Influence of KIR/HLA and FcγRIIIa genotypes on anti-HIV ADCC responses in HIV uninfected and infected slow progressor subjects

Pilar Zanoni
Division of Experimental Medicine
McGill University

A thesis submitted to the
Faculty of Graduate Studies and Research
In partial fulfillment of the requirements of the degree of
Master of Science

March 2013

Influence of KIR/HLA and FcγRIIIa genotypes on anti-HIV ADCC responses in HIV uninfected and infected slow progressor subjects

Pilar Zanoni

Abstract

Thirty-four million people are currently estimated to be infected with HIV. The need for a vaccine against HIV remains urgent. The RV144 vaccine trial reported partial protection against HIV infection. The RV144 vaccine regimen induced non-neutralizing antibodies (Abs) that engage cells of the innate immune system, such as natural killer (NK) cells, which may exert anti-viral activity by mediating antibody-dependent cell-mediated cytotoxicity (ADCC) against HIV-infected cells. The functional response of NK cells is determined by the integration of activating and inhibitory signals delivered to the NK cell through cell surface receptors, which include killer immunoglobulin-like receptors (KIR). Inhibitory KIRs (iKIR) bind human leukocyte antigen (HLA) molecules and are able to detect their downregulated expression on transformed or virally infected cells. Epidemiological evidence has linked certain KIR3DL1/HLA-B (3DL1/HLA-B) genotypes with protective outcomes in HIV-exposed seronegative (HESN) subjects and HIV infected slow progressors (SP). The mechanisms responsible for these protective outcomes remain unknown. Weak degranulation by 3DL1+ NK cells against autologous HIV-infected cells suggests that other activating signals may be required. Cross-linking NK cells with Ab-coated target cells via the NK cell surface Fc-receptor CD16a may provide these stimulatory signals. Using an assay that detects the delivery of Granzyme B (GzB) from NK effector cells to GzB substrate-coated target cells, I found that the source of NK effector cells influenced anti-HIV ADCC potency. In 47 uninfected subjects, NK cells from carriers of a protective 3DL1/Bw4 genotype known as *h/*y+B*57 had higher levels of ADCC activity than those from carriers of other 3DL1/Bw4 combinations or 3DL1/Bw6 homozygotes. This finding implicated NK cell education in ADCC potency. In contrast, in 47 HIV infected SP, NK cells from *h/*y+B*57 carriers had indistinguishable ADCC responses compared to NK cells from carriers of other 3DL1/Bw4 combinations or from 3DL1/Bw6 homozygotes suggesting that ADCC activity was sensitive to dysregulation by HIV infection. Since NK cells within a genotype group exhibited a range of ADCC activity, I examined the role of several factors other than 3DL1/HLA-B genotype that may influence ADCC potency. A polymorphism at amino acid 158 of CD16a results in greater affinity of the V than the F isoform for Ab. I assessed whether genetic variation in CD16a could contribute to differences in ADCC responses and report that percent ADCC target cell killing was independent of CD16a genotype. I also showed that self-iKIR to HLA-C contributed minimally to ADCC responses supporting our contention that interactions between KIR3DL1 and HLA-B*57 stand out from other educationally competent KIR/HLA combinations in educating NK cells for ADCC functional potential. My results suggest that HLA/KIR dependent NK cell education is a determinant of anti-HIV ADCC functional potential in uninfected individuals. Based on the absence of superior ADCC responses in SP carrying *h/*y+B*57 I propose that NK cell mediated ADCC activity is sensitive to and readily extinguished in the context of HIV infection.

Influence des génotypes KIR/HLA et FcγRIIIa sur les réponses ADCC anti-VIH chez des sujets non infectés par le VIH et les sujets infectés progresseurs lents

Pilar Zanoni

Résumé

Il est estimé que 34 millions de personnes sont infectés par le VIH à ce jour. Il est donc urgent de développer un vaccin contre le VIH. Les essais du vaccin RV144 ont rapporté une protection partielle contre l'infection par le VIH. Ce vaccin induit des anticorps (Ac) non-neutralisants qui activent les cellules du système immunitaire inné, telles que les cellules natural killer (NK), qui pour leur part peuvent induire une activité anti-virale par la médiation de la cytotoxicité cellulaire anticorps-dépendante (ADCC) contre les cellules infectées par le VIH. La réponse fonctionnelle des cellules NK est déterminée par l'intégration de signaux activateurs ou inhibiteurs dans la cellule par le biais de récepteurs cellulaires de surface, incluant les récepteurs « killer immunoglobulin-like » (KIR). Les KIRs inhibiteurs (iKIR) se lient aux molécules de l'antigène leukocytaire humain (HLA) et sont capables de détecter leur expression diminuée sur les cellules transformées ou infectées par le virus. Des données épidémiologiques ont relié certains génotypes KIR3DL1/HLA-B (3DL1/HLA-B) à la protection chez des individus séronégatifs exposés au VIH (HESN) et chez des individus infectés au VIH à progression lente (SP). Les mécanismes menant à cette protection demeurent par contre inconnus. La faible dégranulation des cellules autologues infectées par le VIH par les cellules 3DL1 + NK suggère que d'autres signaux activateurs pourraient être requis. Le fait de lier des cellules NK avec des cellules-cibles recouvertes par des Ac via le récepteur de surface Fc-receptor CD16a pourrait induire ces signaux stimulateurs. En utilisant un test qui détecte le transfert du Granzyme B (GzB) des cellules effectrices NK aux cellules-cibles recouvertes du substrat GzB, j'ai découvert que la source de cellules effectrices NK influence le potentiel ADCC anti-VIH. Chez 47 sujets, les cellules NK provenant d'individus porteurs d'un génotype protecteur 3DL1/Bw4 connu (*h/*y+B*57) avaient des niveaux d'activité ADCC plus élevés que ceux observés chez les cellules provenant d'individus porteurs d'autres combinaisons 3DL1/Bw4 ou homozygotes pour 3DL1/Bw6. Ces résultats impliquent l'éducation des cellules NK dans le potentiel ADCC. Par contre, chez 47 individus SP infectés par le VIH, les cellules NK de porteurs de *h/*y+B*57 ont eu des réponses ADCC indétectables comparativement aux cellules NK de porteurs d'autres combinaisons 3DL1/Bw4 ou d'homozygotes pour 3DL1/Bw6, suggérant que l'activité ADCC est sensible à la dysrégulation par l'infection par le VIH. Comme les cellules NK incluses dans un groupe de génotype démontraient une activité ADCC variable, j'ai étudié le rôle de différents facteurs autres que le génotype 3DL1/HLA-B qui pourraient influencer le potentiel ADCC. Un polymorphisme à l'acide aminé 158 du CD16a résulte en une meilleure affinité de l'Ac avec l'isoforme V que l'isoforme F. J'ai donc déterminé si la variation du CD16a contribue aux différences de réponses ADCC et observé que le pourcentage de mort des cellules-cibles par ADCC ne dépendait pas du génotype CD16a. J'ai aussi démontré que les auto-iKIR de HLA-C contribuent un minimum aux réponses ADCC, supportant nos affirmations que les interactions entre KIR3DL1 et HLA-B*57 proviennent de combinaisons KIR/HLA pour l'éducation des cellules NK menant à un potentiel fonctionnel ADCC. Les résultats que j'ai obtenus suggèrent que l'éducation des cellules NK dépendante de HLA/KIR est déterminante pour le potentiel fonctionnel ADCC anti-VIH chez les individus non infectés. En me basant sur l'absence de réponses supérieure ADCC chez des individus SP porteurs de

*h/*y+B*57, je suggère que l'activité ADCC médiée par les cellules NK est sensible à et facilement éliminée dans le contexte de l'infection par le VIH.

Acknowledgements

Above all, I would like to thank my parents for teaching me the equal value of formal education and life experience, and for providing the possibility and opportunity for my pursuit of these. To “mini frundin” Jennifer, for always listening, and to Dave for your patience and strength. This thesis is a product of your influence.

I have had the good fortune of training under my supervisor, Dr. Nicole Bernard, and would like to thank you for providing the resources and guidance that have been integral to the successful completion of this thesis. Your kindness and support have made this endeavour enjoyable and rewarding.

The members of the Bernard lab have enriched my experience with their friendships, and their assistance has been indispensable. Marie-Pierre Boisvert, Tsoarella Mabanga and Xioayan Ni – your daily contributions have not been overlooked. My thanks to Gamze Isitman, Sofia Miconiatis, Benjamin Tallon, Carlos-Melendez Pena and Zoya Padamsi for their various contributions. In particular, I wish to thank Matthew Parsons for your conversation, “caffeination” and counsel.

Finally, I would like to thank those who have generously donated samples.

Table of Contents

Abbreviations

(-)/(+) ssDNA – minus/plus strand strong-stop DNA

aa – amino acid

Ab – antibody

ADCC – antibody dependent cell-mediated cytotoxicity

ADCVI – antibody dependent cell-mediated viral inhibition

AIDS – acquired immune deficiency syndrome

AP-1 – adaptor protein 1

APOBEC - apolipoprotein-B mRNA editing catalytic subunit protein

ARV – AIDS-associated retrovirus

BnAb – broadly neutralizing antibody

CA – capsid

CCR5 – C-C chemokine receptor type 5

cDNA – complementary deoxyribonucleic acid

CMV – cytomegalovirus

CNV – copy number variation

CRF – circulating recombinant forms

CTL – cytotoxic T lymphocytes

CTS – central termination signal

CXCR4 – C-X-C chemokine receptor type 4

DC – dendritic cell

DNA – deoxyribonucleic acid

dNTP – deoxyribonucleoside triphosphates

EBV – Epstein-Barr virus

EC – elite controller

ELISA – enzyme-linked immunosorbent assay

Env – envelope

GALT – gut-associated lymphoid tissue

GRID – gay-related immune deficiency

GVHD – graft-versus-host-disease

GWAS – genome-wide association study

GzB – granzyme B

HAART – highly active antiretroviral therapy

HCV – hepatitis C virus

HCMV – human cytomegalovirus

HESN – HIV-exposed seronegative

HIV – human immunodeficiency virus

HIVIG – HIV Immunoglobulin

HLA – human leukocyte antigen

Hmz - homozygous

HTLV-III – human T-cell lymphotropic virus III

Htz – heterozygous

HSCT – hematopoietic stem cell transplantation

IDU – injection drug user

IFN-α – interferon alpha

IFN-γ – interferon-gamma

Ig – immunoglobulin

iKIR – inhibitory killer immunoglobulin-like receptor

IL – interleukin

IN – integrase

ITAM – immunoreceptor tyrosine-based activation motif

ITIM – immunoreceptor tyrosine-based inhibition motif

KIR – killer immunoglobulin-like receptor

KLR – killer cell lectin-like receptor

LAV – lymphadenopathy associated virus

LILR – leukocyte immunoglobulin-like receptors

LRC – leukocyte receptor complex

LTNP – Long term non-progressor

LTR – long terminal repeats

MA – matrix protein

mAb – monoclonal antibody

MCMV – murine cytomegalovirus

MIC – MHC class I chain-related

MHC – major histocompatibility complex

MMP – Matrix metalloproteinase

mRNA – messenger ribonucleic acid

MSM – men who have sex with men

NC – nucleocapsid

NCR – natural cytotoxicity receptor

NFκB – nuclear factor kappa B

NF-AT – nuclear factor of activated T cells

NHP – nonhuman primate models

NK – natural killer

NLS – nuclear localization signal

PBS – primer binding site

PIC – pre-integration complex

PPT – poly-purine tract

PrEP – pre-exposure prophylaxis

PI – primary infection

Pro – protease

RNA – ribonucleic acid

RT – reverse transcriptase

RTC – reverse transcription complex

RRE – Rev response element

SAMHD1 - SAM domain HD domain-containing protein 1

SCID – severe combined immunodeficiency disease

SHIV – simian immunodeficiency virus

SHP-1 – Src-homology domain-containing tyrosine phosphatase

S-iKIR – self-inhibitory killer immunoglobulin-like receptor

SIV – simian immunodeficiency virus

SHIV – simian-human immunodeficiency virus

SNP – single nucleotide polymorphism

SP – slow progressor

Sp1 – Spacer 1

TNF-α – tumour necrosis factor alpha

TRIM5α – tripartite motif 5 alpha

tRNA – transfer ribonucleic acid

VC – viral controllers

VL – viral load

WT – wild type

List of Tables

Chapter 2: Manuscript - Investigation of the influence of HIV infection, KIR/HLA genotype and FcγRIIIa genotype on NK cell mediated anti-HIV ADCC function

List of Figures

Chapter 1: Introduction and Literature Review

Chapter 2: Manuscript - Investigation of the influence of HIV infection, KIR/HLA genotype and FcγRIIIa genotype on NK cell mediated anti-HIV ADCC function

Chapter 1: Introduction and Literature Review

1.1: The global HIV/AIDS pandemic

The acquired immune deficiency syndrome (AIDS) epidemic was recognized in 1981 when the Center for Disease Control (CDC) in the United States reported unusual clusters of Kaposi's sarcoma and *pneumocystis* (*carinii*) *jiroveci* pneumonia in five homosexual men in Los Angeles. Two publications later that year described a new acquired cellular immunodeficiency which was believed to be a homosexual-only disease and became known as gay-related immune deficiency (GRID)(1;2). Once public health officials reported GRID in Haitians, homosexuals, hemophiliacs and heroin users the term "4H disease" was coined. Once the cellular immunodeficiency began to show up in female sexual partners of infected individuals and among blood transfusion recipients, the terms GRID and "4H disease" were dropped in favor of AIDS in 1982. In 1983, Drs. Luc Montagnier and Françoise Barré-Sinoussi discovered lymphadenopathy associated virus (LAV)(3). The following year Dr. Jay Levy discovered a virus, which he originally called AIDS-associated retrovirus (ARV)(4). That same year, human T-cell lymphotropic virus-III (HTLV-III) was reported by Robert Gallo to be the "probable" cause of AIDS(5-8). In 1986 LAV and HTLV-III were found to be genetically indistinguishable and the virus was renamed the human immunodeficiency virus (HIV). In this thesis, the use of the term HIV will refer to HIV-1. Since its discovery HIV has become a global health problem responsible for the deaths of an estimated 25 million people worldwide, with another estimated 33.3 million living with HIV(9). HIV is highly heterogeneous within infected individuals, likely owing to a number of viral, host and genetic factors. Complex interactions between the genetic variability of the virus and differences in the immune response and genetics of the host influence the outcome of HIV infection in humans(10;11). Effective treatment in the form of highly active antiretroviral therapy (HAART) is available. HAART consists of at least three drugs, often belonging to at least 2 drug classes that are active against HIV. The term drug class is used to refer to related drugs that target different aspects of the HIV replication cycle, such as entry inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and integrase inhibitors. The advent of HAART revolutionized the management of HIV/AIDS therapy for those with access to health care, but remains expensive with more limited access in low to middle income countries (12). HAART is capable of controlling infection with HIV, though it is unable to

eradicate HIV and to date there is no known cure. Furthermore, drug-related toxicities, viral resistance, compliance issues and multiple side effects complicate the use of HAART(13).

Successful treatment of HIV-infected individuals with HAART is defined as reduction of HIV viral loads (VL) to levels below that detected by standard VL assays. One interesting consequence of this is that successful treatment reduces HIV transmission(14;15). A recent trial conducted in serodiscordant couples found that treating HIV-infected partners reduced infection by 96%(15). This test and treat strategy however, will be limited by the barriers to identifying newly infected individuals who have high VL and are particularly infectious(16). HAART is also being introduced for HIV pre-exposure prophylaxis (PrEP) as another potential intervention strategy to reduce HIV transmission(17;18). Other PrEP strategies aimed at reducing the susceptibility of seronegative individuals to HIV infection are in clinical trials, including those using microbicides. PrEP has shown a moderate reduction in the likelihood of HIV infection, ranging from 44% to 73% in those with over 90% adherence(19). Despite this conservative success, the FEM-PrEP clinical study was unable to demonstrate efficacy in preventing HIV infection among high-risk heterosexual women in sub-Saharan Africa(20). It is clear that several issues surrounding PrEP have yet to be resolved, such as refining the populations, which will benefit most, providing adequate education to the public regarding potential risks and benefits of PrEP, and financing and delivery of PrEP globally to those at risk(21).

Given the complex socioeconomic, political and scientific problems, which hamper the efficacy of HAART, and the issues that remain in establishing efficacy and implementing test and treat and PrEP, the design of a preventative or therapeutic vaccine remains an urgent goal for HIV/AIDS research.

1.1.2: The origin of HIV

Two antigenically and evolutionarily distinct lineages of HIV have been identified; HIV-1 and HIV-2. The latter is distantly related to HIV-1 but closely related to a simian immunodeficiency virus (SIV) originating in sooty mangabeys(22). Additional SIVs have been found in various primates from sub-Saharan Africa, including, African green monkeys, mandrills, chimpanzees and others suggesting that HIV in humans emerged as a result of several cross-species transmission events(12). Interestingly, SIVs appear to be largely non-pathogenic in their natural hosts.

HIV-1 has been shown to be of chimpanzee origin, having evolved from two subspecies-specific lineages SIVcpz*Ptt* (*Pan troglodytes troglodytes*) and SIVcpz*Pts* (*Pan troglodytes schweinfurthii*)(12;23). The HIV-1 lineage includes four phylogenetically distinct groups of virus – M (main), N (new or nonmain), O (outlier) and P (putative)(24;25) – with group M being the pandemic form and further divided into nine distinct subtypes or clades (A, B, C, D, F, G, H, J, K), and additional circulating recombinant forms(12). Up to 20% variability in the envelope (Env) protein may exist within a clade and up to 40% variability between clades(26;27).

Unlike the global distribution of HIV-1, since its first discovery in the mid-1980s HIV-2 has remained largely restricted to West Africa with the highest rates of prevalence at 4% reported in Guinea-Bissau(12;26;28). HIV-2 is less pathogenic and less transmissible than HIV-1, with a near complete absence of mother-to-infant transmission(12). Individuals infected with HIV-2 exhibit lower VL than HIV-1 infected individuals and most HIV-2 infected individuals do not progress to AIDS(29). In recent years, HIV-2 prevalence has been declining, particularly among younger people. Given its recent decline, lower transmission efficacy, longer asymptomatic phase and relative low prevalence, most HIV vaccine research has been and remains focused on HIV-1.

1.1.3: Regional epidemics

Clade C viruses are responsible for about 56-60% of all HIV/AIDS cases in the world, predominating in India and China while subtype B predominates in the Americas, Western Europe and Australia(27). Africa shows the greatest subtype diversity. The burden of the pandemic lies in sub-Saharan Africa, which accounts for over 60% of global infections and has the most complex epidemic consisting of rare subtypes and a wide variety of circulating recombinant forms (CRF) without a discernable predominant strain(30). Emerging epidemics have recently been observed in China and Russia among men who have sex with men (MSM) and injection drug users (IDU). Due to the advent of widely available and successful HAART since 1996 in developed countries in North America and Europe, the incidence of HIV in these regions has stabilized.

1.1.4: Transmission

HIV transmission may occur in a number of ways: during sexual activity via the genital tract and/or rectal mucosa, parenteral exposure to contaminated blood, transfusion with contaminated blood and blood products, occupational exposure, and vertically from

mother to child via blood pre- or perinatally or through breast milk(31). Despite its variability in transmission routes, 80% of adults acquire HIV following mucosal exposure and, HIV/AIDS is thus primarily considered a sexually transmitted disease(15;32;33).

As a lentivirus HIV can productively infect non-dividing, terminally differentiated cells as well as dividing cells(34). HIV predominantly infects activated T cells expressing the cellular CD4 receptor and one or both co-receptors, C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 CXCR4(15;35;36). Following establishment of systemic infection, plasma VL peaks which coincides with preferential, rapid, and irreversible depletion of approximately 50% of the total memory CD4+ T cells in lymphoid tissues, particularly the gut-associated lymphoid tissue (GALT) within two to three weeks of infection(37)(Figure 1). Normal levels of CD4+ T cells can fall from ±1000 cells/mm^3 to less than 50 cells/mm^3, and in extreme cases to less than 10 cells/mm^3(38;39). Analysis of CD4+ T cells in treatment-naïve patients has revealed a substantial ongoing exchange of virus between the peripheral blood and plasma compartments during early and chronic HIV infection(40). This generates the potential for productive recombination in HIV-infected individuals carrying at least two genetically distinct viruses, driving immune escape and drug resistance in these subjects.

HIV strains isolated during acute infection are mostly CCR5-tropic, whereas the appearance of CXCR4-tropic isolates coincides with late stages of infection and rapid progression to AIDS. The association between co-receptor usage and stage of infection, however, is not dependent on pathogenicity as CCR5 and CXCR4 isolates are equally cytopathic(41). It is therefore likely that the major determinant in the switch from CCR5-tropic virus in early infection to CXCR4-tropic strains in late stages of infection is the number of target cells expressing the specific co-receptor subtype. Given the varied routes of viral transmission and distinct histological features of the tissues involved, other target cells include dendritic cells (DC), Langerhans cells and macrophages. To date, no vaccine candidate has been able to prevent the massive infection of immune cells and subsequent extensive loss of CD4+ T cells that result from infection with HIV.

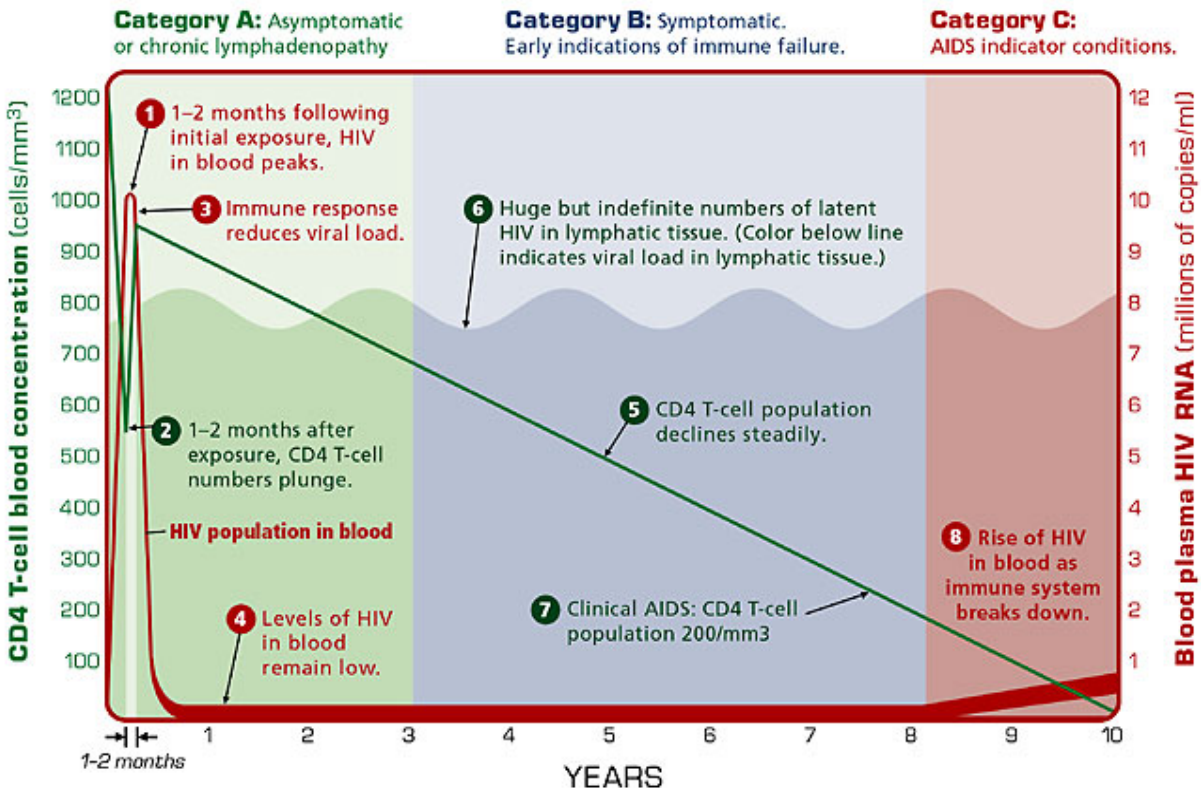


Figure 1: Clinical disease course of HIV/AIDS. Reprinted with permission from Rediscovering Biology and Annenberg Media 2012, www.learner.org/courses/biology/textbook/hiv/hiv_5.html

1.1.5: HIV

HIV is a prototypical retrovirus (*Retroviridae)* consisting of two copies of a single stranded ribonucleic acid (RNA) genome encoding nine genes(42). It belongs to the *Lentivirinae* family so-named to reflect the lengthy incubation period between initial infection and development of disease(43). The HIV genome consists of two copies of positive-sense RNA strands containing nine reading frames encoding three structural proteins and 6 accessory proteins. A mature virion is roughly spherical, with a diameter of 120 nm (44)(Figure 2). The RNA strands are tightly bound to nucleocapsid (NC) proteins and are surrounded by a conical capsid composed of the 5 nm p24 capsid protein (CA)(44). The integrity of the virus particle is maintained by the 7 nm matrix protein (MA) which surrounds the capsid(45). Surrounding the matrix is the bilayer viral envelope, made up of host-derived phospholipids embedded with non-covalently associated viral proteins gp120 and gp41 which make up homotrimeric "viral spikes"(44;45).

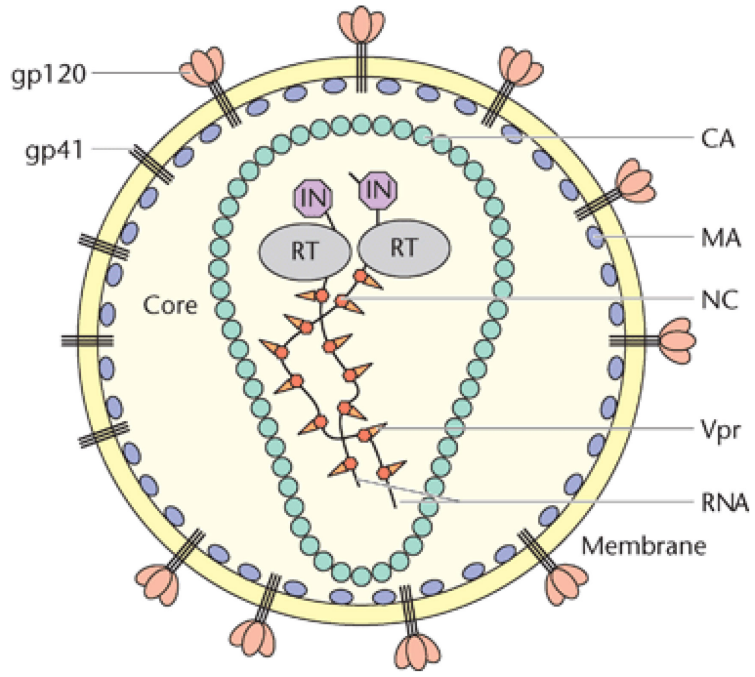


Figure 2. The major structural components of human immunodeficiency virus. Reprinted with permission from Bukrinsky MI. 2010. HIV life cycle and inherited co-receptors. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester

1.1.6: HIV genome structure and replication

1.1.6.1: The viral genome

The HIV genome is stored on two copies of positive-sense RNA strands, approximately 9.7 kilobases in length and flanked at both ends by long terminal repeats (LTR)(44). The HIV-1 genome encodes nine gene products that are divided into three classes (Figure 3);

I. The major structural proteins: Gag, Pol, and Env
II. The regulatory proteins: Tat and Rev
III. The accessory proteins: Vpr, Vpu for HIV-1 and Vpx for HIV-2, Vif and Nef

Further proteolysis of some proteins results in a total of 15 proteins required for the HIV replication cycle.

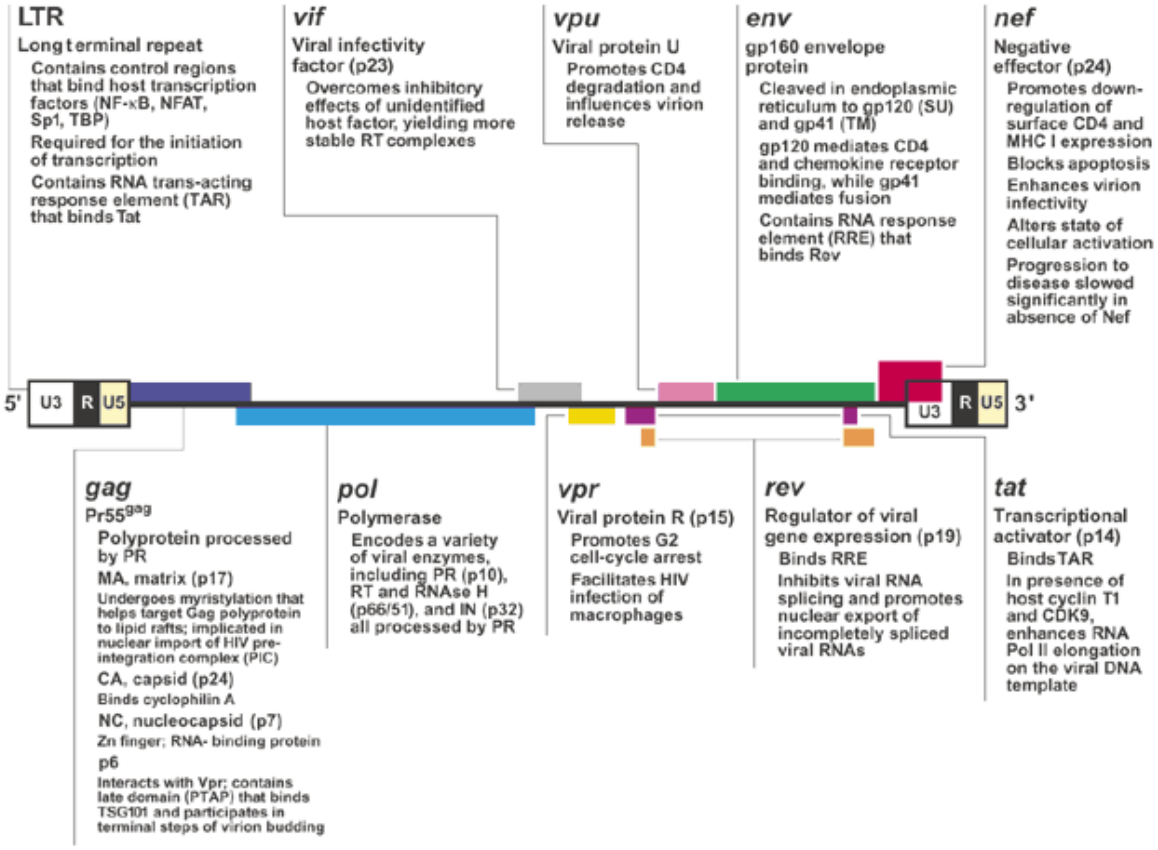


Figure 3. Structure of the HIV genome and function of encoded proteins. Reprinted with permission from Greene WC and Peterlin BM. Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. *Nat Med*, 2002

1.1.6.2: The viral life cycle

Binding and entry: To initiate entry into a target cell, the Env (gp120) trimer spike binds to its primary receptor, CD4, expressed on the surface of T helper cells, monocytes, macrophages, DC and Langerhans cells. This binding induces a conformational change in gp120, increasing its affinity for a co-receptor by exposure of the co-receptor binding site. Env then binds either CCR5 or CXCR4 on target cells which triggers the dissociation of gp120 from gp41, bringing forth a hydrophobic region (fusion peptide) in gp41, which then inserts into the host cell's membrane(42;44). A fusion pore is formed enabling the virus capsid to enter the cell(44). Once a cell has been infected, HIV downregulates cell-surface CD4 to prevent trapping of budding virions through Env

gp120 and cell-surface CD4 interactions, as well as to prevent superinfection of the cell(42).

Uncoating: This process involves release of the viral capsid into the cytoplasm of infected cells. This converts the HIV core to the reverse transcription complex (RTC) and then into the pre-integration complex (PIC), which forms the integrated provirus(42;46).

Reverse transcription: Reverse transcription is catalyzed by reverse transcriptase (RT) and converts the HIV single-positive diploid RNA genome into double-stranded complementary deoxyribonucleic acid (cDNA). The deoxyribonucleic acid (DNA) polymerase activity of RT incorporates deoxyribonucleoside triphosphates (dNTPs) that form 3'-5' phosphodiester bonds with the 3'hydroxyl terminus of the primer to generate nascent DNA(47).

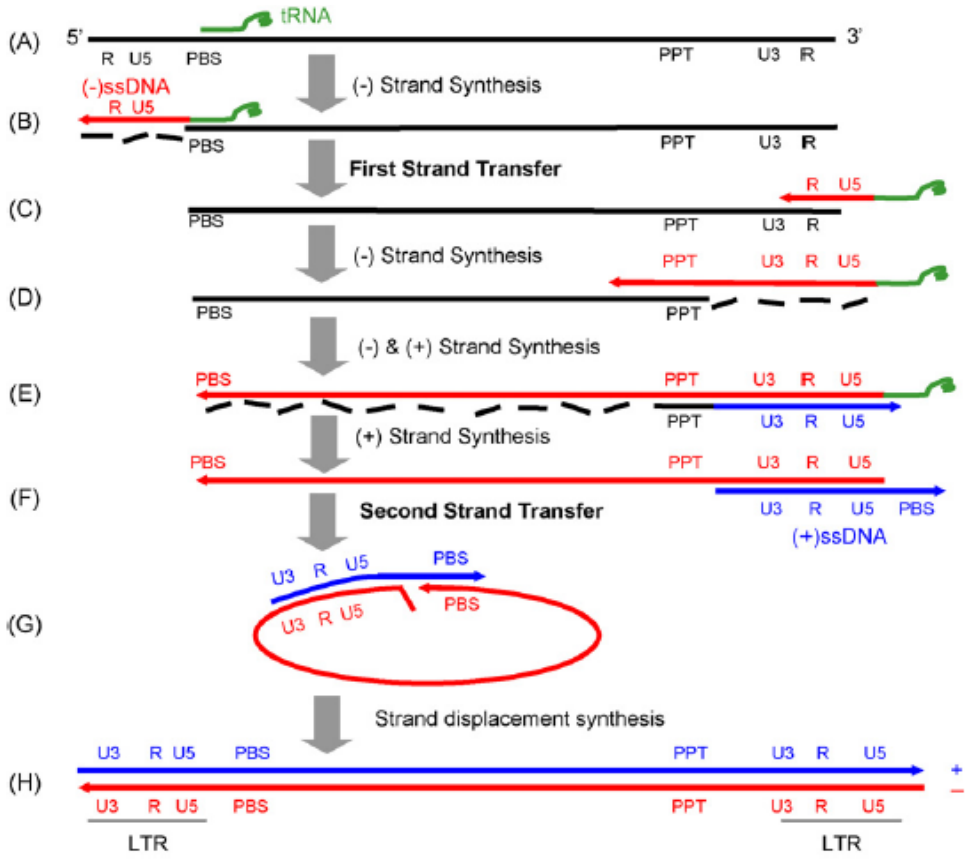


Figure 4: Steps of the reverse transcription process. Reprinted with permission from Basu VP, Song M, Gao L, Rigby ST, Hanson MN, Bambara RA. Strand transfer events during HIV-1 reverse transcription, *Virus Research*, 2008

Steps of HIV reverse transcription process (Figure 4)

(A) Minus strand synthesis: Reverse transcription is initiated from the 3'end of a primer molecule of $tRNA^{lys,3}$ partially annealed to a primer binding site (PBS).

DNA synthesis proceeds to the 5' end of the RNA generating a minus strand strong-stop DNA (-) ssDNA, a DNA/RNA hybrid.

(B) First strand transfer: The 5'-end of the genomic RNA is degraded by the RNase H activity of the RT and (-) ssDNA is translocated to the 3'-end of the genomic RNA.

(C) Minus strand synthesis: A sequence of nucleotides referred to as the 'R' at the 3'-end of the (-) ssDNA binds to a complimentary 'R' region of the genomic RNA allowing for (-) ssDNA synthesis to continue. This forms a hybrid between (-) ssDNA and the RNA genome.

(D) Minus and plus strand synthesis: RNase H activity of RT degrades the RNA strand of the hybrid, releasing any RNA sequences downstream from the 3' R region.

(E) Plus DNA strand synthesis: RNase H degradation of genomic RNA during minus and plus strand synthesis creates the poly-purine tract (PPT) region within the genomic RNA. The plus strand of the DNA template is initiated from the PPT.

(F) Preparation for second strand transfer: Completion of the plus strand of the DNA template results in a copy of the primer transfer $RNA^{lys,3}$ ($tRNA^{lys,3}$) thereby also creating a DNA copy of the PBS at the plus strand strong-stop DNA terminus (+) ssDNA. The action of RNase H removes the primer $tRNA^{lys,3}$.

(G) The complimentary copies of PBS sequences at the 3'-ends of the newly generated (+) ssDNA and the nascent minus strand DNA form base pairs.

(H) Second strand transfer and strand displacement synthesis: A second strand transfer event occurs allowing for minus and plus strand DNA synthesis to resume, with each strand using the other as a template until the double stranded DNA is fully completed creating LTR at either end. An additional termination site referred to as the central termination signal (CTS), located near the center of the genome, causes the displacement of approximately 100 nucleotides resulting in the formation of a DNA flap.

The final product of HIV reverse transcription is linear double stranded cDNA that can be incorporated into the host cell genome. Formation of a central DNA flap results in a PIC, competent for import into the nucleus and integration within the host chromatin.

Nuclear Entry: During reverse transcription the HIV genome is linear, blunt-ended and compacted into the PIC allowing passage through the nuclear pore using cellular nuclear import machinery that normally mediates nuclear import of cellular proteins carrying nuclear localisation signals (NLS)(48). Four viral components contribute to nuclear import of the PIC; 1) integrase (IN), 2) MA, 3) the central DNA flap and 4) Vpr, though its role is controversial as Vpr-deficient viruses retain the capacity to infect non-dividing cells(49). The capacity of HIV to infect non-proliferating cells has been attributed to the presence of NLS in these four factors(50).

Integration: Integration of reverse-transcribed viral DNA into a host genome finalises infection and is mediated IN(42). The integration process occurs in two steps referred to as 3' processing, which takes place in the cytoplasm within the PIC nucleoprotein complex, and strand transfer. During 3' processing IN recognizes a dinucleotide at each 3' end of the viral LTR to expose terminal hydroxyl groups(44). The viral DNA bound to the PIC is transported into the nucleus via NLS in IN, MA and Vpr(44). Strand transfer involves the insertion of processed viral cDNA ends into the host chromosomal DNA. This is accomplished when IN cleaves unpaired dinucleotides from the 5'-ends of the viral DNA and repairs single stranded gaps between the viral and target DNA(44;51;52). The integrated provirus will lay dormant until cellular transcription factors initiate transcription of viral RNAs.

Transcription from provirus: The integrated provirus functions as a cellular gene, regulating HIV gene expression along with cellular transcription factors and Tat. Multiple cellular transcription factors such as Sp1 (spacer 1), NFκB (nuclear factor kappa B), AP-1 (adaptor protein 1) and NF-AT (nuclear factor of activated T cells) bind the LTR(53). As these factors are responsible for T cell activity, T cell activation promotes viral expression(50). LTR promoter activation is primarily driven by Tat to promote transcriptional activity(50).

The fully spliced viral RNA encodes three classes of proteins; i) the multiply spliced mRNA encoding early regulatory proteins such as Nef, Tat and Rev, ii) the singly spliced mRNA that encodes Vpu, Vpr, Vif and Env, and iii) the full-length mRNA encoding the Gag-Pol polyprotein(54). Viral genome transcription and translation occurs in two stages of gene expression, the first of which is Rev-independent and the second Rev-dependent. The translation of Rev, Tat, Vif, Vpr, Vpu and Nef occurs in the Rev-independent phase. Accumulation of Rev results in the nuclear export of unspliced and singly spliced transcripts which encode the HIV structural proteins and carry Rev

response elements (RRE)(50;53). As such, Rev is responsible for the conversion from early to late HIV gene expression and association between Rev and RRE promotes nuclear transport of viral transcripts to the cytosol for assembly, packaging and release(50).

Virion assembly, packaging and release: Virus assembly in T-cells is driven by the Gag protein and is localized to lipid rafts(55). The $Pr55^{Gag}$ polyprotein contains four domains; MA, CA, nucleocapsid (NC) and p6 and is sufficient for viral assembly in the absence of other viral proteins(44).

The N-terminal MA domain of Gag binds to the host cell plasma membrane where Gag-Gag interactions form the structural shell of budding virions, while the MA domain also recruits Env to the plasma membrane to ensure it is incorporated into progeny virus(44). Following this, Gag interacts with components of the host cell endosomal sorting complex to efficiently release virions from the surface of the target cells(44). The prevailing model of virion maturation is that the $Pr55^{Gag}$ and Gag-Pol are cleaved by protease (Pro) to produce mature Gag proteins, which ultimately coordinate the condensation of the inner core, formation of a core shell and conversion of the virus particle into an infectious virion(44;56;57).

During HIV release many host-derived cell surface molecules are incorporated into the HIV viral envelope. These molecules include but are not limited to LFA-1, ICAM-1, CD43, and an abundance of Class I and II major histocompatibility complex (MHC) molecules many of which have been reported to have protective functions for HIV(58;59). Incorporation of such molecules is extensive; they have been demonstrated to retain functionality and have been implicated in HIV pathogenesis(60).

1.1.7: Host response to HIV infection

A vast array of host innate and adaptive immune responses have been identified that can act to restrict or control HIV replication. Type-specific antibodies (Ab) are induced in the first few weeks of acute infection. This initial non-neutralizing Ab response is directed at gp41(61). After about 3 months, neutralizing Abs against Env are observed while broadly neutralizing Abs (BnAbs) appear around 1 year after infection(62;63). BnAbs can mediate neutralization of the virus, although recent passive transfer studies suggest that their efficacy *in vivo* is at least partly dependent upon the ability of effector cells of the innate immune system, such as NK cells, to recognize the Ab constant region (Fc)(64). Both non-neutralizing and neutralizing Abs may complex

with Fcγ receptors on NK cells to mediate effector cell mechanisms, such as antibody dependent cellular cytoxicity (ADCC), against the virus(65). The interaction between Fc domains of predominantly immunoglobulin (Ig)G and some IgA Abs and corresponding receptors on effector cells triggers the release of the content of cytotoxic granules (perforin, granzymes), cytokines and chemokines. Additionally, intrinsic cell-autonomous activities, collectively referred to as host restriction factors are innate immune responders, inducible by interferon (IFN)-α and capable of suppressing viral replication(66). Major classes of host restriction factors include the apolipoprotein-B mRNA editing catalytic subunit (APOBEC) proteins, tripartite motif (TRIM)5α, tetherin and SAM domain HD domain-containing protein 1 (SAMHD1), with new proteins regularly being identified. The APOBEC proteins are cytidine deaminases, which result in viral hypermutation causing replication defects and diminished reverse transcription(66). TRIM5α is a capsid-targeting restriction factor responsible for the inability of HIV to infect many old world monkey cells(67). Tetherin prevents virus release by tethering virus particles to the cell, promoting their endocytosis and degradation(68). SAMHD1, the most recently discovered host restriction factor, is a myeloid specific factor that acts on HIV reverse transcription(69;70). Also capable of inhibiting viral replication in humans and non-human primates are CD8+ T cells.

Several lines of evidence support the role for CD8+ T cell-mediated immune response in the control of HIV. There is a temporal association between the appearance of CD8+ T cells in acute infection with the fall in peak VL(71;72). Certain MHC class I antigens such as HLA-B*27 and B*57 are associated with slow disease progressions while others are associated with rapid progression(10;73-75). This implies a role for CD8+ T cells in HIV control because MHC class I antigens restrict the presentation of HIV epitopes to CD8+ T cells. CD8+ T cell-mediated immune pressure often lead to mutations within epitopes recognized by CD8+ T cells that either result in viral escape or impaired fitness(76-78). The best evidence for the role of CD8+ T cells in control of viremia comes from animal models for human HIV infection, i.e., rhesus macaques infected with SIV; $CD8^+$ lymphocyte depletion leads to increased viral replication and accelerated disease progression in SIV-infected rhesus macaques and African green monkeys(79-82).

Observations that VL set point and rate of disease progression are determined early in HIV and SIV infection, before the establishment of BnAbs, exclude a role for

BnAbs in viral control in acute infection. Although CD8+ T cell responses play an important role in controlling HIV in acute infection, there is also evidence for innate immune mechanisms contributing to early control of viral replication. Indeed, sooty mangabeys, which are natural hosts for SIV and do not develop AIDS, exhibit enhanced levels of NK cells with high functional potential, whereas in rhesus macaques who experience a pathogenic SIV infection exhibit a decline in highly functional NK cells(83). However, these observations do not address the cause and effect, as high viremia may negatively impact NK cell function in sooty mangabeys, rather than SIV infection in rhesus macaques being pathogenic as a result of NK cells with reduced function. Despite this, evidence for a role of NK cells remains supported by the maintenance of highly functional CD8+ NK cells in HIV-infected chimpanzees, which does not lead to an AIDS-like syndrome, while these cells are disrupted in HIV infection in humans(84).

There is epidemiological evidence that NK cells may play a role in controlling HIV infection in humans. The first observations that co-carriage of certain NK receptor and putative HLA ligand combinations are associated with slow time to AIDS were reported in 2002(85). Martin et al. expanded on this finding by reporting several additional NK receptor HLA ligand combinations are associated with time to AIDS(86). Observations of an expansion in the size of the NK cell population in primary and chronic infection, particularly in those with protective NK receptor HLA ligand combinations, suggests that NK cells may exert their effect early in infection(87;88). Together these observations support the idea that NK cells contribute to HIV control and protective outcomes in the context of infection with HIV.

1.1.8: NK cell subsets and functions

NK cells are large, bone marrow-derived granular lymphocytes that comprise 10-15% of the peripheral blood lymphocytes. They may also be found in the spleen, lung, bone marrow, lymph nodes, and liver. NK cells, unlike B and T cells, which belong to the adaptive arm of the immune response, are able to rapidly destroy target cells without prior antigen sensitization through mechanisms such as cytokine release, direct cytolysis and ADCC(89). As innate immune cells, they mediate direct effector functions during the early phase of infection, but may also be essential for inducing downstream adaptive immune responses, as highlighted by their interaction with DC(37;87;90-93). Dendritic cells are a group of bone-marrow-derived leukocytes, with a functional specialisation for antigen uptake, transport, processing and presentation to T cells. An early study by

Fernandez et al. (1999)(93) showed that mice treated with a growth factor promoting DC and NK cell expansion induced an NK-cell-dependent anti-tumour effect. This was the first evidence that DC play a direct role in triggering NK cells *in vivo*. Coinciding with this observation, DCs provide early induction of anti-viral IFN-γ, interleukin (IL)-15 and IL-12 responses, the latter two of which stimulate NK cell proliferation(91). Once activated, NK cells produce IFN-γ, tumour necrosis factor (TNF)-α and other Th-1 type adaptive cytokines to help shape the adaptive T cell response(90;94). As such, NK cells serve as a bridge between innate and adaptive immune responses against transformed and infected cells.

NK cells acquire the potential to function via a process called education that occurs during their development. They can undergo clonal expansion during infection and have recently been suggested to have the ability to develop memory(95-99). The traditional cell-surface phenotype defining human NK cells is an absence of CD3 and CD19/20 and expression of CD56 and/or CD16. Three subsets of NK cells with separate functions have been identified based on their cell-surface density of CD56 and CD16; i) $CD56^{dim}CD16^{bright}$, which comprise 90% of peripheral blood NK cells, are the most cytolytic subset(100-102) ii) $CD56^{bright}$ $CD16^{dim}$ comprise 10% of the peripheral blood NK cells and are more likely to mediate cytokine release than cytolysis(100) and iii) $CD56^{neg}CD16^{high}$ cells constitute a defective, or anergic, NK cell subset which accumulates during chronic viral infection and is characterized by very little cytotoxic activity and no cytokine production(103-105).

1.1.9: NK cell receptors

NK cell function is modulated by a repertoire of germ-line encoded receptors such as the natural cytotoxicity receptors (NCRs), killer cell lectin-like receptors (KLR; C-type lectins), leukocyte immunoglobulin-like receptors (LILR) and killer immunoglobulin-like receptors (KIR)(106-108). These families of inhibitory and activating receptors are expressed in a multifarious manner on the NK cell surface.

Natural cytotoxicity receptors (NCR): The members of this NK cell receptor family include NKp30, NKp44, NKp80 and NKp46. The latter of these is found on all NK cells and along with NKp44 has been demonstrated to recognize the hemagglutinin of influenza virus, activate NK cells in the response against cytomegalovirus (CMV) and play a role in HIV immunopathogenesis(109-114).

Killer cell lectin-like receptors (KLR): The KLR belong to the C-type lectin family. They are highly conserved across mice and humans, and interact with non-classical MHC class I molecules including HLA-E, -G and the stress inducible MHC class I chain-related (MIC)-A and –B(107;115). The NKG2 subfamily of KLR genes exhibits little polymorphism and it is thought that all individuals have all the NKG2 genes(116). These genes encode inhibitory receptors, such as NKG2A, or the activating forms NKG2C, NKG2D and NKG2E(117). Infection and malignancies can upregulate ligands for these receptors and subsequently influence disease outcome.

FcγRIIIa or CD16a: The human-low affinity receptor for the constant region of IgG (eg. FcγRIIIa or CD16a) is expressed on a subset of NK cells(118). CD16a recognizes the constant region of Abs of the IgG1 and/or IgG3 subtypes and signals through a positively charged transmembrane residue, which associates with the immunoreceptor tyrosine-based activation motifs (ITAM)-containing CD3ζ and FCεRI homodimers and/or heterodimers(99;119). Ligation and signalling through CD16a can result in activation of NK cells to mediate cytolysis, through ADCC, and can trigger the secretion of cytokines and chemokines(120;121).

CD16a provides an important mechanism for the function of cytoreductive monoclonal Ab (mAb) therapies(109). Examples of such therapies against cancer include Rituximab, an anti-CD20 Ab used in the treatment of B-cell non-Hodgkin lymphomas, and Trastuzumab, an anti-Her-2 mAb used to treat Her-2-expressing breast cancers(122;123). A polymorphism at base pair 559, a T to G substitution, results in a change of phenylalanine (F) to valine (V) at amino acid (aa) 158(124-126). Igs of the IgG1 and IgG3 subclass have been demonstrated to bind with greater affinity to the isoform with V at aa 158(124-127). Rituximab treatment of lymphoma patients with the homozygous (hmz) V/V CD16a polymorphism results in improved clinical response rates compared with patients possessing the lower affinity V/F or F/F polymorphisms(128-130). Ligation of CD16a by cell-bound Abs has the potential to engage cells of the innate immune system, such as NK cells, suggesting that ADCC may be a relevant mechanism involved in the response against viral infections(131-133).

Killer immunoglobulin-like receptors (KIR): The extensively investigated KIR family of receptors are encoded on human chromosome 19q13.4 within the leukocyte receptor complex (LRC)(74). The KIR region is now recognized to be the most polymorphic gene region followed by the MHC and exhibits extensive population

diversity(116;134). The KIR gene family has rapidly expanded in hominoid species, with primates and cattle being the only mammalian species to show similar diversification of KIR genes(135;136). Discovery of KIR revealed an additional function of the HLA class I molecules, which are encoded by genes within the MHC on chromosome 6 in humans. Independent segregation of the highly polymorphic KIR and HLA gene families creates diverse KIR and HLA gene combinations that are attractive candidates for disease outcome association studies. Host genetic factors, such as genetic polymorphisms within the MHC class I and II loci have been shown to influence HIV disease progression(137). The interaction of certain HLA class I molecules with KIR inhibit NK cell activity to prevent autoaggressive responses, while other KIR stimulate NK effector functions. The observation that KIR come in both inhibitory and activating isotypes coincides with associations of certain KIR in the regulation of NK cell-mediated immune responses.

To date, 15 KIR genes and 2 pseudogenes have been identified within a 100 to 200-kb region of the LRC(138;139). KIR nomenclature is based on the number of their extracellular immunoglobulin (Ig)-like domains (2D – two domains or 3D – three domains) and the length of their intracellular tail (L-long, S-short or P-pseudo, gene)(140;141). For example, a KIR with three extracellular Ig domains and a long cytoplasmic tail is named KIR3DL and given a number as a suffix, such as KIR3DL1, to indicate the order in which the receptors were discovered. Inhibitory KIRs (iKIR) with long tails contain two consecutive immunoreceptor tyrosine-based inhibition motifs (ITIM) in their cytoplasmic domains. Phosphorylation of ITIM results in association with SHP-1 (Src-homology domain-containing tyrosine phosphatase), which inhibits downstream events in the intracellular activation cascade(142). Alternatively, activating KIR have a positive charge in the transmembrane region that allows the receptor to associate with adaptor proteins that carry activation signalling motifs, known as ITAM. A number of adaptor proteins have ITAMs, including DAP-10, DAP-12, CD3ζ, and FCεRI-γ(143;144). Therefore, short-tailed activating KIR have no direct signalling properties but trigger signalling pathways that are associated with the ITAM-mediated signalling pathways to deliver activating signals to the cell upon engaging its HLA ligand(145).

Early work on the identification of NK receptors revealed a family of receptors called “p58”(146). Subsequent studies of the p58 family characterized a distinct cell surface molecule termed NKB1 which was detected by the DX9 mAb. Molecular cloning of NKB1 cDNA indicated that the receptor had three extracellular Ig-like domains, a long cytoplasmic tail and contained two ITIM(147). As the first receptor to be discovered in

the KIR family, NKB1 came to be known by its now widely used alternate name KIR3DL1. KIR3DL1 recognizes HLA-Bw4 molecules as ligands; HLA-Bw4 are found on a subset of HLA-B and –A alleles. HLA-B antigens can be dichotomized into 2 groups based on expressing the highly conserved public epitope, HLA-Bw4 or HLA-Bw6(148;149). HLA-Bw4 and HLA-Bw6 antigens differ from each other in part by the residues present at aa 77-83 of the alpha-1 helix of the HLA heavy chain(150). Approximately 40% of HLA-B alleles belong to the Bw4 group, while the remaining 60% belong to the alternate Bw6 group(108). Bw4 variants can have either an isoleucine at position 80 of the heavy chain (*80I) or a threonine at this position (*80T)(151). The HLA-Bw4*80I (Bw4*80I) variants are superior to *80T variants as ligands for KIR3DL1 receptors(152;153). Though no NK receptor has been identified that interacts with Bw6, the Bw4 epitope is recognized by inhibitory KIR3DL1 receptors on NK cells(151;154).

The KIR3DS/L1 locus encodes the inhibitory KIR3DL1 and activating KIR3DS1 receptors, which segregate as alleles of the same locus(155;156). The highly polymorphic alleles of KIR3DL1 may encode receptors with high (*h: KIR3DL1*001, *002, *008, *009, *015, and *020), intermediate/low (*l: *005, *006, *007, *028, and *053) cell surface expression levels, or *004 with transient expression at the surface(86;157-160). KIR3DL1 genotypes can be separated into 2 groups based on their allelic composition: the *h/*y group includes an *h allele with either a second *h or an *004 allele while the *l/*x group includes an *l allele with either a second *l, an *h or an *004 allele. These groupings have biological relevance because the level of KIR3DL1 receptor levels on NK cells from *h/*y carriers is higher than that on those from *l/*x carriers(158;159).

Also in the p58 family of receptors are three inhibitory receptors KIR2DL1, KIR2DL2 and KIR2DL3 and a related activating receptor, KIR2DS2(161). KIR2DL2 and KIR2DL3 have been shown to segregate as alleles of the same locus(162). Elucidation of the crystal structures of the extracellular domains of KIR2DL1, KIR2DL2 and KIR2DL3 revealed that these receptors are nearly identical, with the major difference present in the hinge angle, which varies from 66° in KIR2DL1 to 81° in KIR2DL2 and 78° in KIR2DL3(163-166). Receptors of the KIR2DL loci display specificity for particular MHC allotypes through variation of a single KIR residue at position 44(166). KIR2DL1 recognizes HLA-C allotypes with Asn^{77} and Lys^{80}, commonly known as the C2 allotype including alleles HLA-Cw2, Cw4, Cw5, Cw6, Cw15, Cw16:02, Cw17 and Cw18(161;167;168). Alternatively, KIR2DL2 and KIR2DL3 recognize C1 allotypes with

Ser^{77} and Asn^{80} which includes the alleles HLA-Cw1, Cw3, Cw7, Cw8, Cw12, Cw14 and Cw16:01(161;167;168).

KIR are expressed in a stochastic manner such that each NK cell clone expresses a variety of activating and inhibitory receptors which yields a broad range of functionally distinct NK cell clones(169). Expression of an individual NK receptor depends on DNA methylation and the set of receptors on an NK cell surface is passed on to daughter cells arising from proliferation(170;171).

1.1.10: NK cell recognition and activation

KIRs expressed on NK cells regulate NK cell function such that NK cell activation is controlled by multiple activating and inhibitory receptors. Engagement of inhibitory receptors protects normal cells expressing self-MHC class I from autologous NK cell attack, thereby mediating what is referred to as tolerance to self. If both activating and inhibitory receptors are engaged, the inhibitory signals will overcome activating signals to inhibit NK cell effector functions and autoaggression(172). Activation of NK cells to mediate effector functions such as degranulation and/or secretion of cytokines and/or chemokines is determined by the net integration of signals received through activating and inhibitory receptors expressed on the NK cell surface. Activating receptors such as CD16a, NKp46 and NKG2D associate with common ITAM-bearing polypeptides such as CD3ζ, DAP-10, DAP-12, or FcεRI-γ(144). Upon encountering a cell with downregulated or altered HLA, the 'missing-self' milieu may result in NK cell activation if an activating receptor is also engaged (Figure 5).

The present understanding of NK cell regulation originated from the observation that wild-type NK cells kill MHC-deficient targets but spare cells expressing sufficient HLA(173). The missing-self hypothesis posits that NK cells recognize and eliminate target cells with downregulated HLA expression(174) (Figure 5). This has been expanded on to account for the requirement for the engagement of an activating NK receptor in addition to the lack of engagement of an inhibitory receptor to activate NK cells, explained by the induced-self hypothesis(106). Ligands for activating receptors, such as NKG2D, are upregulated by stressors such as viral infection or genotoxic damage, and this upregulation was found to be required for NK cell activation(175;176) (Figure 5). Exogenous factors such as cytokines have also been demonstrated to upregulate expression of some NK cell receptors, such as NKp44, on activated NK cells(177).

The ontological process that determines NK cell functional potential has been investigated in recent years. The function of the NK cell population is determined through a process termed education, or licensing, that occurs during NK cell development. The majority of the evidence suggests that NK cell activation is not an on/off switch but rather can be tuned to respond to abnormal autologous cells through interactions between activating and inhibitory receptors with the self-MHC environment. This process, termed "NK cell education" will be discussed in the following section(145).

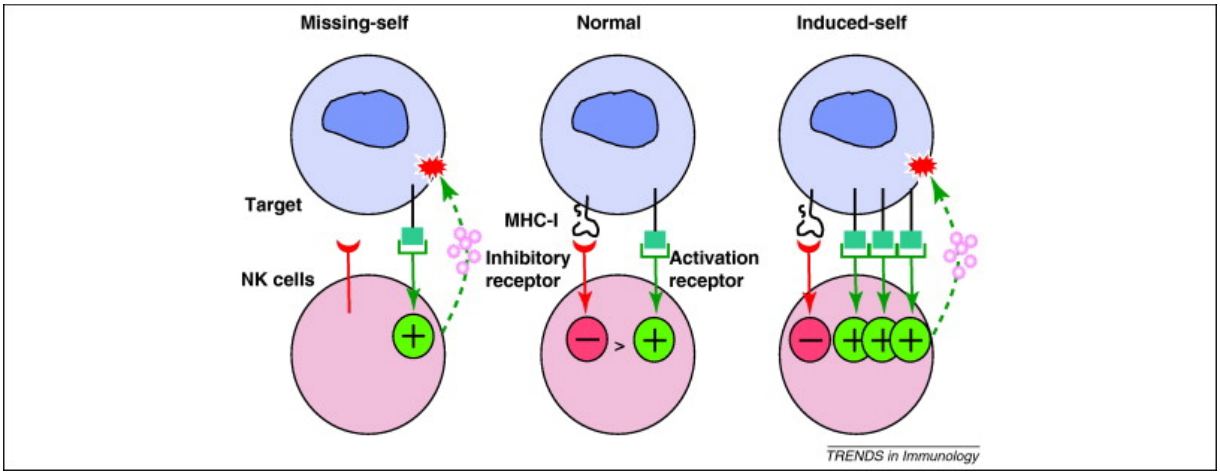


Figure 5. Missing-self hypothesis of NK cell education. Tolerance to self is achieved when interactions between inhibitory receptors on NK cells with their HLA ligands on normal cells overcome activating signals. In the case of "missing self", activation of NK cells occurs as no inhibitory signal is achieved through the inhibitory receptor. In the event of induced self, ligands for activating receptors are upregulated and net stimulatory signals overcome inhibitory interactions resulting in NK cell activation. Reprinted with permission from Elliott JM and Yokoyama WM. Unifying concepts of MHC-dependent natural killer cell education. *Trends in Immunol*, 2011

1.1.11: NK cell education

NK cell responsiveness is the result of the net integration of signals propagated through inhibitory and activating receptors interacting with their ligands. Observations that NK cells from humans and mice with MHC-class-I deficiencies are not autoreactive suggests that additional factors are involved in regulating NK cell functional potential(178-182). Indeed, NK cells expressing inhibitory receptors from MHC class-I-deficient patients, β2m-/-, Tap-/- or MHC class-I-deficient mice do not kill MHC class-I-deficient normal cells(178-180;183;184). While NK cells in MHC class-I-deficient mice have normal expression of activating receptors and are present in normal numbers and distribution, their NK cells are hypofunctional(185-188). Therefore, the absence of self-HLA during NK cell development is not sufficient to activate NK cells. Likewise,

hypofunctional NK cells have been observed in MHC-sufficient mice that have no inhibitory receptors specific for self-MHC(181;182). There appears to be a requirement for at least one iKIR/HLA interaction for the development of functional NK cells in humans. Kim et al. (2005) demonstrated that NK cells acquire functional competence through self-specific receptor-ligand pairings, which educate NK cells through a quantitative process of licensing(189). This process translates into a hierarchy of responsiveness to MHC-class-I-negative cells that is directly proportional to the number of different iKIRs for self-HLA(190-192). The putative requirement of at least one iKIR/HLA interaction for the development of functional NK cells in mice and humans was demonstrated in receptor mutants lacking adaptor proteins that carry inhibitory signalling motifs (i.e. ITIMs) which were unable to confer NK cells with functional potential(189). The number of inhibitory receptors engaged and the strength of the interaction between receptors and ligands influence NK cell licensing for functional potential(190-192).

The rheostat model describes how NK cells are licensed, and serves to further explain several important features of NK cell education. It posits that an NK cell licensed through stronger inhibitory signals during development responds more strongly, with increased frequency of responding cells having a broader range of functions when it encounters MHC-I-deficient targets than NK cells licensed through weaker inhibitory receptor-ligand combinations(192-194) (Figure 6). Indeed, in both humans and mice certain MHC class I variants appear to be more potent than others for licensing NK cells(194-197). In addition, signals received through activating NK cell receptors decrease the chance that an NK cell will respond upon encountering an appropriate target cell(121). Furthermore, the rheostat model proposes that NK cell education is a continuous process. For example, educated NK cells from wild type (WT) mice became hyporesponsive after transfer to MHC-I-deficient hosts, and hyporesponsive NK cells from MHC-I-deficient mice became responsive after transfer to WT mice(198). This demonstrates that the responsiveness of mature, licensed NK cells is not a set feature acquired during development, but rather it is dynamic and can change in response to the MHC class I microenvironment(198).

The current understanding of NK cell ontogeny involves the interaction of NK cells with the normal self-environment to acquire a degree of functional potential such that upon encountering a target cell, the integration of stimulatory and inhibitory signals will determine the response consistent with the education of the NK cell. The association

of NK cell receptors with their ligands is integral to understanding the role of NK cells in the context of disease. The next section will focus on the role of NK cells in disease, followed by a discussion on the association of NK cells with protection from infection and/or progression to AIDS.

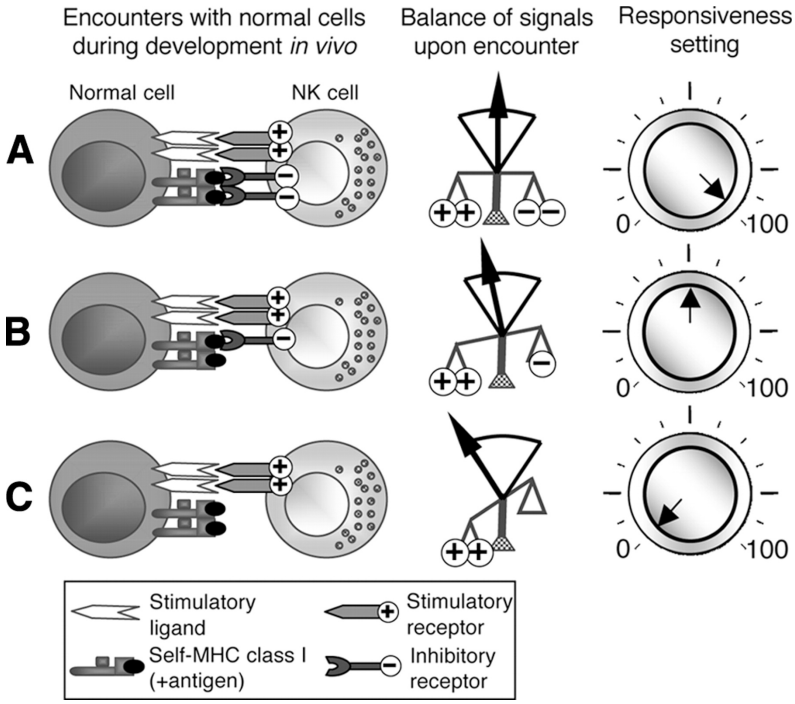


Figure 6. NK cell educational rheostat model whereby NK cell responsiveness is quantitatively tuned through inhibitory and stimulatory interactions during development. (A) A state of high responsiveness is achieved when interactions between inhibitory receptors on NK cells and HLA on normal cells oppose stimulatory signals. (B) An intermediate responsive state is the result of expression of one inhibitory receptor which only partially counters stimulatory signals. (C) An absence of inhibitory receptors induces a state of hyporesponsiveness through persistent activating stimulation. Reprinted with permission from Joncker NT, Fernandez NC, Treiner E, Vivier E and Raulet DH. 2009. NK Cell Responsiveness Is Tuned Commensurate with the Number of Inhibitory Receptors for Self-MHC Class I: The Rheostat Model. *J Immunol*, 182(8): 4572-4580. Copyright 2009. The American Association of Immunologists, Inc.

1.1.12: Relevance of NK cells in disease

NK cells have been implicated in promoting or suppressing inflammatory responses and autoimmune disease development and shown to play an important role in tumor surveillance, hematopoietic stem cell transplantation (HSCT), and viral infection. Observations in mice where elimination of NK cells resulted in a higher incidence of spontaneous tumours and metastasis, and impaired clearance of tumor cells (reviewed in Zamai et al. 2007)(199) suggest an anti-tumor role for NK cells. Indeed, individuals with advanced metastasis show reduced numbers of NK cells with lower functionality, a phenomenon also observed in HIV-infected subjects(200). Furthermore, expansion of

highly cytotoxic NK cells in mice has been shown to eradicate murine acute myeloid leukemia highlighting their importance in immune-surveillance(201).

HSCT has been adopted as a therapy to treat a number of malignant and non-malignant hematological diseases such as leukemia, lymphoma, and severe combined immunodeficiency disease (SCID). Mismatches in MHC between recipients and donors are determinants of transplant outcomes and the specificity of graft rejection are known to be influenced by NK cells(202). The major complication following HSCT is graft-versus-host-disease (GVHD). To minimize this potential reactivity, HSCT is performed between HLA-matched, but not necessarily KIR-matched, donor-recipient pairs. NK cells are the first lymphocyte population to appear in the peripheral blood following HSCT, and consistently express a donor KIR repertoire(108). Alloreactivity of NK cells, whereby donor-derived NK cells lack iKIR for recipient HLA-class I ligands, greatly benefit HSCT outcomes by limiting rates of GVHD, decreasing graft rejection mediated by NK cells, and improving immune reconstitution through NK cell anti-viral activity(108).

1.1.13: Relevance of NK cells in non-HIV viral infections

NK cells have been described to control herpesvirus, poxvirus, papillomavirus, murine CMV (MCMV) and, depending on the virus, their presence or absence is associated with disease progression of influenza in mouse models and in humans(110;203-210). Mouse models have yielded important information about how NK cells function in viral infections. CMVs are herpes viruses that establish asymptomatic, latent and lifelong infections(211). Early stages of infection are characterized by significant NK cell activation, and numerous mechanisms by which MCMV avoids host immune responses have been identified. The activating receptor Ly49H, a C-type lectin NK cell receptor, binds directly to MCMV encoded m157 expressed on the surface of infected cells, serving to activate NK cells(212). To counter this activating signal, m157 has evolved to also bind the inhibitory receptor Ly49I as a mechanism by which virally infected cells can avoid being targeted for destruction by NK cells(212). Similarly, human CMV (HCMV) infected cells upregulate surface expression of MHC class I proteins such as MIC-A and –B, which bind the activating NK receptor NKG2D, while HCMV has evolved strategies to downregulate stress-induced ligands for NKG2D(211;213). The potential importance of NK cells in HCMV has been highlighted in a clinical report of a 3 month old patient exhibiting a unique SCID phenotype, characterized by an absence of T cells, in which 90% of the lymphocyte population were

$CD56^{dim}$ NK cells and the remaining 10% were B cells. The patient recovered without anti-viral therapy after a significant expansion of NK cells, providing direct evidence that NK cells can effectively control CMV infection(214).

NK cell receptors, particularly those belonging to the KIR family, when co-expressed with their putative HLA ligand, have been reported to influence susceptibility or progression to disease. Examples of this include rapid clearance of hepatitis C virus (HCV) in individuals co-carrying KIR2DL3 and its ligand, KIR3DS1, KIR2DS5 and KIR2DL5 with severity of influenza infection, and KIR3DL1-Bw4 with slow time to AIDS, HIV VL control and protection from HIV infection(86;210;215-217). Overwhelming evidence for the importance of NK cells against viral infections comes from patients with NK cell abnormalities and/or deficiencies resulting in susceptibility to severe herpes virus infection and Epstein-Barr virus-driven lymphoproliferative disorders(218;219).

1.1.14: Guilty by association: NK cells in HIV Infection

The importance of NK cells in HIV infection is still being defined, though a growing body of evidence suggests they are involved in protection against initial infection and subsequent disease progression (Figure 7). Higher levels of NK cell function are found in individuals who remain seronegative despite exposure to HIV than in seronegative subjects who eventually seroconvert(220;221). Also, higher levels of functional NK cells are present in non-pathogenic as compared to pathogenic simian immunodeficiency infections(83).

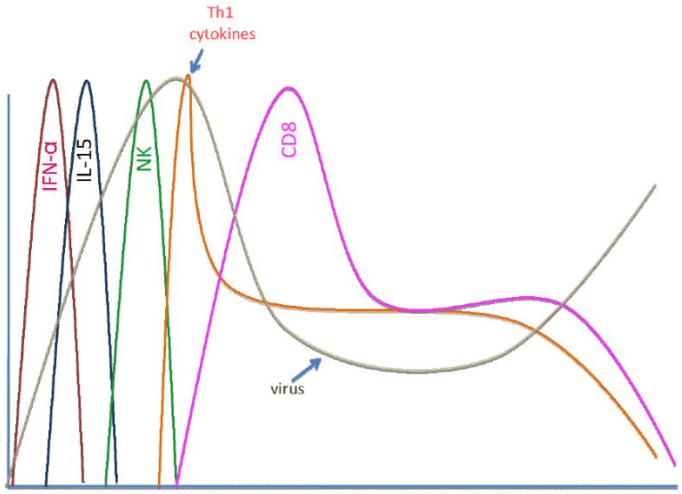


Figure 7: Kinetics of the immune response to viral infection. Reprinted with permission from Alter G and Altfeld M. NK cells in HIV-1 infection: evidence for their role in the control of HIV-1 infection. *J Intern Med*, 2008

Epidemiological studies have found associations between a number of KIR3DL1 genotypes in combination with their putative HLA-Bw4 ligand with protective outcomes upon exposure to and/or infection with HIV(85;86;222). The KIR3DL1/S1 locus was first implicated in HIV disease progression after the observation that carriage of KIR3DS1 and Bw4*80I resulted in slower time to AIDS(85). This combination was subsequently demonstrated to confer protection against some opportunistic infections and associate with lower VL set point in HIV-infected individuals(222). The most potent KIR/HLA genotype combination with respect to protection from infection and/or time to AIDS is co-carriage of KIR3DL1 *h/*y with HLA-B*57 (*h/*y+B*57)(86). A higher frequency of NK cells with multiple functions are present in individuals carrying the *h/*y+B*57 genotype than in carriers of the receptor or genotype alone, or neither(195;196). Carriage of other KIR/HLA genotypes such as KIR3DL1*004+Bw4 and either KIR3DL1 *h/*y or *l/*x with HLA-B*27 (3DL1+B*27) have been reported to have a positive impact on slow time to AIDS and VL control compared to being hmz for Bw6. Bw6hmz have no alleles that interact with KIR3DL1 and therefore, serve as negative controls for the effect of licensing through KIR3DL1 on clinical outcomes in the context of HIV infection(85;86;223). Given that KIR3DL1 and KIR3DS1 variants are 97% homologous in their extracellular domains, and the demonstrated functional interaction between KIR3DL1 and HLA-B, it has been presumed that its activating counterpart KIR3DS1, also binds Bw4*80I antigens. Lending further support to this attractive hypothesis are numerous epidemiological studies showing an association between carriage of KIR3DS1 in the presence of its putative ligand, Bw4*80I, with favourable HIV outcomes(85;140). Indeed, an expansion of both KIR3DS1+ and KIR3DL1+ NK cells in Bw4*80I carriers but not Bw6hmz has been observed in acute infection(224). Despite this, several studies have failed to find an interaction between KIR3DS1 and Bw4*80I(225-227), while others still, have reported no association between carriage of KIR3DS1/Bw4*80I with either protection from HIV infection or disease progression(217;223;228-230).

Although the locus encoding KIR3DL1/S1 has been the one best characterized for its association with favourable outcomes in the context of HIV infection, KIR2DL loci have also been demonstrated to influence HIV infection, though their role is less well characterized than that of KIR3DL1/S1. A polymorphism distal to the ligand-binding site makes KIR2DL2 a stronger receptor for C1 than KIR2DL3(231;232). The potential importance of the KIR2DL loci in HIV infection is not yet clear. Genome-wide association studies (GWAS) have revealed a polymorphism rs9264942 and single

nucleotide deletion in the 5' region and 3' untranslated region, of the HLA-C gene. These single nucleotide polymorphisms (SNP) associate with VL set point and the level of cell surface HLA-C expression(233-235). Imperfect correlation of these associations with control of VL however, suggests that other factors are involved in mediating the observed protective effect. Indeed, licensing of KIR2DL+ NK cells through its HLA-C ligand suggests this KIR/HLA loci may be involved in the protective effect of NK cell responses against HIV infection(182;233;235-237). The relative importance of NK cells and KIR/HLA combinations in the response against HIV infection has been observed in individuals who remain seronegative despite exposure to HIV and cohorts of HIV+ subjects who progress slowly to AIDS in the absence of HAART(85;86;215;238;239). Attempts to elucidate the mechanisms that explain how protective NK receptor and HLA ligand genotypes influence disease outcomes in the context of HIV infection have been made using functional immune assays.

1.1.15: NK cell functional assays

A number of assays may be used to measure the functional potential of NK cells. These include stimulation with HLA-I-null cells, ADCC assays and viral inhibition assays(240-242). Stimulation of NK cells with the HLA-I-devoid cell line K562 reflects the ability of NK cells to recognize cells with transformed or downregulated HLA. This assay allows characterization of NK cell functional potential by measuring secretion of cytokines (IFN-γ and TNF-α) and CD107a expression, a marker for degranulation, through intracellular staining(243;244). Cell surface mobilization of CD107a has been used as a surrogate marker for cytotoxic granule release, and therefore NK cell cytoxicity, as its expression correlates with NK cell-mediated cytokine secretion and cytolytic activity(242;244;245).

ADCC can be assessed using a commercially available GranToxiLux (GTL) assay to measure the killing of target cells(241). Target cells used may include cell lines or primary infected cells, however; due to the small number of primary infected cells killed, cell line targets are more commonly used. The GTL ADCC assay utilizes an NK cell resistant transformed T cell line known as CEM.NKr which expresses CD4, and may be labeled with HIV gp120 and anti-HIV Abs before being mixed with NK cell effectors and a granzyme B (GzB) substrate. GzB is a serine protease released by cytoplasmic granules from NK cells. It is delivered inside the target cells as a result of the Ag-specific Ab-Fc receptor interaction with effector populations. GzB inside the target cells cleaves the GzB

substrate, generating a fluorescent signal that can be quantitatively measured by flow cytometry. Furthermore, the GTL assay correlates with CD107a degranulation as NK resistant targets do not induce CD107a cell surface mobilization, while Ab-coated cells do.

In addition to measuring NK cell functional potential through cytokine release and ADCC; viral inhibition assays measure the ability of NK cells to limit viral replication. Target cells are infected *in vitro* with HIV and incubated with effector cells. Supernatants are taken at several timepoints following co-culture and a quantitative enzyme-linked immunosorbent assay (ELISA) measuring the level of HIV core antigen, p24 secreted into the supernatant, is used to assess inhibition of viral replication(246). The frequency of HIV infected cells can also be measured using intracellular staining for HIV p24(242;247).

These three assays provide essential information about NK cell functional potential by measuring different parameters, namely cytokine release, indirect cytotoxicity and inhibition of viral replication. However, they do not provide any information about the NK cells mediating these effector functions. As such, they may be complemented by flow cytometry assays, which incorporate extracellular phenotyping with intracellular cytokine staining to identify the phenotype of responding NK cell subsets. These approaches have been used to provide the functional basis for several of the observed genetic effects of KIR/HLA interactions and protection upon exposure and/or infection in the context of HIV.

1.1.16: A role for NK cells in HIV infection

A seminal study published in 2002 by Martin et al. reported that KIR3DS1 may play a role in HIV-disease and also demonstrated an association of KIR3DL1/S1 and putative ligand genotype combinations with delayed disease progression(85). Subsequent development of an Ab panel that could distinguish KIR3DS1 and KIR3DL1 made it possible to explore the functional implications of the carriage of these genotypes(140;225;226;248). In a given KIR3DS1+KIR3DL1+Bw4*80I+ individual, it has been demonstrated that the KIR3DS1+KIR3DL1- NK cell subset preferentially responds to HIV-infected targets(242). In 2007, Alter et al. examined the functional ability of NK cells to control HIV replication *in vitro* based on their KIR/HLA types. Indeed, NK cells expressing KIR3DS1 from carriers of Bw4*80I exhibit a superior level of viral inhibition against autologous HIV-infected CD4+ T cell targets compared with

NK cells derived from donors with only one of the two, or neither alleles(242). Since activating KIR interact with HLA-I at a lower affinity than their inhibitory counterparts, it has been postulated that viral infection results in the presentation of viral or stress peptides that increase the affinity of KIR3DS1 for Bw4*80I. Peptides have previously been demonstrated to alter the binding affinity of HLA-I to KIR(107). However, it should be noted that, unlike for KIR3DL1, no functional interaction has been demonstrated between KIR3DS1 and Bw4*80I, nor has a viral epitope been found that increases this affinity(225-227). It is expected that since KIR3DS1hmz exhibit higher frequencies of KIR3DS1+ NK cells than KIR3DL1/S1 heterozygotes, that KIR3DS1+ NK cells, which respond preferentially to infected autologous CD4+ T cells, would mediate potent viral inhibition in KIR3DS1hmz who carry Bw4*80I(226). Although few individuals with this genotype have been tested, NK cells from subjects with this genotype respond very poorly to autologous HIV infected cells(249). Consistent with KIR3DS1+ and KIR3DL1+ NK cell expansion in HIV infection and a role for KIR3DL1 in delayed disease progression, Pelak et al. (2011) report that KIR3DL1/S1+Bw4*80I subjects inhibit viral replication *in vitro* to a much greater extent than KIR3DS1hmz+Bw4*80I individuals(249). The KIR3DS1/L1 locus is subject to copy number variation (CNV), meaning that duplications or deletions can occur at this locus resulting in between zero and four copies of the genes being present. The most robust inhibition of viral replication is mediated by NK cells from subjects with two copies of KIR3DL1 and one copy of KIR3DS1, in the presence of Bw4*80I(249). It should be noted at this point, that the subjects carrying KIR3DS1+Bw4*80I in Alter et al. (2007) were all KIR3DL1/S1 heterozygotes, as were most of the subjects exhibiting slow time to AIDS in the first epidemiological study implicating NK cells in HIV control(85;242). Taken together, these findings suggest that the impact of the KIR3DS1+Bw4*80I combination on anti-HIV activity and slow time to AIDS requires a copy of KIR3DL1, together with the presumed Bw4*80I ligand to provide a signal for NK cell education.

A role for KIR3DL1 HLA receptor ligand combinations in protective outcomes is well documented, and the link between protective genotypes and NK cell functional potential has been investigated in functional studies(74;86;195;217;228;230;250). In carriers of Bw4, expression of KIR3DL1 was associated with higher NK cell cytotoxicity and IFN-γ production upon exposure to HLA-I-devoid target cells than when NK cells are from Bw6hmz(191). The interaction of KIR3DL1 with Bw4 was later demonstrated to license NK cells for ADCC(251). In this study, PBMC from KIR3DL1+ donors

carrying Bw4 mediated significantly higher levels of cytotoxicity against Ab-coated Epstein-Barr Virus (EBV)-transformed B-lymphocyte targets. Additional work by this group showed a role for KIR3DL1-educated NK cells in mediating enhanced anti-HIV ADCC in Bw4 positive but not negative (i.e.Bw6hmz) subjects(252). Dr. Nicole Bernard's lab has demonstrated that KIR3DL1+ NK cells from uninfected carriers of Bw4 respond with increased functional potential in response to stimulation with HLA-I-devoid targets as compared to the KIR3DL1- subset or NK cells from Bw6hmz(230). Particularly, NK cells from subjects carrying the protective *h/*y+B*57 compound genotype express more CD107a and secrete greater levels of IFN-γ and TNF-α than NK cells from carriers of other KIR3DL1 and non-B*57 HLA-Bw4 or Bw6 genotypes(196). These findings were extended to HIV-infected individuals carrying *h/*y+B*57, which respond to stimulation with HLA-devoid K562 cell line with a higher frequency of functional cells exhibiting multiple functions than NK cells from Bw6hmz(195).

1.1.17: Nature's successful immune responses against HIV

Despite a great deal of research on the topic of what constitutes the correlates of immune protection against HIV infection or a slow disease course in those already infected, this question remains an intense area of investigation. Groups of individuals have been identified that provide insight into successful immune responses against HIV. One such group are individuals that are exposed to HIV but remain seronegative. These are now referred to as HIV-exposed seronegative (HESN)(253). Another group that may provide insights into immune responses able to control HIV are the HIV-infected Slow Progressors (SP). Included in the SP classification are elite controllers (EC), which control HIV to levels undetectable by currently used assays, viral controllers (VC) who maintain VL level below 2000 copies/ml plasma and long-term nonprogressors (LTNP) who maintain CD4 counts >450 cells/mm^3 for seven or more years without treatment. EC and VC can also be LTNP(254). A major challenge in defining correlates of protection in humans is that HIV-infected subjects are unable to eradicate the virus(27). Genetic, immunological and environmental factors have all been investigated as potential contributors to favorable outcomes vis-à-vis HIV exposure/infection in these populations. Continued research into host resistance to HIV in HESN is warranted and may offer important insight into mechanisms of protection as we aim to prevent new infections.

1.1.18: HESN

In the 1980s initial reports appeared of individuals who were exposed to HIV yet remained uninfected(255). Since then, a large number of cohorts of HESN subjects have been identified. Resistance to infection in these individuals is defined by lack of seroconversion, and/or the inability to isolate virus and/or detect viral nucleic acid in the blood(256). The route of exposure is heterogeneous in these groups and HESN with exposure through injection drug use, penile/anal sexual intercourse, penile/vaginal sexual intercourse, childbirth and/or breastfeeding have been identified(255;256). Both adaptive and humoral immune responses have been reported as immunological correlates of protection in these individuals, though recent studies have focused on identifying innate immune responses responsible for protection against infection in HESN(257;258). To this end, it has been demonstrated that IDU HESN exhibit enhanced NK cell functionality, as compared to seronegative IDU who eventually seroconvert, and maintain a normal capacity to degranulate in response to HLA-devoid target cell stimulation(220;259). In addition, a number of allelic combinations of NK cell KIR/HLA ligand combinations have been shown to confer enhanced NK cell functionality and occur at higher frequencies in HESN than in HIV susceptible subjects or low risk controls(196;220;230;260;261).

In nearly all other viral infections, examples of naturally occurring immunity have provided the rationale for vaccine design. This may similarly be the case for HIV and warrants continued investigation of the immune correlates of protection from HIV infection in HESN.

1.1.19: Slow disease progressors (SP)

HIV-infected SP constitute approximately 2-5% of the HIV-infected population(262;263). Humoral responses are likely not involved in delayed progression to AIDS in SP. On the other hand cell-mediated immunity, and particularly Gag-specific CD8+ T cell responses are thought to play a role in viral control(76;77;264). Certain HLA class I alleles such as HLA-B*57 and HLA-B*27 are overrepresented among SP(73-75;258;265;266). *Gag*-specific responses in SP carrying HLA-B*57 have been observed to be highly focused on HLA-B*57-restricted peptides(75). Reports that an escape mutation within an HLA-B*57-restricted epitope abrogates binding of KIR3DL1 underlines the potential importance of this KIR/HLA combination in CD8+ T cell responses in these individuals(267). The ability of cytotoxic T lymphocytes (CTL) to

maintain functional HIV-specific responses against primary autologous HIV-infected CD4+ T cells appears to at least partially contribute to the protective responses necessary for nonprogression in these individuals(268). GWAS aimed at identifying SNPs associated with VL control found that the significant associations mapped to chromosome 6, within and near the MHC HLA-B and –C genes(234;266). Of note, the strongest association linked to SP was a polymorphism located in the *hcp5* gene, shown to be in linkage disequilibrium with HLA-B*57:01, and previously reported to relate to VL control and slow progression of HIV disease(234;269).

SP have been reported to have lower levels of neutralizing Abs than progressors, comparable levels of binding Abs and higher levels of ADCC competent Abs. Greater NK cell functional potential has been demonstrated in SP(270;271). This may be related to the higher proportion of SP expressing the protective KIR/HLA *h/*y+ B*57 than found in HIV progressors or uninfected individual. This KIR/HLA genotype is associated with higher NK functional potential than other nonprotective KIR/HLA genotypes(195;196). SP may have better NK function because high HIV VL has a negative impact on NK function(195;272;273).

In summary, the immunological profiles of SP provide several clues as to the mechanisms involved in control of HIV and continue to provide valuable insight into the immune responses associated with slow progression to AIDS.

1.1.20: Major HIV vaccine efficacy trials

There have been two HIV vaccine efficacy phase III trials and 1 phase IIb trial in the last 12 years, testing three major overlapping paradigms(274-279). Initial candidate vaccines aimed to elicit neutralizing Abs by vaccinating with an HIV Env subunit vaccine (VaxGen USA and THAI)(275-278). The second wave focused on stimulation of CD8 T cell responses (STEP trial)(274). The Env subunit vaccine showed no efficacy in preventing infection while the vaccine used in the STEP trial may have increased the risk of HIV infection(274;280). The most recent RV144 Thai trial evaluated four priming injections of a canarypox/envelope protein (ALVAC) followed by two booster injections of a recombinant gp-120 subunit vaccine (AIDSVAX) in 16,402 healthy men and women. A 31% reduction in the frequency of acquisition of HIV infection was observed at the 36 month pre-determined endpoint(279).

Ongoing analysis of the RV144 samples is aimed at understanding which immune responses correlate with protection. A phase II trial of the ALVAC/AIDSVAX vaccine

showed CTL responses in 24% of vaccines and no placebo recipients using a chromium release CTL assay(281). Alternatively, weak CD8+ CTL responses were detected in the RV144 phase III trial using IFN-γ ELISpot assay, and measuring IFN-γ and IL-2 production(279). Also observed were narrowly directed neutralizing Abs with strong CD4+ T cell proliferative responses and considerable titres of non-neutralizing HIV Env binding Abs, which were ADCC competent(282). These have been implicated in the vaccine's protective efficacy(282). Despite an inverse correlation between levels of IgA and IgG titers and protection, vaccine-induced IgA did not enhance infection. Indeed, IgG binding to the variable loop of HIV Env inversely correlated with infection. Based on these observations, it was hypothesized that plasma IgA Ab levels block IgG epitopes and interfere with protective IgG effector functions such as ADCC(282). In the context of HIV infection, IgA blocking of Env epitopes may impede ADCC responses against infected target cells and/or Ab-coated cell-free virions. Although results showed only a modest, non-durable benefit and the degree of efficacy must be improved, this study was the first to demonstrate indirect evidence for ADCC as a correlate of protection in humans. In the work described in this thesis I will show that NK cell licensing, particularly through potent KIR3DL1 and HLA-B*57 interactions influences the strength of NK cell mediated ADCC activity.

1.1.21: HIV and ADCC

ADCC competent Abs appear during the early phase of infection(283-285) (Figure 8), and develop in the majority of patients within a few weeks following HIV infection(286-288). Abs that mediate ADCC have been demonstrated to inversely correlate with the initial decline in VL, slower HIV disease progression and are enriched in EC(289-294). This body of work is consistent with mounting evidence implicating ADCC as a potentially protective response in the context of HIV infection and exposure. In addition, anti-HIV ADCC has been observed as a mechanism of protection from infection in vaccinated humans, HESN, as well as rhesus macaques that have been vaccinated or received passive transfers of a BnAb(64;282;295;296). In addition, high levels of ADCC-mediating Abs have also been associated with delayed progression to AIDS in SP and some SIV-infected and SHIV-infected rhesus macaques(293;297-299).

A role for NK cell licensing in ADCC has been implicated by studies which showed that higher levels of anti-HIV ADCC was mediated by NK cells from uninfected healthy controls who were carriers of KIR3DL1 and at least 1 copy of HLA-Bw4 than by

NK cells from Bw6hmz. The NK cells mediating ADCC activity were KIR3DL1+(252). In contrast to what was observed studying NK cells from uninfected individuals, NK cells from HIV infected subjects who were KIR3DL1hmz and carried at least 1 copy of HLA-Bw4 had poorer ADCC activity than those from Bw6hmz(252). Results described in this thesis extend these findings by testing NK cells from KIR3DL1hmz who are *h/*y+B*57 to determine whether they had superior ADCC activity than those from carriers of other Bw4 alleles or Bw6hmz as would be expected given the superior ability of the *h/*y+B*57 combination to educate NK cells. Results obtained by testing NK cells from both HIV uninfected and HIV positive SP will be described.

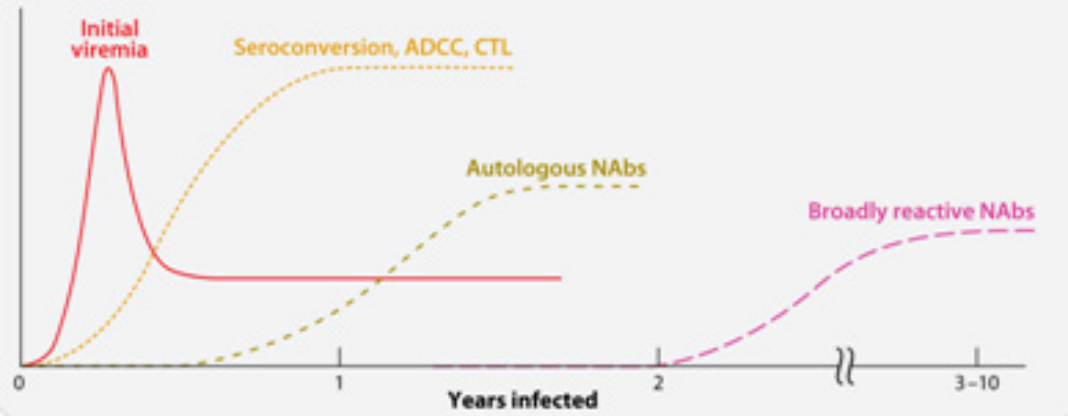


Figure 8: Appearance of antibody responses against infection with HIV. Adapted with permission from Mascola JR and Montefiori DC. The Role of Antibodies in HIV Vaccines, *Ann Rev Immunol*, 2010

1.2: Rationale

The interaction of allelic combinations of KIR3DL1 and HLA-Bw4 during NK cell development determines their functional potential(192-194). Genotypes encoded at the KIR3DL1/S1 locus with or without their putative ligands have been implicated in protection from infection and/or delayed progression to AIDS(85;86;217;230). The strongest level of HIV control has been demonstrated to occur in individuals with the *h/*y+B*57 genotype(86). The combined genotype of *h/*y+B*57 is found at higher frequency in HESN and SP and associates with a reduced risk of infection and/or delayed progression to AIDS(86;230). Coinciding with this observation are reports that NK cells from carriers of this genotype exhibit higher polyfunctional NK cell responses upon stimulation with HLA-devoid target cells whether they originate from HIV uninfected subjects or from HIV infected SP(195;196). Although KIR3DL1^{+} NK mediate poor killing of HIV-infected cells, additional activating signals provided by cross-linking of the NK cell constant region receptor CD16a with anti-viral Abs bound to infected cells

can trigger ADCC(242). A number of studies have implicated anti-HIV ADCC with protection against infection and disease progression, while KIR/HLA combinations have also been shown to educate NK cells for ADCC(182;220;252;282;297;299).

A functional polymorphism in CD16a has been demonstrated to influence IgG binding and ADCC responses, potentially contributing to a successful immune response against HIV(300-302). CD16a genotype is likely to modulate the association between KIR/HLA and ADCC, while variability in responses between individuals may be influenced by the number of iKIR and self-HLA combinations, and the frequency of NK cells. As such, this thesis will present original work that contributes to the knowledge of KIR/HLA in NK cell education and anti-HIV ADCC responses in HIV-uninfected and HIV-infected subjects.

1.2.1: Hypothesis

If ADCC contributes to the protection mediated by allelic combinations of KIR3DL1 and HLA-Bw4, NK cells from carriers of the protective *h/*y+B*57 compound genotype will mediate enhanced anti-HIV ADCC compared to individuals carrying other allelic combinations of KIR3DL1 and HLA-Bw4. Subjects carrying a high affinity CD16a allele will mediate greater ADCC responses compared to individuals who carry the low affinity allele. A higher frequency of total NK cells and KIR3DL1+ NK cells will contribute to greater ADCC responses in KIR3DL1hmz individuals. HIV infection will modulate ADCC activity.

1.2.2: Objectives

This thesis will evaluate the impact of protective KIR/HLA combinations on anti-HIV ADCC responses in HIV-uninfected and HIV-infected SP.

We will investigate:

i) whether NK cell licensing influences the potency of NK cell mediated ADCC,

ii) whether variability in NK cell mediated ADCC within groups constituted based on KIR/HLA could be explained by CD16a polymorphisms and

iii) whether variability in NK cell mediated ADCC within groups constituted based on KIR/HLA could be explained by the number of iKIR to self HLA combinations and/or the percent contribution of NK cells to the total lymphocyte population.

Dr. Bernard's lab has previously published on the association of the *h/*y+B*57 genotype with a reduced risk of HIV infection(230). Furthermore, our lab has provided functional data to support observations of a strong association between the *h/*y+B*57 genetic combination with favourable HIV outcomes. NK cells from HESN and SP co-carrying *h/*y+B*57 had increased polyfunctionality as measured by cytokine secretion and CD107a expression upon stimulation with HLA-devoid target cells compared to participants carrying other Bw4 alleles or Bw6 homozygotes, thereby implicating the *h/*y+B*57 genotype with protection from infection and disease progression(195;196). The protective effect of HLA-B*57 on protective outcomes in HIV disease may be mediated through its interaction with KIR3DL1 alleles to educate NK cells for functional potential. Indeed, the interaction between KIR3DL1 and its HLA-Bw4 ligand has been shown to license NK cells for CD16-mediated effector functions such as ADCC(251). As such, we were interested in whether carriage of the *h/*y+B*57 compound genotype conferred NK cells with enhanced functional potential for anti-HIV ADCC compared to carriers of other Bw4 alleles or Bw6 homozygotes in uninfected subjects and SP. A functional polymorphism at position 158 of CD16a determines the receptor's affinity for Ab(124). We investigated whether CD16a genotype contributed to heterogeneity in ADCC responses between the three KIR/HLA groups studied

Chapter 2: Manuscript – Investigation of the influence of HIV infection, KIR/HLA genotype and FcγRIIIa genotype on NK cell mediated anti-HIV ADCC function

Chapter 2: Manuscript – Investigation of the influence of HIV infection, KIR/HLA genotype and FcγRIIIa genotype on NK cell mediated anti-HIV ADCC function

Pilar Zanoni[1,2,3], Matthew S Parsons[1,2,3], Gamze Isitman[1,2,3], Benjamin Tallon[1,2,3], Sofia Miconiatis[1,2,3], Zoya Padamsi[1], Naglaa H. Shoukry[4], Cécile L. Tremblay[4], Nicole F. Bernard[1,2,3]

[1]Research Institute of the McGill University Health Centre, Montréal, Québec, Canada
[2]Division of Experimental Medicine, McGill University, Montréal, Québec, Canada
[3]Division of Clinical Immunology and Allergy, McGill University Health Centre, Montréal, Québec, Canada
[4]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, Québec, Canada

Mailing address: Dr. Nicole F. Bernard, Research Institute of the McGill University Health
Centre, Montréal General Hospital, 1650 Cedar Ave. Rm C10-160, Montréal, Québec, Canada, H3G 1A4.
Tel: (514) 934-1934 ext: 44584
Fax: (514) 937-1424
E-mail: nicole.bernard@mcgill.ca

Funding: This work was supported by grants from the Canadian Institutes for Health Research (CIHR) #HVI 79515, HOP-86862, MOP-111155, HOP-123800, and the Fonds de la Recherche en Santé du Québec (FRSQ) AIDS and Infectious Diseases Network. MSP is supported by a CIHR Vanier Scholarship. PZ was supported by a CIHR M.Sc. scholarship.

Word count: 9,204

2.1: Author contributions and acknowledgements

PZ and NFB were responsible for study design, data analysis and manuscript preparation. PZ, MSP, GI, BT, SM and ZP designed and performed experiments. NHS and CLT provided subject samples and matched clinical information.

We would like to acknowledge the assistance of numerous members of the Bernard lab for their contributions to this work. In particular, Marie-Pierre Boisvert, Tsoarello Mabanga, and Xioayan Ni for their roles in KIR and HLA genotyping.

2.2: Abstract

Engagement of inhibitory killer immunoglobulin-like receptors (iKIR) by their human leukocyte antigen (HLA) ligands educates natural killer (NK) cells to detect downregulation of HLA on the surface of virally-infected cells. Integration of activating and inhibitory signals delivered to the NK cell determines their functional potential. Certain KIR3DL1/HLA-B genotypes are associated with protective outcomes in HIV-exposed seronegative (HESN) subjects and HIV infected slow progressors (SP). The mechanisms responsible for these protective outcomes remain unknown. Weak degranulation by 3DL1+ NK cells to autologous HIV-infected cell stimuli suggest other activating signals, such as cross-linking NK cells with antibody(Ab)-coated target cells via the NK cell surface Fc-receptor CD16a to induce antibody-dependent cell-mediated cytotoxicity (ADCC) may be required. Using an ADCC assay that detects delivery of Granzyme B from NK to target cells, we found that NK cells from uninfected carriers of the HIV protective *h/*y+B*57 genotype had higher levels of ADCC activity than those from carriers of other Bw4 alleles or Bw6 homozygotes. In contrast, NK cells from SP carrying the same KIR/HLA genotype combinations exhibited no between group differences in ADCC activity, suggesting NK cell mediated ADCC activity was sensitive to dysregulation by HIV infection. Heterogeneity in ADCC responses led us to examine the role of other factors that may influence ADCC potency. A polymorphism at amino acid 158 of CD16a results in greater affinity of the V than the F isoform for Ab. Genetic variation in CD16a did not contribute to differences in ADCC responses in our study. Also, iKIR to self-HLA-C contributed minimally to ADCC responses. Results generated for this thesis support a role for potent NK cell educational signals influencing ADCC potency and that this activity is sensitive to extinction in the context of HIV infection.

2.3: Introduction

Mounting evidence from epidemiological studies suggests that natural killer (NK) cells are involved in mediating protective outcomes upon exposure to and/or infection with human immunodeficiency virus (HIV)(1-7). NK cells are among the earliest responders to viral infection, mediating effector functions such as direct cytolysis, cytokine and chemokine release and antibody-dependent cell-mediated cytotoxicity (ADCC)(8;9). NK cell activation is predominantly under the control of inhibitory NK cell receptors that bind major histocompatibility complex (MHC) class I human leukocyte antigen (HLA) molecules, which serve as markers of self on normal cells(10). A group of HLA-specific inhibitory NK cell receptors that has been the focus of many studies examining NK cell regulation are encoded by the polygenic and polymorphic killer immunoglobulin-like receptor (KIR) gene family(11).

NK cells gain functional potential during ontogeny through an HLA-dependent process called education, or licensing(12;13). Interactions between inhibitory KIR (iKIR) and self-HLA educates NK cells and induces tolerance to self, whereas NK cells lacking iKIR remain unlicensed and hyporesponsive(12;13). Consequently, NK cells are educated to recognize virally infected or transformed cells that have downregulated HLA class I (HLA-I) ligands for inhibitory receptors provided some of their activating receptors are also engaged by ligands on target cells. It has been proposed that the strength and number of signals received through iKIR, such as KIR3DL1 or KIR2DL1/2/3 following ligation with their respective ligand is directly related to the strength of an NK cell's functional potential(10;13-15).

Epidemiological studies have linked the carriage of certain KIR3DL1/HLA-B genotypes encoding receptor ligand pairs with protective outcomes in HIV infection. All HLA-B molecules can be separated into those expressing the HLA-Bw4 or HLA-Bw6 public epitope(16;17). HLA-Bw4 can also be found on a subset of HLA-A molecules(16;18). HLA-Bw4 variants are ligands for KIR3DL1 NK receptors, while Bw6 variants do not interact with any KIR3DL1 receptors(16;17;19;20). Some HLA-B alleles expressing the Bw4 motif, such as HLA-B*57 and HLA-B*27, have been associated with control of viral replication and slower time to acquired immune deficiency syndrome (AIDS)(1;16;21-24). Previous work has shown that HLA-B*57 mediates its effect on delayed progression to AIDS and viral load (VL) set point, at least partly through the adaptive arm of the immune response via CD8+ T cells that recognize HLA-B*57 restricted epitopes in HIV Gag(24-28). This most protective allele, HLA-B*57, is present

in about 40% of elite controllers (EC), which represents a group of slow progressors (SP) who control viremia to below the limit of detection without antiretroviral therapy(29). A study examining the impact of combinations of KIR3DL1 encoded NK receptor HLA ligand genotypes on time to AIDS in HIV infected individuals found that the combination associated with the most potent VL control and slowest time to AIDS was HLA-B*57 co-carried with KIR3DL1 homozygous (hmz) genotypes with no low expression alleles; a compound genotype designated as *h/*y+B*57, where the *h/*y indicates carriage of a high expression allele in the absence of a low expression allele(1). This protective genotype is also associated with protection against HIV infection in a largely Caucasian injection drug user (IDU) group and slower progression to AIDS in an African population(3;30). The influence of HLA-B*57 in protective outcomes with respect to HIV thus likely also involve the NK cell response, as HLA-Bw4 molecules serve as ligands for KIR on NK cells and have the capacity to render NK cells functionally competent through NK cell education(31;32).

The NK cell education model predicts that KIR3DL1+ NK cells from individuals also carrying Bw4 are more likely to respond to cells that have downregulated HLA ligands than are KIR3DL1+ NK cells from individuals not carrying Bw4 (ie. Bw6hmz). Increased responsiveness of KIR3DL1+ NK cells was demonstrated in donors carrying two HLA-Bw4 genes, as compared to only one or no Bw4 genes suggesting a role for KIR3DL1/HLA-Bw4 in licensing NK cells(33). Additionally, signals received through KIR3DL1 upon ligation of Bw4 result in more potent educational interactions, conferring KIR3DL1+ NK cells with enhanced ability to mediate polyfunctional responses(31;32;34). The combined genotype *h/*y+B*57 has been associated with a reduced risk of HIV infection, coinciding with observations that a higher frequency of NK cells from HIV-exposed seronegative (HESN) individuals carrying *h/*y+B*57 respond to stimulation with HLA-devoid K562 cell line with higher polyfunctional responses than from Bw6hmz(3;32). Stimulation of NK cells with HLA-devoid targets induced a higher frequency of trifunctional NK cells contributing to the overall response in SP carrying KIR3DL1/HLA-Bw4 receptor ligand pairs as compared to Bw6hmz, with the most enhanced responses observed in carriers of *h/*y+B*57(31). Despite the functional potential conferred to KIR3DL1+ NK cells in the presence of its Bw4 ligand, these cells only weakly degranulate when stimulated with autologous HIV-infected CD4+ T cells suggesting additional stimulatory signals are required for sufficient activation of KIR3DL1+ NK cells(35).

Ligation of the activating immunoglobulin G (IgG) constant region receptor (FcγRIIIa or CD16a) on NK cells by anti-viral IgG antibodies (Ab) recognizing epitopes on infected cells has the potential to provide the additional stimulatory signals needed to activate NK cells for cytolysis. IgG Abs against the HIV Envelope gp160 can induce NK-mediated ADCC of HIV-infected cells(36-38). Indeed, ADCC responses in HIV-infected patients and SIV-infected macaques have been correlated with slower disease progression and lower VL(39-45). Induction of ADCC competent Abs has been reported to correlate with the moderate protection from HIV infection observed in the RV144 vaccine trial(38;46-48). NK cell education may also play a role in this since NK cells from carriers of KIR3DL1 and HLA-Bw4 genotype combinations were shown to confer NK cells with enhanced functional potential in uninfected subjects(34).

Interactions between the Fc segment of Ab and Fc receptors on effector cells such as NK cells determine much of the biological activity of Abs. FcyRIIIa/CD16a is a low-affinity receptor for IgG Fc involved in ADCC by cross-linking NK cells with IgG-sensitized target cells. A functional allelic dimorphism in the FcyRIIIa gene which generates allotypes with either a phenylalanine (F) or valine (V) at amino acid (aa) position 158 has been described(49;50). The V isoform has been reported to have greater affinity for Igs of the IgG1 and IgG3 subclass(49;51-54). The CD16a polymorphism is associated with rate of HIV infection and disease progression(55-57) and has been demonstrated to influence virus neutralization, antibody-dependent cell-mediated viral inhibition (ADCVI), and ADCC(58-61).

Using a recently developed flow cytometric GranToxiLux (GTL) ADCC assay based on the hydrolysis of Granzyme B (GzB) in target cells, we investigated the role of NK cell licensing in NK mediated ADCC activity by assessing the ability of NK cells from carriers of *h/*y+B*57 to mediate anti-HIV ADCC as compared to individuals carrying other combinations of KIR3DL1 and HLA-Bw4 or Bw6hmz. Since previous work from our group found that NK cells from SP educated through certain KIR3DL1/HLA-Bw4 combinations had higher NK cell functional potential than those from Bw6hmz, we questioned whether they were also able to mediate superior anti-HIV ADCC than Bw6hmz. Because FcyRIIIa polymorphisms affect IgG binding and exert immunomodulating effects upon exposure to and in the context of HIV infection, we investigated whether ADCC responses were associated with phenotypic differences in CD16a genotype.

Here we report that the educational advantage of the protective *h/*y+B*57 compound genotype in uninfected subjects confers NK cells with enhanced anti-HIV ADCC functional potential but this advantage is abrogated in HIV infection. We observed no influence of CD16a genotype or other KIR/HLA combinations on ADCC responses in uninfected subjects and SP.

2.4: Methods

2.4.1: Study Populations: The study included a total of 47 KIR3DL1hmz who were HIV uninfected (Table 1) and 47 KIR3DL1hmz SP (Table 2). Informed consent was obtained from all study participants and research adhered to the ethical guidelines of the authors' institutions.

2.4.2: Cells: Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation (Ficoll-Paque, Pharmacia Upsala, Sweden) from whole blood obtained by venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA) or by leukapheresis. The cells were then cryopreserved in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St-Louis, MO) with 90% fetal bovine serum (FBS, Wisent, St-Bruno, Quebec, Canada).

Thawed PBMC were suspended at $1x10^6$ cells/ml in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 IU penicillin and 100ug/ml streptomycin (R10 media; all from Wisent). The CEM.NKr.CCR5 cell line (NIH AIDS Research and Reference Reagent Program, from Dr. Alexandra Trkola) was used as a target cell in ADCC assays and was maintained in R10 media in logarithmic phase by diluting the cultures 1:5 every 48hr.

2.4.3: KIR region genotyping: All subjects were typed for the presence of the KIR2DL1, KIR2DL2, KIR2DL3 and KIR3DL1/S1 generic genotypes based on the presence of KIR3DL1 and/or KIR3DS1. Only KIR3DL1 homozygotes were included in this study. The rationale for this is that KIR3DL1 and HLA-Bw4 interact as receptor ligand pairs to license NK cells for functional potential. We excluded carriers of KIR3DS1 because it is unclear whether this molecule interacts with HLA-Bw4 or Bw4*80I antigens and what effect carriage of KIR3DS1 may have on NK function. Genomic DNA was extracted from PBMC or Epstein–Barr virus (EBV)-transformed cells using a QIAamp DNA blood kit (QIAGEN, Inc., Mississauga, ON). KIR region typing was performed using either a GenProbe KIR-SSO kit (GenProbe Inc., San Diego, CA) or a OneLambda KIR-SSO Genotyping kit (OneLambda Inc., Canoga Park, CA). Briefly, genomic DNA was amplified by polymerase chain reaction (PCR) with primers targeting the single KIR gene locus of interest on chromosome 19q13.4 according to manufacturer's specifications. Amplification was performed on a Biometra TGradient thermocycler (Biometra GmbH, Göttingen, Germany), using primers supplied by the

manufacturer and Platinum Taq (Invitrogen Inc., Carlsbad CA). Following amplification and confirmation of expected amplicon size by gel electrophoresis, fluorescence-labelled sequence-specific oligonucleotides (SSO), specific for individual KIR genes, were annealed to the amplification product using a Biometra TGradient thermocycler. Following the addition of streptavidin-phycoerythrin (PE) (OneLambda Inc.), the product was transferred to UNIPLATE™ 96-well polystyrene flat-bottom plate (Whatman, Piscataway, NJ) for data acquisition on a Luminex 100IS instrument (Luminex Corp., Austin, TX). Genotypes were assigned using analysis software provided by the manufacturer (either LIFECODES LifeMatch v.2.4.2 or HLA Fusion [Research] v.2.0.0.27232-SP2). Any ambiguities were resolved by amplification of genomic DNA using primers specific for individual KIR genes and visualization of amplicons of expected sizes on agarose gels(62;63).

2.4.4: KIR3DL1 and HLA allotyping: KIR3DL1 allotyping was done by sequencing KIR3DL1 exons as previously described(3). Single nucleotide polymorphisms (SNP) corresponding to the 3DL1 alleles were identified by aligning the sequenced DNA to a reference consensus sequence consisting of a contig of 3DL1 cDNA sequences. In this study KIR3DL1 high expression (*h) subtypes included KIR3DL*001, *002, *008, *009, *015 and *020 the KIR3DL1 low expression (*l) subtypes included KIR3DL1*005 and *007. All subjects were typed for HLA class I alleles by sequence-based typing using kits from Atria Genetics (South San Francisco, CA). Assign v. 3.5+ was used to interpret sequence information for allele typing (Conexio Genetics, Perth, Australia). Bw6 homozygotes were defined by the lack of any HLA-Bw4 epitope at the HLA-A and -B locus.

For the purposes of this study the following iKIR/HLA receptor ligand pairs were considered: 1) KIR2DL1 with HLA-C2, 2) KIR2DL2/3 with HLA-C1 alleles and, 3) KIR3DL1 with HLA-Bw4 alleles. HLA-B alleles were assigned to the Bw4 or Bw6 public specificities based on amino acids (aa) at positions 77 to 83(16). Individuals who were Bw6hmz served as controls for the effect of NK signalling through KIR3DL1 on NK responses(17;19;20). The HLA-C1 allele subset has an asparagine at position 80 and includes HLA-Cw1, Cw3, Cw7, Cw8, Cw12, Cw14 and Cw16:01. The HLA-C2 allele subset has a lysine at position 80 and includes HLA-Cw2, Cw4, Cw5, Cw6, Cw15, Cw16:02, Cw17 and Cw18(64).

2.4.5: CD16 genotyping: The CD16 gene has a SNP in exon 4 at position 559 such that the protein it encodes has either a valine (V) or a phenylalanine (F) at aa 158(50). The CD16 genotype of study subjects was assigned by PCR genotyping. The allele-specific common forward primer FcγRIIIa R 5'- TCC AAA AGC CAC ACT CAA AGA C-3' and reverse primers FcγRIIIa F1 (V) 5' – CTG AAG ACA CAT TTT TAC TCC CAA C– 3' and FcγRIIIa F2 (F) 5' – CTG AAG ACA CAT TTT TAC TCC CAA– 3' were used. The first nucleotide (nt) at the 3′-end of the allele-specific primers was designed to anneal specifically with the polymorphic nt-559 of the FcγRIIIa gene. This rendered each reverse primer, FcγRIIIa F1 and FcγRIIIa F2 capable of only amplifying the gene if the V or F allele, respectively, was present.

Primer mixes at 5uM were prepared for the V allele, the F allele and a FcγRIIa control primer mix. Reactions were performed with 100ng of genomic DNA in a 20µl reaction volume and AmpliTaq Stoffel Fragment (Invitrogen) was used for amplification. PCR conditions were: denaturation for 5 min at 95°C; 30 cycles of 94°C for 30 seconds, 62°C for 30 seconds and 72° for 30 seconds, and 8 min at 72°C followed by cool down to 4°C. PCR products were run on a 3% agarose gel at 70V for 50min and visualized for the presence of absence of a 73bp amplicon specific for the V and F allele.

2.4.6: CD16 sequencing: CD16a genotyping was verified by sequencing. For sequencing, the allele-specific forward primer FcγRIIIa R 5'-TCC AAA AGC CAC ACT CAA AGA C-3' and CD16Seq R1 reverse primer at 5uM were used to generate a 371bp band of DNA.

Reactions were performed with 100ng of genomic DNA in a 40ul reaction volume. PCR conditions were: denaturation for 5 min at 95°C; 30 cycles of 94°C for 20 seconds, 54°C for 30 seconds and 72°C for 45 seconds, and 5 min at 72°C followed by cool down to 4°C. PCR products were run on a 2% agarose gel and amplified PCR products identified as a 371bp band.

Amplified PCR products were sequenced. The presence of SNPs in exon 4 that encodes a protein with either a V or a F at aa 158 were identified from sequencing results.

2.4.7: GranToxiLux ADCC assay: The functional potential of NK cells from 47 uninfected subjects and 47 SP carrying allelic combinations of KIR3DL1 and HLA-Bw4 was evaluated using the commercially available GranToxiLux PLUS! ADCC assay kit

(OncoImmunin, Inc., Gaithersburg, MD). Briefly, CEM.NKr.CCR5 target cells were coated with 5ug/10^6 cells HIV-1_{BaL} gp120 (NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-1_{BaL} gp120 from DAIDS, NIAID) for 90 minutes at 4°C. Next, target cells were labelled with a fluorescent target cell marker TFL4 and a viability marker NFL1 (both from OncoImmunin, Inc.) for 15 min at 37°C in a humidified 5% CO_2 incubator. PBMC effector cells and target cells were mixed at a ratio of 30:1 in a final volume of 200ul containing $1.5x10^6$ effectors and $5x10^4$ targets. Samples were centrifuged for 7 minutes at 1500rpm after which 75ul GzB substrate (OncoImmunin, Inc.) was added. After a 5 min incubation at RT, 25ul of anti-HIV ADCC competent plasma or plasma from an HIV seronegative subject as a control were added to the target/effector cell suspension and then incubated for 15 min at RT. Flow tubes were centrifuged for 7 min at 1500rpm and incubated for 1h at 37°C and 5% CO_2. During this incubation, GzB is delivered inside the target cells as a result of the antigen (Ag)-specific Ab-Fc receptor interaction with effector cell populations. GzB inside the target cells cleaves the GzB substrate, generating a fluorescent signal that can be quantitatively measured by flow cytometry. After one wash, cells were acquired with the FacsCanto flow cytometer (BD, Franklin Lakes, NJ) within 5 hours. A minimum of $2x10^5$ events representing viable GzB-positive target cells was acquired for each sample.

Data analysis was performed using FlowJo version 9.4 software (Tree Star Inc., Ashland, OR). The results are expressed as percent GzB activity, representing the percentage of target cells recognized by the effector cells, and therefore, fluorescent due to the presence of cleaved GzB substrate. The gating strategy used to assess the percent of GzB positive cells is shown in Figure 1A. Background was represented by the percent of GzB activity observed in tubes that contained effector and target cells in the absence of any source of human Ab. Final results are expressed after subtracting background from the percent GzB activity observed under conditions containing effector and target cell populations in the presence of plasma.

2.4.8: Antibody purification and (Fab')$_2$ fragment preparation: HIV Ig (HIVIG) (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: Catalog #3957, HIV-IG from NABI and NHLBI) (Fab')2 fragments were generated and purified using a commercially available (Fab')$_2$ Fragmentation (Micro) Kit according to the manufacturer's instructions (G-Biosciences, St. Louis, MO, USA). Briefly, HIVIG was applied to immobilized pepsin and centrifuged at 1000g for 2 min to collect IgG

solution. The IgG solution was applied to the immobilized papain spin column, sealed and incubated at 37°C in a high speed shaking waterbath for 2 hours. Following incubation, the sample was centrifuged at 5000g for 1 min to collect the digested Ab. Two subsequent washes of the spin column with 1X PBS were combined with the digested Ab sample. For $(Fab')_2$ fragmentation, the digested Ab sample was applied to a Protein A spin column, sealed and mixed end-over-end at room temperature for 15 min. Following mixing, the Protein A spin column was centrifuged at 1000g for 1 min. to collect the $(Fab')_2$. For maximum $(Fab')_2$ recovery, the column was washed twice with 1X PBS and combined with the initial $(Fab')_2$ fragment flow through.

2.4.9: NK cell phenotypic staining: $1x10^6$ PBMC were stained for cell surface markers with anti-CD56-allophycocyanin (APC), anti-CD3-peridinin chlorophyll protein complex (PerCP), anti-CD16-pacific blue (PB) and anti-CD158e1-phycoerythrin (PE) (anti-KIR3DL1/NKB1; clone DX9) (all from Biolegend, San Diego, CA) for 30 minutes in the dark at RT. After washing with R10 media, cells were fixed with a 1% paraformaldehyde solution (Fischer Scientific, Ottawa, ON) and stored in the dark at 4°C until acquisition. In parallel, a condition in which PBMC were not stained for KIR3DL1 (DX9-PE) were included for each subject.

Between 300,000 and 400,000 events were collected per sample on a FACSCanto II instrument (BD). Data analysis was performed using FlowJo version 9.4.11 software (Tree Star Inc.). For multicolour compensation and gating, unstained, single colour controls were used. NK cells were defined as $CD3^-CD56^+CD16^+$. The proportion of NK cells in PBMC and frequency of $CD3^-CD56^{dim}KIR3DL1^+$ NK cells were determined by the gating strategy shown in Figure 12.

2.4.10: Statistics: Statistical analysis and graphical presentation were performed using Prism 5.03. Unpaired t-tests and one way ANOVA, or their nonparametric equivalents with multiple comparisons post tests were used to compare percent GzB activity by NK cells between 2 and more than 2 KIR/HLA groups, to assess the influence of CD16a polymorphism on ADCC responses and the impact of other iKIR/HLA combinations on ADCC in uninfected study subjects and SP. Where necessary, a Bonferroni correction for multiple comparisons was applied (corrected p-value). Fisher's exact test was used to compare proportions of selected CD16a genotypes between uninfected and SP subjects. A two-tailed chi-squared (χ^2) test was used to examine

differences in the distribution of genotypes between uninfected subjects and SP, and uninfected HIV seronegative historical datasets previously described in the literature. Hardy-Weinberg equilibrium was evaluated using a χ^2 test. Spearman correlation coefficients were used to assess correlations between percent GzB activity and number of iKIR to self HLA combinations, and between percent GzB activity and VL in SP. A coefficient of variation (CV) was calculated to assess assay reproducibility. A p-value of less than 0.05 was considered significant.

2.5: Results

2.5.1: The GTL Assay measures ADCC activity

ADCC activity was measured using the GTL assay(65). Figure 1A shows the gating strategy that was used to analyze the generated data. Target cells were CEM.NKr.CCR5 cells coated with gp120, which were treated with the cellular TFL4 and the NFL1 viability dyes. Live target cells (i.e., $TFL4^{+}NFL1^{-}$) were analyzed to gauge the percentage of cells containing hydrolyzed GzB, reflecting the delivery of a lethal dose of GzB. To ensure cytolysis was dependent on the presence of Ab the %GzB delivery in the presence and the absence of plasma from an HIV positive subject were compared (Fig. 1A). In the absence of plasma, GzB activity was 5.14%, while in the presence of plasma from an HIV infected subject GzB activity was 12.7% (Figure 1A). Figure 1B shows that GzB delivery is dependent on the specificity of the Ab, as plasma from an HIV negative subject yields lower GzB delivery than plasma from an HIV infected subject. To show GzB delivery required recognition of the IgG constant region by CD16, $(Fab')_2$ fragments of HIVIG were prepared. Utilization of these fragments resulted in GzB delivery similar to background (Figure 1C). These results demonstrate that the GTL assay measured ADCC, as the Fc region of IgG is necessary for GzB delivery.

2.5.2: Impact of HIV infection on NK cell mediated ADCC

Compromised NK cell function during HIV infection has been reported several times(31;34;66-68). HIV infected SP with low viral load (VL) have NK cells able to secrete IFN-γ, TNF-α and express CD107a in response to stimulation with HLA-null cells(31). Although NK cells from individuals in the chronic phase of HIV infection have poor NK cell mediated ADCC activity(34), little information is available on if NK cells from HIV infected SP also exhibit compromised ADCC activity(43;45). We therefore investigated whether NK cells from uninfected individuals mediate more potent anti-HIV ADCC responses than NK cells from SP. We measured %GzB activity in 47 uninfected subjects and 47 SP using gp120-labelled CEM.NKr.CCR5 target cells and ADCC competent Abs from an HIV infected SP. The results for %GzB activity were reported after subtracting the background. The mean ± standard deviation of the %GzB activity was significantly higher in uninfected subjects compared to SP (8.0±1.2 vs. 4.3±0.8, respectively, p=0.018, Mann-Whitney test) (Figure 2A).

*2.5.3: NK cells from HIV uninfected carriers of high expression 3DL1 homozygous genotypes co-carried with HLA-B*57 have higher anti-HIV ADCC responses than those co-carried with other Bw4 or Bw6 alleles*

Interaction between inhibitory NK cell receptors and their ligands is required for NK cell education(10-12;14). The strength of these interactions is directly related to NK cell activation achieved upon encountering target cells with ligands for activating receptors but reduced levels of ligands for inhibitory receptors(14;69). As little information is available on the role of NK cell education in ADCC activity, it was investigated if carriage of KIR3DL1 in the presence or absence of its ligand influenced ADCC responses. Subjects were grouped according to if they were KIR3DL1hmz and carried either a HLA-Bw4 ligand or were HLA-Bw6hmz. There is no interaction between Bw6 and KIR3DL1, allowing HLA-Bw6hmz to serve as controls(17;20). The %GzB activity was higher for NK cells from the 37 individuals carrying KIR3DL1 and HLA-Bw4 compared to the 10 HLA-Bw6 homozygotes in the uninfected group (8.9±1.4 vs. 5.7±1.7). However, this difference did not achieve statistical significance (p=0.15, Mann-Whitney test) (Figure 2B). ADCC responses were not significantly higher in 36 SP carrying KIR3DL1 and HLA-Bw4 compared to 11 HLA-Bw6 homozygotes (4.1±0.8 vs. 4.8±2.0, p=0.96, Mann-Whitney test) (Figure 2B).

Previous work from the Bernard lab reported NK cells from carriers of highly expressed KIR3DL1hmz genotypes with HLA-B*57 (*h/*y+B*57) had higher functionality in terms of non-ADCC functions, such as cytokine secretion and CD107a expression, than those from carriers of KIR3DL1hmz genotypes with other HLA-Bw4 or HLA-Bw4*80I alleles(31;32). HLA-Bw4 carriers in the uninfected group were separated into those carrying the protective *h/*y+B*57 combination and other KIR3DL1/HLA-Bw4 combinations. These groups were then compared to each other and to Bw6 homozygotes for NK cell mediated ADCC activity, resulting in the observation of between group differences (p=0.02, Kruskal-Wallis test) (Figure 2C). NK cells from subjects carrying *h/*y+B*57 had significantly higher %GzB activity (13.4±2.4) than Bw6 homozygotes (4.7±1.7, p>0.05). Percent GzB activity in *h/*y+B*57 uninfected subjects was also higher than that mediated by carriers of other KIR3DL1/HLA-Bw4 combinations (6.8±1.6), but this difference was not statistically significant (p<0.05, Dunn's multiple comparisons test) (Figure 2C). Interestingly, NK cells from *h/*y+B*57 SP did not mediate higher %GzB activity (2.1±0.4) than those carrying other KIR3DL1/HLA-Bw4 combinations (5.1±1.2) or HLA-Bw6hmz (4.8±2.0) (p=0.57,

Kruskal-Wallis test) (Figure 2D). Perhaps most intriguing, NK cells from uninfected *h/*y+B*57 subjects mediated significantly higher ADCC responses than those from *h/*y+B*57 SP (13.4±2.4 vs. 2.1±3.1, p=0.0002, Mann-Whitney test) (Figure 2D). These results suggest that carriage of *h/*y+B*57 in HIV uninfected individuals, which confers enhanced NK cell direct functional potential, also enhances ADCC functional potential(31;32). However, HIV infection, even the well-controlled infection observed in SP appears to abrogate the educational advantage of carriage of *h/*y+B*57 on ADCC activity.

*2.5.4: Receptor ligand requirements for increased ADCC activity in *h/*y+B*57 carriers*

Previous work from Dr. Bernard's lab has shown NK cells from HIV uninfected individuals with the *h/*y+B*57 genotype to have a higher percentage of NK cells with trifunctional potential as measured by cytokine secretion and CD107a expression in response to an HLA-null stimulus, than those from carriers of the *h/*y+Bw4*80I, *h/*y+Bw4 genotypes or Bw6 homozygotes(32). Another study from the Bernard lab demonstrated that co-carriage of the *h/*y KIR3DL1 receptor genotype and the HLA-B*57 ligand is required for elevated trifunctional NK cell responses in SP(31).

To address the receptor ligand requirements for enhanced %GzB activity by NK cells from uninfected *h/*y+B*57 carriers, NK cell mediated %GzB activity was compared between 12 *h/*y+B*57 carriers, 3 carriers of *l/*x+B*57 and 16 carriers of *h/*y+other Bw4 alleles. No statistically significant differences were observed between carriers of the *h/*y+B*57 and *l/*x+B*57 genotypes (13.35±2.4 vs. 17.1±4.3) (Figure 2E). However, we did observe significantly higher %GzB activity in carriers of *h/*y+B*57 compared to individuals carrying *h/*y+other non-B*57 alleles (13.8±1.9 vs. 3.9±1.3, p<0.01) (p=0.0026, Kruskal-Wallis test, followed by Dunn's multiple comparison test) (Figure 2E). The low number of carriers of *l/*x+B*57 tested precluded the ability to formally determine if elevated %GzB activity from uninfected *h/*y+B*57 carriers required both the highly expression KIR3DL1 receptor genotype and HLA-B*57. These observations suggest the combined *h/*y+B*57 genotype confers increased %GzB activity. It is possible that carriage of HLA-B*57 with either KIR3DL1hmz genotype group (i.e. *h/*y and *l/*x) is sufficient to confer high NK cell mediated ADCC activity.

2.5.5: Influence of HIV VL on NK cell-mediated anti-HIV ADCC killing of target cells

Dysregulated NK cell function and subset redistribution is correlated with viremia levels in HIV infected subjects(31;66;67). As viremia was present in a subset of the SP in the current study, viremia was assessed in conjunction %GzB activity to determine if a relationship between VL and NK cell mediated anti-HIV ADCC responses exists. No relationship between these two parameters was observed (Spearman rho= -0.14, p=0.16) (Figure 3). Therefore, our finding that ADCC responses in SP do not differ between carriers of *h/*y+B*57, KIR3DL1hmz with other Bw4 alleles and Bw6 homozygotes (Figure 2D), cannot be readily explained by differential VL control in these groups. As well, no significant between group differences were detected in %GzB activity between EC with VL below the level of detection by standard assays (6.9±2.2) and non-EC (3.1±6.4) (p=0.26, Mann-Whitney test) (Figure 4). The lack of association between VL set point and %GzB activity provides further support for a loss in functional potential of NK cells in SP with the *h/*y+B*57 compound genotype.

2.5.6: Distribution of CD16a genotypes in uninfected and SP subjects

Variation in %GzB activity was observed within each KIR3DL1/HLA-B genotype combination group. ADCC requires cross-linking of CD16a on NK cells with an Ab-coated target cell. A functional polymorphism of CD16a that influences the affinity of this receptor for IgG has been described(58). The effect of NK cells in the presence of antibody is primarily due to ADCC, thus we questioned whether this CD16a polymorphism could account for some of the within group variability in %GzB activity. Of the 46 uninfected subjects genotyped for the CD16a polymorphism, none were V/Vhmz, 27 (59%) were heterozygous (htz) for the V/F genotype and 19 (41%) were F/Fhmz (Table 3). The 47 SP were also genotyped. It was found that 3 (6%) were V/Vhmz, 26 (55%) V/Fhtz and 18 (38%) F/Fhmz (Table 3). Comparison of the frequencies for CD16a genotypes (V/V, V/F and F/F) in uninfected versus SP subjects revealed no significant difference (χ^2=3.04, p=0.2192, degree of freedom [df]=2) (Table 3). While the distribution of CD16a genotypes in SP did not deviate statistically from Hardy-Weinberg (HW) equilibrium (χ^2=2.5, p=0.11, df=1) that in the uninfected population did (χ^2=7.9, p=0.0048, df=1). We questioned if the skewed distribution of CD16a genotype in our uninfected subjects was observed by others working with HIV uninfected Caucasian populations. A literature review found 3 previously published

datasets(49;52;70). Comparisons of the distribution of CD16a genotype in our HIV uninfected study population to the three HIV seronegative Caucasian low-risk historical datasets previously published in the literature revealed no significant differences in the distribution of CD16a genotypes(49;52;70) (Table 4). Furthermore, all three comparator populations were found to be in HW equilibrium for the small nucleotide polymorphism (SNP) encoding the three possible genotypes for CD16a (Table 4). We conclude that the absence of subjects with the V/V CD16a genotype in our uninfected population that leads to skewing of the HW equilibrium is due to chance and is an observation not replicated in other uninfected Caucasian populations.

2.5.7: No effect of CD16a genotype on anti-HIV ADCC responses

No effect was observed for the CD16a genotype on the ability of NK cells to mediate ADCC in uninfected subjects (p=0.31, Mann-Whitney test) and SP (p=0.40, Kruskal-Wallis,) (Figure 5A). To assess if CD16a genotype explained the variation in ADCC responses observed within KIR/HLA combinations, we examined ADCC responses between carriers of the V/F and F/F genotype in each KIR/HLA group. No significant between group differences were observed in *h/*y+B*57 carriers (p=0.37, Mann-Whitney test), other Bw4 carriers (p=0.62, Mann-Whitney test), or Bw6 homozygotes (p=0.91, Mann-Whitney test) in the uninfected population (Figure 5B). Similarly, no significant between group differences in %GzB activity were observed between carriers of V/V, V/F and F/F CD16a SNPs in SP that carried *h/*y+B*57 (p=0.29, Mann-Whitney test), other Bw4 allele (p=0.38, Kruskal-Wallis test), or Bw6hmz (p=0.1, Kruskal-Wallis test) (Figure 5C).

*2.5.8: Distribution of inhibitory KIR to self-HLA-C (S-iKIR to HLA-C) in carriers of *h/*y+B*57, other Bw4 alleles and Bw6 homozygotes*

The KIR genetic region is polygenic as well as polymorphic. The former term means that individuals differ from each other in the number of KIR region genes they carry(62). KIR2DL1 is carried by over 99% of Caucasians. All of the 46 uninfected subjects (Table 1) and 44 of 46 SP (Table 2) for which KIR2D typing was available were positive for a KIR2DL1 gene. KIR2DL2 and KIR2DL3 segregate as alleles of the KIR2DL2/3 locus, which is carried by all KIR haplotypes(62;71). HLA-C1 antigens are ligands for KIR2DL2/3 while HLA-C2 antigens are ligands for KIR2DL1(72-74). The uninfected and SP study populations in this thesis research are all KIR3DL1hmz such

that, depending on if a subject was HLA-C1 or HLA-C2hmz versus HLA-C1/C2htz, they each carry 1 or 2 S-iKIR to HLA-C if they are Bw6hmz and 2 or 3 S-iKIR to HLA-C and HLA-B if they are a Bw4 carrier. If these iKIR are co-expressed with their cognate ligand, NK cells from KIR3DL1/Bw4 carriers may also be educated through the interaction of inhibitory KIR2DL receptors that recognize self HLA-C1 and/or C2(13;75). As NK cell education can be cumulative(14;69), it was questioned if the higher %GzB activity in carriers of *h/*y+B*57 could be attributed to this group containing more S-iKIR to HLA-C than KIR3DL1hmz carriers of other Bw4 alleles or Bw6hmz. For this analysis if the combination of KIR2DL1 with C2 and KIR2DL2/3 with C1 were both present they were counted as 2 HLA-C/KIR2DL1/2/3 combinations, while if only 1 was present they were counted as 1 HLA-C/KIR2DL1/2/3 combination. No between group differences were observed in the number of S-iKIR to HLA-C in uninfected subjects (p=0.78, Kruskal-Wallis test) (Figure 6). The same analysis was performed in SP. Although there appeared to be more carriers of 2 S-iKIR to HLA-C in KIR3DL1hmz carriers of other Bw4 alleles than in carriers of *h/*y+B*57 or in Bw6 homozygotes (p=0.031, Kruskal-Wallis test), 2 by 2 post-test analyses revealed no significant between group differences (Dunn's multiple comparison test) (Figure 6). This analysis demonstrates that enhanced %GzB activity in *h/*y+B*57 uninfected participants is not due to the presence of more educationally competent S-iKIR to HLA-C.

*2.5.9: Enhanced NK cell functional potential in carriers of the protective *h/*y+B*57 compound genotype is not due to the number of S-iKIR to HLA-C*

The interaction of KIR3DL1 with Bw4 has been demonstrated to license NK cells for ADCC(75). Others have shown that KIR2DL/HLA-C combinations educate KIR2D+ NK cells for ADCC(13). Carriage of S-iKIR to HLA-C does not appear to enhance non-ADCC functionality of NK cells as measured by cytokine secretion and CD107a expression in carriers of the S-iKIR to HLA-Bw4 including those positive for the *h/*y+B*57 genotype(76). To further investigate if carriage of educationally competent S-iKIR/HLA-C combinations influenced ADCC, KIR3DL1hmz uninfected subjects and SP were categorized according to the number and type of S-iKIR they carried. Group 1 included Bw6hmz subjects with only 1 S-iKIR to either an HLA-C1 or C2 allele. Group 2 included Bw6hmz subjects with 2 S-iKIR to both an HLA-C1 and C2 allele. Group 3 included subjects with an S-iKIR to a non-HLA-B*57 HLA-Bw4 allele

and 1 S-iKIR to either HLA-C1 or C2. Group 4 included subjects with an S-iKIR to a non-HLA-B*57 HLA-Bw4 allele and 2 S-iKIR to both HLA-C1 and C2. Group 5 included KIR3DL1*h/*y subjects with an S-iKIR to HLA-B*57 and 1 S-iKIR to either a HLA-C1 or C2 and Group 6 included KIR3DL1*h/*y subjects with an S-iKIR to HLA-B*57 and 2 S-iKIR to both an HLA-C1 and C2.

The degree of NK cell activation upon target cell stimulation is proportional to the education signals received during development. It was hypothesized that if the S-iKIR to HLA-C were educationally competent, NK cells from individuals with 2 S-iKIR to HLA-C would have higher %GzB activity than those with 1 S-iKIR to HLA-C in groups categorized according to whether or not they had a S-iKIR to HLA-B*57, other Bw4 antigens or were Bw6hmz. No significant between-group differences were observed in the %GzB activity between Groups 1 and 2 (4.95±2.17 vs. 4.3±3.1, p=1.00, Mann-Whitney test), between Groups 3 and 4 (5.9±1.9 vs. 6.7±2.7, p=0.79, Mann-Whitney test), or between Groups 5 and 6 (14.7±2.5 vs. 10.7±5.4, p=0.46, Mann-Whitney test) in uninfected subjects. Similarly, no between group differences were observed between Group 3 and 4 (4.5±1.5 vs. 5.7±2.0, p=0.56, Mann-Whitney test). A small number of SP in Group 2 and 6 precluded formal analysis between Group 1 and 2 (5.3±2.2 vs. 0±0), and between Group 5 and 6 (1.9±0.57 vs. 3.1±0.1), however no between group differences were observed in all the SP groups (p=0.57, Kruskal-Wallis) (Figure 7). Taken together this suggests that the carriage of 1 versus 2 S-iKIR to HLA-C did not influence anti-HIV ADCC activity in our study populations.

Next, a correlation analysis was performed between classification within groups 1-6 as described earlier and %GzB activity. It was assumed that having an S-iKIR to HLA-B*57 resulted in more potent educational signalling than having a S-iKIR to other HLA-Bw4 antigens, which was more potent than having no S-iKIR to HLA-Bw4 (i.e. Bw6hmz). It was also assumed that within these categories having 2 S-iKIR to HLA-C conferred a stronger educational signal than having 1 S-iKIR to HLA-C. As such, it was hypothesized that if the number and type of S-iKIR to HLA-C and Bw4 combinations contributed to NK cell education, and if the level of NK cell education influenced ADCC activity, that there would be a correlation between S-iKIR number and potency of KIR/HLA interactions with %GzB activity. A Spearman rank correlation test detected no significant correlation between hypothesized educational potential and %GzB activity in SP (Spearman rho= -0.05, p=0.73), but did show a correlation between the number and assumed potency of S-iKIR to HLA-C and Bw4 and ADCC responses in uninfected

subjects (Spearman rho=0.36, p=0.01) (Figure 8). If carriers of HLA-B*57 (Groups 5 and 6) were removed from this analysis, however, %GzB activity no longer correlated with carriage or not of an S-iKIR to HLA-Bw4 and having 1 versus 2 S-KIR to HLA-C within these categories in uninfected subjects (Spearman rho=0.11, p=0.55) or in SP (Spearman rho=0.09, p=0.61) (Figure 9). This finding indicates that the positive correlation observed in the analysis described in Figure 8 is driven by the high %GzB activity generated by NK cells from carriers of KIR3DL1*h/*y genotypes with HLA-B*57.

We also categorized subjects according to carriage of 1 (Group 1) or 2 (Group 2) S-iKIR to HLA-C only, or 1 S-iKIR to HLA-Bw4 and either 1 (Groups 3 and 5) or 2 (Groups 4 and 6) S-iKIR to HLA-C. No correlation between %GzB activity and the number of S-iKIR to HLA-C or Bw4 combinations was observed in uninfected subjects (Spearman rho=0.10, p=0.50) or SP (Spearman rho=0.12, p=0.42) (Figure 10). Together these results suggest that in the context of the experimental approach used in this thesis 1) NK cell educational signals provided by S-iKIR to HLA-C and non HLA-B*57 HLA-Bw4 antigens are contributing minimally to NK cell functional potential measured by ADCC, 2) that the influence of signals through S-iKIR to HLA-C and HLA-Bw4 on NK cell functional potential measured by ADCC is not cumulative and, 3) that interactions between KIR3DL1 and HLA-B*57 stand out from other S-iKIR to self HLA in educating NK cells for functional potential measured by ADCC.

2.5.10: No influence of proportion of NK cells and frequency of KIR3DL1+ NK cells in PBMC on ADCC activity

The frequency of NK cells in PBMC may impact observed %GzB activity, as PBMC with higher frequencies of total NK cells or KIR3DL1+ NK cells would be expected to mediate higher levels of ADCC(34). To address this possibility, we randomly selected 10 carriers of *h/*y+B*57, 12 KIR3DL1hmz carrying other HLA-Bw4 alleles and 10 Bw6hmz uninfected subjects for testing in a second set of anti-HIV ADCC assays with concurrent surface staining for NK cell markers CD3, CD56, CD16 and KIR3DL1. We defined NK cells as $CD3^{-}CD56^{+}CD16^{+}$, and $KIR3DL1^{+}$ NK cells as $CD3^{-}CD56^{dim}KIR3DL1^{+}$, as KIR are primarily expressed on NK cells with the $CD56^{dim}$ phenotype(77;78). A representative gating strategy is shown in Figure 11. No between group differences in the proportion of total $CD3^{-}CD56^{+}CD16^{+}$ NK cells were observed (p=0.39, Kruskal-Wallis) (Figure 12). There were also no between group differences in the frequency of $CD3^{-}CD56^{dim}KIR3DL1^{+}$ NK cells (p=0.38, Kruskal-Wallis) (Figure 13).

Therefore, there is no evidence that the frequency of total NK cells or the frequency of NK cells expressing KIR3DL1 differs based on which of the 3 KIR3DL1/HLA-B genotype groups they originate from. Similar experiments have been conducted by others in the Bernard lab, as well as other research groups, with similar results(75;79). Based on these findings it is unlikely that between group differences in the frequency of NK cells or KIR3DL1+ NK cells contributed to differences in the %GzB activity observed in our uninfected study population.

2.5.11: Inter- and intra-assay reproducibility of the GTL ADCC assay

In ADCC experiments reported in Figure 2C, on 47 uninfected subjects significantly higher ADCC responses were seen in individuals carrying the *h/*y+B*57 protective genotype as compared to KIR3DL1hmz individuals carrying other Bw4 alleles or Bw6 homozygotes. Assessment of %GzB activity in the second set of assays obtained different results (Figure 14). Samples from 10 carriers of *h/*y+B*57, 12 KIR3DL1 homozygotes co-carrying other Bw4 alleles, and 10 Bw6hmz uninfected study subjects were included in the second set of experiments. Cells from the same timepoint were used for each sample in the replicate assay as in the first set of experiments. No significant between group differences were observed between carriers of *h/*y+B*57 (8.4±2.2) and individuals with other Bw4 alleles (17.3±2.9) or Bw6 homozygotes (14.7±2.4) (p=0.08, Kruskal-Wallis, followed by Dunn's multiple comparison test) (Figure 14). This led us to question the reproducibility of the GTL assay. To address this issue an analysis of inter-assay variability was performed by measuring %GzB activity in the above mentioned subset of uninfected subjects taken from each of the 3 KIR/HLA groups. The coefficient of variation (CV) revealed inter-assay variability ranging from 64.2% to 88.3% (Figure 15). This CV was in the range seen when ADCC results from all study participants were included irrespective of KIR3DL1/HLA-B genotype (72.9%). This demonstrates that inter-assay variability was not influenced by differences in %GzB responses between KIR/HLA genotype groups.

In light of the results from our replicate ADCC experiment, we further evaluated the reproducibility of this assay with samples from one uninfected *h/*y+B*57 carrier (UI1001; Table 1) and one Bw6 homozygote (UI3009; Table 1). Experiments were performed in triplicate on 3 occasions in order to obtain information on intra- and inter-assay CV as a measure of assay variability. We observed an intra-assay variability of 22.73±2.2% and an inter-assay variability of 22.73%. The inter-assay variability observed

in this study are in the same range as previously reported inter-assay variability of less than 25% when using the same CEM.NKr.CCR5 cells as targets, a single source of gp120 to coat targets and a single source of anti-gp120 antibody - as was used in the experiments described in this thesis(65).

2.6: Discussion

Here we report that NK cells from uninfected individuals carrying the *h/*y+B*57 combined genotype mediate higher anti-HIV ADCC responses than those from individuals carrying KIR3DL1hmz genotypes with other HLA-Bw4 alleles or from HLA-Bw6 homozygotes. In contrast, the enhanced functional potential conferred to NK cells by carriage of *h/*y+B*57 is abrogated in HIV infected SP. We report that a CD16a polymorphism that influences IgG binding to this receptor does not impact on frequency of anti-HIV ADCC target cells in uninfected and SP subjects in our study. The percent of NK cells and KIR3DL1+ NK cells did not differ between the 3 KIR3DL1/HLA-B genotype groups studied. iKIR to self HLA-C have also been reported to educate NK cells for functional potential. Despite this, we found no evidence that the quantity and type of iKIR to self HLA-C contributed to elevated ADCC activity observed in uninfected carriers of *h/*y+B*57, nor did they appear to contribute to higher ADCC activity of NK cells from either uninfected or infected SP individuals carrying other KIR3DL1/HLA-B combinations.

Although a second set of GTL ADCC experiments utilizing the same subject samples from the same timepoint revealed very different results than the first dataset, follow up experiments indicated a percent inter- and intra-assay variation within the range previously reported in the literature(65). The reasons for the discrepant results are being investigated and have yet to be resolved. For the purpose of this thesis, I used the results generated in the first set of experiments to address the research questions and build the discussion of these results.

The 3DL1/HLA-Bw4 combination with the most potent effect on time to AIDS and VL control is the *h/*y+B*57 genotype(1). This genotype combination occurs at higher frequencies in HESN compared with HIV infected individuals enrolled in a primary infection (PI) cohort(3). Our lab has previously demonstrated in HIV-uninfected subjects that NK cells from carriers of *h/*y+B*57 had higher trifunctional potential than those from carriers of *h/*y+Bw4*80I, *h/*y+Bw4, or Bw6hmz genotypes(32). It was reported that individuals carrying 3DL1*h/*y without B*57 had lower NK cell trifunctional responses when compared with the *h/*y+B*57 group(31;32). KIR3DL1/HLA-Bw4 genotype is a determinant of NK cell functional potential as measured by the ability of these cells to mediate polyfunctional responses. Our results suggest the *h/*y+B*57 genotype is also a potent licensing combination for ADCC. In this thesis I demonstrate for the first time that the protective *h/*y+B*57 compound

genotype confers NK cells from HIV uninfected individuals with an enhanced ability to mediate anti-HIV ADCC (Figure 2C and 2E). The low number of carriers of the *l/*x+B*57 genotype (n=3) in the current study is a limitation to making conclusions as to whether elevated ADCC functional potential of NK cells requires the presence of both the high expression KIR3DL1 NK receptors and HLA-B*57 ligands. Differences in ADCC responses between carriers of *h/*y+B*57 and carriers of *h/*y+other Bw4 implicate the requirement for HLA-B*57 but it is less certain that high expression KIR3DL1 receptors is required for the observed effect of greater NK cell mediated ADCC in the *h/*y+B*57 group (Figure 2E).

Several studies have demonstrated a detrimental effect of HIV infection on NK cell function(66-68). NK cells from individuals in the chronic phase of HIV infection had significantly lower anti-HIV ADCC activity as measured by CD107a and IFN-γ expression than those from uninfected subjects(34). A study of NK cell function in HIV infected populations reported less NK cell dysfunction in SP compared with subjects in the chronic phase of infected, treated or not(66). The frequency of HLA-B*57 is higher among HIV infected SP than typical progressors(24) and thus, so is the frequency of carriers of the *h/*y+B*57 genotype in this population. Since carriage of this genotype favors elevated ADCC activity in uninfected subjects we were able to examine whether it also confers higher ADCC activity to NK cells from individuals who are HIV infected but SP with better VL control and limited immune damage. NK cells from HIV infected SP carriers of the *h/*y+B*57 protective genotype supported ADCC response levels that were lower than those of NK cells from uninfected *h/*y+B*57 subjects and indistinguishable from those from 3DL1hmz SP carrying other HLA-B alleles (Figure 2D, left panel). Thus, not only do NK cells from treated or untreated individuals in chronic phase HIV infection exhibit dysregulated ADCC function but so do NK cells from subjects with the more benign HIV disease course observed in SP. This contrasts with what was found for other NK cell functions, namely HLA-null cell stimulated NK cell activity. NK cells from SP who are *h/*y+B*57 maintain their ability to mediate polyfunctional responses characterized by secretion of IFN-γ and TNF-α and expression of CD107a(31). Although the level of these responses negatively correlated with VL, NK cell response levels also depended on which 3DL1/HLA-B genotype combinations were carried, with *h/*y+B*57 carriage supporting the highest level of trifunctional NK cell potential(31). In the work presented in this thesis we found no association between VL and anti-HIV ADCC levels in the SP population (Figures 3-4). The *h/*y+B*57 SP

group included a single EC with undetectable HIV VL whose NK cells also mediated low level ADCC activity.

Taken together these results suggest that NK cell activation for ADCC activity is abrogated in HIV infected carriers of 3DL1/HLA-Bw4, even those who belong to the SP group with a benign disease course and carry the *h/*y+B*57 genotype that confers high ADCC activity in uninfected individuals. Others have proposed a hierarchy of the relative strengths required to achieve induction of different NK cell responses(80). One interpretation of these results is that NK cell mediated ADCC activity is particularly sensitive to extinction in the context of HIV disease, more so than IFN-γ and TNF-α secretion, degranulation and direct cytotoxicity. Engagement of CD16 on NK cells in the context of HIV infection may not be sufficient to overcome the threshold of activating stimuli required for induction of ADCC. Weak ADCC responses in SP may reflect differences in licensing interactions and signal strengths required for induction of specific NK effector functions. Alternately, in HIV infected SP the presence of ADCC competent Abs that can bind the CD16a receptor may lead to its downmodulation or desensitization as an activating receptor for ADCC.

In addition to the established influence of KIR/HLA genotypes on protective outcomes upon exposure to or infection with HIV, FcγRIIIa polymorphisms have been associated with susceptibility to HIV infection and progression to AIDS(45;55-57;59). There is disagreement in the literature as to whether carriage of the high affinity V allele at aa position 158 is associated with protection from infection and progression to AIDS(55;58;59;81;82). An overrepresentation of the V/V genotype was observed in HIV progressors suggesting an association of the V allele with disease progression(56). Interestingly, the same study reported a higher frequency of F/F homozygotes in the uninfected population than HIV seropositive subjects suggesting an association of this genotype with protection from infection(56). The frequency of the V allele has been reported to be lower than the F allele in populations of various ethnic backgrounds(49;51-53). We also observed a lower frequency of the V compared to the F allele in both our predominantly Caucasian study populations (Table 3). Although the distribution of CD16a genotypes in uninfected subjects was not in HW equilibrium in our study, the frequency of each genotype was consistent with previously published reports(49;53;55). Comparison with 3 low-risk healthy control Caucasian historical datasets supports our

interpretation that the differences in CD16a allele frequencies in our uninfected study population are due to chance (Table 4).

Both the V and F alleles have been reported to be risk factors for HIV infection and progression, however no consistent pattern has emerged. Forthal et al. (2007) observed significantly higher ADCVI responses in V/Vhmz HIV seropositive subjects, but report a reduced benefit in protection from infection in carriers of the V allele(59). Later work by Forthal et al. (2012) showed a trend towards increased risk of infection for V/Vhmz low-risk vaccinees in the VAX004 trial(57). Brown et al. (2012) reported higher levels of viral inhibition in carriers of the V allele, with a loss of neutralization upon NK cell depletion(58). Polymorphism at this locus is likely to modulate the association between KIR/HLA and NK cell mediated ADCC, though to date little work has been carried out to elucidate its role in the context of HIV infection. We observed no influence of CD16a genotype on anti-HIV ADCC functional potential between carriers of *h/*y+B*57, 3DL1hmz with other Bw4 alleles or Bw6 homozygotes (Figure 5A-C). These results are consistent with the observations of Johansson et al. (2011) who despite observing ADCC-inducing Env Abs in a group of controllers, found no major influence of CD16a genotype on NK cell ADCC activity(81). The contradictory evidence surrounding the potential impact of CD16a genotype on outcomes upon exposure to or infection with HIV suggests factors other than differences in binding affinity may contribute to the activity of this Fc receptor on effector cells.

Inhibitory KIR to self-HLA other than KIR3DL1 in combination with Bw4 have been demonstrated to differ in their contribution to NK cell functional potential(15;76;83;84). It was recently demonstrated that education signals can be mediated through the interaction of HLA-C with KIR2DL1/2/3(84) and others report that HLA-C contributes to NK cell education and functional potential in HIV uninfected individuals(13). Therefore, in theory variations in the levels of anti-HIV ADCC activity in carriers of a particular KIR3DL1 and HLA-Bw4 genotype group may be due to inhibitory signals propagated through S-iKIR to HLA-C. It has also been reported that the quantity of S-iKIR influences the potency of licensing(15;33). In our study, carriage of more than one S-iKIR to HLA-C did not lead to higher ADCC responses in KIR3DL1hmz uninfected or SP subjects carrying or not carrying HLA-Bw4, indicating that enhanced ADCC in carriers of *h/*y+B*57 was not due to carriage of more educationally competent S-iKIR to HLA-C than subjects with other Bw4 alleles or Bw6

homozygotes (Figure 6). Dr. Bernard's lab has previously shown that carriage of S-iKIR to HLA-C did not alter the frequencies of trifunctional NK cell responses to stimulation with HLA class I-devoid K562 targets in 3DL1/HLA-Bw4 carriers(76). Here, we also showed that carriage of more than one S-iKIR to HLA-C did not result in higher ADCC responses in our study populations, nor did the number of S-iKIR to HLA-C confer heightened functional potential as measured by ADCC to NK cells from carriers of *h/*y+B*57 compared to subjects carrying other Bw4 alleles or Bw6 homozygotes (Figures 7-10). The limited contribution of S-iKIR to HLA-C in HIV infected SP may be attributed to functional exhaustion of NK cells licensed through this interaction(85-88). The same findings in uninfected subjects suggests that signalling through S-iKIR to HLA-C has limited influence on NK cell education and functional potential as measured by ADCC compared to the potency of licensing conferred through interactions between KIR3DL1 and HLA-B*57. The level of HLA-C expression is much lower than HLA-A and –B(89;90). As such, it has been proposed that higher HLA-B expression may result in more effective NK cell education through KIR3DL1 than inhibitory KIR2D receptors with HLA-C(91). If differential surface expression of HLA influences the potency of NK cell educational signals, this may contribute to observations that carriage of S-iKIR to HLA-C did not influence ADCC responses in carriers of KIR3DL1/HLA-B genotypes.

Differences in the frequency of total NK cells or a differential presence of licensed NK cells in humans may contribute to associations between KIR/HLA combinations and protective outcomes. We found no evidence that the frequency of total NK cells or the frequency of NK cells expressing KIR3DL1 differs between the 3 KIR/HLA genotype groups studied (Figures 12-13). These data support our conclusion that neither total NK cell frequency nor frequency of KIR3DL1+ NK cells significantly contributes to differences in ADCC responses observed in our uninfected study population.

The GTL assay is a recently developed technique for evaluation of ADCC(65). Using cells from the same study subjects and timepoint, we performed a second set of experiments to evaluate the reproducibility of this assay in measuring the effect KIR/HLA genotype on anti-HIV ADCC. Although we were unable to replicate the results from our first set of experiments, more rigorous evaluation using two uninfected samples in triplicate over 3 experiments indicated an intra-assay variability of 22.73±2.2% and an

inter-assay variability of 22.73%. These results fall within the range of less than 25% inter-assay variability previously reported in the literature(65). Future experiments to investigate the reasons for disparate results between our first and second set of experiments are required.

2.7: Figure Legends

Table 1: HIV Uninfected Study Population Characteristics

Table 2: Slow Progressor Study Population Characteristics

Figure 1: *Representative gating strategy utilized for the GTL assay.* (A) This procedure involved gating on total target and effector cells, identifying target cells (i.e., $TFL4^{+}$), excluding target cells that were dead prior to incubation (i.e., $NFL1^{+}$), and identifying target cells that received a lethal hit of Granzyme B (GzB) during incubation with PBMC in the absence or presence of plasma from an HIV-infected individual. (B) The percentage of Granzyme B positive target cells is shown for 1 individual following a 1 hour incubation of PBMC effectors and BaL-gp120-coated CEM.NKr.CCR5 targets at a ratio of 30:1 in the absence of plasma (left panel), presence of plasma from an HIV negative subject (middle panel), and plasma from an HIV positive subject at a 1:1000 dilution (right panel). The y-axis for these plots is the side scatter and the x-axis the mean fluorescent intensity of the GTL readout. The anti-HIV ADCC activity is the percentage of GzB positive target cells obtained in the presence of plasma from an HIV infected individual minus the percentage of GzB positive target cells obtained in the absence of plasma. (C) The percentage of Granzyme B positive target cells is shown for 1 individual following a 1 hour incubation of PBMC effectors and BaL-gp120-coated CEM.NKr.CCR5 targets at a ratio of 30:1 in the presence of whole HIVIG(left panel), and the presence of HIVIG Fab2 fragments (right panel). The y-axis for these plots is the side scatter and the x-axis the mean fluorescent intensity of the GTL readout. The anti-HIV ADCC activity is the percentage of GzB positive target cells obtained in the presence of plasma from an HIV infected individual minus the percentage of GzB positive target cells obtained in the absence of plasma.

Figure 2: *Uninfected but not slow progressor (SP) carriers of the *h/*y+B*57 compound genotype mediate higher anti-HIV ADCC responses than carriers of other Bw4 alleles or Bw6 homozygotes.* (A) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1 homozygous (hmz) uninfected subjects (n=47) compared to that mediated by KIR3DL1hmz SP (n=47). Between group differences were assessed with a Mann-Whitney test. (B) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz HLA-Bw4 carriers compared to that mediated by KIR3DL1hmz HLA-Bw6 homozygotes. Between group differences were assessed with a Mann-Whitney test. (C) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz individuals that co-carry the *h/*y+B*57 genotype, other Bw4 ligands or are Bw6hmz. Between group differences were assessed with a Kruskal-Wallis test, followed by Dunn's multiple comparison test. (D) (Left panel) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz SP carrying the *h/*y+B*57 combined genotype, other Bw4 antigens or Bw6 homozygotes. Between group differences were assessed with a Kruskal-Wallis test. (Right panel) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz uninfected subjects and SP carrying the *h/*y+B*57 combined genotype. Between group differences were assessed with a Mann-Whitney test. (E) The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz individuals that co-carry the

*h/*y+B*57 genotype, the *l/*x+B*57 genotype, KIR3DL1*h/*y with other Bw4 ligands or are Bw6hmz. Between group differences were assessed with a Kruskal-Wallis test, followed by Dunn's multiple comparison test.

Figure 3: *No correlation between HIV viral load (VL) and percent GzB activity in SP.* A Spearman rank correlation was used to assess the relationship between the percent GzB activity and VL in SP.

Figure 4: *No between group differences in percent GzB activity in elite controllers (EC) compared to non-EC.* Between group differences were evaluated using a Mann-Whitney test.

Table 3: *Genotype distribution of FcγRIIIa (CD16a) in uninfected and SP study populations.* Frequency of CD16a genotypes in uninfected subjects and SP are shown. Fisher's exact test was used to compare proportions of selected genotypes between uninfected and SP. A two-tailed chi-squared (χ^2) test was used to examine differences in the distribution of genotypes between uninfected and SP subjects.

Table 4. *CD16a genotype distribution in three historical low-risk HIV seronegative comparator datasets*. Frequency of CD16a genotypes in uninfected subjects three low-risk HIV seronegative Caucasion datasets from the literature are shown. Each population was analyzed for Hardy-Weinberg (HW) equilibrium.

Figure 5: (A) *No effect of CD16a genotype on anti-HIV ADCC responses in uninfected subjects and SP.* The scatter plot depicts the percent GzB activity of KIR3DL1hmz individuals with the V/F or F/F genotype of the CD16a polymorphism in uninfected (left panel) and SP subjects (right panel). Between group differences in the left panel were assessed with a Mann-Whitney test (left panel) and a Kruskal-Wallis test (right panel). (B) The scatter plot depicts the percent GzB activity of NK cells from KIR3DL1hmz uninfected individuals with the V/F or F/F CD16a genotype within groups constituted according to KIR/HLA combinations *h/*y+B*57, other Bw4 alleles and Bw6hmz. Between group differences were assessed with a Mann-Whitney test. (C) The scatter plot depicts the percent GzB activity mediated by NK cells from KIR3DL1hmz SP with the V/V, V/F or F/F CD16a genotypes within groups composed of KIR/HLA combinations *h/*y+B*57, other Bw4 alleles and Bw6hmz. Between group differences were assessed with a Mann-Whitney test (left panel) and a Kruskal-Wallis test (center and right panel).

Figure 6: *The number of educationally competent inhibitory KIR to self (S-iKIR) to HLA-C do not contribute to the educational advantage of *h/*y+B*57 in KIR3DL1hmz.* The scatter plot depicts the number of HLA-C combinations with cognate KIR2DL1/2/3 NK cell receptors (S-iKIR to HLA-C) in KIR3DL1hmz individuals that co-carry the *h/*y+B*57 genotype, other Bw4 ligands or are Bw6hmz. Between group differences were assessed with a Kruskal-Wallis test.

Figure 7: *Inhibitory KIR to self (S-iKIR) HLA-C do not influence percent GzB activity in uninfected subjects or SP categorized according to whether or not they carry a S-iKIR to HLA-B.* The scatter plots depict the percent GzB activity of NK cells from KIR3DL1 homozygous HIV uninfected subjects (left panel) and HIV infected SP (right panel). Groups 1, 3 and 5 include subjects with only one S-iKIR to either an HLA-C1 or C2 allele, Groups 2, 4 and 6 include subjects with two S-iKIR to both an HLA-C1 and C2

allele, Groups 1 and 2 are Bw6 homozygotes, Groups 3 and 4 carry an S-iKIR to Bw4 alleles other than HLA-B*57 and Groups 5 and 6 include KIR3DL1*h/*y subjects with an S-iKIR to HLA-B*57. A Mann-Whitney test was used to assess between group differences of Groups 1 and 2, 3 and 4, and 5 and 6 in uninfected subjects. A small number of SP in Group 2 and 6 precluded formal analysis between Group 1 and 2, and between Group 5 and 6 in SP. A Kruskal-Wallis test was used to test the significance of between group means in SP subjects.

Figure 8: *Increasing potency of NK educational signals received through iKIR to self HLA-Bw4 (S-iKIR to HLA-Bw4) and increasing S-iKIR to HLA-C within the 3 categories assessed correlated with percent GzB activity in uninfected subjects, but not SP.* The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz individuals in uninfected subjects (left panel) and SP (right panel). Groups 1 to 6 are as described in Figure 7. A Spearman rank correlation was used to assess the association of percent GzB activity with increasing potency of potential NK cell educational signals.

Figure 9: *Increasing S-iKIR to HLA-C and potency of NK educational signals received through S-iKIR to HLA-Bw4 does not correlate with percent GzB in the absence of *h/*y+B*57 in uninfected subjects or SP.* Scatter plots depict percent GzB activity of KIR3DL1hmz individuals in uninfected subjects (left panel) and SP (right panel). Group 1 includes subjects with only one S-iKIR to either an HLA-C1 or C2 allele, Group 2 includes subjects with only two S-iKIR to both an HLA-C1 and C2 allele, Group 3 includes subjects with S-iKIR to HLA-Bw4 and either a HLA-C1 or C2 allele and Group 4 includes subjects with a S-iKIR to HLA-Bw4 and both an HLA-C1 and C2 allele. A Spearman rank correlation was used to assess the relationship between groups.

Figure 10: *NK cell educational signals received through S-iKIR to HLA-Bw4 and increasing number of S-iKIR to HLA-C do not correlate with percent GzB activity in uninfected subjects or SP.* Scatter plots showing the percent GzB activity of KIR3DL1hmz uninfected subjects (left panel) and SP (right panel) categorized into two groups according to the type and number of S-iKIR. Group 1 includes subjects with only one S-iKIR to either an HLA-C1 or C2 allele, Group 2 includes subjects with only two S-iKIR to both an HLA-C1 and C2 allele, Group 3 and 5 includes subjects with S-iKIR to HLA-Bw4 and either a HLA-C1 or C2 allele, and subjects with S-iKIR to HLA-B*57 and either a HLA-C1 or C2 allele, Group 4 and 6 includes subjects with a S-iKIR to HLA-Bw4 and both an HLA-C1 and C2 allele, and subjects with S-iKIR to HLA-B*57 and both an HLA-C1 and C2 allele. A Spearman rank correlation was used to assess the relationship between groups.

Figure 11: *Representative gating strategy for identifying frequency of $CD3^-CD56^+CD16^+$ cells and $CD3^-CD56^{dim}KIR3DL1^+$ cells in whole PBMC.* This procedure involved gating on total lymphocytes, $CD3^-$ cells and $CD56^+CD16^+$ cells (top panel) or $CD56^{dim}KIR3DL1^+$ cells (bottom panel).

Figure 12: *No difference in the frequency of $CD3^-CD56^+CD16^+$ NK cells between carriers of *h/*y+B*57, other Bw4 ligands or Bw6 homozygotes.* The scatter plot depicts the frequency of $CD3^-CD56^+CD16^+$ NK cells in PBMC in KIR3DL1hmz individuals that co-carry the *h/*y+B*57 genotype, other Bw4 ligands or are Bw6hmz. Between group differences were assessed with a Kruskal-Wallis test.

Figure 13: *No difference in the frequency CD3$^-$CD56^{dim}KIR3DL1$^+$ NK cells between carriers of *h/*y+B*57, other Bw4 alleles or are Bw6hmz.* The scatter plot depicts the frequency of CD3$^-$CD56^{dim}KIR3DL1$^+$ NK cells in PBMC in KIR3DL1hmz individuals that co-carry the *h/*y+B*57 genotype, other Bw4 ligands or are Bw6hmz. Between group differences were assessed with a Kruskal-Wallis test.

Figure 14: *No difference in anti-HIV ADCC responses mediated by NK cells from carriers of *h/*y+B*57, KIR3DL1 homozygotes (hmz) carrying other Bw4 alleles or Bw6hmz.* The scatter plot depicts the percent GzB activity mediated by PBMC from KIR3DL1hmz *h/*y+B*57 (n=10) carriers, other HLA-Bw4 allele carriers (n=12), and HLA-Bw6hmz (n=10). Each point represents percent GzB activity from a second GTL experiment. Between group differences were assessed with a Kruskal-Wallis, followed by Dunn's multiple comparison test.

Figure 15: *Assay reproducibility*. The reproducibility of the assay was tested using BaL-gp120-coated target cells. The assay was performed twice using PBMC effector cells from the same donor and time point at E:T = 30:1. Each figure reports the percent GzB activity after background subtraction for both replicate assays. The coefficient of variation (%CV) represents the percent of inter-assay variation within each KIR/HLA group.

Table 1

Subject ID	Ethnicity	CD16 genotype	HLA-A	HLA-B	HLA-C	HLA-C Allotype	Bw4/Bw6	KIR3DL1	KIR3DL1 allotype	KIR/HLA genotype	2DL1:C2 [a]	2DL2:C1 [a]	2DL3:C1 [a]	3DL1:Bw4 [a]	S-iKIR #
UI1001	Caucasian	V/F	01:01, 26:01	38:01, 57:01	06:02, 12:03	C2, C1	Bw4hmz	001:01, 001:01	*h/*y	1	yes	no	yes	yes	3
UI1002	Caucasian	V/F	02:01, 02:01	51:01, 57:01	06:02, 15:02	C2, C2	Bw4hmz	015:02, 004:01	*h/*y	1	yes	no	no	yes	2
UI1003	Caucasian	F/F	02:22, 03:01	44:02, 57:01	05:01, 06:02	C2, C2	Bw4hmz	001:01, 001:01	*h/*y	1	yes	no	no	yes	2
UI1004	Caucasian	V/F	29:01, 68:011	44:031, 57:01	16:01, 18:01	C1, C2	Bw4hmz	002, 004:01	*h/*y	1	yes	no	yes	yes	3
UI1005	Caucasian	V/F	01:01, 02:01	15:01, 57:01	05:01, 06:02	C2, C2	Bw4/Bw6	001:01, 001:01	*h/*y	1	yes	no	no	yes	2
UI1005	Carribean	N/A	01:01, 23:01	37:01, 57:01	06:04, 18:01	C2, C2	Bw4/Bw6	001:01, 001:01	*h/*y	1	yes	no	no	yes	2
UI1006	Caucasian	F/F	01:01, 23:01	37:01, 57:01	06:02, 06:02	C2, C2	Bw4/Bw6	001:01, 001:01	*h/*y	1	yes	no	no	yes	2
UI1007	Caucasian	V/F	01:01, 02:01	38:01, 57:01	06:02, 12:03	C2, C1	Bw4hmz	002, 004:01	*h/*y	1	yes	no	yes	yes	3
UI1008	Caucasian	F/F	24:02, 25:01	37:01, 57:01	06:02, 06:04	C2, C2	Bw4hmz	002, 015:02	*h/*y	1	yes	no	no	yes	2
UI1009	Caucasian	V/F	02:01, 02:01	57:01, 57:01	03:03, 07:01	C1, C1	Bw4hmz	001:01, 008	*h/*y	1	no	no	yes	yes	2
UI1010	Caucasian	V/F	01:01, 33:01	14:02, 57:01	06:01, 06:11	C2, C1	Bw4/Bw6	001:01, 008	*h/*y	1	yes	no	yes	yes	3
UI1011	Caucasian	V/F	02:01, 02:01	07:02, 57:01	05:01, 06:02	C2, C2	Bw4/Bw6	001:01, 001:01	*h/*y	1	yes	no	no	yes	2
UI2001	African	V/F	30:01, 33:01	42:01, 53:01	17:01, 04:011	C2, C2	Bw4/Bw6	015:02, 031	*l/*x	2	yes	no	no	yes	2
UI2002	African	F/F	29:01, 36:01	07:021, 53:01	04:011, 07:02	C2, C1	Bw4/Bw6	015:01, 015:02	*h/*y	2	yes	yes	no	yes	3
UI2003	Caucasian	V/F	02:011, 24:021	51:011, unknown	04:01, 05:01	C2, C2	Bw4hmz	009, 009	*h/*y	2	yes	no	no	yes	2
UI2004	Caucasian	V/F	24:021, unknown	39:011, 49:01	07:01, 12:03	C1, C1	Bw4/Bw6	001:01, 005:01	*l/*x	2	no	no	yes	yes	2
UI2005	Caucasian	V/F	02:011, 23:01	37:01, 49:01	06:02, 07:011	C2, C1	Bw4hmz	001:01, 004:01	*h/*y	2	yes	yes	yes	yes	3
UI2006	Caucasian	F/F	01:01, 29:01	44:031, 18:01	07:011, 16:01	C1, C1	Bw4/Bw6	001:01, 001:01	*h/*y	2	no	yes	yes	yes	2
UI2007	Caucasian	V/F	26:01, 68:01	07:02, 38:01	07:02, 12:03	C1, C1	Bw4/Bw6	001:01, 001:01	*h/*y	2	no	no	yes	yes	2
UI2008	Caucasian	V/F	01:01, 11:01	51:01, 55:01	01:02, 03:03	C1, C1	Bw4/Bw6	002, 007	*l/*x	2	no	no	yes	yes	2
UI2009	Asian	F/F	11:01, 11:01	35:01, 51:09	03:03, 14:02	C1, C1	Bw4/Bw6	005:01, 007	*l/*x	2	no	no	yes	yes	2
UI2010	Caucasian	V/F	01:01, 01:01	08:01, 44:02	05:01, 07:01	C2, C1	Bw4/Bw6	001:01, 001:01	*h/*y	2	yes	yes	yes	yes	3
UI2011	Caucasian	V/F	02:011, unknown	15:011, 51:011	01:02, 03:031	C1, C1	Bw4/Bw6	004:01, 004:01	*l/*x	2	no	yes	no	yes	2
UI2012	Caucasian	F/F	11, 24	78:01, 60	03:04, 16:02	C1, C1	Bw4/Bw6	001:01, 004:01	*h/*y	2	no	no	yes	yes	2
UI2013	Caucasian	V/F	3, 30	44, 35	04:011, 04:011	C2, C2	Bw4/Bw6	001:01, 004:01	*h/*y	2	yes	no	no	yes	2
UI2014	Caucasian	V/F	29:01, 31:01	40:01, 4:02	04:04, 16:01	C1, C1	Bw4/Bw6	001:01, 004:01	*h/*y	2	no	no	yes	yes	2
UI2015	Caucasian	F/F	03:01, 26:01	14:02, 52:01	07:01, 08:02	C1, C1	Bw4/Bw6	004:01, 004:01	*l/*x	2	no	yes	yes	yes	2
UI2016	Caucasian	V/F	01:01, 03:01	35:02, 51:01	03:02, 15:021	C1, C2	Bw4/Bw6	001:01, 004:01	*h/*y	2	yes	no	yes	yes	3
UI2017	Caucasian	F/F	02:011, 31:012	27:05, 40:011	03:02, 15:021	C1, C2	Bw4/Bw6	004:01, 008	*h/*y	2	yes	no	yes	yes	3
UI2018	Caucasian	F/F	02:011, 25:01	18:01, 55:01	03:031, 12:03	C1, C1	Bw4/Bw6	004:01, 015:01	*h/*y	2	no	yes	no	yes	2
UI2019	Caucasian	F/F	23:01, 68:011	37:01, 44:031	04:011, 06:03	C2, C2	Bw4hmz	009, 009	*h/*y	2	yes	no	no	yes	2
UI2020	Caucasian	F/F	01:01, 03:01	08:01, 44:02	05:01, 07:01	C2, C1	Bw4/Bw6	001:01, 004:01	*h/*y	2	yes	yes	yes	yes	3
UI2021	Western European	N/A	23:01, 26:01	15:17, 57:01	06:02, 07:01	C2, C1	Bw4/Bw6	004:01, 005:01	*l/*x	2	N/A	N/A	N/A	yes	N/A
UI2022	Caucasian	F/F	02:01, 02:01	57:01, 57:01	06:02, 07:02	C2, C1	Bw4hmz	021, 015:02	*l/*x	2	yes	no	yes	yes	3
UI2023	Caucasian	F/F	24:02, 29:02	07:02, 35:01	04:04, 07:02	C2, C1	Bw4/Bw6	015:02, 017:01	*h/*y	2	yes	yes	yes	yes	3
UI2024	Caucasian	V/F	01:01, 24:02	07:02, 18:01	05:01, 07:02	C2, C1	Bw4/Bw6	001:01, 015:02	*h/*y	2	yes	yes	yes	yes	3
UI2025	Caucasian	V/F	24:02, 26:01	15:01, 57:01	05:01, 06:02	C2, C1	Bw4/Bw6	005:01, 005:01	*l/*x	2	yes	no	yes	yes	3
UI3001	Caucasian	F/F	02:011, 29:011	39:011, 50:01	06:02, 12:031	C2, C1	Bw6hmz	002, 004:01	*h/*y	3	yes	yes	yes	no	2
UI3002	Caucasian	F/F	02:01, 03:01	07:02, 40:01	03:02, 07:10	C1, C1	Bw6hmz	001:01, 004:01	*h/*y	3	no	yes	yes	no	1
UI3003	Caucasian	V/F	2, 30	7, 8	07:01, 07:02	C1, C1	Bw6hmz	001:01, 002	*h/*y	3	no	no	yes	no	1
UI3004	Caucasian	F/F	01:011, 02:011	08:01, 40:011	03:021, 07:011	C1, C1	Bw6hmz	007, 007	*l/*x	3	no	yes	yes	no	1
UI3005	Caucasian	V/F	02:01, 03:01	18:01, 35:01	04:01, 08:02	C2, C1	Bw6hmz	005:01, 007	*l/*x	3	yes	no	yes	no	2
UI3006	Caucasian	V/F	02:011, unknown	18:011, 40:011	03:04, 07:011	C1, C1	Bw6hmz	001:01, 004:01	*h/*y	3	no	no	yes	no	1
UI3007	Caucasian	V/F	02:01, 03:01	07:02, 18:01	07:01, 04:01	C1, C1	Bw6hmz	001:01, 007	*l/*x	3	no	yes	yes	no	1
UI3008	Caucasian	V/F	01:011, 02:011	08:01, 15:011	03:031, 07:011	C1, C1	Bw6hmz	001:01, 004:01	*h/*y	3	no	yes	no	no	1
UI3009	Caucasian	V/F	03:011, 11:01	07:021, 35:011	04:011, 07:02	C2, C1	Bw6hmz	001:01, 001;01	*h/*y	3	yes	no	yes	no	2
UI3010	Caucasian	F/F	02:01, 02:01	07:02, 08:01	07:01, 07:02	C1, C1	Bw6hmz	001:01, 015:02	*h/*y	3	no	yes	yes	no	1

a: yes = present, no = absence

N/A = not available

KIR/HLA genotype: 1 = *h/*y+B*57, 2 = other Bw4, 3 = Bw6hmz

Table 2

Slow Progressor	Ethnicity	CD16 genotype	HLA-A	HLA-B	HLA-C	HLA-C Allotype	Bw4/Bw6	KIR3DL1 allotype	KIR3DL1 expression	Log_{10} Viral	CD4 Count[a]	KIR3DL1 genotyp A	2DL1:C 2[b]	2DL2:C1 [b]	2DL3:C1 [b]	3DL1:Bw 4[b]	S-iKIR #
SP1001	Caucasian	F/F	02:01, 02:01	44:02, 57:01	05:01, 15:02	C2, C2	Bw4hmz	00101, 002	*h/*y	57093	630	1	yes	no	no	yes	2
SP1002	Caucasian	F/F	01:01, 68:02	27:03, 57:01	02:02, 06:02	C2, C2	Bw4hmz	002, 01502	*h/*y	3456	340	1	yes	no	no	yes	2
SP1003	Black Haitian	F/F	01:01, 68:01	57:03, 58:01	07:01, 07:01	C1, C1	Bw4hmz	002, 008	*h/*y	21278	397	1	no	yes	yes	yes	2
SP1004	N/A	V/F	02:01, 32:01	07:02, 57:01	06:02, 07:02	C2, C1	Bw4/Bw6	002, 00401	*h/*y	32846	420	1	yes	no	yes	yes	3
SP1005	Black Haitian	V/F	01:01, 31:02	51:01, 57:01	06:02, 15:02	C2, C2	Bw4hmz	00101, 00401	*h/*y	62	620	1	yes	no	no	yes	2
SP1006	Black African	V/F	02:02, 33:03	35:01, 57:01	12:03, 18:01	C1, C2	Bw4/Bw6	00101, 01502	*h/*y	40	1190	1	N/A	N/A	N/A	yes	N/A
SP1007	Black African	V/F	01:01, 68:02	57:03, 73:01	15:05, 17:01	C2, C2	Bw4/Bw6	00101, 00401	*h/*y	616	670	1	yes	no	no	yes	2
SP1008	Black African	F/F	23:01, 23:01	41:01, 57:03	07:01, 07:01	C1, C2	Bw4/Bw6	01501, 01501	*h/*y	130355	518	1	no	yes	yes	yes	2
SP1009	Black Haitian	F/F	02:01, 30:01	13:02, 57:01	06:02, 06:02	C2, C2	Bw4hmz	00101, 01501	*h/*y	470	560	1	yes	no	no	yes	2
SP1010	Caucasian	F/F	03:01, 03:01	40:02, 57:01	02:02, 06:02	C2, C2	Bw4/Bw6	002, 002	*h/*y	20996	540	1	yes	no	no	yes	2
SP1011	Caucasian	V/F	01:01, 30:01	27:03, 57:01	05:01, 06:02	C2, C2	Bw4hmz	00101, 002	*h/*y	9304	690	1	yes	no	no	yes	2
SP1012	Other	V/F	02:02, 30:02	53:01, 57:03	04:01, 08:02	C2, C1	Bw4hmz	00101, 01501	*h/*y	193	605	1	yes	yes	no	yes	3
SP2001	Caucasian	V/V	02:01, 02:01	40:02, 52:01	02:01, 02:02	C2, C2	Bw4/Bw6	00101, 00101	*h/*y	49772	500	2	yes	no	no	yes	2
SP2002	N/A	V/F	02:01, 02:01	27:05, 40:02	02:02, 15:02	C2, C2	Bw4/Bw6	002, 00401	*h/*y	70	920	2	yes	no	no	yes	2
SP2003	Black Haitian	F/F	03:01, 03:02	27:03, 51:01	07:01, 16:01	C1, C1	Bw4hmz	00501, 01501	*l/*x	50	720	2	no	yes	yes	yes	2
SP2004	Caucasian	F/F	02:01, 26:01	44:02, 52:01	05:01, 12:02	C2, C1	Bw4hmz	00101, 00101	*h/*y	474	270	2	yes	no	yes	yes	3
SP2005	Black Haitian	V/F	02:01, 02:01	15:03, 44:02	02:02, 05:01	C2, C2	Bw4/Bw6	01501, 01501	*h/*y	3844	500	2	yes	no	no	yes	2
SP2006	Black Haitian	V/F	02:01, 02:05	53:01, 58:01	04:01, 07:01	C2, C1	Bw4hmz	007, 01501	*l/*x	50	600	2	yes	yes	no	yes	3
SP2007	Black Haitian	V/F	02:05, 74:02	27:03, 49:01	02:02, 07:01	C2, C1	Bw4hmz	007, 01501	*l/*x	55	634	2	yes	yes	yes	yes	3
SP2008	Black Haitian	V/F	03:01, 34:02	44:03, 53:01	04:01, 04:01	C2, C2	Bw4hmz	007, 01501	*l/*x	1328	480	2	yes	no	no	yes	2
SP2009	Black Haitian	F/F	02:05, 23:01	49:01, 53:01	04:01, 07:01	C2, C1	Bw4hmz	008, 020	*h/*y	322	550	2	yes	yes	yes	yes	3
SP2010	Caucasian	V/F	02:01, 02:01	44:02, 44:02	05:01, 05:01	C2, C2	Bw4hmz	00401, 00101	*h/*y	40	790	2	yes	no	no	yes	2
SP2011	Caucasian	F/F	03:01, 24:02	14:02, 27:05	02:02, 08:02	C2, C1	Bw4/Bw6	01501, 01502	*h/*y	40	780	2	yes	yes	yes	yes	3
SP2012	Caucasian	F/F	24:03, 32:01	18:01, 44:02	05:01, 12:03	C2, C1	Bw4/Bw6	00101, 002	*h/*y	50	480	2	yes	yes	yes	yes	3
SP2013	Caucasian	V/F	03:01, 11:01	51:01, 51:01	03:03, 14:02	C1, C1	Bw4hmz	008, 01502	*h/*y	1968	552	2	no	no	yes	yes	2
SP2014	Caucasian	V/F	01:01, 02:01	27:05, 51:01	01:02, 15:02	C1, C2	Bw4hmz	00401, 00501	*l/*x	45	712	2	yes	yes	yes	yes	3
SP2015	Native American	F/F	02:01, 24:02	15:01, 27:05	02:02, 03:03	C2, C1	Bw4/Bw6	00101, 01502	*h/*y	40	760	2	yes	yes	yes	yes	3
SP2016	Caucasian	V/F	02:01, 26:01	07:02, 27:05	01:02, 07:02	C1, C1	Bw4/Bw6	00501, 00501	*l/*x	2136	530	2	no	no	yes	yes	2
SP2017	N/A	V/F	29:01, 66:02	14:02, 58:01	07:01, 08:02	C1, C1	Bw4/Bw6	00101, 01501	*h/*y	1760	600	2	no	yes	yes	yes	2
SP2018	Caucasian	V/F	03:01, 68:02	53:01, 53:01	02:02, 04:01	C2, C2	Bw4hmz	002, 01502	*h/*y	40	1160	2	yes	no	no	yes	2
SP2019	Black African	V/F	02:01, 23:01	15:03, 53:01	02:10, 06:02	C2, C2	Bw4/Bw6	01501, 01501	*h/*y	3713	469	2	yes	no	no	yes	2
SP2020	Black African	V/F	02:02, 30:02	49:01, 58:02	06:02, 07:01	C2, C1	Bw4hmz	01501, 01501	*h/*y	3389	470	2	yes	no	yes	yes	3
SP2021	Caucasian	V/V	02:01, 02:01	07:02, 51:01	07:02, 14:02	C1, C1	Bw4/Bw6	00101, 00401	*h/*y	1426	380	2	no	no	yes	yes	2
SP2022	Caucasian	V/F	03:01, 25:01	35:01, 39:01	04:01, 12:03	C2, C1	Bw4/Bw6	00101, 008	*h/*y	49936	432	2	yes	no	yes	yes	3
SP2023	Caucasian	V/F	02:011, 03:011	7, 13	06:02, 07:021	C2, C1	Bw4/Bw6	00401, 008	*h/*y	3387	1307	2	yes	yes	yes	yes	3
SP2024	Black Haitian	F/F	66:02, 74:01	53:01, 58:01	04:01, 07:01	C2, C1	Bw4hmz	01501, 034	*h/*y	4691	554	2	yes	yes	yes	yes	3
SP3001	Caucasian	F/F	02:01, 03:01	49:01, 53:01	07:01, 07:02	C1, C1	Bw6hmz	002, 00501	*l/*x	50	480	3	no	no	yes	no	1
SP3002	Black Haitian	F/F	68:02, 74:02	15:10, 41:01	03:04, 07:01	C1, C1	Bw6hmz	007, 01502	*l/*x	50	360	3	no	yes	yes	no	1
SP3003	Black Haitian	F/F	03:01, 68:02	07:02, 14:02	07:02, 08:02	C1, C1	Bw6hmz	002, 00501	*l/*x	362	810	3	no	yes	yes	no	1
SP3004	Other	F/F	02:01, 11:01	14:02, 40:06	04:01, 15:02	C2, C2	Bw6hmz	01502, 01502	*h/*y	39	720	3	yes	no	no	no	1
SP3005	Caucasian	V/F	01:01, 03:01	07:02, 14:02	07:02, 08:02	C1, C1	Bw6hmz	00101, 00401	*h/*y	501	371	3	no	no	yes	no	1
SP3006	Caucasian	V/F	02:01, 33:01	07:02, 14:02	07:02, 08:02	C1, C1	Bw6hmz	00401, 00401	*l/*x	1553	404	3	no	yes	yes	no	1
SP3007	N/A	V/F	01:01, 30:02	08:02, 81:01	07:01, 18:01	C1, C2	Bw6hmz	007, 01501	*l/*x	8428	438	3	yes	yes	yes	no	2
SP3008	Caucasian	F/F	02:06, 31:01	15:01, 15:01	01:02, 01:02	C1, C1	Bw6hmz	01502, 01502	*h/*y	40	950	3	no	no	yes	no	1
SP3009	Black Haitian	V/F	02:06, 31:01	40:02, 48:01	03:04, 08:03	C1, C1	Bw6hmz	01502, 01502	*h/*y	793	480	3	no	yes	no	no	1
SP3010	Caucasian	V/F	02:01, 03:01	07:02, 48:01	07:02, 08:03	C1, C1	Bw6hmz	00101, 00401	*h/*y	88	1002	3	no	yes	yes	no	1
SP3011	Black Haitian	V/V	02:011, 30:01	07:021, 42:01	04:011, 17:01	C2, C2	Bw6hmz	007, 01501	*l/*x	50	623	3	yes	no	no	no	1

a: Cells per mm^3

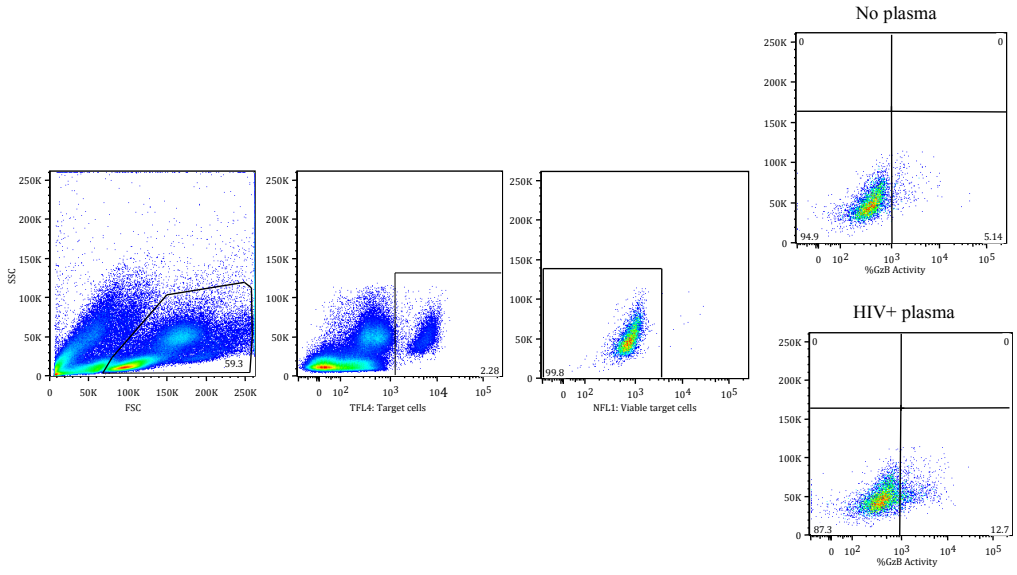
b: yes = presence, no = absence

N/A = not available

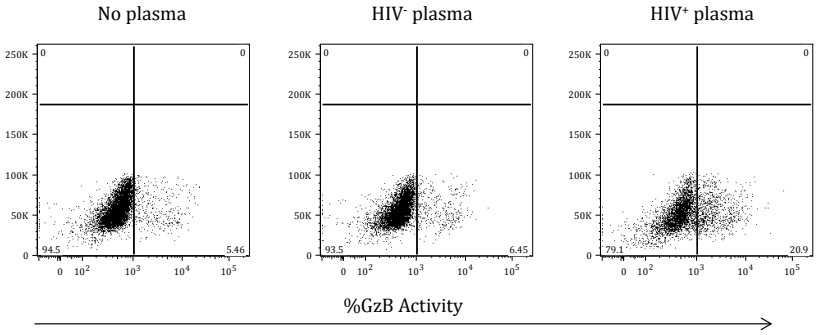
KIR/HLA genotype: 1 = *h/*y+B*57, 2 = other Bw4, 3 = Bw6hmz

Figure 1

A


No plasma
HIV+ plasma
SSC
FSC
TFL4: Target cells
NFL1: Viable target cells
%GzB Activity
59.3
2.28
99.8
94.9
5.14
87.3
12.7

B


No plasma
HIV- plasma
HIV+ plasma
94.5
5.46
93.5
6.45
79.1
20.9
%GzB Activity

C

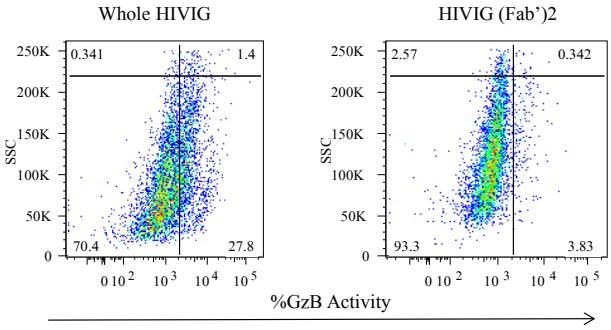

Whole HIVIG
HIVIG (Fab')2
SSC
0.341
1.4
70.4
27.8
2.57
0.342
93.3
3.83
%GzB Activity

Figure 2

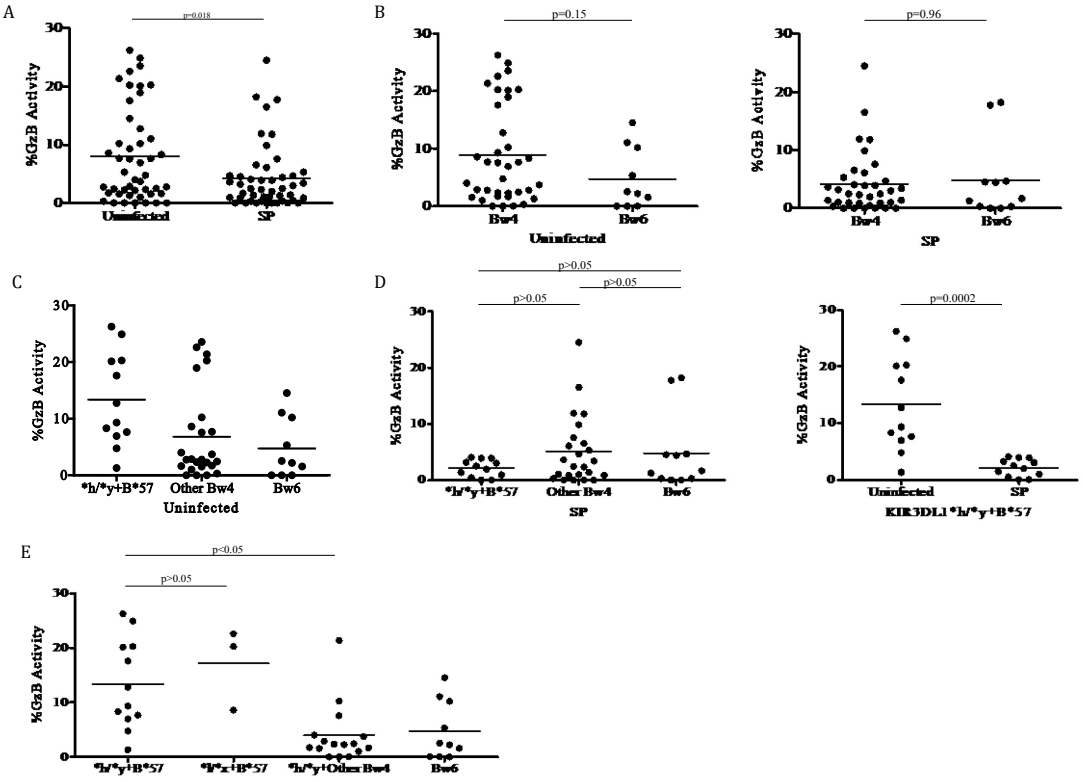

A
p=0.018
%GzB Activity
Uninfected
SP
B
p=0.15
%GzB Activity
Bw4
Bw6
Uninfected
p=0.96
%GzB Activity
Bw4
Bw6
SP
C
%GzB Activity
*h/*y+B*57
Other Bw4
Bw6
Uninfected
D
p>0.05
p>0.05
p>0.05
%GzB Activity
*h/*y+B*57
Other Bw4
Bw6
SP
p=0.0002
%GzB Activity
Uninfected
SP
KIR3DL1 *h/*y+B*57
E
p<0.05
p>0.05
%GzB Activity
*h/*y+B*57
*h/*x+B*57
*h/*y+Other Bw4
Bw6

Figure 3

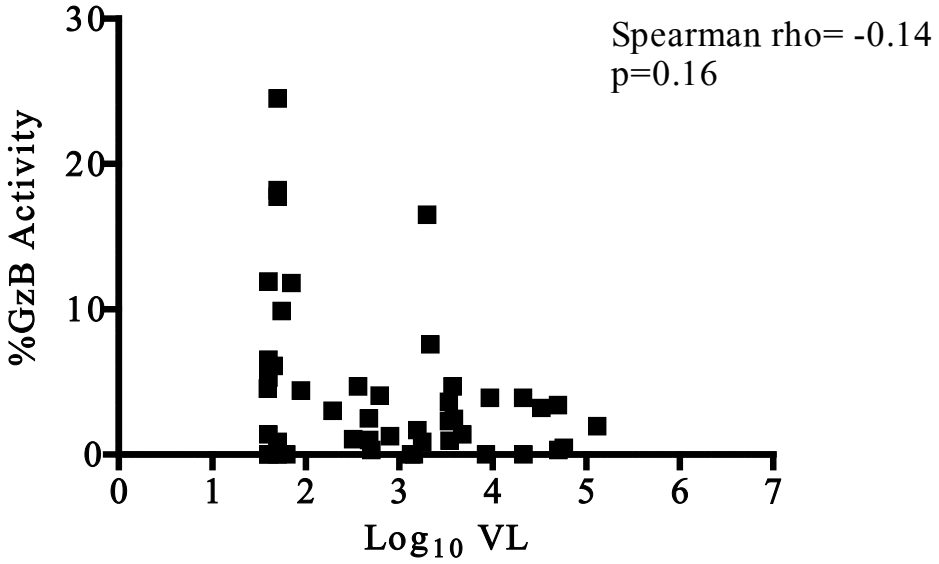

Spearman rho= -0.14
p=0.16
%GzB Activity
30
20
10
0
0
1
2
3
4
5
6
7
Log10 VL

Figure 4

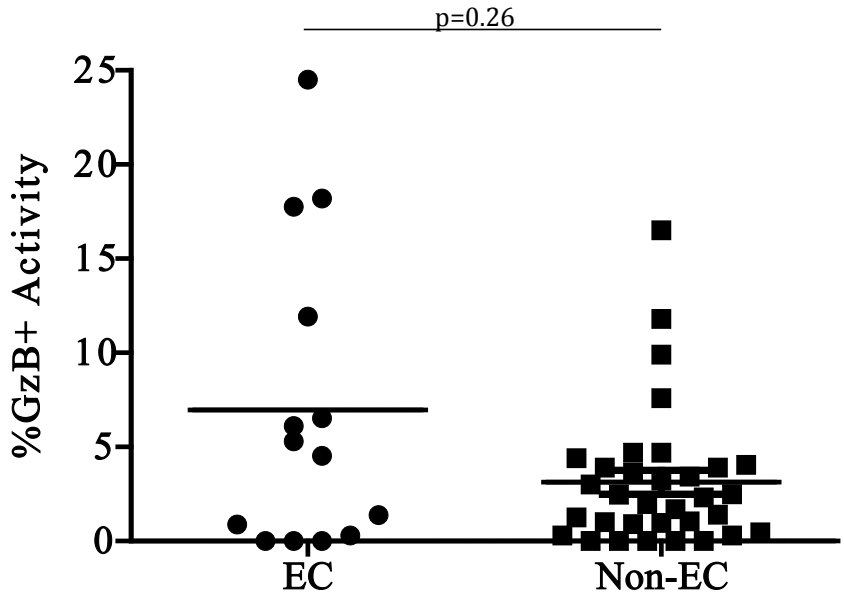


Table 3

Genetic factor	Uninfected (%) (n=46)	SP (%) (n=47)	OR	95% CI	p
V/V	0 (0)	3 (6)	0.1377	0.006775 - 2.797	0.2373
V/F	27 (59)	26 (55)	0.2327	0.006539 - 2.809	0.2327
F/F	19 (41)	18 (38)	0.9383	0.4246 - 2.279	1.0000
3x2 χ^2 test	n/a	n/a	n/a	n/a	0.2192

Table 4

Genetic factor	Uninfected (%) (n=46)	Healthy donors (%) (n=87)[1]	Dutch donors (%) (n=104)[2]	Norweigen donors (%) (n=96)[3]
V/V	0 (0)	15 (17)	17 (10)	13 (15)
V/F	27 (59)	44 (51)	85 (48)	32 (37)
F/F	19 (41)	28 (32)	74 (42)	41 (48)
χ^2	7.9	0.1	1.1	2.41
p	0.0048	0.75	0.29	0.12
HW equilibrium	no	yes	yes	yes

[1] Koene et al. 1997

[2] Leppers van de Straat et al. 2000

[3] Van den Berg et al. 2001

Figure 5

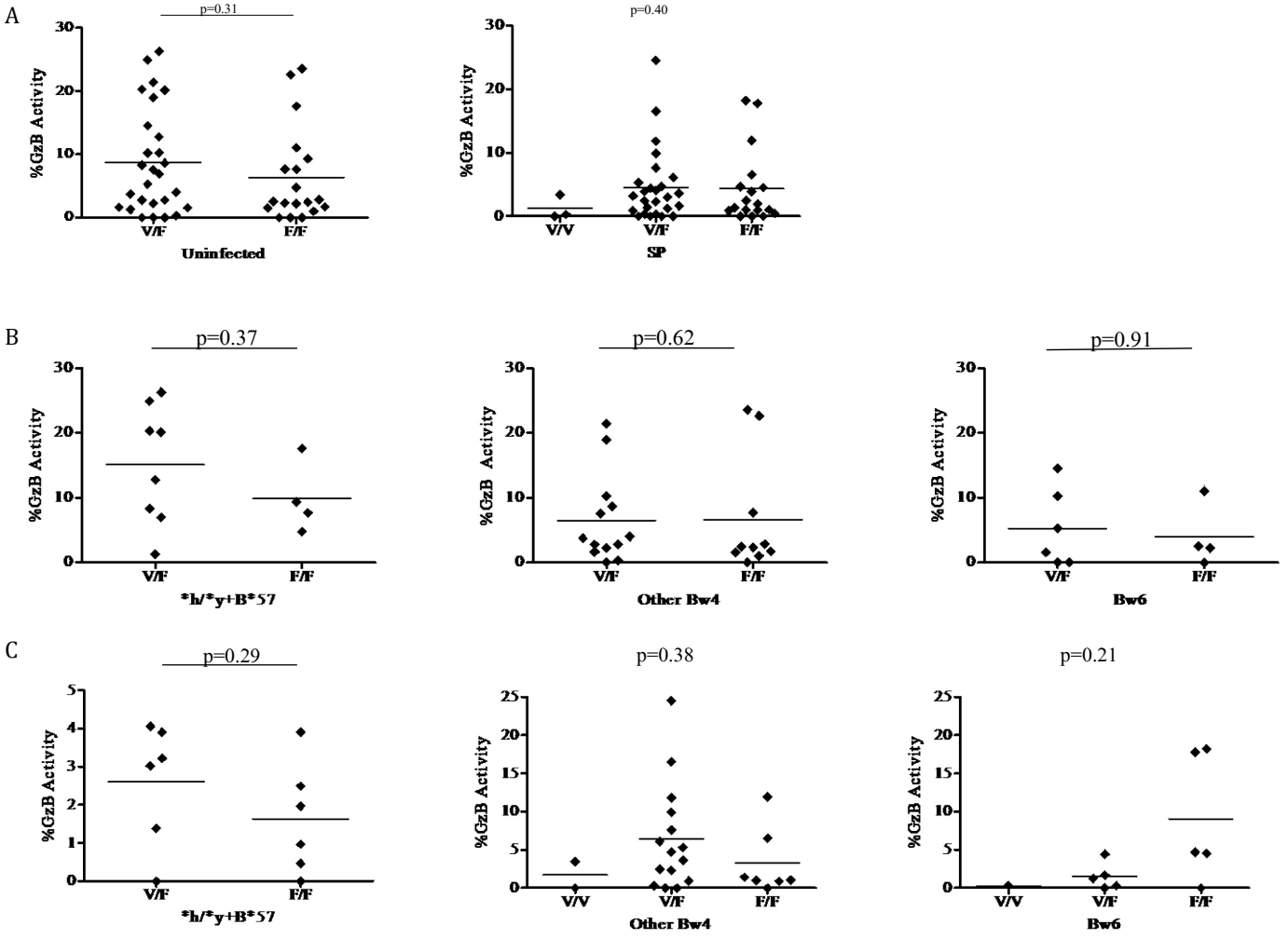

A
p=0.31
%GzB Activity
30
20
10
0
V/F
F/F
Uninfected
p=0.40
%GzB Activity
30
20
10
0
V/V
V/F
F/F
SP
B
p=0.37
%GzB Activity
30
20
10
0
V/F
F/F
*h/*y+B*57
p=0.62
%GzB Activity
30
20
10
0
V/F
F/F
Other Bw4
p=0.91
%GzB Activity
30
20
10
0
V/F
F/F
Bw6
C
p=0.29
%GzB Activity
5
4
3
2
1
0
V/F
F/F
*h/*y+B*57
p=0.38
%GzB Activity
25
20
15
10
5
0
V/V
V/F
F/F
Other Bw4
p=0.21
%GzB Activity
25
20
15
10
5
0
V/V
V/F
F/F
Bw6

Figure 6

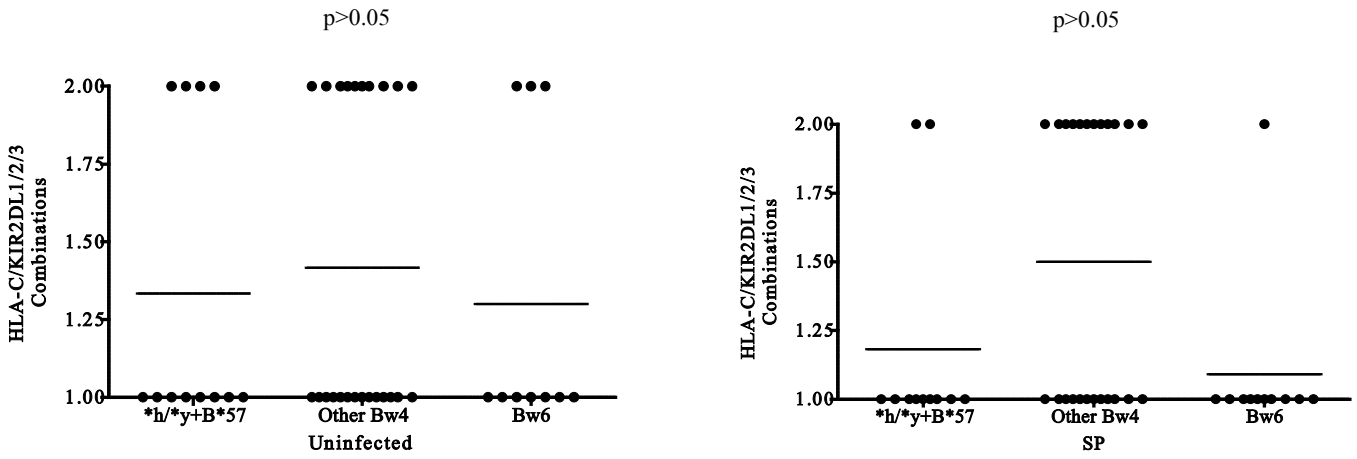

p>0.05
HLA-C/KIR2DL1/2/3 Combinations
2.00
1.75
1.50
1.25
1.00
*h/*y+B*57
Other Bw4
Bw6
Uninfected
p>0.05
HLA-C/KIR2DL1/2/3 Combinations
2.00
1.75
1.50
1.25
1.00
*h/*y+B*57
Other Bw4
Bw6
SP

Figure 7

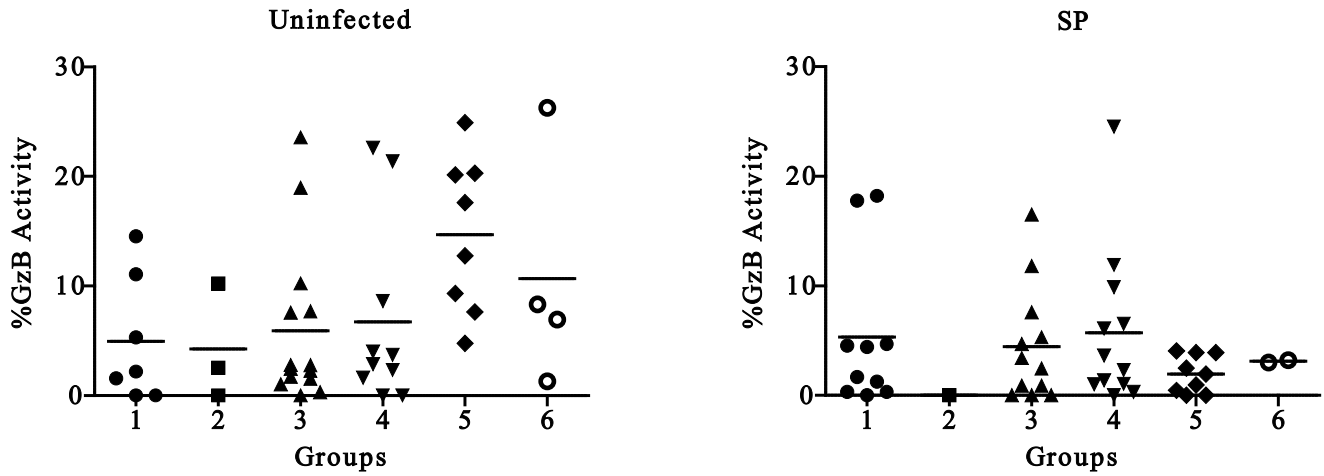

Uninfected
%GzB Activity
30
20
10
0
1
2
3
4
5
6
Groups
SP
%GzB Activity
30
20
10
0
1
2
3
4
5
6
Groups

Figure 8

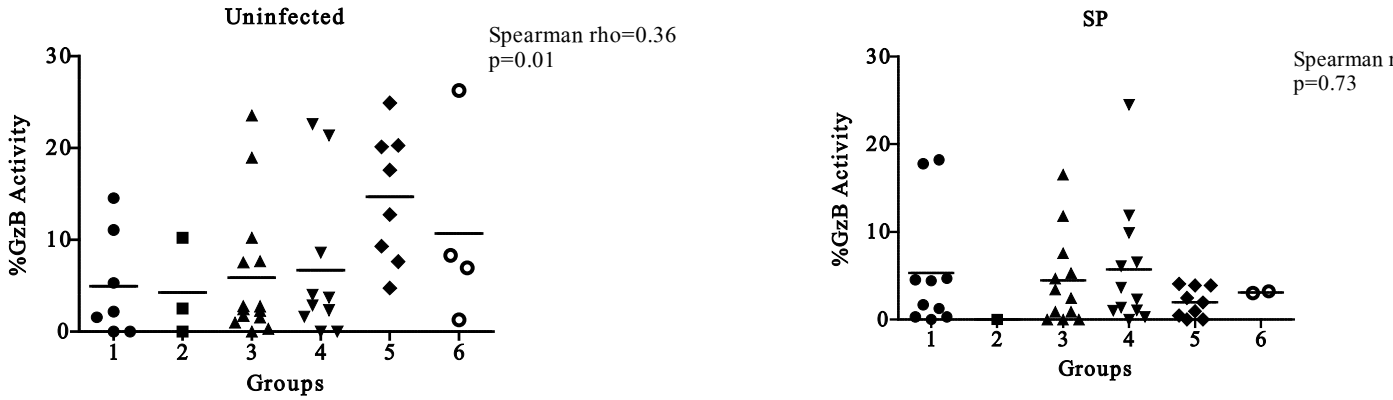

Uninfected
Spearman rho=0.36
p=0.01
%GzB Activity
30
20
10
0
1
2
3
4
5
6
Groups
SP
Spearman r
p=0.73
%GzB Activity
30
20
10
0
1
2
3
4
5
6
Groups

Figure 9

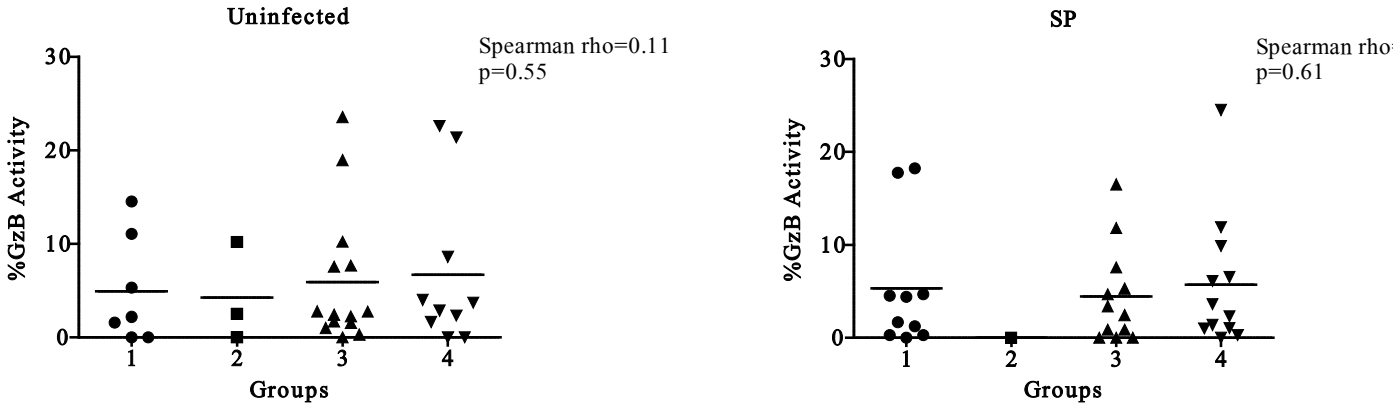

Uninfected
Spearman rho=0.11
p=0.55
%GzB Activity
30
20
10
0
1
2
3
4
Groups
SP
Spearman rho
p=0.61
%GzB Activity
30
20
10
0
1
2
3
4
Groups

Figure 10

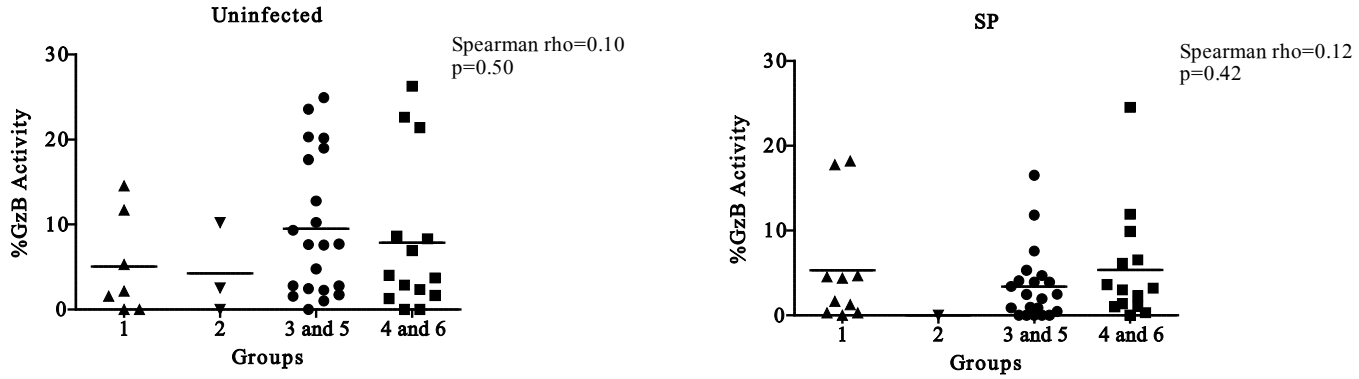

Uninfected
Spearman rho=0.10
p=0.50
%GzB Activity
30
20
10
0
1
2
3 and 5
4 and 6
Groups
SP
Spearman rho=0.12
p=0.42
%GzB Activity
30
20
10
0
1
2
3 and 5
4 and 6
Groups

Figure 11

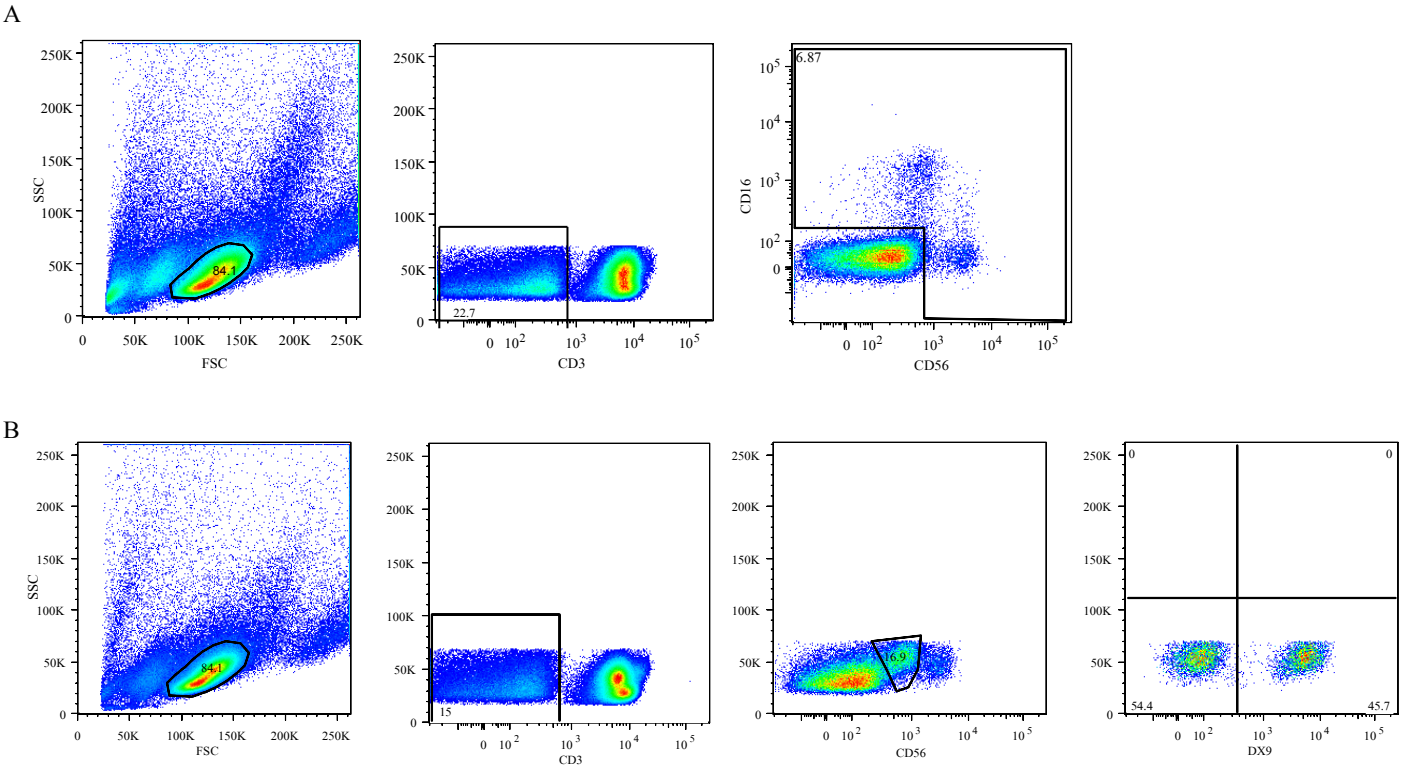

A
SSC
FSC
84.1
CD3
22.7
CD16
CD56
6.87
B
SSC
FSC
84.1
CD3
15
CD56
16.9
DX9
54.4
45.7

Figure 12

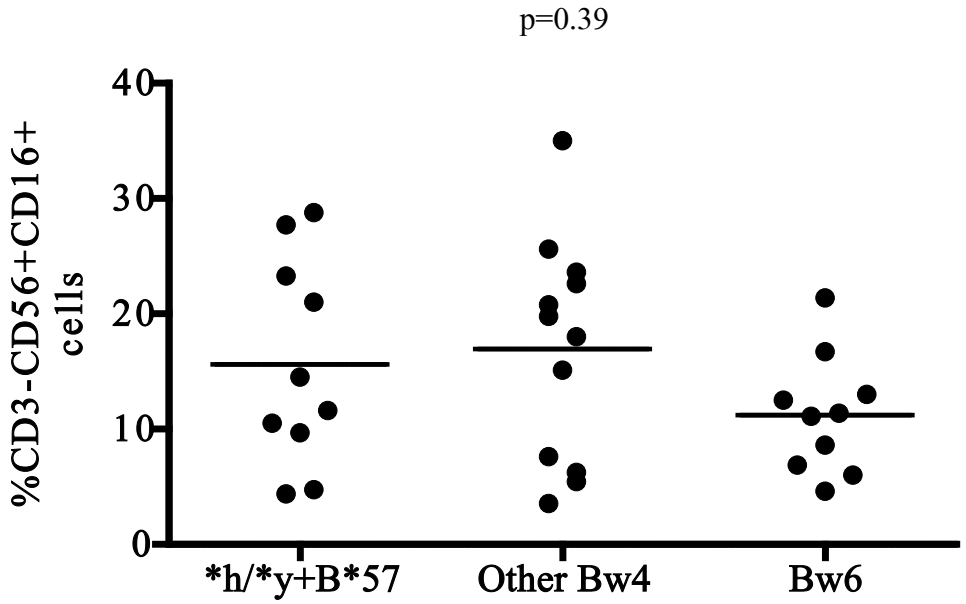


Figure 13

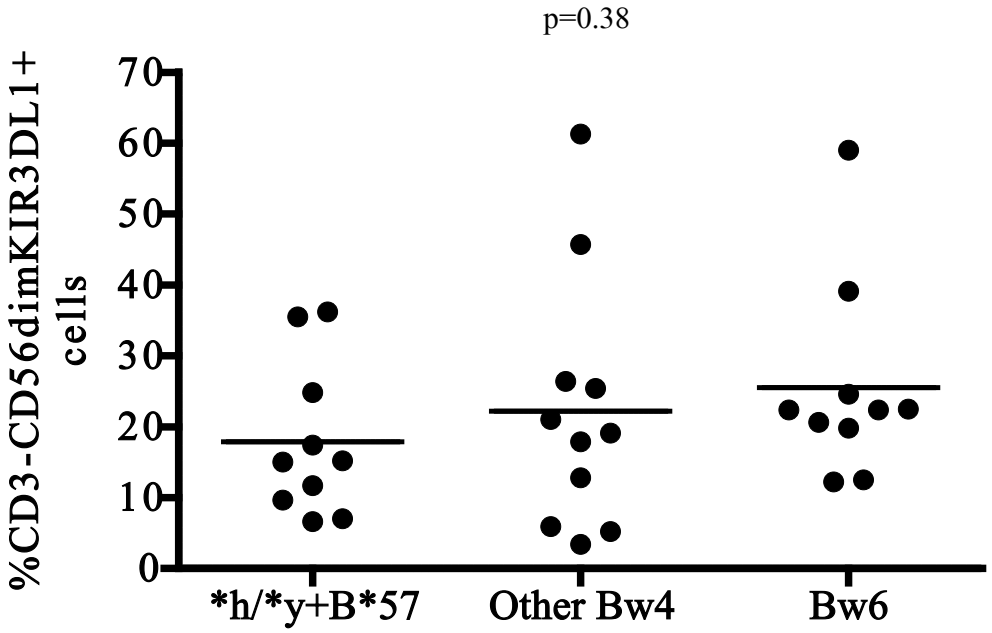

p=0.38
%CD3-CD56dimKIR3DL1+ cells
70
60
50
40
30
20
10
0
*h/*y+B*57
Other Bw4
Bw6

Figure 14

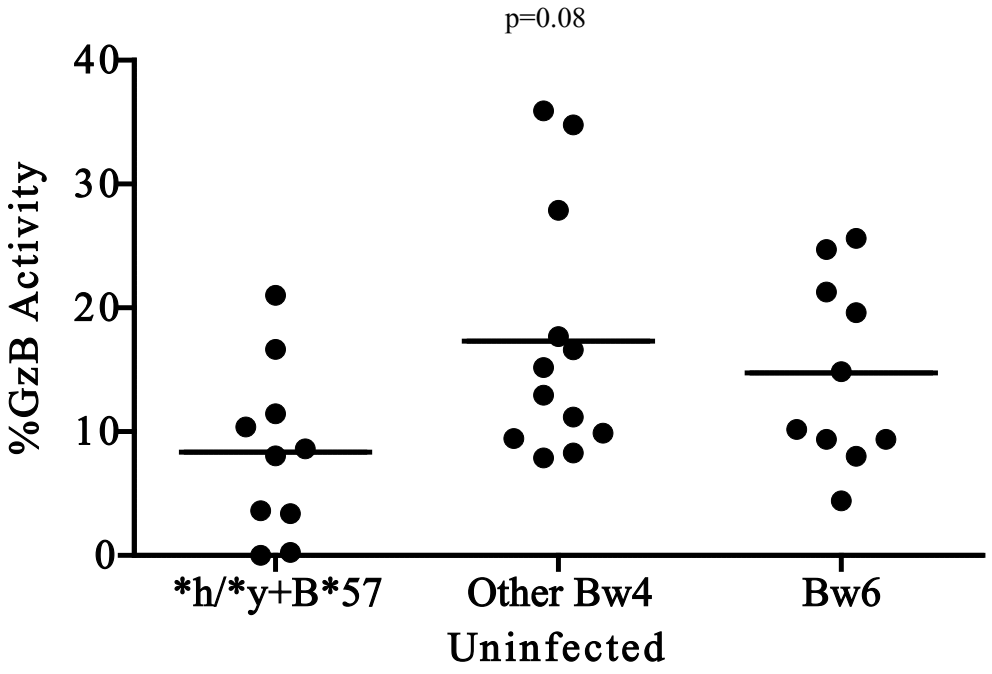
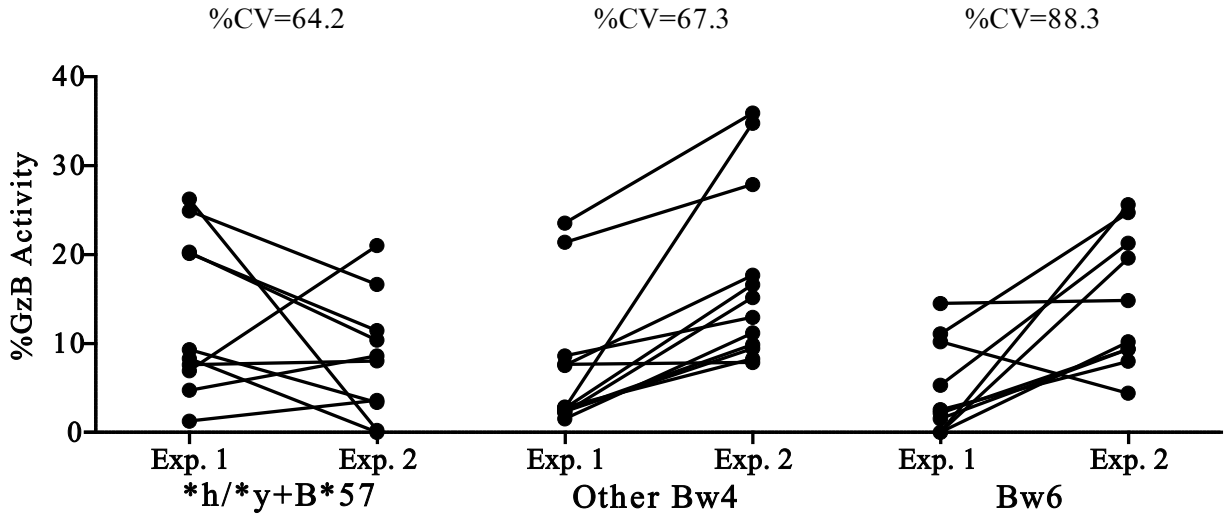

p=0.08
40
30
20
10
0
%GzB Activity
*h/*y+B*57
Other Bw4
Bw6
Uninfected

Figure 15


%CV=64.2
%CV=67.3
%CV=88.3
%GzB Activity
40
30
20
10
0
Exp. 1
Exp. 2
Exp. 1
Exp. 2
Exp. 1
Exp. 2
*h/*y+B*57
Other Bw4
Bw6

3.13: Reference List

(1) Martin MP, Qi Y, Gao X, Yamada E, Martin JN, Pereyra F, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 2007 Jun;39(6):733-40.

(2) Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 2002 Aug;31(4):429-34.

(3) Boulet S, Kleyman M, Kim JY, Kamya P, Sharafi S, Simic N, et al. A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS 2008 Jul 31;22(12):1487-91.

(4) Boulet S, Sharafi S, Simic N, Bruneau J, Routy JP, Tsoukas CM, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS 2008 Mar 12;22(5):595-9.

(5) Pelak K, Need AC, Fellay J, Shianna KV, Feng S, Urban TJ, et al. Copy number variation of KIR genes influences HIV-1 control. PLoS Biol 2011 Nov;9(11):e1001208.

(6) Jennes W, Verheyden S, Demanet C, Menten J, Vuylsteke B, Nkengasong JN, et al. Low CD4+ T cell counts among African HIV-1 infected subjects with group B KIR haplotypes in the absence of specific inhibitory KIR ligands. PLoS ONE 2011;6(2):e17043.

(7) Ravet S, Scott-Algara D, Bonnet E, Tran HK, Tran T, Nguyen N, et al. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. Blood 2007 May 15;109(10):4296-305.

(8) Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 2010 Mar 18;115(11):2167-76.

(9) Mandelboim O, Malik P, Davis DM, Jo CH, Boyson JE, Strominger JL. Human CD16 as a lysis receptor mediating direct natural killer cell cytotoxicity. Proc Natl Acad Sci U S A 1999 May 11;96(10):5640-4.

(10) Hoglund P, Brodin P. Current perspectives of natural killer cell education by MHC class I molecules. Nat Rev Immunol 2010 Oct;10(10):724-34.

(11) Lanier LL. NK cell recognition. Annu Rev Immunol 2005;23:225-74.

(12) Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 2005 Aug 4;436(7051):709-13.

(13) Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, et al. Human NK cell education by inhibitory receptors for MHC class I. Immunity 2006 Aug;25(2):331-42.

(14) Brodin P, Lakshmikanth T, Johansson S, Karre K, Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. Blood 2009 Mar 12;113(11):2434-41.

(15) Yu J, Heller G, Chewning J, Kim S, Yokoyama WM, Hsu KC. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. J Immunol 2007 Nov 1;179(9):5977-89.

(16) Wan AM, Ennis P, Parham P, Holmes N. The primary structure of HLA-A32 suggests a region involved in formation of the Bw4/Bw6 epitopes. J Immunol 1986 Dec 1;137(11):3671-4.

(17) Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M. NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. J Exp Med 1994 Oct 1;180(4):1235-42.
(18) Foley BA, De SD, Van BE, Lathbury LJ, Christiansen FT, Witt CS. The reactivity of Bw4+ HLA-B and HLA-A alleles with KIR3DL1: implications for patient and donor suitability for haploidentical stem cell transplantations. Blood 2008 Jul 15;112(2):435-43.
(19) Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, et al. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. J Immunol 1997 Jun 1;158(11):5237-41.
(20) Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J Exp Med 1995 Mar 1;181(3):1133-44.
(21) Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med 2003;54:535-51.
(22) Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, Cirulli ET, et al. Common genetic variation and the control of HIV-1 in humans. PLoS Genet 2009 Dec;5(12):e1000791.
(23) Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. Science 2007 Aug 17;317(5840):944-7.
(24) Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A 2000 Mar 14;97(6):2709-14.
(25) Altfeld M, Kalife ET, Qi Y, Streeck H, Lichterfeld M, Johnston MN, et al. HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1. PLoS Med 2006 Oct;3(10):e403.
(26) Goulder PJ, Bunce M, Krausa P, McIntyre K, Crowley S, Morgan B, et al. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS Res Hum Retroviruses 1996 Dec 10;12(18):1691-8.
(27) Leslie AJ, Pfafferott KJ, Chetty P, Draenert R, Addo MM, Feeney M, et al. HIV evolution: CTL escape mutation and reversion after transmission. Nat Med 2004 Mar;10(3):282-9.
(28) Miura T, Brockman MA, Schneidewind A, Lobritz M, Pereyra F, Rathod A, et al. HLA-B57/B*5801 human immunodeficiency virus type 1 elite controllers select for rare gag variants associated with reduced viral replication capacity and strong cytotoxic T-lymphocyte [corrected] recognition. J Virol 2009 Mar;83(6):2743-55.
(29) Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, Rathod A, et al. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis 2008 Feb 15;197(4):563-71.
(30) Lopez-Vazquez A, Mina-Blanco A, Martinez-Borra J, Njobvu PD, Suarez-Alvarez B, Blanco-Gelaz MA, et al. Interaction between KIR3DL1 and HLA-B*57 supertype alleles influences the progression of HIV-1 infection in a Zambian population. Hum Immunol 2005 Mar;66(3):285-9.
(31) Kamya P, Boulet S, Tsoukas CM, Routy JP, Thomas R, Cote P, et al. Receptor-ligand requirements for increased NK cell polyfunctional potential in slow

progressors infected with HIV-1 coexpressing KIR3DL1*h/*y and HLA-B*57. J Virol 2011 Jun;85(12):5949-60.
(32) Boulet S, Song R, Kamya P, Bruneau J, Shoukry NH, Tsoukas CM, et al. HIV protective KIR3DL1 and HLA-B genotypes influence NK cell function following stimulation with HLA-devoid cells. J Immunol 2010 Feb 15;184(4):2057-64.
(33) Kim S, Sunwoo JB, Yang L, Choi T, Song YJ, French AR, et al. HLA alleles determine differences in human natural killer cell responsiveness and potency. Proc Natl Acad Sci U S A 2008 Feb 26;105(8):3053-8.
(34) Parsons MS, Wren L, Isitman G, Navis M, Stratov I, Bernard NF, et al. HIV infection abrogates the functional advantage of natural killer cells educated through KIR3DL1/HLA-Bw4 interactions to mediate anti-HIV antibody-dependent cellular cytotoxicity. J Virol 2012 Apr;86(8):4488-95.
(35) Alter G, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med 2007 Nov 26;204(12):3027-36.
(36) Forthal DN, Landucci G, Keenan B. Relationship between antibody-dependent cellular cytotoxicity, plasma HIV type 1 RNA, and CD4+ lymphocyte count. AIDS Res Hum Retroviruses 2001 Apr 10;17(6):553-61.
(37) Ahmad A, Morisset R, Thomas R, Menezes J. Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. J Acquir Immune Defic Syndr 1994 May;7(5):428-37.
(38) Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 2012 Nov;86(21):11521-32.
(39) Forthal DN, Landucci G, Haubrich R, Keenan B, Kuppermann BD, Tilles JG, et al. Antibody-dependent cellular cytotoxicity independently predicts survival in severely immunocompromised human immunodeficiency virus-infected patients. J Infect Dis 1999 Oct;180(4):1338-41.
(40) Banks ND, Kinsey N, Clements J, Hildreth JE. Sustained antibody-dependent cell-mediated cytotoxicity (ADCC) in SIV-infected macaques correlates with delayed progression to AIDS. AIDS Res Hum Retroviruses 2002 Nov 1;18(16):1197-205.
(41) Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, Rinaldo C, et al. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. J Immunol 1996 Sep 1;157(5):2168-73.
(42) Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 2005 Feb 15;174(4):2185-9.
(43) Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. AIDS 2009 May 15;23(8):897-906.
(44) Barouch DH. Challenges in the development of an HIV-1 vaccine. Nature 2008 Oct 2;455(7213):613-9.
(45) Jia M, Li D, He X, Zhao Y, Peng H, Ma P, et al. Impaired natural killer cell-induced antibody-dependent cell-mediated cytotoxicity is associated with human immunodeficiency virus-1 disease progression. Clin Exp Immunol 2013 Jan;171(1):107-16.

(46) Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012 Apr 5;366(14):1275-86.
(47) Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009 Dec 3;361(23):2209-20.
(48) Karnasuta C, Paris RM, Cox JH, Nitayaphan S, Pitisuttithum P, Thongcharoen P, et al. Antibody-dependent cell-mediated cytotoxic responses in participants enrolled in a phase I/II ALVAC-HIV/AIDSVAX B/E prime-boost HIV-1 vaccine trial in Thailand. Vaccine 2005 Mar 31;23(19):2522-9.
(49) Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de HM. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 1997 Aug 1;90(3):1109-14.
(50) Wu J, Edberg JC, Redecha PB, Bansal V, Guyre PM, Coleman K, et al. A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest 1997 Sep 1;100(5):1059-70.
(51) Lehrnbecher T, Foster CB, Zhu S, Leitman SF, Goldin LR, Huppi K, et al. Variant genotypes of the low-affinity Fcgamma receptors in two control populations and a review of low-affinity Fcgamma receptor polymorphisms in control and disease populations. Blood 1999 Dec 15;94(12):4220-32.
(52) Van Den Berg L, Myhr KM, Kluge B, Vedeler CA. Fcgamma receptor polymorphisms in populations in Ethiopia and Norway. Immunology 2001 Sep;104(1):87-91.
(53) Deepe RN, Kistner-Griffin E, Martin JN, Deeks SG, Pandey JP. Epistatic interactions between Fc (GM) and FcgammaR genes and the host control of human immunodeficiency virus replication. Hum Immunol 2012 Mar;73(3):263-6.
(54) Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 2009 Apr 16;113(16):3716-25.
(55) Lehrnbecher TL, Foster CB, Zhu S, Venzon D, Steinberg SM, Wyvill K, et al. Variant genotypes of FcgammaRIIIA influence the development of Kaposi's sarcoma in HIV-infected men. Blood 2000 Apr 1;95(7):2386-90.
(56) Poonia B, Kijak GH, Pauza CD. High affinity allele for the gene of FCGR3A is risk factor for HIV infection and progression. PLoS ONE 2010;5(12):e15562.
(57) Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. Blood 2012 Oct 4;120(14):2836-42.
(58) Brown BK, Wieczorek L, Kijak G, Lombardi K, Currier J, Wesberry M, et al. The role of natural killer (NK) cells and NK cell receptor polymorphisms in the assessment of HIV-1 neutralization. PLoS ONE 2012;7(4):e29454.
(59) Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007 May 15;178(10):6596-603.
(60) Hatjiharissi E, Xu L, Santos DD, Hunter ZR, Ciccarelli BT, Verselis S, et al. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism. Blood 2007 Oct 1;110(7):2561-4.

(61) Bowles JA, Wang SY, Link BK, Allan B, Beuerlein G, Campbell MA, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. Blood 2006 Oct 15;108(8):2648-54.
(62) Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity 1997 Dec;7(6):753-63.
(63) Kulkarni S, Martin MP, Carrington M. KIR genotyping by multiplex PCR-SSP. Methods Mol Biol 2010;612:365-75.
(64) Mandelboim O, Reyburn HT, Vales-Gomez M, Pazmany L, Colonna M, Borsellino G, et al. Protection from lysis by natural killer cells of group 1 and 2 specificity is mediated by residue 80 in human histocompatibility leukocyte antigen C alleles and also occurs with empty major histocompatibility complex molecules. J Exp Med 1996 Sep 1;184(3):913-22.
(65) Pollara J, Hart L, Brewer F, Pickeral J, Packard BZ, Hoxie JA, et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. Cytometry 2011;79A(8):603-12.
(66) Alter G, Teigen N, Davis BT, Addo MM, Suscovich TJ, Waring MT, et al. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. Blood 2005 Nov 15;106(10):3366-9.
(67) Mavilio D, Lombardo G, Benjamin J, Kim D, Follman D, Marcenaro E, et al. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proc Natl Acad Sci U S A 2005 Feb 22;102(8):2886-91.
(68) Mavilio D, Benjamin J, Daucher M, Lombardo G, Kottilil S, Planta MA, et al. Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. Proc Natl Acad Sci U S A 2003 Dec 9;100(25):15011-6.
(69) Brodin P, Karre K, Hoglund P. NK cell education: not an on-off switch but a tunable rheostat. Trends Immunol 2009 Apr;30(4):143-9.
(70) Leppers-van de Straat FG, van der Pol WL, Jansen MD, Sugita N, Yoshie H, Kobayashi T, et al. A novel PCR-based method for direct Fc gamma receptor IIIa (CD16) allotyping. J Immunol Methods 2000 Aug 28;242(1-2):127-32.
(71) Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. Immunol Rev 2002 Dec;190:40-52.
(72) Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc Natl Acad Sci U S A 1993 Dec 15;90(24):12000-4.
(73) Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, et al. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 1993 Aug 1;178(2):597-604.
(74) Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati MS, Vitale M, et al. Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity 1995 May;2(5):439-49.

(75) Parsons MS, Zipperlen K, Gallant M, Grant M. Killer cell immunoglobulin-like receptor 3DL1 licenses CD16-mediated effector functions of natural killer cells. J Leukoc Biol 2010 Nov;88(5):905-12.
(76) Kamya P, Tallon B, Melendez-Pena C, Parsons MS, Migueles SA, Connors M, et al. Inhibitory Killer Immunoglobulin-like Receptors to self HLA-B and HLA-C ligands contribute differentially to Natural Killer cell functional potential in HIV infected slow progressors. Clin Immunol 2012 Jun;143(3):246-55.
(77) Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, et al. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. Blood 2010 Nov 11;116(19):3853-64.
(78) Beziat V, Descours B, Parizot C, Debre P, Vieillard V. NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. PLoS ONE 2010;5(8):e11966.
(79) Eller MA, Koehler RN, Kijak GH, Eller LA, Guwatudde D, Marovich MA, et al. Human immunodeficiency virus type 1 infection is associated with increased NK cell polyfunctionality and higher levels of KIR3DL1+ NK cells in ugandans carrying the HLA-B Bw4 motif. J Virol 2011 May;85(10):4802-11.
(80) Bryceson YT, Chiang SC, Darmanin S, Fauriat C, Schlums H, Theorell J, et al. Molecular mechanisms of natural killer cell activation. J Innate Immun 2011;3(3):216-26.
(81) Johansson SE, Rollman E, Chung AW, Center RJ, Hejdeman B, Stratov I, et al. NK cell function and antibodies mediating ADCC in HIV-1-infected viremic and controller patients. Viral Immunol 2011 Oct;24(5):359-68.
(82) Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature 2012 Feb 2;482(7383):89-93.
(83) Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. Blood 2008 Sep 15;112(6):2369-80.
(84) Charoudeh HN, Schmied L, Gonzalez A, Terszowski G, Czaja K, Schmitter K, et al. Quantity of HLA-C surface expression and licensing of KIR2DL+ natural killer cells. Immunogenetics 2012 Oct;64(10):739-45.
(85) Bolanos FD, Tripathy SK. Activation receptor-induced tolerance of mature NK cells in vivo requires signaling through the receptor and is reversible. J Immunol 2011 Mar 1;186(5):2765-71.
(86) Tripathy SK, Keyel PA, Yang L, Pingel JT, Cheng TP, Schneeberger A, et al. Continuous engagement of a self-specific activation receptor induces NK cell tolerance. J Exp Med 2008 Aug 4;205(8):1829-41.
(87) Coudert JD, Scarpellino L, Gros F, Vivier E, Held W. Sustained NKG2D engagement induces cross-tolerance of multiple distinct NK cell activation pathways. Blood 2008 Apr 1;111(7):3571-8.
(88) Coudert JD, Zimmer J, Tomasello E, Cebecauer M, Colonna M, Vivier E, et al. Altered NKG2D function in NK cells induced by chronic exposure to NKG2D ligand-expressing tumor cells. Blood 2005 Sep 1;106(5):1711-7.
(89) Snary D, Barnstable CJ, Bodmer WF, Crumpton MJ. Molecular structure of human histocompatibility antigens: the HLA-C series. Eur J Immunol 1977 Aug;7(8):580-5.

(90) Neisig A, Melief CJ, Neefjes J. Reduced cell surface expression of HLA-C molecules correlates with restricted peptide binding and stable TAP interaction. J Immunol 1998 Jan 1;160(1):171-9.
(91) Bashirova AA, Thomas R, Carrington M. HLA/KIR Restraint of HIV: Surviving the Fittest. Annu Rev Immunol 2011 Mar 22;29(1):295-317.

Chapter 3: Discussion

3.1: Novel contributions of this work to advancing knowledge in the field

Previous reports have demonstrated that the HIV protective *h/*y+B*57 genotype confers NK cells with heightened functional potential for secretion of cytokines and degranulation(195;196). The results in this thesis show that the *h/*y+B*57 compound genotype also confers NK cells with enhanced functional potential for ADCC. We observed that NK cells from uninfected individuals carrying *h/*y+B*57 mediate higher anti-HIV ADCC responses than individuals carrying other 3DL1/HLA-Bw4 combinations or Bw6hmz. In line with the reported NK cell dysfunction in HIV infection, we show that although NK cells from uninfected subjects carrying *h/*y+B*57 have enhanced ADCC responses compared to carriers of other KIR3DL1/HLA-B genotypes, NK cells from HIV infected SP subjects carrying this genotype combination do not. We find no evidence that CD16a polymorphism contributes to variations in anti-HIV ADCC responses in uninfected subjects and SP carrying *h/*y+B*57 (or other genotypes). While S-iKIR to HLA-C combinations have been reported to have the capacity to educate NK cells for functional potential, the combination of *h/*y+B*57 stood out in its ability to confer NK cells with enhanced ADCC functional potential in uninfected carriers. No differences were observed in the frequency of total NK cells or KIR3DL1+ NK cells between the 3 KIR/HLA genotype groups studied suggesting a higher proportion of NK cells or differential presence of NK cells licensed through KIR3DL1 was not responsible for between-group differences in the NK cell mediated ADCC activity.

Despite a loss of significance between ADCC responses in subjects carrying *h/*y+B*57 compared to donors with other Bw4 alleles or Bw6 homozygotes in a second set of ADCC experiments, we observed inter-assay variability in the range of what was previously reported in the literature(241). Future GranToxiLux ADCC experiments are required to resolve the discrepancy between our results and evaluate the effect of KIR/HLA on NK cell education using the commercially available GranToxiLux ADCC assay.

3.2: Does education confer NK cells with functional potential to mediate protective outcomes upon exposure to and/or infection with HIV?

As previously discussed, epidemiological studies have demonstrated an association between carriage of certain allelic combinations of KIR3DL1 with HLA-Bw4 ligands and protection from HIV infection as well as slow time to AIDS and VL control in those who are HIV infected(85;86;217;222;228;230). Relevant to this thesis, the KIR/HLA compound genotype conferring the most potent protection from infection and HIV disease progression is co-carriage of KIR3DL1*h/*y and HLA-B*57, as compared to homozygosity for HLA-Bw6(86;230). HLA-Bw6 is unable to participate in NK cell education through KIR3DL1 as it does not bind any KIR3DL1 receptor(151;154). NK cell education requires the interaction of ligands with inhibitory receptors(181;182;189). Functional competence is acquired through self-specific receptor-ligand pairings, such that the level of NK cell activation upon encounter of a target cell with reduced ligands for inhibitory receptors is directly proportional to the number of different iKIRs for self-HLA provided ligands for activating receptors are present(190-192). Evidence from both human and murine studies suggest a hierarchy of licensing whereby NK cells educated through stronger inhibitory signals during development respond more strongly, with an increased frequency and a broader range of functions(192-194). Studies from our lab on NK cell functional potential as measured by stimulation with HLA-null cells show that the *h/*y+B*57 combination is more potent for conferring functional potential than other 3DL1/HLA-Bw4 combinations or Bw6hmz(195;196). Others have reported that an increased percentage of NK cells from carriers of 3DL1/Bw4 produced IFN-y upon stimulation with anti-HLA class I-coated ADCC targets than NK cells from Bw6hmz(251).

Results presented in this thesis addressed if NK cells from carriers of *h/*y+B*57, which would include NK cells educated through KIR3DL1 high expression receptors interacting with HLA-B*57 would mediate enhanced ADCC against gp120-coated target cells compared to those from carriers of other 3DL1/Bw4 combinations and 3DL1/Bw6 homozygotes. The latter 2 genotypes would be expected to support either weaker or no educational signals through KIR3DL1, respectively. We found that NK cells from uninfected individuals with the HIV protective *h/*y+B*57 genotype had

superior ADCC responses to those from subjects who carried different 3DL1/Bw4 combinations or were HLA-Bw6hmz. We interpret these results to mean that NK cell education is a determinant of NK mediated ADCC function.

HIV uninfected individuals, particularly those at low risk for HIV exposure, would not be expected to have Abs to HIV Env. Therefore, it is unlikely that NK cell mediated ADCC activity plays a role in protection from infection, irrespective of KIR3DL1/HLA-B genotype. Where our thesis results may be informative is in vaccine trial design or in the interpretation of results arising from HIV vaccine trials, such as the RV144 Thai trial that employ a vaccine regimen able to induce non-neutralizing anti-HIV Env Abs(279;282). Such Abs have been proposed to support NK cell mediated ADCC activity(303). Future studies should consider stratifying participants into the KIR3DL1/HLA-B genotype combinations studied here in order to ascertain whether vaccine recipients who developed anti-HIV Env Abs and carried the *h/*y+B*57 genotype were more likely to remain uninfected than Ab positive carriers of other KIR3DL1/HLA-B genotypes.

3.3: Why do NK cells from HIV infected SP carrying the *h/*y+B*57 genotype have lower ADCC activity than those from uninfected subjects.

Several studies have demonstrated that HIV infection has a detrimental effect on NK cell functional potential(103-105;252;273). In this thesis we show that NK cells from HIV infected SP co-carrying *h/*y+B*57 have anti-HIV ADCC activity that is not significantly different than those from SP carrying other 3DL1/Bw4 combinations or Bw6 homozygotes. NK cells from SP with the *h/*y+B*57 genotype mediated significantly lower anti-HIV ADCC responses compared to those from uninfected carriers of this genotype combination. Our results are consistent with a recent report showing that the ADCC functional advantage of NK cells from HIV uninfected subjects educated through 3DL1/Bw4 is not present in NK cells originating from HIV infected individuals(252). NK cells from HIV infected progressors with high VL and/or low CD4+ T cell counts exhibit decreased ADCC potential and lysis of HLA-null target cells(104;291). The studies cited above examined ADCC activity in NK cells from HIV infected individuals in the chronic phase of infection, some of whom were treated and some not. One impetus for examining ADCC activity in SP is that this population

includes a high percentage of carriers of the *h/*y+B*57 genotype allowing us to address the effect of HIV infection on ADCC function in individuals carrying matched 3DL1/HLA-B combinations. Our finding that NK cell mediated ADCC activity is low in SP *h/*y+B*57 carrier was unexpected because NK cells from SP carrying the *h/*y+B*57 genotype exhibited significantly higher trifunctional potential for cytokine secretion and degranulation upon stimulation with HLA-null target cells compared to those from SP co-carrying other 3DL1/Bw4 combinations or Bw6 homozygotes(195). One possible interpretation of our findings is that NK mediated ADCC may be more sensitive to extinction in the context of HIV infection than degranulation and cytokine secretion.

Induction of various NK cell effector functions may require different strengths and durations of activating stimuli(144;304). A hierarchy has been proposed whereby induction of chemokines such as MIP-1β requires a moderate activating stimulus, while degranulation has a higher threshold, and production of cytokines such as TNF-α and IFN-γ display the highest threshold for activation(144). In this hierarchy, a high activation threshold for ADCC may make this function more readily extinguishable in the context of HIV infection than degranulation or secretion of cytokines. A hierarchy of functional extinction has been observed for T cell functions in the context of viral diseases that are characterized by different antigen (viral) loads(305-308). T cell exhaustion occurs in a stepwise manner whereby IL-2 production is among the first effector activities extinguished, followed by TNF-α production, while IFN-γ secretion persists the longest(305;307;308). A stepwise model of NK cell activation for induction of effector functions with different thresholds would reconcile observations by our lab and others that have demonstrated intact polyfunctional responses by NK cells in SP and chronically infected subjects with the loss of anti-HIV ADCC activity in SP carrying protective 3DL1/Bw4 combinations described in this thesis(88;195;196;252).

While there is an association between NK function and HIV disease status, it is unclear whether NK cell function controls VL in SP or whether low VL preserves NK cell function, particularly in EC. Within our SP study population, no differences in VL was observed between the three KIR/HLA groups suggesting that VL did not contribute to an absence of anti-HIV ADCC responses in carriers of *h/*y+B*57. Furthermore there was no evidence that NK cells from EC with undetectable VL had higher ADCC function

than SP with detectable VL. This contrasts with what was found for NK cell functional potential as measured by HLA-null cell stimulated cytokine secretion and CD107a expression; in this study VL was negatively correlated with NK cell trifunctional potential(195).

There is evidence from studies in humans and mice that NK cell tolerance may be induced by constitutive engagement of self-specific activating receptors(304;309-313). Indeed, overexpression of ligands for activating Ly49 or NKG2D led to a decreased responsiveness of NK cells(310;313;314). Stimulation through the activating receptor CD16 results in downregulation of the receptor on the NK cell surface by matrix metalloproteinases (MMP)(102;315;316). Inhibition of MMPs has been demonstrated to increase Fc receptor expression on NK cells and may serve to reconstitute ADCC function in HIV disease(316;317). Indeed, a recent study reports compromised NK cell mediated ADCC in a macaque model of SIV/SHIV infection was directly correlated with CD16 expression(316). MMP inhibitors significantly increased CD16 expression on macaque NK cells and enhanced ADCC function in MMP inhibitor-treated macaques as compared to untreated macaques(316). However, in the context of HIV infection educated NK cells may be rendered hyporesponsive by signaling through CD16 resulting in a loss of ADCC functional potential. In this scenario, increased CD16 expression may contribute to loss of NK cell ADCC functional potential. It has been reported that SP have high levels of non-neutralizing anti-Env Abs(293;299). If these engage CD16a on NK cells, this phenomenon may provide the signal needed for CD16 donwmodulation and/or NK cell hyporesponsiveness. A future direction of these studies would be to measure and compare CD16a levels on the NK cells of SP versus uninfected subjects, and examine the functional potential of NK cells to mediate ADCC.

In summary, we observed a lower level of ADCC activity in NK cells from SP than uninfected individuals carrying the HIV protective *h/*y+B*57 combination. This may reflect an abrogation of NK cell education-conferred functional potential and/or indicate that ADCC is a more readily extinguishable NK cell function than are cytokine secretion and CD107a expression, even in the context of the benign course of infection observed in SP.

3.4. Role of S-iKIR to HLA-C in NK cell licensing and function.

NK cell education for functional potential requires signals received through inhibitory receptors on NK cells upon ligation of self-HLA(181;182;189). Signaling

through inhibitory receptors such as KIR3DL1 and KIR2DL1/2/3 following ligation of HLA-Bw4, and HLA-C1/C2, respectively has been shown to license NK cells(182;236;251).

Provided ligands for activating receptors are present, the degree of NK cell activation upon target cell stimulation is directly proportional to the number of iKIRs for self-HLA(190-192;197). One study showed that NK cells from donors carrying two HLA-Bw4 alleles had higher licensing levels compared to NK cells from carriers of one or no HLA-Bw4 alleles(191). The NK functions assessed in the work cited above was HLA-null stimulated IFN-γ secretion and/or direct target lysis. In contrast to these findings, results from the Bernard lab found no evidence for the contribution of KIR2DL1/2/3/HLA-C1/C2 combination over and above that of KIR3DL1/HLA-Bw4 genotypes on NK functions characterized by any of the permutations of IFN-γ and TNF-α secretion and CD107a expression(318). Nor did they find that having 2 HLA-Bw4 alleles co-carried with KIR3DL1hmz genotype licence NK cells more potently than having 1 HLA-Bw4 allele (N Bernard, unpublished observations).

In this thesis we investigated whether carriage of one or two S-iKIR to HLA-C combinations contributed to quantitative differences in the ADCC functional potential of NK cells from carriers of 3 KIR/HLA genotype groups. No effect of S-iKIR to HLA-C on ADCC was observed in KIR3DL1hmz co-carrying or not HLA-Bw4 in uninfected or SP subjects, including carriers of *h/*y+B*57. We found in our uninfected and SP populations that carrying one or two S-iKIR to HLA-C did not significantly contribute to NK cell functional potential for ADCC in KIR3DL1hmz carriers of one or no HLA-Bw4 ligands, including *h/*y+B*57 subjects. This is consistent with previous work by Dr. Bernard's lab for HLA-null cell-stimulated NK functions(318).

No combinations of specific KIR and HLA-C allotypes have been associated with controlling HIV infection. The level of HLA-C expression has been associated with slower progression to AIDS and improved control of VL(233;234). This may be mediated through better CTL recognition of peptide-HLA-C combinations that are expressed at higher levels on the cell surface. In HIV infected cells Nef downmodulates HLA-B and HLA-A, but not HLA-C(319). This phenomenon may negatively impact CTL recognition of HIV epitopes restricted by HLA-A and -B antigens on autologous HIV-infected CD4+ T cells. In contrast, maintenance of HLA-C and increased HLA-E surface expression levels may provide targets for CTL recognition while inhibiting NK cells from direct cytotoxicity and ADCC(319-321). Blocking the interaction between HLA-C and –E with

their inhibitory receptors allows NK cell lysis of autologous HIV-infected CD4+ T cells. This research suggests that although licensing through KIR3DL1 would result in NK cell activation upon stimulation with a target cell with decreased HLA-B expression, education of NK cells in the same donor through S-iKIR to HLA-C would impede the capacity of NK cells to destroy HIV-infected cells expressing HLA-C. The extent to which HLA-A and -B downmodulation and cell surface HLA-C retention favor NK cell stimulation and CTL recognition is complicated by the following observation. HLA-C is expressed at an average 10-fold lower density on the cell surface than HLA-B; HIV Nef more effectively downmodulates HLA-B than HLA-A and may not downmodulate HLA-A and B enough to abrogate inhibitory signals through iKIR(322;323). In summary, more work needs to be done to understand how HIV infection affects NK target cell recognition.

Since the strength of inhibitory signals during education have been demonstrated to determine the level of NK cell responsiveness(194), differences in NK cell education between high and low HLA-C expressers may be reflected in NK cell mediated effector functions against HIV, similar to the effects of KIR3DL1 expression on NK cell function demonstrated in this thesis and by others(86;195;230;236). Resistance to HIV infection has been associated with the presence of inihibitory KIR2DL receptors in the absence of their HLA ligands(324). Recently, Jennes et al. (2013) examined the KIR2DL1/2/3 and HLA-C1/C2 combinations carried by HIV serodiscordant and seroconcordant couples(325). They found that when recipient partners carried KIR2DL1/2/3/HLA-C1/C2 combinations that licenced NK cells in a manner that permitted recognition of the HLA-C allele of the index (transmitting) HIV positive partner, transmission was more frequent than when it did not. The implication is that recognition of HLA-C on incoming HIV infected cells inhibits recipient NK cells, while absence of HLA-C recognition permits NK cell activation in a manner that limits or prevents transmission of HIV infection.

The work presented in this thesis found no evidence that educational signals propagated through S-iKIR to HLA-C contributed to variations in anti-HIV ADCC in carriers of 3DL1/Bw4 genotype combinations that did influence anti-HIV ADCC. It may be that the influence of S-iKIR to HLA-C on NK cell education was too subtle compared to that on S-iKIR to HLA-B to detect the influence of the former KIR/HLA combinations. This may be related to expression levels of HLA-C versus HLA-B. Indeed, high expression HLA-C alleles have been associated with better VL control compared to low expression alleles in ART naïve seroconverter cohorts(11). Future studies could

stratify S-iKIR to HLA-C combinations according to HLA-C expression levels to determine whether those with high expression levels may have a bigger impact on NK cell education and subsequent function.

3.5: Role of KIR and HLA on potency of NK cell education: What are the receptor-ligand requirements for increased anti-HIV ADCC in uninfected carriers of *h/*y+B*57?

Associations between KIR3DL1/HLA-B genotypes and protection against infection and disease progression are likely influenced by the differential presence of licensed NK cells in humans. It has been hypothesized that the potency of NK cell educational signals received through inhibitory receptors upon ligation of their HLA ligands is directly related with the strength of cell functional potential(145). The receptor-ligand requirements for enhanced NK cell mediated ADCC in carriers of *h/*y+B*57 have not yet been elucidated. Although some research support the idea that both highly expressed variants of KIR3DL1 in combination with HLA-B*57 are required to favor the highest levels of cytokine secretion and degranulation as well as the strongest effect on slow time to AIDS and VL control(86;195;230), our results suggest it is possible that carriage of HLA-B*57 is sufficient to confer NK cells with increased functional potential in Bw4 positive individuals that carry either KIR3DL1hmz genotype (i.e. *h/*y and *l/*x).

Differences in educational potency of MHC ligands have been demonstrated in mice and humans(191;197;326). In humans, some HLA allotypes confer strong NK cell education while others have minimal educational capacity. For instance, HLA-Cw*07 confers strong education whereas Cw*14:02 ligation provides a weak education signal(326). Binding analysis of inhibitory KIR2DL2/3 receptors demonstrates interactions with allotypes of HLA-C1 and –C2(232;327). KIR2DL2*001 has greater avidity for both HLA-C1 and –C2 than KIR2DL3*001, suggesting stronger educational signals may be achieved through the interaction of KIR2DL2*001 with HLA-C(232). Within a particular KIR3DL1/HLA-B ligand group there is a variation in the capacity for NK cell education as a result of different binding affinities between variably expressed KIR3DL1 alleles and HLA-Bw4 molecules(159;326). It is possible that there is similar variation in the capacity of highly and low expressed alleles of KIR3DL1 to educate NK cells. Indeed, Dr. Peter Parham's lab compared IFN-γ production by NK cells from donors with KIR3DL1 allotypes expressed at different levels and demonstrated that binding of HLA-B to either low or high expressing allotypes delivers inhibitory signals

with comparable functional effect(328). If high and low expressed alleles of KIR3DL1 confer similar NK cell functional potential, this may account for the lack of difference in responses between carriers of KIR3DL1*h/*y and KIR3DL1*l/*x in the presence of HLA-B*57. Although our data suggest that both highly expressed variants and HLA-B*57 are required for enhanced ADCC functional potential, we cannot rule out that carriage of HLA-B*57 is sufficient to confer NK cells with increased functional potential irrespective of KIR3DL1hmz genotype (i.e. *h/*y and *l/*x).

Of course it is important to note that NK cells from only 3 individuals were available having the *l/*x+B*57 genotype combination, which limits our ability to make firm conclusions on this point. Future studies that increase the size of *l/*x+B*57 group will aid in teasing out whether carriage of B*57 is sufficient to confer high NK cell mediate ADCC activity or whether the KIR3DL1 receptor allotype also plays a role.

3.6: NK cell dysfunction in HIV

NK cells mediate protection against HIV by direct lysis of HIV infected target cells, secretion of chemokines and proinflammatory cytokines and ADCC. *Ex vivo* studies have shown that reduced NK cell function is associated with poor prognostic markers(104;286). NK cell dysfunction in HIV infection manifests as NK cell subset redistribution, changes in the patterns of receptor expression and altered functionality.

In HIV uninfected healthy controls, NK cells are divided into two subsets; $CD56^{dim}$ $CD16^{bright}$ which readily mediate cytolysis and secrete low levels of cytokines, and $CD56^{bright}CD16^{dim/neg}$, recognized for high levels of cytokine secretion and poor cytolysis upon activation(100). Despite an increase in the absolute number of NK cells during acute HIV infection, chronic infection is marked by a preferential decline in the cytolytic $CD56^{dim}CD16^{bright}$ NK cell subset and an expansion of an anergic subset characterized by a loss of CD56 expression(105;273;329;330). The association of the pathologic NK cell subset redistribution with VL is confirmed by reports from treatment-naïve, HIV infected controllers who either do not display an expansion or have a minimal expansion of the $CD56^{neg}CD16^{high}$ subset(195;271;331). Following initiation of successful HAART in HIV infected individuals, the frequency of this defective NK cell subset decreases(332).

A second level of NK dysfunction is altered patterns of receptor expression. In the presence of high viremia an increased proportion of NK cells express inhibitory natural killer receptors, including KIR, resulting in NK cells that are less likely to become activated(90;333;334). In addition, a loss of activation reduces cytokine secretion which

impairs the interaction of NK cells with cellular components of the adaptive immune system and disturbs priming of DCs(333).

Phenotypic alterations in HIV infection are accompanied with a reduced functionality upon receiving activating stimuli. Decreased ADCC potential has been observed by NK cells from HIV progressors in response to Ab stimulation through CD16a(335;336). Indeed, an inverse correlation has been reported between titers of HIV-specific ADCC Abs and the stage of infection(336). Furthermore, NK cells from progressors with high VL and low CD4 counts showed reduced ability to lyse HLA-devoid class-I target cells(104). Direct interactions between HIV and NK cells, such as infection of NK cells or binding of viral components to NK cell surface receptors may also contribute to altered functionality observed in infected individuals(273;333).

Evidence for the importance of NK cell functional potential and HIV disease status is supported by studies in non-human primate (NHP) models. Natural hosts for SIV who do not develop AIDS exhibit enhanced levels of NK cell functional potential, whereas rhesus macaques exhibit a decline in functional NK cells and experience a pathogenic SIV infection(83). Furthermore, HIV-infected chimpanzees do not develop AIDS but maintain highly functional CD8+ NK cells; these cells are disrupted in HIV infection in humans(84). Successful treatment with antiretrovirals suppresses viremia, restores CD56 expression on NK cells similar to levels observed in uninfected individuals, decreases inhibitory natural killer receptor expression on NK cells and normalizes NK cell functionality(103;104).

Epidemiological observations associating NK cell functionality with HIV disease status have been corroborated by studies of NK cell function in HIV infected SP, including LTNP, EC, and VC, and patients successfully treated with HAART. Studies on the effect of HAART on NK cells have revealed that successful treatment, defined by suppression of viral replication, is associated with partial restoration of NK cell functionality(104). Although post-HAART counts of CD4+ T cells are associated with normalization of NK cell functionality, partial immune reconstitution following HAART has not been found to correlate with restoration of ADCC functional potential for NK cells educated through KIR3DL1 in the presence of Bw4(252). Coupled with the association of NK cell dysfunction with HIV disease status, these data suggest that alterations in NK cell function could contribute to viral replication and disease progression.

3.7: ADCC in HIV infection: protection or progression?

The role of NK cell mediated ADCC in the context of exposure to or infection with HIV has been the subject of extensive investigation following its identification as a possible correlate of immunity in NHP and human vaccine trials(27;64;282).

ADCC-mediating Abs are among the first anti-viral Ab responses to develop following infection and, have been observed as early as 48 days after acute HIV infection (337). Interestingly, HIV-specific ADCC competent Abs are detected in the serum of a large majority of HIV-infected individuals(338). ADCC responses in HIV-infected patients and SIV-infected rhesus macaques have been associated with slower progression to AIDS and/or lower VL(291;297;339). Similarly, macaque vaccine trials have been shown to induce ADCC responses and were associated with protection from infection and/or reduced viremia(27;285;295;340;341). As HIV disease progresses, the ability of NK cells to mediate ADCC decreases(336). Low ADCC titers correlate with disease progression and CD4+ T cell decline(293). Compromised NK cell mediated ADCC has also recently been demonstrated in a macaque model of SIV/SHIV infection which was associated with reduced expression of CD16 on NK cells(316). *In vitro* experiments of chronic ADCC stimulation through CD16 resulted in NK cells developing a similar phenotype as those of NK cells from HIV infected subjects, characterized by a reduction in CD16 and CD56 expression(342). Despite NK cell dysfunction in HIV infection, SP who maintain a limited pool of dysfunctional NK cells have high serum ADCC titers in comparison to HIV progressors(195;271;293;331). Indeed, sustained ADCC responses have been associated with delayed progression to AIDS in SIV-infected macaques(297). Furthermore, HIV replication has been negatively correlated with ADCC responses in some studies(294;343), though others have demonstrated a lack of correlation(344). The discrepancy in these reports may arise from a small sample size of HIV controllers(343) and differences in the outcomes measured; using an intracellular cytokine staining ADCC assay to determine the ability of ADCC-competent plasma to activate NK cells in the presence of HIV Ag versus measuring the frequency of target cells killed in the presence of effector cells and ADCC Abs.

Although high NK cell activity delays time to AIDS, NK cell activation has been shown to result in shedding of CD16 which may contribute to HIV disease pathogenesis. Increased levels of soluble CD16 correlate with disease progression in HIV infected individuals(345). Anti-HIV ADCC also has the potential to contribute to HIV disease pathogenesis. Shedding of unstable HIV Env from the viral membrane may bind to CD4

on T cells, making them a target for ADCC if Env is recognized by anti-HIV Abs and NK cells(346). Consequently, anti-HIV ADCC would contribute to the loss of CD4+ T cells in HIV infection. Indeed, high NK cell activity has been shown to lyse activated uninfected CD4+ T cells(113;114). It is therefore plausible that NK cells conferred with enhanced functionality through the education process have the potential to contribute to HIV disease progression by mediating ADCC against uninfected target cells.

NK cell activation through CD16a interactions with Ab has been demonstrated to overcome KIR-mediated inhibition(347), though Ward et al. (2004) report that interactions between inhibitory receptors and HLA-C and –E inhibit NK cells from lysing autologous HIV-infected CD4+ T cells in the presence of ADCC-competent Abs(320). However, NK cells educated through KIR3DL1 can overcome inhibitory signals through KIR3DL1 interacting with HLA-Bw4 and respond to autologous cells coated with HIV Env peptides and anti-HIV Abs(252). In addition, a common sequence mutation selected for by CD8+ T cells has been shown to prevent the binding of KIR3DL1 to HLA-B such that disinhibition of NK cells could render infected cells susceptible to NK cell lysis(348). Similar modulation of NK cell activity has been observed for HLA-C binding peptides and KIR2DL2/3(349).

Observations that HIV infection is associated with a decline in the ability of NK cells to mediate ADCC corroborates our observation of loss of ADCC functional potential in SP. Variant viral peptides alter the repertoire of peptides bound by HLA-B and HLA-C(348;349), therefore; in the context of HIV infection, the NK cell functional potential for ADCC conferred by co-carriage of *h/*y+B*57 may partly depend on sequence mutations selected for by HIV.

3.8: Lost in translation: is there an influence of FcyRIIIa genotype on ADCC responses in the context of HIV infection?

A functional polymorphism at position 158 of FcγRIIIa protein influences the ability of NK cells to mediate effector functions through CD16a(124;126;127;301;350). There is disagreement regarding what influence FcyRIIIa polymorphism has on the effector functions of Fc-bearing immune cells such as NK cells. Both the V and F isoform have been associated with susceptibility to HIV infection and progression to AIDS(286;301;351;352). Despite a higher affinity for Abs of the IgG1 and IgG3 subclass, the V allele has been implicated as a risk factor for HIV disease progression(301;351;352). We observed no influence of CD16a genotype on differences

in ADCC responses between KIR/HLA groups in uninfected subjects or SP corroborating work by others that showed no differences in HIV-specific NK cell activation based on CD16a genotype(343).

Increased cell surface CD16 expression on NK cells may contribute to heterogeneity of ADCC responses observed between subjects. The potency of ADCC function has been correlated with CD16 expression on NK cells(317). Carriers of at least one V allele have been reported to show increased cell surface expression of CD16a on NK cells however, another study failed to attribute an increased expression of the V allotype to enhanced anti-CD16a mAb binding(130;300). Work by others has suggested that the functional effect of CD16a polymorphism on ADCC responses may in part be dependent on Fc gene polymorphisms and the nature of the binding Ab. Genetic variants of the IgG γ (GM allotypes) and κ chain (KM allotypes) have been associated with host control of HIV infection(126). The GM5 allotype has been associated with host control of HIV replication in controllers(126). As interpreted by the authors, this may indicate a greater affinity of GM5-expressing anti-HIV IgG for the V allele resulting in enhanced ADCC against infected cells. Furthermore, carbohydrate composition has long been recognized to influence the affinity of IgG for FcγRIIIa(353-355). Fucose depletion on Abs has been demonstrated to improve FcγRIIIa binding(350;356-358). Although a non-fucosylated variant of the mAb b12 had higher binding affinity for FcγRIIIa and was more effective in ADCC-mediated killing of target cells, it did not enhance protection in vivo against challenge with a simian-human immunodeficiency virus (SHIV)(359). NK cells deficient for signaling adaptor FcyR with intact CD16 expression and robust Ab-dependent immune functions through CD3ζ have recently been identified(360). NK cells of this immature phenotype were restricted to the CD56dim subset and expressed KIRs at very low levels, or not at all. These 'unlicensed' FcyR-deficient NK cells may be associated with viral infection providing an explanation for some of the diversity in results reported in ADCC functionality.

Despite evidence that CD16a polymorphisms may modulate ADCC activity, functional variation in CD16a did not explain heterogeneity of ADCC responses between KIR/HLA groups in our study. It is possible the effect of CD16a polymorphism on ADCC and clinical outcomes relies on a series of more complex factors such as levels of CD16a surface expression on NK cells, affinity of the Ab for CD16a, and binding properties of the Ab, which were not investigated in this thesis. The single source of

ADCC competent Ab used in these studies would control the possibility of a role for ADCC Ab titers and IgG subtypes as factors in between subject variation in ADCC activity. Finally, the ability of CD16a to harness components of the innate immune system to shape adaptive immune responses makes the receptor an attractive therapeutic target.

3.9: Two is better than one: ADCC Abs and educated NK cells

ADCC Abs provide activating signals to NK cells that have been demonstrated to overcome inhibition through KIR/HLA(347). The potential for ADCC as a protective mechanism against exposure to HIV has been propelled by several human and primate vaccine and passive transfer studies. Hessell et al. (2007) showed that the Fc binding capacity of the BnAb b12 was required to protect macaques against mucosal and intravenous challenge with SHIV(64). Alternatively, a b12 variant defective in complement binding but FcyR competent had no effect on protection in these passive transfer studies(64). This study was followed by a vaccine trial testing several permutations of poxvirus/adenovirus based vaccine vectors(27). Following multiple low dose intra-rectal challenges of neutralization resistant SIV_{mac251}, examination of 35 immune parameters revealed protection from infection was associated with vaccine induced Env Ab responses including ADCC. A subsequent macaque vaccine study also suggested that ADCC was a correlate of protection(361). Recently, the RV144 Thai HIV vaccine trial provided a modest level of protection against HIV acquisition(282). The vaccine regimen induced high titers of ADCC competent Abs, but did not induce immune responses such as BnAbs or CTL - traditionally considered important for protection from HIV infection(281). Although ADCC correlated with a lower incidence of HIV acquisition, secondary analysis of immune correlates from this trial has shown an inverse correlation between levels of IgA and IgG titers and protection(282). Investigators speculate that plasma IgA Abs compete with anti-HIV IgG for binding to ADCC epitopes and interfere with IgG effector functions such as ADCC.

At this point we see that ADCC may provide protection upon exposure to HIV if an effective vaccine can induce anti-HIV Env Abs. Upon exposure to HIV, anti-HIV Env Abs could coat infected allogeneic and/or autologous cells and mediate their lysis by NK cells, whose function would not yet have been abrogated by HIV infection. Studies demonstrating the importance of NK cell education for ADCC functional potential suggest that ADCC competent Abs most likely require licensed NK cells to mediate

protective effector functions (Parsons et al. 2010 JLB; Parsons et al. 2012). Future analysis of vaccine trials should examine the KIR/HLA background of vaccinees to further elucidate the role of NK cell education in anti-HIV ADCC responses.

3.10: Why the discrepancy? Evaluating inter- and intra-assay variability of the GranToxiLux ADCC assay

We report that uninfected individuals carrying the *h/*y+B*57 protective genotype mediated significantly higher anti-HIV ADCC responses as compared to KIR3DL1hmz individuals carrying other Bw4 alleles or Bw6 homozygotes. However, a second set of ADCC experiments using cells from the same timepoint revealed no significant between KIR/HLA genotype group differences. An analysis of inter-assay variability between these 2 datasets revealed a CV of 72.9%, a value similar to that seen between subjects. This result prompted a more rigorous evaluation of CV for the GTL ADCC assay consisting of experiments run on 2 subjects in triplicate on three occasions. The intra- and inter-assay CV for these experiments were both below 25%, as previously reported in the literature(241).

In line with the use of this assay in other published reports, we used the same CEM.NKr.CCR5 cells as targets, eliminating the possibility that alterations to target cells would impact the results of our experiments. Furthermore, we used a single source of HIV-1_{BaL} recombinant gp120 to coat targets, as well as a single source of anti-gp120 Ab titrated to an optimal dilution of 1:1000 - as was used in the experiments described by Pollara et al. (2011)(241). Multiple freeze-thaw cycles may increase damage to components in plasma such as Abs. In our experiments, plasma was aliquoted and freeze-thaw cycles were limited to less than 5, though no significant differences have been reported in the levels of analytes in plasma after up to 20 repeated freeze-thaw cycles(362-365). Additionally, plasma samples were mixed before each use to ensure proteins and serum were evenly distributed. A source of variation may be the lot of the gp120 used in the first and second set of experiments, which differed one from the other. The first lot of gp120 is depleted and could not be compared to the second lot in terms of binding to targets or Ab recognition.

The source of gp120 used to label target cells differed between our experiments and those previously reported by Pollara et al. (2011)(241). Therefore, we considered that poor binding of the gp120 to CD4 on target cells used in our experiments may have resulted in reduced recognition by anti-gp120 Ab. We acquired gp120 from Immune

Technology Corp (the source used by Pollara et al.) to do a side by side comparison with the gp120 we used from the NIH AIDS Research and Reference Reagent Program in a competition assay for CD4 binding and GTL ADCC assay for Ab binding. Although we observed an overall slightly better binding of the Immune Technology Corp gp120 for CD4, the gp120 used in our experiments did not demonstrate significantly poorer CD4 binding. A side by side comparison of both gp120s in a GTL ADCC assay using two samples in triplicate also revealed no differences in binding of Ab for gp120. The results of these experiments do not rule out the possibility that degradation of gp120 used in the second set of experiments could have contributed to the discrepant results, however, we demonstrate intact binding of our gp120 source to CD4 and recognition by Ab to mediate ADCC. Thus, variations in CD4 binding and Ab recognition are unlikely to have impacted on differences in the results between our first and second set of experiments. Future experiments are required to address whether the gp120 used in our experiments is subject to degradation over time affecting its ability to bind CD4 on targets and compromises the ability to measure NK cell mediated anti-HIV ADCC using the GTL assay.

Despite the discrepancy in results between our first and second set of experiments, numerous epidemiological and functional studies support our findings from the first set of experiments that allelic combinations of KIR3DL1/HLA-Bw4 are determinants of NK cell functional potential. The KIR/HLA genotype associated with the most potent control of VL and slow time to AIDS is *h/*y+B*57(86). This combination has also been associated with protection from HIV infection in HESN(230). Carriage of *h/*y+B*57 has been demonstrated to confer NK cells with heightened functional potential as measured by secretion of IFN-γ and TNF-γ, and expression of CD107a, that is used as a surrogate marker for cytotoxic granule release(195;196;242;244;245). Although we observed high inter-assay variability between dataset 1 and 2, our more rigorous evaluation of inter- and intra-assay variability revealed variation within the range of that previously reported(241). These results together with those reported by Parsons et al. (2012)(252) showing a role for NK cell education in ADCC activity levels were the basis for our decision to use the first set of results as the platform for the analyses presented in this thesis. Future GTL ADCC experiments evaluating KIR/HLA combinations as determinants of NK cell functional potential should use samples in triplicates and be carried out in conjunction with assays that measured cytokine secretion and degranulation.

3.11: Limitations and future directions

A limitation of the present study is that it did not examine the level of CD16a expression on NK cells. Increased CD16a expression may contribute to heterogeneity of ADCC responses observed between KIR/HLA groups(130). Furthermore, this study did not characterize NK cell subsets in SP to determine whether a decrease in cytolytic $CD56^{dim}CD16^{bright}$ NK cells and/or an expansion of anergic $CD56^{neg}CD16^{dim}$ NK cells contributed to the absence of anti-HIV ADCC responses observed in carriers of the HIV protective *h/*y+B*57 genotype. Furthermore, a limitation of the GTL assay is that it does not permit phenotypic evaluation of the effector NK cells or assessment of activated KIR^{+} NK cell populations.

While no effect of carriage of S-iKIR to HLA-C was observed, GWAS have identified a SNP that associated with expression of HLA-C and control of viral replication(234;269). Increased HLA-C expression has been shown to educate NK cells for functional potential(236). A differential effect of high- and low-expressing HLA-C might educate NK cells through KIR2DL receptors for greater functional potential against aberrant target cells. A more rigorous evaluation of the effect S-iKIR to HLA-C have on anti-HIV ADCC responses would involve examining whether HLA-C expression enhances NK cell activity through a licensing process. Future research investigating other KIR/HLA interactions and their importance in NK cell education and NK cell mediated ADCC and other effector functions are required to elucidate the mechanisms important for viral control.

The ADCC experiments used to generate the results presented in this thesis utilized an NK cell resistant transformed human T cell line. While these cells express normal levels of CD4 and CXCR4, and thus can be coated with HIV gp120 and anti-HIV Abs, they are highly resistant to infection with HIV. Although this relative resistance can be overcome with spinoculation and improved using a dextran exposure(285), this approach as well as labeling with gp120 do not represent productive infections. The use of more physiologically relevant HIV-infected autologous CD4+ T cells as targets in future experiments may improve our understanding of NK cell education in the context of HIV infection, NK cell anti-viral responses and would inform the design of more effective HIV vaccines.

3.12: Conclusions

The data presented in this thesis demonstrates that KIR3DL1/HLA-Bw4 interactions determine the functional potential of NK cells to mediate anti-HIV ADCC. We showed that the allelic combination of *h/*y+B*57, associated with protection against HIV infection and/or disease progression(86;230), confers NK cells with enhanced functional potential for anti-HIV ADCC. Enhanced functionality of NK cells from carriers of *h/*y+B*57 has previously been reported(195;196). Taken together with recent reports from an HIV vaccine trial implicating ADCC as an immune correlate of protection, these data suggest that HIV infection may be prevented by vaccines designed to harness the potential of the innate immune system to induce adaptive immune responses(282). However, we present evidence that NK cell mediated anti-HIV ADCC functional potential is extinguished in the context of HIV infection. This finding is in agreement with a report of abrogated NK cell functional potential in HIV-infected individuals carrying KIR3DL1/HLA-Bw4 combinations and reflects the NK cell dysfunction observed during HIV infection(103;104;252;273;332). Our results raise questions surrounding the role of Ab-dependent NK cell mediated mechanisms in slowed progression to AIDS. Our observations went on to show that differences in NK cell mediated ADCC activity within groups constituted based on KIR/HLA background were not affected by a functional polymorphism in FcγRIIIa that influences binding of the receptor to Ab or carriage of educationally competent S-iKIR to HLA-C. In agreement with our data, differences in HIV-specific NK cell activation have not been attributed to CD16a genotype(343), nor have they been associated with reduced rates of HIV acquisition following recombinant gp120 vaccination(351). Our results suggest that KIR3DL1/HLA-Bw4 combinations support stronger educational signals than interactions between KIR2DL and HLA-C. The observation that ADCC is dependent on KIR/HLA-mediated NK cell education calls into question the universal applicability of vaccines designed to induce ADCC responses as KIR/HLA background will modulate the magnitude of responses by anti-viral Abs in different individuals.

In conclusion, the data presented in this thesis are consistent with reports that KIR/HLA combinations are a determinant of NK cell functional potential that provide protection against HIV infection, and support observations of aberrant NK cell function in HIV infected individuals.

Reference List

(1) Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, et al. Pneumocystis carinii Pneumonia and Mucosal Candidiasis in Previously Healthy Homosexual Men. N Engl J Med 1981 Dec 10;305(24):1425-31.

(2) Masur H, Michelis MA, Greene JB, Onorato I, Vande Stouwe RA, Holzman RS, et al. An Outbreak of Community-Acquired Pneumocystis carinii Pneumonia. N Engl J Med 1981 Dec 10;305(24):1431-8.

(3) Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983 May 20;220(4599):868-71.

(4) Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM, Oshiro LS. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 1984 Aug 24;225(4664):840-2.

(5) Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 1984 May 4;224(4648):506-8.

(6) Schupbach J, Popovic M, Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 1984 May 4;224(4648):503-5.

(7) Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984 May 4;224(4648):497-500.

(8) Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 1984 May 4;224(4648):500-3.

(9) UNAIDS. Report on the Global AIDS Epidemic 2010. Geneva 2010:1-359. Available from: URL: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf

(10) Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, Goedert JJ, et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science 1999 Mar 12;283(5408):1748-52.

(11) Bashirova AA, Thomas R, Carrington M. HLA/KIR Restraint of HIV: Surviving the Fittest. Annu Rev Immunol 2011 Mar 22;29(1):295-317.

(12) Sharp PM, Hahn BH. Origins of HIV and the AIDS Pandemic. Cold Spring Harb Perspect Med 2011 Sep;1(1):a006841.

(13) Bourinbaiar AS, Abulafia-Lapid R. Autoimmunity, alloimmunization and immunotherapy of AIDS. Autoimmun Rev 2005 Jul;4(6):403-9.

(14) Chun TW, Justement JS, Moir S, Hallahan CW, Maenza J, Mullins JI, et al. Decay of the HIV reservoir in patients receiving antiretroviral therapy for extended periods: implications for eradication of virus. J Infect Dis 2007 Jun 15;195(12):1762-4.

(15) Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. N Engl J Med 2011 May 18;364(20):1943-54.

(16) Brenner BG, Roger M, Stephens D, Moisi D, Hardy I, Weinberg J, et al. Transmission clustering drives the onward spread of the HIV epidemic among men who have sex with men in Quebec. J Infect Dis 2011 Oct 1;204(7):1115-9.

(17) Aaron E, Cohan D. Pre-exposure Prophylaxis for the Prevention of HIV Transmission to Women in the United States. AIDS 2012 Aug 18.

(18) Okwundu CI, Uthman OA, Okoromah CA. Antiretroviral pre-exposure prophylaxis (PrEP) for preventing HIV in high-risk individuals. Cochrane Database Syst Rev 2012;7:CD007189.
(19) Abdool KQ, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science 2010 Sep 3;329(5996):1168-74.
(20) Van DL, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure prophylaxis for HIV infection among African women. N Engl J Med 2012 Aug 2;367(5):411-22.
(21) Brooks RA, Kaplan RL, Lieber E, Landovitz RJ, Lee SJ, Leibowitz AA. Motivators, concerns, and barriers to adoption of preexposure prophylaxis for HIV prevention among gay and bisexual men in HIV-serodiscordant male relationships. AIDS Care 2011 Sep;23(9):1136-45.
(22) Wertheim JO, Worobey M. Dating the age of the SIV lineages that gave rise to HIV-1 and HIV-2. PLoS Comput Biol 2009 May;5(5):e1000377.
(23) Guindon Sp, Gascuel O. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. Systematic Biology 2003 Oct 1;52(5):696-704.
(24) Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemee V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med 2009 Aug;15(8):871-2.
(25) Vallari A, Holzmayer V, Harris B, Yamaguchi J, Ngansop C, Makamche F, et al. Confirmation of Putative HIV-1 Group P in Cameroon. Journal of Virology 2011 Feb 1;85(3):1403-7.
(26) Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. Clin Infect Dis 2011 Mar 15;52(6):780-7.
(27) Barouch DH. Challenges in the development of an HIV-1 vaccine. Nature 2008 Oct 2;455(7213):613-9.
(28) Peterson K, Jallow S, Rowland-Jones SL, de Silva TI. Antiretroviral Therapy for HIV-2 Infection: Recommendations for Management in Low-Resource Settings. AIDS Res Treat 2011;2011:463704.
(29) Rowland-Jones SL, Whittle HC. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? Nat Immunol 2007 Apr;8(4):329-31.
(30) Chen L, Jha P, Stirling B, Sgaier SK, Daid T, Kaul R, et al. Sexual risk factors for HIV infection in early and advanced HIV epidemics in sub-Saharan Africa: systematic overview of 68 epidemiological studies. PLoS ONE 2007;2(10):e1001.
(31) Munier CM, Andersen CR, Kelleher AD. HIV vaccines: progress to date. Drugs 2011 Mar 5;71(4):387-414.
(32) Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proceedings of the National Academy of Sciences 2008 May 27;105(21):7552-7.
(33) Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. Nat Rev Immunol 2008 Jun;8(6):447-57.
(34) Shah VB, Aiken C. HIV Nuclear Entry: Clearing the Fog. Viruses 2010 May;2(5):1190-4.
(35) Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002 May 2;417(6884):95-8.

(36) Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. Nature 2010 Mar 11;464(7286):217-23.
(37) Lehner T, Wang Y, Whittall T, Seidl T. Innate immunity and HIV-1 infection. Adv Dent Res 2011 Apr;23(1):19-22.
(38) Graziosi C, Pantaleo G, Butini L, Demarest JF, Saag MS, Shaw GM, et al. Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. Proceedings of the National Academy of Sciences 1993 Jul 15;90(14):6405-9.
(39) Pedersen C, Lindhardt BO, Jensen BL, Lauritzen E, Gerstoft J, Dickmeiss E, et al. Clinical course of primary HIV infection: consequences for subsequent course of infection. BMJ 1989 Jul 15;299(6692):154-7.
(40) Josefsson L, King MS, Makitalo B, Br+ñnnstr+Âm J, Shao W, Maldarelli F, et al. Majority of CD4+ T cells from peripheral blood of HIV-1ΓÇôinfected individuals contain only one HIV DNA molecule. Proceedings of the National Academy of Sciences 2011 Jun 20.
(41) Grivel JC, Margolis LB. Nat Med 1999 Mar;5(3):344-6.
(42) Freed EO. HIV-1 Replication. Somatic Cell and Molecular Genetics 2001 Nov 1;26(1):13-33.
(43) Greene WC. The molecular biology of human immunodeficiency virus type 1 infection. N Engl J Med 1991 Jan 31;324(5):308-17.
(44) Tavassoli A. Targeting the protein-protein interactions of the HIV lifecycle. Chem Soc Rev 2011 Mar;40(3):1337-46.
(45) Freed EO. HIV-1 Gag Proteins: Diverse Functions in the Virus Life Cycle. Virology 1998 Nov 10;251(1):1-15.
(46) Arhel N. Revisiting HIV-1 uncoating. Retrovirology 2010;7:96.
(47) Basu VP, Song M, Gao L, Rigby ST, Hanson MN, Bambara RA. Strand transfer events during HIV-1 reverse transcription. Virus Research 2008 Jun;134(1ΓÇô2):19-38.
(48) Bukrinsky MI, Haggerty S, Dempsey MP, Sharova N, Adzhubei A, Spitz L, et al. A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. Nature 1993 Oct 14;365(6447):666-9.
(49) Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P. HIV-1 Genome Nuclear Import Is Mediated by a Central DNA Flap. Cell 101[2], 173-185. 14-4-2000.
(50) Wu Y, Marsh JW. Gene transcription in HIV infection. Microbes Infect 2003 Sep;5(11):1023-7.
(51) Engelman A, Mizuuchi K, Craigie R. HIV-1 DNA integration: Mechanism of viral DNA cleavage and DNA strand transfer. Cell 67[6], 1211-1221. 20-12-1991.
(52) Farnet CM, Haseltine WA. Integration of human immunodeficiency virus type 1 DNA in vitro. Proceedings of the National Academy of Sciences 1990 Jun 1;87(11):4164-8.
(53) Karn J, Stoltzfus CM. Transcriptional and Posttranscriptional Regulation of HIV-1 Gene Expression. Cold Spring Harb Perspect Med 2012 Feb;2(2):a006916.
(54) Pollard VW, Malim MH. THE HIV-1 REV PROTEIN. Annu Rev Microbiol 1998 Oct 1;52(1):491-532.
(55) Ono A, Freed EO. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc Natl Acad Sci U S A 2001 Nov 20;98(24):13925-30.
(56) Kaplan AH. Assembly of the HIV-1 core particle. AIDS Rev 2002 Apr;4(2):104-11.

(57) Kaplan AH, Manchester M, Swanstrom R. The activity of the protease of human immunodeficiency virus type 1 is initiated at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. J Virol 1994 Oct;68(10):6782-6.
(58) Cantin R, Fortin JF, Lamontagne G, Tremblay M. The presence of host-derived HLA-DR1 on human immunodeficiency virus type 1 increases viral infectivity. Journal of Virology 1997 Mar 1;71(3):1922-30.
(59) Tremblay MJ, Fortin JF, Cantin R. The acquisition of host-encoded proteins by nascent HIV-1. Immunology today 19[8], 346-351. 1-8-1998.
(60) CANTIN R+, FORTIN JF, TREMBLAY MICH. The Amount of Host HLA-DR Proteins Acquired by HIV-1 Is Virus Strain- and Cell Type-Specific. Virology 1996 Apr 15;218(2):372-81.
(61) Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, Chavez LL, et al. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J Virol 2008 Dec;82(24):12449-63.
(62) Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature 2003 Mar 20;422(6929):307-12.
(63) Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. Annu Rev Immunol 2010;28:413-44.
(64) Hessell AJ, Hangartner L, Hunter M, Havenith CEG, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature 2007 Sep 6;449(7158):101-4.
(65) Tudor D, Bomsel M. The broadly neutralizing HIV-1 IgG 2F5 elicits gp41-specific antibody-dependent cell cytotoxicity in a FcgammaRI-dependent manner. AIDS 2011 Mar 27;25(6):751-9.
(66) Neil S, Bieniasz P. Human immunodeficiency virus, restriction factors, and interferon. J Interferon Cytokine Res 2009 Sep;29(9):569-80.
(67) Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 2004 Feb 26;427(6977):848-53.
(68) Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature 2008 Jan 24;451(7177):425-30.
(69) Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 2011 Jun 30;474(7353):658-61.
(70) Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 2011 Jun 30;474(7353):654-7.
(71) Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994 Jul;68(7):4650-5.
(72) Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol 1994 Sep;68(9):6103-10.
(73) Kaslow RA, Carrington M, Apple R, Park L, Munoz A, Saah AJ, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat Med 1996 Apr;2(4):405-11.

(74) Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med 2003;54:535-51.
(75) Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A 2000 Mar 14;97(6):2709-14.
(76) Brockman MA, Brumme ZL, Brumme CJ, Miura T, Sela J, Rosato PC, et al. Early selection in Gag by protective HLA alleles contributes to reduced HIV-1 replication capacity that may be largely compensated for in chronic infection. J Virol 2010 Nov;84(22):11937-49.
(77) Miura T, Brockman MA, Schneidewind A, Lobritz M, Pereyra F, Rathod A, et al. HLA-B57/B*5801 human immunodeficiency virus type 1 elite controllers select for rare gag variants associated with reduced viral replication capacity and strong cytotoxic T-lymphocyte [corrected] recognition. J Virol 2009 Mar;83(6):2743-55.
(78) Allen TM, Altfeld M, Geer SC, Kalife ET, Moore C, O'Sullivan KM, et al. Selective escape from CD8+ T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution. J Virol 2005 Nov;79(21):13239-49.
(79) Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999 Feb 5;283(5403):857-60.
(80) Gaufin T, Ribeiro RM, Gautam R, Dufour J, Mandell D, Apetrei C, et al. Experimental depletion of CD8+ cells in acutely SIVagm-infected African Green Monkeys results in increased viral replication. Retrovirology 2010;7:42.
(81) Mueller YM, Do DH, Boyer JD, Kader M, Mattapallil JJ, Lewis MG, et al. CD8+ cell depletion of SHIV89.6P-infected macaques induces CD4+ T cell proliferation that contributes to increased viral loads. J Immunol 2009 Oct 15;183(8):5006-12.
(82) Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999 Mar 15;189(6):991-8.
(83) Pereira LE, Johnson RP, Ansari AA. Sooty mangabeys and rhesus macaques exhibit significant divergent natural killer cell responses during both acute and chronic phases of SIV infection. Cell Immunol 2008;254(1):10-9.
(84) Rutjens E, Mazza S, Biassoni R, Koopman G, Ugolotti E, Fogli M, et al. CD8+ NK cells are predominant in chimpanzees, characterized by high NCR expression and cytokine production, and preserved in chronic HIV-1 infection. Eur J Immunol 2010 May;40(5):1440-50.
(85) Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 2002 Aug;31(4):429-34.
(86) Martin MP, Qi Y, Gao X, Yamada E, Martin JN, Pereyra F, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 2007 Jun;39(6):733-40.
(87) Alter G, Teigen N, Ahern R, Streeck H, Meier A, Rosenberg ES, et al. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. J Infect Dis 2007 May 15;195(10):1452-60.
(88) Eller MA, Koehler RN, Kijak GH, Eller LA, Guwatudde D, Marovich MA, et al. Human immunodeficiency virus type 1 infection is associated with increased NK cell polyfunctionality and higher levels of KIR3DL1+ NK cells in ugandans carrying the HLA-B Bw4 motif. J Virol 2011 May;85(10):4802-11.

(89) Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, et al. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. J Exp Med 2003 Oct 6;198(7):1069-76.
(90) Alter G, Altfeld M. NK cells in HIV-1 infection: evidence for their role in the control of HIV-1 infection. J Intern Med 2009 Jan;265(1):29-42.
(91) Ferlazzo G, Pack M, Thomas D, Paludan C, Schmid D, Strowig T, et al. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. Proc Natl Acad Sci U S A 2004 Nov 23;101(47):16606-11.
(92) Ferlazzo G, Morandi B, D'Agostino A, Meazza R, Melioli G, Moretta A, et al. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. Eur J Immunol 2003 Feb;33(2):306-13.
(93) Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M, et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat Med 1999 Apr;5(4):405-11.
(94) Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. Nat Immunol 2004 Dec;5(12):1260-5.
(95) Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. Proceedings of the National Academy of Sciences 2009 Feb 10;106(6):1915-9.
(96) Sun JC, Beilke JN, Lanier LL. Immune memory redefined: characterizing the longevity of natural killer cells. Immunol Rev 2010 Jul;236:83-94.
(97) Paust S, Gill HS, Wang BZ, Flynn MP, Moseman EA, Senman B, et al. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. Nat Immunol 2010 Dec;11(12):1127-35.
(98) Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL. NK cells and immune "memory". J Immunol 2011 Feb 15;186(4):1891-7.
(99) Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or Adaptive Immunity? The Example of Natural Killer Cells. Science 2011 Jan 7;331(6013):44-9.
(100) Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001 Nov;22(11):633-40.
(101) Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. J Immunol 1989 Nov 15;143(10):3183-91.
(102) Grzywacz B, Kataria N, Verneris MR. CD56(dim)CD16(+) NK cells downregulate CD16 following target cell induced activation of matrix metalloproteinases. Leukemia 2007 Feb;21(2):356-9.
(103) Mavilio D, Lombardo G, Benjamin J, Kim D, Follman D, Marcenaro E, et al. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proc Natl Acad Sci U S A 2005 Feb 22;102(8):2886-91.
(104) Mavilio D, Benjamin J, Daucher M, Lombardo G, Kottilil S, Planta MA, et al. Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. Proc Natl Acad Sci U S A 2003 Dec 9;100(25):15011-6.

(105) Alter G, Teigen N, Davis BT, Addo MM, Suscovich TJ, Waring MT, et al. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. Blood 2005 Nov 15;106(10):3366-9.
(106) Lanier LL. NK cell recognition. Annu Rev Immunol 2005;23:225-74.
(107) Fadda L, Alter G. KIR/HLA: genetic clues for a role of NK cells in the control of HIV. Adv Exp Med Biol 2011;780:27-36.
(108) Rajalingam R. Human diversity of killer cell immunoglobulin-like receptors and disease. Korean J Hematol 2011 Dec;46(4):216-28.
(109) Orange JS, Ballas ZK. Natural killer cells in human health and disease. Clin Immunol 2006 Jan;118(1):1-10.
(110) Brandstadter JD, Yang Y. Natural killer cell responses to viral infection. J Innate Immun 2011;3(3):274-9.
(111) Fausther-Bovendo H, Sol-Foulon N, Candotti D, Agut H, Schwartz O, Debre P, et al. HIV escape from natural killer cytotoxicity: nef inhibits NKp44L expression on CD4+ T cells. AIDS 2009 Jun 1;23(9):1077-87.
(112) Vieillard V, Dereuddre-Bosquet N, Mangeot-Mederle I, Le GR, Debre P. An HIVgp41 vaccine protects CD4 central memory T cells in SHIV-infected macaques. Vaccine 2012 Sep 16.
(113) Vieillard V, Le GR, Dausset J, Debre P. A vaccine strategy against AIDS: an HIV gp41 peptide immunization prevents NKp44L expression and CD4+ T cell depletion in SHIV-infected macaques. Proc Natl Acad Sci U S A 2008 Feb 12;105(6):2100-4.
(114) Vieillard V, Strominger JL, Debre P. NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand. Proc Natl Acad Sci U S A 2005 Aug 2;102(31):10981-6.
(115) Pegram HJ, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH. Activating and inhibitory receptors of natural killer cells. Immunol Cell Biol 2011 Feb;89(2):216-24.
(116) Williams AP, Bateman AR, Khakoo SI. Hanging in the balance. KIR and their role in disease. Mol Interv 2005 Aug;5(4):226-40.
(117) Khakoo SI, Brooks CR. MHC class I receptors on natural killer cells: on with the old and in with the new. Clin Sci (Lond) 2003 Aug;105(2):127-40.
(118) Selvaraj P, Carpen O, Hibbs ML, Springer TA. Natural killer cell and granulocyte Fc gamma receptor III (CD16) differ in membrane anchor and signal transduction. J Immunol 1989 Nov 15;143(10):3283-8.
(119) Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 2008 May;9(5):495-502.
(120) Mandelboim O, Malik P, Davis DM, Jo CH, Boyson JE, Strominger JL. Human CD16 as a lysis receptor mediating direct natural killer cell cytotoxicity. Proc Natl Acad Sci U S A 1999 May 11;96(10):5640-4.
(121) Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 2010 Mar 18;115(11):2167-76.
(122) Barth M, Raetz E, Cairo MS. The future role of monoclonal antibody therapy in childhood acute leukaemias. Br J Haematol 2012 Oct;159(1):3-17.
(123) Nordstrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcgamma receptor binding properties. Breast Cancer Res 2011;13(6):R123.

(124) Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de HM. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 1997 Aug 1;90(3):1109-14.
(125) Lehrnbecher T, Foster CB, Zhu S, Leitman SF, Goldin LR, Huppi K, et al. Variant genotypes of the low-affinity Fcgamma receptors in two control populations and a review of low-affinity Fcgamma receptor polymorphisms in control and disease populations. Blood 1999 Dec 15;94(12):4220-32.
(126) Deepe RN, Kistner-Griffin E, Martin JN, Deeks SG, Pandey JP. Epistatic interactions between Fc (GM) and FcgammaR genes and the host control of human immunodeficiency virus replication. Hum Immunol 2012 Mar;73(3):263-6.
(127) Van Den Berg L, Myhr KM, Kluge B, Vedeler CA. Fcgamma receptor polymorphisms in populations in Ethiopia and Norway. Immunology 2001 Sep;104(1):87-91.
(128) Weng WK, Czerwinski D, Levy R. Humoral immune response and immunoglobulin G Fc receptor genotype are associated with better clinical outcome following idiotype vaccination in follicular lymphoma patients regardless of their response to induction chemotherapy. Blood 2007 Feb 1;109(3):951-3.
(129) Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003 Nov 1;21(21):3940-7.
(130) Hatjiharissi E, Xu L, Santos DD, Hunter ZR, Ciccarelli BT, Verselis S, et al. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism. Blood 2007 Oct 1;110(7):2561-4.
(131) Smalls-Mantey A, Doria-Rose N, Klein R, Patamawenu A, Migueles SA, Ko SY, et al. Antibody-dependent cellular cytotoxicity against primary HIV-infected CD4+ T cells is directly associated with the magnitude of surface IgG binding. J Virol 2012 Aug;86(16):8672-80.
(132) Kohl S. Role of antibody-dependent cellular cytotoxicity in defense against herpes simplex virus infections. Rev Infect Dis 1991 Jan;13(1):108-14.
(133) Hashimoto G, Wright PF, Karzon DT. Antibody-dependent cell-mediated cytotoxicity against influenza virus-infected cells. J Infect Dis 1983 Nov;148(5):785-94.
(134) Jiang W, Johnson C, Jayaraman J, Simecek N, Noble J, Moffatt MF, et al. Copy number variation leads to considerable diversity for B but not A haplotypes of the human KIR genes encoding NK cell receptors. Genome Res 2012 Oct;22(10):1845-54.
(135) McQueen KL, Wilhelm BT, Harden KD, Mager DL. Evolution of NK receptors: a single Ly49 and multiple KIR genes in the cow. Eur J Immunol 2002 Mar;32(3):810-7.
(136) Storset AK, Slettedal IO, Williams JL, Law A, Dissen E. Natural killer cell receptors in cattle: a bovine killer cell immunoglobulin-like receptor multigene family contains members with divergent signaling motifs. Eur J Immunol 2003 Apr;33(4):980-90.
(137) Malhotra U, Holte S, Dutta S, Berrey MM, Delpit E, Koelle DM, et al. Role for HLA class II molecules in HIV-1 suppression and cellular immunity following antiretroviral treatment. J Clin Invest 2001 Feb;107(4):505-17.

(138) Martin AM, Freitas EM, Witt CS, Christiansen FT. The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. Immunogenetics 2000 Apr;51(4-5):268-80.
(139) Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. Proc Natl Acad Sci U S A 2000 Apr 25;97(9):4778-83.
(140) Pascal V, Yamada E, Martin MP, Alter G, Altfeld M, Metcalf JA, et al. Detection of KIR3DS1 on the cell surface of peripheral blood NK cells facilitates identification of a novel null allele and assessment of KIR3DS1 expression during HIV-1 infection. J Immunol 2007 Aug 1;179(3):1625-33.
(141) Gardiner CM. Killer cell immunoglobulin-like receptors on NK cells: the how, where and why. Int J Immunogenet 2008 Feb;35(1):1-8.
(142) Peterson ME, Long EO. Inhibitory receptor signaling via tyrosine phosphorylation of the adaptor Crk. Immunity 2008 Oct 17;29(4):578-88.
(143) Lanier LL, Corliss BC, Wu J, Leong C, Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature 1998 Feb 12;391(6668):703-7.
(144) Bryceson YT, Chiang SC, Darmanin S, Fauriat C, Schlums H, Theorell J, et al. Molecular mechanisms of natural killer cell activation. J Innate Immun 2011;3(3):216-26.
(145) Hoglund P, Brodin P. Current perspectives of natural killer cell education by MHC class I molecules. Nat Rev Immunol 2010 Oct;10(10):724-34.
(146) Litwin V, Gumperz J, Parham P, Phillips JH, Lanier LL. NKB1: a natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules. J Exp Med 1994 Aug 1;180(2):537-43.
(147) D'Andrea A, Chang C, Franz-Bacon K, McClanahan T, Phillips JH, Lanier LL. Molecular cloning of NKB1. A natural killer cell receptor for HLA-B allotypes. J Immunol 1995 Sep 1;155(5):2306-10.
(148) Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J Exp Med 1995 Mar 1;181(3):1133-44.
(149) Wan AM, Ennis P, Parham P, Holmes N. The primary structure of HLA-A32 suggests a region involved in formation of the Bw4/Bw6 epitopes. J Immunol 1986 Dec 1;137(11):3671-4.
(150) Lutz CT, Smith KD, Greazel NS, Mace BE, Jensen DA, McCutcheon JA, et al. Bw4-reactive and Bw6-reactive antibodies recognize multiple distinct HLA structures that partially overlap in the alpha-1 helix. J Immunol 1994 Nov 1;153(9):4099-110.
(151) Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M. NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. J Exp Med 1994 Oct 1;180(4):1235-42.
(152) Luque I, Solana R, Galiani MD, Gonzalez R, Garcia F, Lopez de Castro JA, et al. Threonine 80 on HLA-B27 confers protection against lysis by a group of natural killer clones. Eur J Immunol 1996 Aug;26(8):1974-7.
(153) Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, et al. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. J Immunol 1997 Jun 1;158(11):5237-41.

(154) Vivian JP, Duncan RC, Berry R, O'Connor GM, Reid HH, Beddoe T, et al. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. Nature 2011 Nov 17;479(7373):401-5.
(155) Gardiner CM, Guethlein LA, Shilling HG, Pando M, Carr WH, Rajalingam R, et al. Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism. J Immunol 2001 Mar 1;166(5):2992-3001.
(156) Middleton D, Meenagh A, Gourraud PA. KIR haplotype content at the allele level in 77 Northern Irish families. Immunogenetics 2007 Feb;59(2):145-58.
(157) Pando MJ, Gardiner CM, Gleimer M, McQueen KL, Parham P. The protein made from a common allele of KIR3DL1 (3DL1*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. J Immunol 2003 Dec 15;171(12):6640-9.
(158) Yawata M, Yawata N, Draghi M, Little AM, Partheniou F, Parham P. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. J Exp Med 2006 Mar 20;203(3):633-45.
(159) Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, et al. Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol 2007 Jan 1;178(1):33-7.
(160) Taner SB, Pando MJ, Roberts A, Schellekens J, Marsh SG, Malmberg KJ, et al. Interactions of NK cell receptor KIR3DL1*004 with chaperones and conformation-specific antibody reveal a functional folded state as well as predominant intracellular retention. J Immunol 2011 Jan 1;186(1):62-72.
(161) Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, et al. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 1993 Aug 1;178(2):597-604.
(162) Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity 1997 Dec;7(6):753-63.
(163) Fan QR, Mosyak L, Winter CC, Wagtmann N, Long EO, Wiley DC. Structure of the inhibitory receptor for human natural killer cells resembles haematopoietic receptors. Nature 1997 Sep 4;389(6646):96-100.
(164) Snyder GA, Brooks AG, Sun PD. Crystal structure of the HLA-Cw3 allotype-specific killer cell inhibitory receptor KIR2DL2. Proc Natl Acad Sci U S A 1999 Mar 30;96(7):3864-9.
(165) Maenaka K, Juji T, Stuart DI, Jones EY. Crystal structure of the human p58 killer cell inhibitory receptor (KIR2DL3) specific for HLA-Cw3-related MHC class I. Structure 1999 Apr 15;7(4):391-8.
(166) Boyington JC, Brooks AG, Sun PD. Structure of killer cell immunoglobulin-like receptors and their recognition of the class I MHC molecules. Immunol Rev 2001 Jun;181:66-78.
(167) Colonna M, Borsellino G, Falco M, Ferrara GB, Strominger JL. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. Proc Natl Acad Sci U S A 1993 Dec 15;90(24):12000-4.
(168) Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati MS, Vitale M, et al. Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity 1995 May;2(5):439-49.

(169) Raulet DH, Vance RE, McMahon CW. Regulation of the natural killer cell receptor repertoire. Annu Rev Immunol 2001;19:291-330.
(170) Santourlidis S, Trompeter HI, Weinhold S, Eisermann B, Meyer KL, Wernet P, et al. Crucial role of DNA methylation in determination of clonally distributed killer cell Ig-like receptor expression patterns in NK cells. J Immunol 2002 Oct 15;169(8):4253-61.
(171) Chan HW, Kurago ZB, Stewart CA, Wilson MJ, Martin MP, Mace BE, et al. DNA methylation maintains allele-specific KIR gene expression in human natural killer cells. J Exp Med 2003 Jan 20;197(2):245-55.
(172) Watzl C, Stebbins CC, Long EO. NK cell inhibitory receptors prevent tyrosine phosphorylation of the activation receptor 2B4 (CD244). J Immunol 2000 Oct 1;165(7):3545-8.
(173) Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature 1986 Feb 20;319(6055):675-8.
(174) Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 1990 Jul;11(7):237-44.
(175) Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999 Jul 30;285(5428):727-9.
(176) Zafirova B, Wensveen FM, Gulin M, Polic B. Regulation of immune cell function and differentiation by the NKG2D receptor. Cell Mol Life Sci 2011 Nov;68(21):3519-29.
(177) Vitale M, Bottino C, Sivori S, Sanseverino L, Castriconi R, Marcenaro E, et al. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. J Exp Med 1998 Jun 15;187(12):2065-72.
(178) Bix M, Liao NS, Zijlstra M, Loring J, Jaenisch R, Raulet D. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. Nature 1991 Jan 24;349(6307):329-31.
(179) Hoglund P, Ohlen C, Carbone E, Franksson L, Ljunggren HG, Latour A, et al. Recognition of beta 2-microglobulin-negative (beta 2m-) T-cell blasts by natural killer cells from normal but not from beta 2m- mice: nonresponsiveness controlled by beta 2m- bone marrow in chimeric mice. Proc Natl Acad Sci U S A 1991 Nov 15;88(22):10332-6.
(180) Vitale M, Zimmer J, Castriconi R, Hanau D, Donato L, Bottino C, et al. Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor(s) that prevent lysis of normal autologous cells. Blood 2002 Mar 1;99(5):1723-9.
(181) Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood 2005 Jun 1;105(11):4416-23.
(182) Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, et al. Human NK cell education by inhibitory receptors for MHC class I. Immunity 2006 Aug;25(2):331-42.
(183) Furukawa H, Yabe T, Watanabe K, Miyamoto R, Miki A, Akaza T, et al. Tolerance of NK and LAK activity for HLA class I-deficient targets in a TAP1-deficient patient (bare lymphocyte syndrome type I). Hum Immunol 1999 Jan;60(1):32-40.

(184) Zimmer J, Donato L, Hanau D, Cazenave JP, Tongio MM, Moretta A, et al. Activity and phenotype of natural killer cells in peptide transporter (TAP)-deficient patients (type I bare lymphocyte syndrome). J Exp Med 1998 Jan 5;187(1):117-22.
(185) Held W, Dorfman JR, Wu MF, Raulet DH. Major histocompatibility complex class I-dependent skewing of the natural killer cell Ly49 receptor repertoire. Eur J Immunol 1996 Oct;26(10):2286-92.
(186) Salcedo M, Diehl AD, Olsson-Alheim MY, Sundback J, Van KL, Karre K, et al. Altered expression of Ly49 inhibitory receptors on natural killer cells from MHC class I-deficient mice. J Immunol 1997 Apr 1;158(7):3174-80.
(187) Liao NS, Bix M, Zijlstra M, Jaenisch R, Raulet D. MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. Science 1991 Jul 12;253(5016):199-202.
(188) Belanger S, Tu MM, Rahim MM, Mahmoud AB, Patel R, Tai LH, et al. Impaired natural killer cell self-education and "missing-self" responses in Ly49-deficient mice. Blood 2012 Jul 19;120(3):592-602.
(189) Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 2005 Aug 4;436(7051):709-13.
(190) Yu J, Heller G, Chewning J, Kim S, Yokoyama WM, Hsu KC. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. J Immunol 2007 Nov 1;179(9):5977-89.
(191) Kim S, Sunwoo JB, Yang L, Choi T, Song YJ, French AR, et al. HLA alleles determine differences in human natural killer cell responsiveness and potency. Proc Natl Acad Sci U S A 2008 Feb 26;105(8):3053-8.
(192) Joncker NT, Fernandez NC, Treiner E, Vivier E, Raulet DH. NK cell responsiveness is tuned commensurate with the number of inhibitory receptors for self-MHC class I: the rheostat model. J Immunol 2009 Apr 15;182(8):4572-80.
(193) Brodin P, Karre K, Hoglund P. NK cell education: not an on-off switch but a tunable rheostat. Trends Immunol 2009 Apr;30(4):143-9.
(194) Brodin P, Lakshmikanth T, Johansson S, Karre K, Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. Blood 2009 Mar 12;113(11):2434-41.
(195) Kamya P, Boulet S, Tsoukas CM, Routy JP, Thomas R, Cote P, et al. Receptor-ligand requirements for increased NK cell polyfunctional potential in slow progressors infected with HIV-1 coexpressing KIR3DL1*h/*y and HLA-B*57. J Virol 2011 Jun;85(12):5949-60.
(196) Boulet S, Song R, Kamya P, Bruneau J, Shoukry NH, Tsoukas CM, et al. HIV protective KIR3DL1 and HLA-B genotypes influence NK cell function following stimulation with HLA-devoid cells. J Immunol 2010 Feb 15;184(4):2057-64.
(197) Johansson S, Johansson M, Rosmaraki E, Vahlne G, Mehr R, Salmon-Divon M, et al. Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. J Exp Med 2005 Apr 4;201(7):1145-55.
(198) Joncker NT, Shifrin N, Delebecque F, Raulet DH. Mature natural killer cells reset their responsiveness when exposed to an altered MHC environment. J Exp Med 2010 Sep 27;207(10):2065-72.

(199) Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, et al. NK cells and cancer. J Immunol 2007 Apr 1;178(7):4011-6.
(200) Whiteside TL, Herberman RB. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol 1994 Mar;1(2):125-33.
(201) Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res 2009 May 1;69(9):4010-7.
(202) Hoglund P, Ljunggren HG, Ohlen C, Ahrlund-Richter L, Scangos G, Bieberich C, et al. Natural resistance against lymphoma grafts conveyed by H-2Dd transgene to C57BL mice. J Exp Med 1988 Oct 1;168(4):1469-74.
(203) Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 1999;17:189-220.
(204) Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med 1989 Jun 29;320(26):1731-5.
(205) Ballas ZK, Turner JM, Turner DA, Goetzman EA, Kemp JD. A patient with simultaneous absence of "classical" natural killer cells (CD3-, CD16+, and NKH1+) and expansion of CD3+, CD4-, CD8-, NKH1+ subset. J Allergy Clin Immunol 1990 Feb;85(2):453-9.
(206) Pietra G, Semino C, Cagnoni F, Boni L, Cangemi G, Frumento G, et al. Natural killer cells lyse autologous herpes simplex virus infected targets using cytolytic mechanisms distributed clonotypically. J Med Virol 2000 Nov;62(3):354-63.
(207) Fang M, Lanier LL, Sigal LJ. A role for NKG2D in NK cell-mediated resistance to poxvirus disease. PLoS Pathog 2008 Feb 8;4(2):e30.
(208) Parker AK, Parker S, Yokoyama WM, Corbett JA, Buller RM. Induction of natural killer cell responses by ectromelia virus controls infection. J Virol 2007 Apr;81(8):4070-9.
(209) Martinez J, Huang X, Yang Y. Direct action of type I IFN on NK cells is required for their activation in response to vaccinia viral infection in vivo. J Immunol 2008 Feb 1;180(3):1592-7.
(210) Aranda-Romo S, Garcia-Sepulveda CA, Comas-Garcia A, Lovato-Salas F, Salgado-Bustamante M, Gomez-Gomez A, et al. Killer-cell immunoglobulin-like receptors (KIR) in severe A (H1N1) 2009 influenza infections. Immunogenetics 2012 Sep;64(9):653-62.
(211) Revilleza MJ, Wang R, Mans J, Hong M, Natarajan K, Margulies DH. How the virus outsmarts the host: function and structure of cytomegalovirus MHC-I-like molecules in the evasion of natural killer cell surveillance. J Biomed Biotechnol 2011;2011:724607.
(212) Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. Science 2002 May 17;296(5571):1323-6.
(213) Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 2001 Feb;14(2):123-33.
(214) Kuijpers TW, Baars PA, Dantin C, van den Burg M, van Lier RA, Roosnek E. Human NK cells can control CMV infection in the absence of T cells. Blood 2008 Aug 1;112(3):914-5.

(215) Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science 2004 Aug 6;305(5685):872-4.
(216) Knapp S, Warshow U, Hegazy D, Brackenbury L, Guha IN, Fowell A, et al. Consistent beneficial effects of killer cell immunoglobulin-like receptor 2DL3 and group 1 human leukocyte antigen-C following exposure to hepatitis C virus. Hepatology 2010 Apr;51(4):1168-75.
(217) Boulet S, Sharafi S, Simic N, Bruneau J, Routy JP, Tsoukas CM, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS 2008 Mar 12;22(5):595-9.
(218) Jawahar S, Moody C, Chan M, Finberg R, Geha R, Chatila T. Natural Killer (NK) cell deficiency associated with an epitope-deficient Fc receptor type IIIA (CD16-II). Clin Exp Immunol 1996 Mar;103(3):408-13.
(219) Eidenschenk C, Dunne J, Jouanguy E, Fourlinnie C, Gineau L, Bacq D, et al. A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. Am J Hum Genet 2006 Apr;78(4):721-7.
(220) Scott-Algara D, Truong LX, Versmisse P, David A, Luong TT, Nguyen NV, et al. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. J Immunol 2003 Dec 1;171(11):5663-7.
(221) Naranbhai V, Altfeld M, Abdool KQ, Ndung'u T, Abdool Karim SS, Carr WH. Natural killer cell function in women at high risk for HIV acquisition: insights from a microbicide trial. AIDS 2012 Sep 10;26(14):1745-53.
(222) Qi Y, Martin MP, Gao X, Jacobson L, Goedert JJ, Buchbinder S, et al. KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. PLoS Pathog 2006 Aug;2(8):e79.
(223) Gaudieri S, DeSantis D, McKinnon E, Moore C, Nolan D, Witt CS, et al. Killer immunoglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression. Genes Immun 2005 Dec;6(8):683-90.
(224) Alter G, Rihn S, Walter K, Nolting A, Martin M, Rosenberg ES, et al. HLA class I subtype-dependent expansion of KIR3DS1+ and KIR3DL1+ NK cells during acute human immunodeficiency virus type 1 infection. J Virol 2009 Jul;83(13):6798-805.
(225) Carr WH, Rosen DB, Arase H, Nixon DF, Michaelsson J, Lanier LL. Cutting Edge: KIR3DS1, a gene implicated in resistance to progression to AIDS, encodes a DAP12-associated receptor expressed on NK cells that triggers NK cell activation. J Immunol 2007 Jan 15;178(2):647-51.
(226) O'Connor GM, Guinan KJ, Cunningham RT, Middleton D, Parham P, Gardiner CM. Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. J Immunol 2007 Jan 1;178(1):235-41.
(227) Gillespie GM, Bashirova A, Dong T, McVicar DW, Rowland-Jones SL, Carrington M. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8+ T cell epitopes. AIDS Res Hum Retroviruses 2007 Mar;23(3):451-5.
(228) Lopez-Vazquez A, Mina-Blanco A, Martinez-Borra J, Njobvu PD, Suarez-Alvarez B, Blanco-Gelaz MA, et al. Interaction between KIR3DL1 and HLA-B*57 supertype alleles influences the progression of HIV-1 infection in a Zambian population. Hum Immunol 2005 Mar;66(3):285-9.

(229) Barbour JD, Sriram U, Caillier SJ, Levy JA, Hecht FM, Oksenberg JR. Synergy or independence? Deciphering the interaction of HLA Class I and NK cell KIR alleles in early HIV-1 disease progression. PLoS Pathog 2007 Apr;3(4):e43.
(230) Boulet S, Kleyman M, Kim JY, Kamya P, Sharafi S, Simic N, et al. A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS 2008 Jul 31;22(12):1487-91.
(231) Hilton HG, Vago L, Older Aguilar AM, Moesta AK, Graef T, Abi-Rached L, et al. Mutation at positively selected positions in the binding site for HLA-C shows that KIR2DL1 is a more refined but less adaptable NK cell receptor than KIR2DL3. J Immunol 2012 Aug 1;189(3):1418-30.
(232) Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, Parham P. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. J Immunol 2008 Mar 15;180(6):3969-79.
(233) Thomas R, Apps R, Qi Y, Gao X, Male V, O'hUigin C, et al. HLA-C cell surface expression and control of HIV/AIDS correlate with a variant upstream of HLA-C. Nat Genet 2009 Dec;41(12):1290-4.
(234) Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. Science 2007 Aug 17;317(5840):944-7.
(235) Kulkarni S, Savan R, Qi Y, Gao X, Yuki Y, Bass SE, et al. Differential microRNA regulation of HLA-C expression and its association with HIV control. Nature 2011 Apr 28;472(7344):495-8.
(236) Charoudeh HN, Schmied L, Gonzalez A, Terszowski G, Czaja K, Schmitter K, et al. Quantity of HLA-C surface expression and licensing of KIR2DL+ natural killer cells. Immunogenetics 2012 Oct;64(10):739-45.
(237) Alter G, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, et al. HIV-1 adaptation to NK-cell-mediated immune pressure. Nature 2011 Aug 4;476(7358):96-100.
(238) Zuniga J, Romero V, Azocar J, Terreros D, Vargas-Rojas MI, Torres-Garcia D, et al. Protective KIR-HLA interactions for HCV infection in intravenous drug users. Mol Immunol 2009 Aug;46(13):2723-7.
(239) Seich Al Basatena NK, Macnamara A, Vine AM, Thio CL, Astemborski J, Usuku K, et al. KIR2DL2 enhances protective and detrimental HLA class I-mediated immunity in chronic viral infection. PLoS Pathog 2011 Oct;7(10):e1002270.
(240) Ravet S, Scott-Algara D, Bonnet E, Tran HK, Tran T, Nguyen N, et al. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. Blood 2007 May 15;109(10):4296-305.
(241) Pollara J, Hart L, Brewer F, Pickeral J, Packard BZ, Hoxie JA, et al. High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. Cytometry 2011;79A(8):603-12.
(242) Alter G, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med 2007 Nov 26;204(12):3027-36.
(243) Peters PJ, Borst J, Oorschot V, Fukuda M, Krahenbuhl O, Tschopp J, et al. Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. J Exp Med 1991 May 1;173(5):1099-109.
(244) Betts MR, Brenchley JM, Price DA, De Rosa SC, Douek DC, Roederer M, et al. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow

cytometric assay for degranulation. J Immunol Methods 2003 Oct 1;281(1-2):65-78.
(245) Bryceson YT, March ME, Barber DF, Ljunggren HG, Long EO. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. J Exp Med 2005 Oct 3;202(7):1001-12.
(246) Povolotsky J, Gold JW, Darminin P, Chein N, Baron P, Armstrong D. An ELISA-inhibition assay for antibody to human immunodeficiency virus core antigen (p24). J Virol Methods 1990 Oct;30(1):109-14.
(247) Davis ZB, Ward JP, Barker E. Preparation and use of HIV-1 infected primary CD4+ T-cells as target cells in natural killer cell cytotoxic assays. J Vis Exp 2011;(49).
(248) Trundley A, Frebel H, Jones D, Chang C, Trowsdale J. Allelic expression patterns of KIR3DS1 and 3DL1 using the Z27 and DX9 antibodies. Eur J Immunol 2007 Mar;37(3):780-7.
(249) Pelak K, Need AC, Fellay J, Shianna KV, Feng S, Urban TJ, et al. Copy number variation of KIR genes influences HIV-1 control. PLoS Biol 2011 Nov;9(11):e1001208.
(250) Parsons MS, Boulet S, Song R, Bruneau J, Shoukry NH, Routy JP, et al. Mind the gap: lack of association between KIR3DL1*004/HLA-Bw4-induced natural killer cell function and protection from HIV infection. J Infect Dis 2010 Nov 1;202 Suppl 3:S356-S360.
(251) Parsons MS, Zipperlen K, Gallant M, Grant M. Killer cell immunoglobulin-like receptor 3DL1 licenses CD16-mediated effector functions of natural killer cells. J Leukoc Biol 2010 Nov;88(5):905-12.
(252) Parsons MS, Wren L, Isitman G, Navis M, Stratov I, Bernard NF, et al. HIV infection abrogates the functional advantage of natural killer cells educated through KIR3DL1/HLA-Bw4 interactions to mediate anti-HIV antibody-dependent cellular cytotoxicity. J Virol 2012 Apr;86(8):4488-95.
(253) Meyers AF, Fowke KR. International symposium on natural immunity to HIV: a gathering of the HIV-exposed seronegative clan. J Infect Dis 2010 Nov 1;202 Suppl 3:S327-S328.
(254) Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. Immunity 2007 Sep;27(3):406-16.
(255) Horton RE, McLaren PJ, Fowke K, Kimani J, Ball TB. Cohorts for the Study of HIV-1 Exposed but Uninfected Individuals: Benefits and Limitations. Journal of Infectious Diseases 2010 Nov 1;202(Supplement 3):S377-S381.
(256) Shacklett B. Understanding the "lucky few": The conundrum of HIV-exposed, seronegative individuals. Current Infectious Disease Reports 2006 Jun 1;8(3):248-53.
(257) Miyazawa M, Lopalco L, Mazzotta F, Lo CS, Veas F, Clerici M. The 'immunologic advantage' of HIV-exposed seronegative individuals. AIDS 2009 Jan 14;23(2):161-75.
(258) Piacentini L, Biasin M, Fenizia C, Clerici M. Genetic correlates of protection against HIV infection: the ally within. J Intern Med 2009 Jan;265(1):110-24.
(259) Tomescu C, Duh FM, Lanier MA, Kapalko A, Mounzer KC, Martin MP, et al. Increased plasmacytoid dendritic cell maturation and natural killer cell activation in HIV-1 exposed, uninfected intravenous drug users. AIDS 2010 Sep 10;24(14):2151-60.

(260) Tiemessen CT, Shalekoff S, Meddows-Taylor S, Schramm DB, Papathanasopoulos MA, Gray GE, et al. Cutting Edge: Unusual NK cell responses to HIV-1 peptides are associated with protection against maternal-infant transmission of HIV-1. J Immunol 2009 May 15;182(10):5914-8.
(261) Montoya CJ, Velilla PA, Chougnet C, Landay AL, Rugeles MT. Increased IFN-gamma production by NK and CD3+/CD56+ cells in sexually HIV-1-exposed but uninfected individuals. Clin Immunol 2006 Aug;120(2):138-46.
(262) Okulicz JF, Marconi VC, Landrum ML, Wegner S, Weintrob A, Ganesan A, et al. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. J Infect Dis 2009 Dec 1;200(11):1714-23.
(263) Mandalia S, Westrop SJ, Beck EJ, Nelson M, Gazzard BG, Imami N. Are long-term non-progressors very slow progressors? Insights from the Chelsea and Westminster HIV cohort, 1988-2010. PLoS ONE 2012;7(2):e29844.
(264) Kiepiela P, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, Moodley E, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med 2007 Jan;13(1):46-53.
(265) Klein MR, van der Burg SH, Hovenkamp E, Holwerda AM, Drijfhout JW, Melief CJ, et al. Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. J Gen Virol 1998 Sep;79 (Pt 9):2191-201.
(266) Pereyra F, Jia X, McLaren PJ, Telenti A, de Bakker PI, Walker BD, et al. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science 2010 Dec 10;330(6010):1551-7.
(267) Brackenridge S, Evans EJ, Toebes M, Goonetilleke N, Liu MK, di GK, et al. An early HIV mutation within an HLA-B*57-restricted T cell epitope abrogates binding to the killer inhibitory receptor 3DL1. J Virol 2011 Jun;85(11):5415-22.
(268) Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity 2008 Dec 19;29(6):1009-21.
(269) Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, Cirulli ET, et al. Common genetic variation and the control of HIV-1 in humans. PLoS Genet 2009 Dec;5(12):e1000791.
(270) O'Connor GM, Holmes A, Mulcahy F, Gardiner CM. Natural Killer cells from long-term non-progressor HIV patients are characterized by altered phenotype and function. Clin Immunol 2007 Sep;124(3):277-83.
(271) Vieillard V, Fausther-Bovendo H, Samri A, Debre P. Specific phenotypic and functional features of natural killer cells from HIV-infected long-term nonprogressors and HIV controllers. J Acquir Immune Defic Syndr 2010 Apr;53(5):564-73.
(272) Alter G, Malenfant JM, Delabre RM, Burgett NC, Yu XG, Lichterfeld M, et al. Increased natural killer cell activity in viremic HIV-1 infection. J Immunol 2004 Oct 15;173(8):5305-11.
(273) Brunetta E, Hudspeth KL, Mavilio D. Pathologic natural killer cell subset redistribution in HIV-1 infection: new insights in pathophysiology and clinical outcomes. J Leukoc Biol 2010 Dec;88(6):1119-30.
(274) Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study):

a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008 Nov 29;372(9653):1881-93.
(275) Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 2005 Mar 1;191(5):654-65.
(276) Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van GF, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 2006 Dec 15;194(12):1661-71.
(277) Gilbert PB, Ackers ML, Berman PW, Francis DP, Popovic V, Hu DJ, et al. HIV-1 virologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. J Infect Dis 2005 Sep 15;192(6):974-83.
(278) Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 2005 Mar 1;191(5):666-77.
(279) Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009 Dec 3;361(23):2209-20.
(280) Rolland M, Tovanabutra S, Decamp AC, Frahm N, Gilbert PB, Sanders-Buell E, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nat Med 2011 Mar;17(3):366-71.
(281) Karnasuta C, Paris RM, Cox JH, Nitayaphan S, Pitisuttithum P, Thongcharoen P, et al. Antibody-dependent cell-mediated cytotoxic responses in participants enrolled in a phase I/II ALVAC-HIV/AIDSVAX B/E prime-boost HIV-1 vaccine trial in Thailand. Vaccine 2005 Mar 31;23(19):2522-9.
(282) Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 2012 Apr 5;366(14):1275-86.
(283) Chung AW, Isitman G, Navis M, Kramski M, Center RJ, Kent SJ, et al. Immune escape from HIV-specific antibody-dependent cellular cytotoxicity (ADCC) pressure. Proceedings of the National Academy of Sciences 2011 Apr 18.
(284) Sawyer LA, Katzenstein DA, Hendry RM, Boone EJ, Vujcic LK, Williams CC, et al. Possible beneficial effects of neutralizing antibodies and antibody-dependent, cell-mediated cytotoxicity in human immunodeficiency virus infection. AIDS Res Hum Retroviruses 1990 Mar;6(3):341-56.
(285) Alpert MD, Harvey JD, Lauer WA, Reeves RK, Piatak M, Jr., Carville A, et al. ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protection against SIV(mac)251 challenge. PLoS Pathog 2012 Aug;8(8):e1002890.
(286) Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007 May 15;178(10):6596-603.
(287) Connick E, Marr DG, Zhang XQ, Clark SJ, Saag MS, Schooley RT, et al. HIV-specific cellular and humoral immune responses in primary HIV infection. AIDS Res Hum Retroviruses 1996 Aug 10;12(12):1129-40.
(288) Ojo-Amaize EA, Nishanian P, Keith DE, Jr., Houghton RL, Heitjan DF, Fahey JL, et al. Antibodies to human immunodeficiency virus in human sera induce cell-

mediated lysis of human immunodeficiency virus-infected cells. J Immunol 1987 Oct 1;139(7):2458-63.
(289) Forthal DN, Moog C. Fc receptor-mediated antiviral antibodies. Curr Opin HIV AIDS 2009 Sep;4(5):388-93.
(290) Forthal DN, Landucci G, Daar ES. Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. J Virol 2001 Aug;75(15):6953-61.
(291) Forthal DN, Landucci G, Keenan B. Relationship between antibody-dependent cellular cytotoxicity, plasma HIV type 1 RNA, and CD4+ lymphocyte count. AIDS Res Hum Retroviruses 2001 Apr 10;17(6):553-61.
(292) Brenner BG, Gryllis C, Wainberg MA. Role of antibody-dependent cellular cytotoxicity and lymphokine-activated killer cells in AIDS and related diseases. J Leukoc Biol 1991 Dec;50(6):628-40.
(293) Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, Rinaldo C, et al. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. J Immunol 1996 Sep 1;157(5):2168-73.
(294) Ahmad R, Sindhu ST, Toma E, Morisset R, Vincelette J, Menezes J, et al. Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. J Clin Immunol 2001 May;21(3):227-33.
(295) Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 2005 Feb 15;174(4):2185-9.
(296) Hessell AJ, Rakasz EG, Poignard P, Hangartner L, Landucci G, Forthal DN, et al. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog 2009 May;5(5):e1000433.
(297) Banks ND, Kinsey N, Clements J, Hildreth JE. Sustained antibody-dependent cell-mediated cytotoxicity (ADCC) in SIV-infected macaques correlates with delayed progression to AIDS. AIDS Res Hum Retroviruses 2002 Nov 1;18(16):1197-205.
(298) Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, et al. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. Nature 2012 Feb 2;482(7383):89-93.
(299) Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, Parks RJ, et al. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. AIDS 2009 May 15;23(8):897-906.
(300) Congy-Jolivet N, Bolzec A, Ternant D, Ohresser M, Watier H, Thibault G. Fc gamma RIIIa expression is not increased on natural killer cells expressing the Fc gamma RIIIa-158V allotype. Cancer Res 2008 Feb 15;68(4):976-80.
(301) Lehrnbecher TL, Foster CB, Zhu S, Venzon D, Steinberg SM, Wyvill K, et al. Variant genotypes of FcgammaRIIIA influence the development of Kaposi's sarcoma in HIV-infected men. Blood 2000 Apr 1;95(7):2386-90.
(302) Brown BK, Wieczorek L, Kijak G, Lombardi K, Currier J, Wesberry M, et al. The role of natural killer (NK) cells and NK cell receptor polymorphisms in the assessment of HIV-1 neutralization. PLoS ONE 2012;7(4):e29454.
(303) Bonsignori M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, et al. Antibody-dependent cellular cytotoxicity-mediating antibodies from an HIV-1

vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 2012 Nov;86(21):11521-32.
(304) Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaelsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. Blood 2010 Feb 11;115(6):1166-74.
(305) Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol 2003 Apr;77(8):4911-27.
(306) Harari A, Vallelian F, Meylan PR, Pantaleo G. Functional heterogeneity of memory CD4 T cell responses in different conditions of antigen exposure and persistence. J Immunol 2005 Jan 15;174(2):1037-45.
(307) Fuller MJ, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. J Immunol 2004 Apr 1;172(7):4204-14.
(308) Fuller MJ, Zajac AJ. Ablation of CD8 and CD4 T cell responses by high viral loads. J Immunol 2003 Jan 1;170(1):477-86.
(309) Bolanos FD, Tripathy SK. Activation receptor-induced tolerance of mature NK cells in vivo requires signaling through the receptor and is reversible. J Immunol 2011 Mar 1;186(5):2765-71.
(310) Tripathy SK, Keyel PA, Yang L, Pingel JT, Cheng TP, Schneeberger A, et al. Continuous engagement of a self-specific activation receptor induces NK cell tolerance. J Exp Med 2008 Aug 4;205(8):1829-41.
(311) Coudert JD, Scarpellino L, Gros F, Vivier E, Held W. Sustained NKG2D engagement induces cross-tolerance of multiple distinct NK cell activation pathways. Blood 2008 Apr 1;111(7):3571-8.
(312) Coudert JD, Zimmer J, Tomasello E, Cebecauer M, Colonna M, Vivier E, et al. Altered NKG2D function in NK cells induced by chronic exposure to NKG2D ligand-expressing tumor cells. Blood 2005 Sep 1;106(5):1711-7.
(313) Sun JC, Lanier LL. Tolerance of NK cells encountering their viral ligand during development. J Exp Med 2008 Aug 4;205(8):1819-28.
(314) Oppenheim DE, Roberts SJ, Clarke SL, Filler R, Lewis JM, Tigelaar RE, et al. Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. Nat Immunol 2005 Sep;6(9):928-37.
(315) Bowles JA, Wang SY, Link BK, Allan B, Beuerlein G, Campbell MA, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. Blood 2006 Oct 15;108(8):2648-54.
(316) He X, Li D, Luo Z, Liang H, Peng H, Zhao Y, et al. Compromised NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity in Chronic SIV/SHIV Infection. PLoS ONE 2013;8(2):e56309.
(317) Liu Q, Sun Y, Rihn S, Nolting A, Tsoukas PN, Jost S, et al. Matrix metalloprotease inhibitors restore impaired NK cell-mediated antibody-dependent cellular cytotoxicity in human immunodeficiency virus type 1 infection. J Virol 2009 Sep;83(17):8705-12.
(318) Kamya P, Tallon B, Melendez-Pena C, Parsons MS, Migueles SA, Connors M, et al. Inhibitory Killer Immunoglobulin-like Receptors to self HLA-B and HLA-C ligands contribute differentially to Natural Killer cell functional potential in HIV infected slow progressors. Clin Immunol 2012 Jun;143(3):246-55.

(319) Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, Strominger JL, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity 1999 Jun;10(6):661-71.
(320) Ward JP, Bonaparte MI, Barker E. HLA-C and HLA-E reduce antibody-dependent natural killer cell-mediated cytotoxicity of HIV-infected primary T cell blasts. AIDS 2004 Sep 3;18(13):1769-79.
(321) Bonaparte MI, Barker E. Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. Blood 2004 Oct 1;104(7):2087-94.
(322) Blais ME, Dong T, Rowland-Jones S. HLA-C as a mediator of natural killer and T-cell activation: spectator or key player? Immunology 2011 May;133(1):1-7.
(323) Brodin P, Lakshmikanth T, Karre K, Hoglund P. Skewing of the NK cell repertoire by MHC class I via quantitatively controlled enrichment and contraction of specific Ly49 subsets. J Immunol 2012 Mar 1;188(5):2218-26.
(324) Jennes W, Verheyden S, Demanet C, Adje-Toure CA, Vuylsteke B, Nkengasong JN, et al. Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands. J Immunol 2006 Nov 15;177(10):6588-92.
(325) Jennes W, Verheyden S, Mertens JW, Camara M, Seydi M, Dieye TN, et al. Inhibitory KIR/HLA incompatibility between sexual partners confers protection against HIV-1 transmission. Blood 2013 Feb 14;121(7):1157-64.
(326) Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. Blood 2008 Sep 15;112(6):2369-80.
(327) Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol 1998 Jul 15;161(2):571-7.
(328) Draghi M, Yawata N, Gleimer M, Yawata M, Valiante NM, Parham P. Single-cell analysis of the human NK cell response to missing self and its inhibition by HLA class I. Blood 2005 Mar 1;105(5):2028-35.
(329) Hu PF, Hultin LE, Hultin P, Hausner MA, Hirji K, Jewett A, et al. Natural killer cell immunodeficiency in HIV disease is manifest by profoundly decreased numbers of CD16+CD56+ cells and expansion of a population of CD16dim. J Acquir Immune Defic Syndr Hum Retrovirol 1995 Nov 1;10(3):331-40.
(330) Hong HS, Eberhard JM, Keudel P, Bollmann BA, Ballmaier M, Bhatnagar N, et al. HIV infection is associated with a preferential decline in less-differentiated CD56dim CD16+ NK cells. J Virol 2010 Jan;84(2):1183-8.
(331) Barker E, Martinson J, Brooks C, Landay A, Deeks S. Dysfunctional natural killer cells, in vivo, are governed by HIV viremia regardless of whether the infected individual is on antiretroviral therapy. AIDS 2007 Nov 12;21(17):2363-5.
(332) Brunetta E, Fogli M, Varchetta S, Bozzo L, Hudspeth KL, Marcenaro E, et al. The decreased expression of Siglec-7 represents an early marker of dysfunctional natural killer-cell subsets associated with high levels of HIV-1 viremia. Blood 2009 Oct 29;114(18):3822-30.
(333) Fauci AS, Mavilio D, Kottilil S. NK cells in HIV infection: paradigm for protection or targets for ambush. Nat Rev Immunol 2005 Nov;5(11):835-43.

(334) Mantegani P, Tambussi G, Galli L, Din CT, Lazzarin A, Fortis C. Perturbation of the natural killer cell compartment during primary human immunodeficiency virus 1 infection primarily involving the CD56 bright subset. Immunology 2010 Feb;129(2):220-33.
(335) Tyler DS, Stanley SD, Nastala CA, Austin AA, Bartlett JA, Stine KC, et al. Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. J Immunol 1990 May 1;144(9):3375-84.
(336) Ahmad A, Morisset R, Thomas R, Menezes J. Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. J Acquir Immune Defic Syndr 1994 May;7(5):428-37.
(337) Pollara J, Landucci G, Trac C, White AC, Yates NL, Kappes JC, et al. Early appearance of ADCC- and ADCVI-mediating antibody responses against autologous HIV-1 transmitted/founder virus. AIDS Res.Hum.Retroviruses 26[10], A-12. 2010.
(338) Chung AW, Rollman E, Center RJ, Kent SJ, Stratov I. Rapid degranulation of NK cells following activation by HIV-specific antibodies. J Immunol 2009 Jan 15;182(2):1202-10.
(339) Ahmad A, Menezes J. Antibody-dependent cellular cytotoxicity in HIV infections. FASEB J 1996 Feb;10(2):258-66.
(340) Patterson LJ, Malkevitch N, Venzon D, Pinczewski J, Gomez-Roman VR, Wang L, et al. Protection against mucosal simian immunodeficiency virus SIV(mac251) challenge by using replicating adenovirus-SIV multigene vaccine priming and subunit boosting. J Virol 2004 Mar;78(5):2212-21.
(341) Xiao P, Zhao J, Patterson LJ, Brocca-Cofano E, Venzon D, Kozlowski PA, et al. Multiple vaccine-elicited nonneutralizing antienvelope antibody activities contribute to protective efficacy by reducing both acute and chronic viremia following simian/human immunodeficiency virus SHIV89.6P challenge in rhesus macaques. J Virol 2010 Jul;84(14):7161-73.
(342) Jewett A, Cavalcanti M, Giorgi J, Bonavida B. Concomitant killing in vitro of both gp120-coated CD4+ peripheral T lymphocytes and natural killer cells in the antibody-dependent cellular cytotoxicity (ADCC) system. J Immunol 1997 Jun 1;158(11):5492-500.
(343) Johansson SE, Rollman E, Chung AW, Center RJ, Hejdeman B, Stratov I, et al. NK cell function and antibodies mediating ADCC in HIV-1-infected viremic and controller patients. Viral Immunol 2011 Oct;24(5):359-68.
(344) Dalgleish A, Sinclair A, Steel M, Beatson D, Ludlam C, Habeshaw J. Failure of ADCC to predict HIV-associated disease progression or outcome in a haemophiliac cohort. Clin Exp Immunol 1990 Jul;81(1):5-10.
(345) Khayat D, Soubrane C, Andrieu JM, Visonneau S, Eme D, Tourani JM, et al. Changes of soluble CD16 levels in serum of HIV-infected patients: correlation with clinical and biologic prognostic factors. J Infect Dis 1990 Mar;161(3):430-5.
(346) Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. J Leukoc Biol 2008 Jul;84(1):27-49.
(347) Lang P. Clinical scale isolation of T cell-depleted CD56+ donor lymphocytes in children. Pfeiffer M HRSMDBSSKTKUKSND, editor. Bone Marrow Transplant 29[6], 497-502. 2002.

(348) Fadda L, O'Connor GM, Kumar S, Piechocka-Trocha A, Gardiner CM, Carrington M, et al. Common HIV-1 peptide variants mediate differential binding of KIR3DL1 to HLA-Bw4 molecules. J Virol 2011 Jun;85(12):5970-4.
(349) Fadda L, Borhis G, Ahmed P, Cheent K, Pageon SV, Cazaly A, et al. Peptide antagonism as a mechanism for NK cell activation. Proc Natl Acad Sci U S A 2010 Jun 1;107(22):10160-5.
(350) Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 2009 Apr 16;113(16):3716-25.
(351) Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB. Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. Blood 2012 Oct 4;120(14):2836-42.
(352) Poonia B, Kijak GH, Pauza CD. High affinity allele for the gene of FCGR3A is risk factor for HIV infection and progression. PLoS ONE 2010;5(12):e15562.
(353) Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Glycosylation and the immune system. Science 2001 Mar 23;291(5512):2370-6.
(354) Jefferis R, Lund J, Pound JD. IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. Immunol Rev 1998 Jun;163:59-76.
(355) Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001;19:275-90.
(356) Okazaki A, Shoji-Hosaka E, Nakamura K, Wakitani M, Uchida K, Kakita S, et al. Fucose depletion from human IgG1 oligosaccharide enhances binding enthalpy and association rate between IgG1 and FcgammaRIIIa. J Mol Biol 2004 Mar 5;336(5):1239-49.
(357) Peipp M, Lammerts van Bueren JJ, Schneider-Merck T, Bleeker WW, Dechant M, Beyer T, et al. Antibody fucosylation differentially impacts cytotoxicity mediated by NK and PMN effector cells. Blood 2008 Sep 15;112(6):2390-9.
(358) Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 2002 Jul 26;277(30):26733-40.
(359) Moldt B, Shibata-Koyama M, Rakasz EG, Schultz N, Kanda Y, Dunlop DC, et al. A nonfucosylated variant of the anti-HIV-1 monoclonal antibody b12 has enhanced FcgammaRIIIa-mediated antiviral activity in vitro but does not improve protection against mucosal SHIV challenge in macaques. J Virol 2012 Jun;86(11):6189-96.
(360) Hwang I, Zhang T, Scott JM, Kim AR, Lee T, Kakarla T, et al. Identification of human NK cells that are deficient for signaling adaptor FcRgamma and specialized for antibody-dependent immune functions. Int Immunol 2012 Dec;24(12):793-802.
(361) Brocca-Cofano E, McKinnon K, Demberg T, Venzon D, Hidajat R, Xiao P, et al. Vaccine-elicited SIV and HIV envelope-specific IgA and IgG memory B cells in rhesus macaque peripheral blood correlate with functional antibody responses and reduced viremia. Vaccine 2011 Apr 12;29(17):3310-9.
(362) Fipps DR, Damato JJ, Brandt B, Burke DS. Effects of multiple freeze thaws and various temperatures on the reactivity of human immunodeficiency virus antibody using three detection assays. J Virol Methods 1988 Jun;20(2):127-32.
(363) Aziz N, Nishanian P, Mitsuyasu R, Detels R, Fahey JL. Variables that affect assays for plasma cytokines and soluble activation markers. Clin Diagn Lab Immunol 1999 Jan;6(1):89-95.

(364) Wang GR, Yang JY, Lin TL, Chen HY, Horng CB. Temperature effect on the sensitivity of ELISA, PA and WB to detect anti-HIV-1 antibody and infectivity of HIV-1. Zhonghua Yi Xue Za Zhi (Taipei) 1997 Jun;59(6):325-33.
(365) Laeyendecker O, Latimore A, Eshleman SH, Summerton J, Oliver AE, Gamiel J, et al. The effect of sample handling on cross sectional HIV incidence testing results. PLoS ONE 2011;6(10):e25899.