BrdU (T.C.I.) was dissolved in sterile PBS and injected into mice intraperitoneally 4 hours prior to sacrifice.

For BrdU I.H.C, a BrdU Immunohistochemistry Kit (CHEMICON MilliPore) was used on 5um thick paraffin-embedded formalin-fixed duodenal tissue sections.

pSTAT3 immunohistochemistry

Immunohistochemistry for phosphorylated STAT3 (residue Tyr705) was performed on 5um thick paraffin-embedded formalin-fixed duodenal tissue sections. Antigen retrieval was performed using a 1:10 dilution of 10X Citrate Buffer (Sigma). Phospho-STAT3 (Tyr705, clone D3A7) Rabbit mAb (Cell Signaling Technology) was used for as primary antibody. Secondary antibody was Anti-Rabbit EnVision+ System-HRP (Dako).

Arginase1 RNAscope

In situ hybridization for arginase1 was performed on 5um thick paraffin-embedded formalin-fixed duodenal sections using RNAscope 2.5 HD Assay – BROWN kit (Advanced Cell Diagnostics), with probe for Mm-arginase1 (ref: 403431), according to the manufacturer's instructions.

Immunofluorescence Staining and Confocal Microscopy

Duodenal tissue was cut open length-wise, cleared of worms and debris, rolled, and frozen in OCT. 8um thick live-frozen sections were cut onto superfrost slides, and fixed in ice-cold fixing solution (75%acetone/25% ethanol). Following fixation, sections were washed with PBS, and blocked for 1 hour in blocking buffer containing BSA, anti-Fc receptor, and mouse serum in PBS. Antibody staining was performed for 2 hours at room temperature, followed by a 20 minute DAPI stain at room temperature.

Naïve CD4+ T Cell Stimulation

For *in vitro* experiments, Naive CD62L+CD4+ T cells from peripheral lymph nodes were isolated using the EasySep™ Mouse Naive CD4+ T Cell Isolation Kit as per the manufacturer's protocol (STEMCELL). Cells were subsequently stimulated with plate bound anti-CD3 (1ug/ml), anti-CD28 (2ug/ml) and the following reagents: anti-IFNγ (1ug/ml), IL-2 (5ng/ml) and IL-4

(10ng/ml). All antibodies were purchased from Thermofisher while all cytokines were purchased from PeproTech or Biolegend.

IL4/13/10 ELISAs

IL-13 was quantified using Mouse IL-13 ELISA Ready-SET-Go! From eBioscience, IL-10 was quantified using Mouse IL-10 Uncoated ELISA Kit (Invitrogen), and IL-4 was quantified using Mouse IL-4 Uncoated ELISA Kit (Invitrogen).

Cell Culture

For BMDM cultures, femurs from C57BL/6-WT mice were harvested and cleared of surrounding muscle in cold PBS. Bone marrow cells were flushed in non-differentiating media (RPMI 1640 containing 10%FBS and 1% Pencillin/Streptomycin), and centrifuged at 1800rpm for 5 minutes at 4°C. Red blood cells were lysed with 1X Red Blood Cell Lysis Buffer for 2-3 minutes, cells were washed, and resuspended. 5-10x10^6 bone marrow cells were seeded in a 10cm Petri Dish containing 15mL of differentiating media (RPMI 1640 containing 10%FBS, 1% Penicillin/Streptomycin, and 20% L929 Cell Conditioned Medium (LCCM)). On the 3rd day of culture, dishes were supplemented with 10mL of differentiating media. On the 6th day of culture, differentiated BMDMs were washed with PBS several times, detached using Cell Stripper, and plated in the desired format for stimulation in non differentiation media. Cells were allowed to adhere overnight prior to stimulations.

To obtain LCCM media, L929 fibroblasts were cultured in L929 cell media (RPMI 1640 containing 10%FBS, 1% Penicillin/Streptomycin, and 1% L-Glutamine). Cells were passaged up to T75 flasks, and left in culture for 2-4 weeks. Afterwards, media was collected, centrifuged, and subjected to 0.2um filtration before storage at -20°C, and use in BMDM cultures.

Arginase Activity Assay

Arginase activity of BMDMs was assessed using the Arginase Activity Assay Kit (Sigma). $1.5x10^6$ BMDMs were stimulated in 6-well plates with the indicated cytokines (10ng/mL IL4, 10ng/mL IL-10, or both) for 24 hours. After the stimulation period, cells were washed with PBS and lysed in fresh protein lysis buffer, and cell lysates were assayed for arginase activity according to the manufacturer's instructions. One unit of arginase is the amount of enzyme that will convert 1.0 μmole of L-arginine to ornithine and urea per minute at pH 9.5 and 37°C.

Bioenergetic Flux analyser (Seahorse) Assay

C57BL/6-WT BMDMs were seeded in 96-well plates from Seahorse Biosciences (Agilent Technologies), at 100,000 cells/well. Cells were incubated at 37°C 5% CO2 overnight in RPMI 1640 containing 10%FBS and 1%Penicillin/Streptomycin. Cells were stimulated the following day with either 10ng/mL IL-4, 10ng/mL IL-10, or both, for 24 hours. After 24 hours, cells were washed and resuspended in Seahorse media. The Searhose Bioenergetic flux analyser (Agilent) was used to measure the OCR and ECAR. Mitochondrial inhibitors were added in 10 minute intervals in the following order: oligomycin A (1.5uM), FCCP (2uM), and Antimycin A (2uM).

Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed by a two-tailed Student t test or one-way ANOVA as appropriate using the GraphPad Prism program. A p value < 0.05 is considered significant.

References

Akiho, H., Blennerhassett, P., Deng, Y., and Collins, S.M. (2002). Role of IL-4, IL-13, and STAT6 in inflammation-induced hypercontractility of murine smooth muscle cells. Am J Physiol Gastrointest Liver Physiol *282*, G226-232. 10.1152/ajpgi.2002.282.2.G226.

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Stadecker, M.J., and Gause, W.C. (2007). Protective immune mechanisms in helminth infection. Nat Rev Immunol *7*, 975-987. 10.1038/nri2199.

Anthony, R.M., Urban, J.F., Alem, F., Hamed, H.A., Rozo, C.T., Boucher, J.L., Van Rooijen, N., and Gause, W.C. (2006). Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med *12*, 955-960. 10.1038/nm1451.

Artis, D., Wang, M.L., Keilbaugh, S.A., He, W., Brenes, M., Swain, G.P., Knight, P.A., Donaldson, D.D., Lazar, M.A., Miller, H.R., et al. (2004). RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. Proc Natl Acad Sci U S A *101*, 13596-13600. 10.1073/pnas.0404034101.

Bain, C.C., and Schridde, A. (2018). Origin, Differentiation, and Function of Intestinal Macrophages. Front Immunol *9*, 2733. 10.3389/fimmu.2018.02733.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., and Hotez, P.J. (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet *367*, 1521-1532. 10.1016/S0140-6736(06)68653-4.

Borkow, G., and Bentwich, Z. (2006). HIV and helminth co-infection: is deworming necessary? Parasite Immunol *28*, 605-612. 10.1111/j.1365-3024.2006.00918.x.

Bosurgi, L., Cao, Y.G., Cabeza-Cabrerizo, M., Tucci, A., Hughes, L.D., Kong, Y., Weinstein, J.S., Licona-Limon, P., Schmid, E.T., Pelorosso, F., et al. (2017). Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. Science *356*, 1072-1076. 10.1126/science.aai8132.

Campbell, L., Hepworth, M.R., Whittingham-Dowd, J., Thompson, S., Bancroft, A.J., Hayes, K.S., Shaw, T.N., Dickey, B.F., Flamar, A.L., Artis, D., et al. (2019). ILC2s mediate systemic innate protection by priming mucus production at distal mucosal sites. J Exp Med *216*, 2714-2723. 10.1084/jem.20180610.

Cardoso, V., Chesné, J., Ribeiro, H., García-Cassani, B., Carvalho, T., Bouchery, T., Shah, K., Barbosa-Morais, N.L., Harris, N., and Veiga-Fernandes, H. (2017). Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. Nature *549*, 277-281. 10.1038/nature23469.

Chan, M.S. (1997). The global burden of intestinal nematode infections--fifty years on. Parasitol Today *13*, 438-443. 10.1016/s0169-4758(97)01144-7.

Cimmino, L., Martins, G.A., Liao, J., Magnusdottir, E., Grunig, G., Perez, R.K., and Calame, K.L. (2008). Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. J Immunol *181*, 2338-2347. 10.4049/jimmunol.181.4.2338.

Cliffe, L.J., Humphreys, N.E., Lane, T.E., Potten, C.S., Booth, C., and Grencis, R.K. (2005). Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. Science *308*, 1463-1465. 10.1126/science.1108661.

Corraliza, I.M., Soler, G., Eichmann, K., and Modolell, M. (1995). Arginase induction by suppressors of nitric oxide synthesis (IL-4, IL-10 and PGE2) in murine bone-marrow-derived macrophages. Biochem Biophys Res Commun *206*, 667-673. 10.1006/bbrc.1995.1094.

Crotty, S., Johnston, R.J., and Schoenberger, S.P. (2010). Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat Immunol *11*, 114-120. 10.1038/ni.1837.

Dabbagh, K., Takeyama, K., Lee, H.M., Ueki, I.F., Lausier, J.A., and Nadel, J.A. (1999). IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. J Immunol *162*, 6233-6237.

Druilhe, P., Tall, A., and Sokhna, C. (2005). Worms can worsen malaria: towards a new means to roll back malaria? Trends Parasitol *21*, 359-362. 10.1016/j.pt.2005.06.011.
Duque-Correa, M.A., Karp, N.A., McCarthy, C., Forman, S., Goulding, D., Sankaranarayanan, G., Jenkins, T.P., Reid, A.J., Cambridge, E.L., Ballesteros Reviriego, C., et al. (2019). Exclusive dependence of IL-10Rα signalling on intestinal microbiota homeostasis and control of whipworm infection. PLoS Pathog *15*, e1007265. 10.1371/journal.ppat.1007265.
Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., Gause, W.C., Seitz, A., Verbeek, J.S., and Harris, N.L. (2013). Antibodies trap tissue migrating helminth larvae and prevent tissue damage by driving IL-4Rα-independent alternative differentiation of macrophages. PLoS Pathog *9*, e1003771. 10.1371/journal.ppat.1003771.
Esser-von Bieren, J., Volpe, B., Sutherland, D.B., Bürgi, J., Verbeek, J.S., Marsland, B.J., Urban, J.F., and Harris, N.L. (2015). Immune antibodies and helminth products drive CXCR2-dependent macrophage-myofibroblast crosstalk to promote intestinal repair. PLoS Pathog *11*, e1004778. 10.1371/journal.ppat.1004778.
Gentile, M.E., Li, Y., Robertson, A., Shah, K., Fontes, G., Kaufmann, E., Polese, B., Khan, N., Parisien, M., Munter, H.M., et al. (2019). NK cell recruitment limits tissue damage during an enteric helminth infection. Mucosal Immunol. 10.1038/s41385-019-0231-8.
Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., et al. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature *529*, 226-230. 10.1038/nature16527.
Harris, N.L., and Loke, P. (2017). Recent Advances in Type-2-Cell-Mediated Immunity: Insights from Helminth Infection. Immunity *47*, 1024-1036. 10.1016/j.immuni.2017.11.015.
Hasnain, S.Z., Evans, C.M., Roy, M., Gallagher, A.L., Kindrachuk, K.N., Barron, L., Dickey, B.F., Wilson, M.S., Wynn, T.A., Grencis, R.K., and Thornton, D.J. (2011). Muc5ac: a critical component mediating the rejection of enteric nematodes. J Exp Med *208*, 893-900. 10.1084/jem.20102057.
He, K., Hettinga, A., Kale, S.L., Hu, S., Xie, M.M., Dent, A.L., Ray, A., and Poholek, A.C. (2020). Blimp-1 is essential for allergen-induced asthma and Th2 cell development in the lung. J Exp Med *217*. 10.1084/jem.20190742.
Herbert, D.R., Yang, J.Q., Hogan, S.P., Groschwitz, K., Khodoun, M., Munitz, A., Orekov, T., Perkins, C., Wang, Q., Brombacher, F., et al. (2009). Intestinal epithelial cell secretion of RELM-beta protects against gastrointestinal worm infection. J Exp Med *206*, 2947-2957. 10.1084/jem.20091268.
Hewitson, J.P., Filbey, K.J., Esser-von Bieren, J., Camberis, M., Schwartz, C., Murray, J., Reynolds, L.A., Blair, N., Robertson, E., Harcus, Y., et al. (2015). Concerted activity of IgG1 antibodies and IL-4/IL-25-dependent effector cells trap helminth larvae in the tissues following vaccination with defined secreted antigens, providing sterile immunity to challenge infection. PLoS Pathog *11*, e1004676. 10.1371/journal.ppat.1004676.
Ho, L.H., Ohno, T., Oboki, K., Kajiwara, N., Suto, H., Iikura, M., Okayama, Y., Akira, S., Saito, H., Galli, S.J., and Nakae, S. (2007). IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. J Leukoc Biol *82*, 1481-1490. 10.1189/jlb.0407200.
Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., and Jacobson, J. (2008). Helminth infections: the great neglected tropical diseases. J Clin Invest *118*, 1311-1321. 10.1172/JCI34261.
Howitt, M.R., Lavoie, S., Michaud, M., Blum, A.M., Tran, S.V., Weinstock, J.V., Gallini, C.A., Redding, K., Margolskee, R.F., Osborne, L.C., et al. (2016). Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science *351*, 1329-1333. 10.1126/science.aaf1648.
Huang, S.C., Everts, B., Ivanova, Y., O'Sullivan, D., Nascimento, M., Smith, A.M., Beatty, W., Love-Gregory, L., Lam, W.Y., O'Neill, C.M., et al. (2014). Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol *15*, 846-855. 10.1038/ni.2956.

Jia, T.W., Melville, S., Utzinger, J., King, C.H., and Zhou, X.N. (2012). Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. PLoS Negl Trop Dis *6*, e1621. 10.1371/journal.pntd.0001621.
Johnston, C.J.C., Smyth, D.J., Kodali, R.B., White, M.P.J., Harcus, Y., Filbey, K.J., Hewitson, J.P., Hinck, C.S., Ivens, A., Kemter, A.M., et al. (2017). A structurally distinct TGF-β mimic from an intestinal helminth parasite potently induces regulatory T cells. Nat Commun *8*, 1741. 10.1038/s41467-017-01886-6.
Johnston, R.J., Poholek, A.C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., Dent, A.L., Craft, J., and Crotty, S. (2009). Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science *325*, 1006-1010. 10.1126/science.1175870.
Kallies, A., Hawkins, E.D., Belz, G.T., Metcalf, D., Hommel, M., Corcoran, L.M., Hodgkin, P.D., and Nutt, S.L. (2006). Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. Nat Immunol *7*, 466-474. 10.1038/ni1321.
Kaplan, M.H., Schindler, U., Smiley, S.T., and Grusby, M.J. (1996). Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity *4*, 313-319. 10.1016/s1074-7613(00)80439-2.
Katona, I.M., Urban, J.F., and Finkelman, F.D. (1988). The role of L3T4+ and Lyt-2+ T cells in the IgE response and immunity to Nippostrongylus brasiliensis. J Immunol *140*, 3206-3211.
Kepka-Lenhart, D., Mistry, S.K., Wu, G., and Morris, S.M. (2000). Arginase I: a limiting factor for nitric oxide and polyamine synthesis by activated macrophages? Am J Physiol Regul Integr Comp Physiol *279*, R2237-2242. 10.1152/ajpregu.2000.279.6.R2237.
Khan, W.I., Abe, T., Ishikawa, N., Nawa, Y., and Yoshimura, K. (1995). Reduced amount of intestinal mucus by treatment with anti-CD4 antibody interferes with the spontaneous cure of Nippostrongylus brasiliensis-infection in mice. Parasite Immunol *17*, 485-491. 10.1111/j.1365-3024.1995.tb00919.x.
Klose, C.S.N., Mahlakõiv, T., Moeller, J.B., Rankin, L.C., Flamar, A.L., Kabata, H., Monticelli, L.A., Moriyama, S., Putzel, G.G., Rakhilin, N., et al. (2017). The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. Nature *549*, 282-286. 10.1038/nature23676.
Koyama, K., Tamauchi, H., and Ito, Y. (1995). The role of CD4+ and CD8+ T cells in protective immunity to the murine nematode parasite Trichuris muris. Parasite Immunol *17*, 161-165. 10.1111/j.1365-3024.1995.tb01018.x.
Kurowska-Stolarska, M., Kewin, P., Murphy, G., Russo, R.C., Stolarski, B., Garcia, C.C., Komai-Koma, M., Pitman, N., Li, Y., Niedbala, W., et al. (2008). IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. J Immunol *181*, 4780-4790. 10.4049/jimmunol.181.7.4780.
Latour, Y.L., Gobert, A.P., and Wilson, K.T. (2020). The role of polyamines in the regulation of macrophage polarization and function. Amino Acids *52*, 151-160. 10.1007/s00726-019-02719-0.
Loke, P., MacDonald, A.S., Robb, A., Maizels, R.M., and Allen, J.E. (2000). Alternatively activated macrophages induced by nematode infection inhibit proliferation via cell-to-cell contact. Eur J Immunol *30*, 2669-2678. 10.1002/1521-4141(200009)30:9<2669::AID-IMMU2669>3.0.CO;2-1.
Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., and Allen, J.E. (2004). Helminth parasites--masters of regulation. Immunol Rev *201*, 89-116. 10.1111/j.0105-2896.2004.00191.x.
Makita, N., Hizukuri, Y., Yamashiro, K., Murakawa, M., and Hayashi, Y. (2015). IL-10 enhances the phenotype of M2 macrophages induced by IL-4 and confers the ability to increase eosinophil migration. Int Immunol *27*, 131-141. 10.1093/intimm/dxu090.
Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol *229*, 176-185. 10.1002/path.4133.
Martins, G.A., Cimmino, L., Shapiro-Shelef, M., Szabolcs, M., Herron, A., Magnusdottir, E., and Calame, K. (2006). Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol *7*, 457-465. 10.1038/ni1320.

McCoy, K.D., Stoel, M., Stettler, R., Merky, P., Fink, K., Senn, B.M., Schaer, C., Massacand, J., Odermatt, B., Oettgen, H.C., et al. (2008). Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection. Cell Host Microbe *4*, 362-373. 10.1016/j.chom.2008.08.014.
Meli, A.P. (2019). Dissecting mechanisms of T follicular helper cell and T helper type 2 cell differentiation during parasitic helminth infection. Doctor of Philosophy (McGill University).
Meli, A.P., Fontés, G., Avery, D.T., Leddon, S.A., Tam, M., Elliot, M., Ballesteros-Tato, A., Miller, J., Stevenson, M.M., Fowell, D.J., et al. (2016). The Integrin LFA-1 Controls T Follicular Helper Cell Generation and Maintenance. Immunity *45*, 831-846. 10.1016/j.immuni.2016.09.018.
Meli, A.P., Fontés, G., Leung Soo, C., and King, I.L. (2017). T Follicular Helper Cell-Derived IL-4 Is Required for IgE Production during Intestinal Helminth Infection. J Immunol *199*, 244-252. 10.4049/jimmunol.1700141.
Minutti, C.M., Drube, S., Blair, N., Schwartz, C., McCrae, J.C., McKenzie, A.N., Kamradt, T., Mokry, M., Coffer, P.J., Sibilia, M., et al. (2017). Epidermal Growth Factor Receptor Expression Licenses Type-2 Helper T Cells to Function in a T Cell Receptor-Independent Fashion. Immunity *47*, 710-722.e716. 10.1016/j.immuni.2017.09.013.
Minutti, C.M., Modak, R.V., Macdonald, F., Li, F., Smyth, D.J., Dorward, D.A., Blair, N., Husovsky, C., Muir, A., Giampazolias, E., et al. (2019). A Macrophage-Pericyte Axis Directs Tissue Restoration via Amphiregulin-Induced Transforming Growth Factor Beta Activation. Immunity *50*, 645-654.e646. 10.1016/j.immuni.2019.01.008.
Mohrs, K., Wakil, A.E., Killeen, N., Locksley, R.M., and Mohrs, M. (2005). A two-step process for cytokine production revealed by IL-4 dual-reporter mice. Immunity *23*, 419-429. 10.1016/j.immuni.2005.09.006.
Nadjsombati, M.S., McGinty, J.W., Lyons-Cohen, M.R., Jaffe, J.B., DiPeso, L., Schneider, C., Miller, C.N., Pollack, J.L., Nagana Gowda, G.A., Fontana, M.F., et al. (2018). Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. Immunity *49*, 33-41.e37. 10.1016/j.immuni.2018.06.016.
Nurieva, R.I., Chung, Y., Martinez, G.J., Yang, X.O., Tanaka, S., Matskevitch, T.D., Wang, Y.H., and Dong, C. (2009). Bcl6 mediates the development of T follicular helper cells. Science *325*, 1001-1005. 10.1126/science.1176676.
Ouyang, W., Löhning, M., Gao, Z., Assenmacher, M., Ranganath, S., Radbruch, A., and Murphy, K.M. (2000). Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. Immunity *12*, 27-37. 10.1016/s1074-7613(00)80156-9.
Ouyang, W., and O'Garra, A. (2019). IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. Immunity *50*, 871-891. 10.1016/j.immuni.2019.03.020.
Poholek, A.C., Jankovic, D., Villarino, A.V., Petermann, F., Hettinga, A., Shouval, D.S., Snapper, S.B., Kaech, S.M., Brooks, S.R., Vahedi, G., et al. (2016). IL-10 induces a STAT3-dependent autoregulatory loop in T. Sci Immunol *1*.
Reynolds, L.A., Filbey, K.J., and Maizels, R.M. (2012). Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. Semin Immunopathol *34*, 829-846. 10.1007/s00281-012-0347-3.
Rodig, S.J., Meraz, M.A., White, J.M., Lampe, P.A., Riley, J.K., Arthur, C.D., King, K.L., Sheehan, K.C., Yin, L., Pennica, D., et al. (1998). Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell *93*, 373-383. 10.1016/s0092-8674(00)81166-6.
Saha, S., Aranda, E., Hayakawa, Y., Bhanja, P., Atay, S., Brodin, N.P., Li, J., Asfaha, S., Liu, L., Tailor, Y., et al. (2016). Macrophage-derived extracellular vesicle-packaged WNTs rescue intestinal stem cells and enhance survival after radiation injury. Nat Commun *7*, 13096. 10.1038/ncomms13096.
Salgame, P., Yap, G.S., and Gause, W.C. (2013). Effect of helminth-induced immunity on infections with microbial pathogens. Nat Immunol *14*, 1118-1126. 10.1038/ni.2736.
Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T.K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., et al. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related

protein ST2 and induces T helper type 2-associated cytokines. Immunity *23*, 479-490. 10.1016/j.immuni.2005.09.015.
Schopf, L.R., Hoffmann, K.F., Cheever, A.W., Urban, J.F., and Wynn, T.A. (2002). IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. J Immunol *168*, 2383-2392. 10.4049/jimmunol.168.5.2383.
Setiawan, T., Metwali, A., Blum, A.M., Ince, M.N., Urban, J.F., Elliott, D.E., and Weinstock, J.V. (2007). Heligmosomoides polygyrus promotes regulatory T-cell cytokine production in the murine normal distal intestine. Infect Immun *75*, 4655-4663. 10.1128/IAI.00358-07.
Shea-Donohue, T., Sullivan, C., Finkelman, F.D., Madden, K.B., Morris, S.C., Goldhill, J., Piñeiro-Carrero, V., and Urban, J.F. (2001). The role of IL-4 in Heligmosomoides polygyrus-induced alterations in murine intestinal epithelial cell function. J Immunol *167*, 2234-2239. 10.4049/jimmunol.167.4.2234.
Shimokawa, C., Kanaya, T., Hachisuka, M., Ishiwata, K., Hisaeda, H., Kurashima, Y., Kiyono, H., Yoshimoto, T., Kaisho, T., and Ohno, H. (2017). Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. Immunity *46*, 863-874.e864. 10.1016/j.immuni.2017.04.017.
Svetić, A., Madden, K.B., Zhou, X.D., Lu, P., Katona, I.M., Finkelman, F.D., Urban, J.F., and Gause, W.C. (1993). A primary intestinal helminthic infection rapidly induces a gut-associated elevation of Th2-associated cytokines and IL-3. J Immunol *150*, 3434-3441.
Takeda, K., Tanaka, T., Shi, W., Matsumoto, M., Minami, M., Kashiwamura, S., Nakanishi, K., Yoshida, N., Kishimoto, T., and Akira, S. (1996). Essential role of Stat6 in IL-4 signalling. Nature *380*, 627-630. 10.1038/380627a0.
Urban, J.F., Katona, I.M., and Finkelman, F.D. (1991). Heligmosomoides polygyrus: CD4+ but not CD8+ T cells regulate the IgE response and protective immunity in mice. Exp Parasitol *73*, 500-511. 10.1016/0014-4894(91)90074-7.
Urban, J.F., Maliszewski, C.R., Madden, K.B., Katona, I.M., and Finkelman, F.D. (1995). IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. J Immunol *154*, 4675-4684.
Van de Velde, L.A., Subramanian, C., Smith, A.M., Barron, L., Qualls, J.E., Neale, G., Alfonso-Pecchio, A., Jackowski, S., Rock, C.O., Wynn, T.A., and Murray, P.J. (2017). T Cells Encountering Myeloid Cells Programmed for Amino Acid-dependent Immunosuppression Use Rictor/mTORC2 Protein for Proliferative Checkpoint Decisions. J Biol Chem *292*, 15-30. 10.1074/jbc.M116.766238.
Vats, D., Mukundan, L., Odegaard, J.I., Zhang, L., Smith, K.L., Morel, C.R., Wagner, R.A., Greaves, D.R., Murray, P.J., and Chawla, A. (2006). Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab *4*, 13-24. 10.1016/j.cmet.2006.05.011.
von Moltke, J., Ji, M., Liang, H.E., and Locksley, R.M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature *529*, 221-225. 10.1038/nature16161.
Wallrapp, A., Riesenfeld, S.J., Burkett, P.R., Abdulnour, R.E., Nyman, J., Dionne, D., Hofree, M., Cuoco, M.S., Rodman, C., Farouq, D., et al. (2017). The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. Nature *549*, 351-356. 10.1038/nature24029.
Weber-Nordt, R.M., Riley, J.K., Greenlund, A.C., Moore, K.W., Darnell, J.E., and Schreiber, R.D. (1996). Stat3 recruitment by two distinct ligand-induced, tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain. J Biol Chem *271*, 27954-27961. 10.1074/jbc.271.44.27954.
Wong, C.K., Li, P.W., and Lam, C.W. (2007). Intracellular JNK, p38 MAPK and NF-kappaB regulate IL-25 induced release of cytokines and chemokines from costimulated T helper lymphocytes. Immunol Lett *112*, 82-91. 10.1016/j.imlet.2007.07.002.
Yao, Y., Xu, X.H., and Jin, L. (2019). Macrophage Polarization in Physiological and Pathological Pregnancy. Front Immunol *10*, 792. 10.3389/fimmu.2019.00792.

Zhao, A., McDermott, J., Urban, J.F., Gause, W., Madden, K.B., Yeung, K.A., Morris, S.C., Finkelman, F.D., and Shea-Donohue, T. (2003). Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. J Immunol *171*, 948-954. 10.4049/jimmunol.171.2.948.
Zhao, A., Urban, J.F., Anthony, R.M., Sun, R., Stiltz, J., van Rooijen, N., Wynn, T.A., Gause, W.C., and Shea-Donohue, T. (2008). Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. Gastroenterology *135*, 217-225.e211. 10.1053/j.gastro.2008.03.077.
Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell *89*, 587-596. 10.1016/s0092-8674(00)80240-8.
Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol *28*, 445-489. 10.1146/annurev-immunol-030409-101212.
Ziegler, S.F., and Artis, D. (2010). Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol *11*, 289-293. 10.1038/ni.1852.