From the Department of Medicine, Huddinge
Karolinska Institutet, Stockholm, Sweden

INNATE-LIKE T CELLS IN THE IMMUNOPATHOGENESIS OF HIV-1 INFECTION

Kerri Guth Lal

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Innate-like T cells in the immunopathogenesis of HIV-1 infection

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Kerri Guth Lal

Principal Supervisor:

Professor Johan K. Sandberg
Karolinska Institutet
Department of Medicine, Huddinge
Center for Infectious Medicine

Co-supervisor:

Principal Investigator Michael A. Eller
U.S. Military HIV Research Program | HJF
Walter Reed Army Institute of Research

Opponent:

Professor Paul Klenerman
University of Oxford
Department of Clinical Medicine
Division of Experimental Medicine

Examination Board:

Professor Francesca Chiodi
Karolinska Institutet
Department of Microbiology,
Tumor, and Cell Biology

Professor Mikael Karlsson
Karolinska Institutet
Department of Microbiology,
Tumor, and Cell Biology

Professor Susanna Cardell
University of Gothenburg
Institute of Biomedicine
Department of Microbiology
and Immunology

For my family

ABSTRACT

The immunodynamics of HIV infection have been studied for over three decades in the hopes of determining underlying mechanisms that contribute to the sustained immune dysfunction observed even in individuals on therapy. Conventional and adaptive T cells are both the target of the virus and considered the main players in controlling viral burden. However, there are unconventional T cells with innate-like, rapid effector functions that are also altered in HIV infection. Among these are iNKT cells and MAIT cells, which both decline in frequency, are dysfunctional, and have an altered phenotype in the blood in chronic HIV infection. The dynamics of their loss, phenotype, and dysfunction are a major focus of this thesis. In order to study MAIT and iNKT cell dynamics in acute HIV-1 infection, the unique acute HIV infection cohort, RV217, was used with donor matched pre- and post- HIV infection blood samples in the acute infection stage. An additional study where ART is initiated in the acute infection stage, RV254, which collected both blood and rectosigmoid biopsy samples, was also used. This cohort allowed for both the study colonic MAIT and iNKT cell dynamics in acute HIV infection, as well as the impact of early ART initiation on blood and colonic iNKT cells. In **Paper I**, an optimized multiparameter immunofluorescence panel (OMIP) was generated to measure the frequency and phenotype of iNKT and MAIT cells in individuals from RV217. In **Paper II**, detailed studies of the RV217 cohort revealed that MAIT cells express markers of activation and exhaustion, and briefly expand alongside an elevated functional capacity in the acute infection stage. However, by 3 months post-infection the MAIT cell response to *E. coli* stimulation *in vitro* declined. Total transcriptome analysis showed that MAIT cells upregulate an innate-like transcriptional signature. This finding, coupled with the expansion of a subset of MAIT cells that express the NK-cell receptor CD56 and produce more of the antiviral cytokine, IFNγ, in response to innate cytokine stimulation IL-12 and IL-18 *in vitro*, suggests that MAIT cells become progressively more innate-like during the course of acute HIV infection. The OMIP was also used to study iNKT cells in acute HIV-1 infection in **Paper III**. The RV217 cohort showed that iNKT cells are preferentially depleted in the blood in acute HIV infection, particularly CD4+ iNKT cells. Remaining iNKT cells express markers of activation and become dysfunctional during acute infection. Colonic samples revealed that CD4+ iNKT cells are depleted more substantially than in the blood in the acute infection stage. The early ART treatment cohort, RV254, demonstrated that early ART initiation is able to prevent iNKT cell activation and depletion in the blood, but unable to stop their depletion in the colon during acute HIV infection. The work in **Paper IV**, shows that conventional adaptive CD8 T cells can develop an innate-like functionality mediated by the FcγRIIIA (CD16) during chronic stages of HIV infection. Expression of CD16 occurs in late-stage differentiated CD8 T cells, is associated with expression of the *IKZF2* transcription factor (Helios)

and an NKp80+IL-7Rα- surface phenotype. Interestingly, this CD8 T cell subset mediates antibody-dependent cellular cytotoxicity (ADCC) against HIV protein coated target cells at a level on par with NK cells on a per cell basis. In summary, the work in this thesis sheds new light on the behavior and responses of unconventional T cell subsets during acute HIV infection. Furthermore, the work defines a novel function of conventional adaptive CD8 T cells in chronic HIV infection. Altogether, this thesis provides new insights on the role of innate-like functions in the human T cell compartment in the immunopathogenesis of HIV-1 infection.

LIST OF SCIENTIFIC PAPERS

I. OMIP-046: Characterization of Invariant T Cell Subset Activation in Humans. **Lal KG**, Leeansyah E, Sandberg JK, Eller MA. **Cytometry A. 2018;93(5):499-503.**

II. Dynamic MAIT cell response with progressively enhanced innateness during acute HIV-1 infection. **Lal KG**, Kim D, Costanzo MC, Creegan M, Leeansyah E, Dias J, Paquin-Proulx D, Eller LA, Krebs SJ, Slike BM, Kibuuka H, Maganga L, Nitayaphan S, Sawe FK, Bolton DL, Ake JA, Michael NL, Shacklett BL, Robb ML, Eller MA, and Sandberg JK. **Nature Communications. 2020 Jan 14;11(1):272.**

III. Preferential loss of colonic CD4+ iNKT cells in early acute HIV-1 infection. Paquin-Proulx D, **Lal KG**, Creegan M, Tokarev A, Alrubayyi A, Phuang-Ngern Y, Kroon E, Slike BM, Bolton DL, Krebs SJ, Eller L, Rerknimitrv R, Chomchey N, Phanuphak N, Nitayaphan S, Michael NL, Robb ML, Ananworanich J, Sandberg JK, Eller MA, and Schuetz A. Manuscript under consideration.

IV. Terminal Effector CD8 T Cells Defined by an IKZF2+IL7R- Transcriptional Signature Express FcγRIIIA, Expand in HIV Infection, and Mediate Potent HIV-Specific Antibody-Dependent Cellular Cytotoxicity. Naluyima P, **Lal KG**, Costanzo MC, Kijak GH, Gonzalez VD, Blom K, Eller LA, Creegan M, Hong T, Quinn TC, Björkström NK, Ljunggren H, Serwadda DM, Katabira ET, Sewankambo NK, Gray RH, Baeten JM, Michael NL, Wabwire-Mangen F, RobbML, Bolton DL, Sandberg JK, Eller MA. **Journal of Immunology. 2019 Oct 15;203(8):2210-2221.**

CONTENTS

LIST OF ABBREVIATIONS

Ab	Antibody
ADCC	Antibody-dependent cellular cytoxicity
AHI	Acute HIV infection
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
ART	Antiretorviral therapy
BCR	B cell receptor
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CD1d	Cluster of differentiation 1-d
CHI	Chronic HIV infection
CRP	C-reactive protein
CT	Cycle threshold
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CXCR	CXC chemokine receptors
DC	Dendritic cell
DN	Double negative
ECHO	Early Capture HIV Cohort Study
EndoCAb	Endotoxin core antibody
Eomes	Eomesodermin
GSEA	Gene set enrichment analysis
GI	Gastrointestinal
GrzB	Granzyme B
HAND	HIV-associated neurocognitive disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
HTLV-1	Human T-lymphotropic virus-1
I-FABP	Intestinal fatty-acid binding protein
IFN	Interferon
iGb3	Isoglobotrihexosylceramide
IL	Interleukin
iNKT	Invariant natural killer T
IP-10	IFNγ inducible protein 10
KIR	Killer cell immunoglobulin-like receptors
KLRB1	Killer Cell Lectin Like Receptor B1 (CD161)

KLRG1	Killer cell lectin like receptor G1
LAG3	Lymphocyte activation gene 3
LBP	Lipopolysaccharide binding protein
LCMV	Lymphocytic choriomeningitis virus
LPS	Lipopolysaccharides
MAIT	Mucosa-associated invariant T
MAPK	Mitogen-activated protein kinase
MHC	Major histocompabability complex
MHRP	U.S. Military HIV Research Program
MIP	Macrophage inflammatory protein
MR1	Major histocompability complex class Ib-like molecule 1
MSM	Men who have sex with men
NCAM	Neural cell adhesion molecule
Nef	Negative Regulatory Factor
NHP	Nonhuman primate
NK	Natural killer
OMIP	Optimized Multicolor Immunofluorescence Panel
PAMP	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PCA	Principal component analysis
PD1	Programmed cell death protein 1
pDC	Plasmacytoid dendritic cell
PLZF	Promyelocytic leukemia zinc finger
PRR	Pattern recognition receptors
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
sCD14	Soluble CD14
SIV	Simmian immunodeficiency virus
TB	Tuberculosis
TCR	T cell receptor
TEMRA	Terminally differentiated effector memory T cell re-expressing CD45RA
TIGIT	T cell immunoreceptor with immunoglobulin and ITIM domains
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRA	T cell receptor alpha
TRB	T cell receptor beta
VL	Viral load
Vpu	Viral protein U
αGalCer	Alpha-galactosylceramide
5-OP-RU	5-(2-oxopropylideneamino)-6-D-ribitylaminouracil

1 INTRODUCTION

1.1 The innate and adaptive immune system

The human immune system works as an elegant, evolutionarily selected system to protect the body against the assault humans undergo daily from pathogens. This collection of cells, molecules, and protective physical barriers that contribute to the immune system are often categorized into two subsystems; adaptive/acquired and innate immunity. Innate immunity is the first to respond to pathogens and respond within hours of infiltration. Days to weeks later, a highly antigen specific adaptive immune response emerges. Several hundred million years older than the adaptive immune system, innate immunity is highly evolutionarily conserved across vertebrates (*1, 2*). The human innate immune system consists of physical barriers like skin, cellular components such as phagocytic and granular cells including macrophages, dendritic cells, and neutrophils, and humoral components like complement. Innate immune cells may recognize non-self molecules through pattern recognition receptors (PRRs) that bind to conserved pathogen-associated molecular patterns (PAMPs), such as viral RNA or bacterially-derived lipopolysaccharide (LPS) (*3*). Toll-like receptors (TLRs) are PRRs expressed in a variety of myeloid cell types such as monocytes. Cell surface expression of TLR4 on monocytes, for instance, can recognize bacterially-derived LPS and elicit the transcription and production of pro-inflammatory cytokines and type-1 interferon from monocytes, thus engaging the recruitment of other immune cells to combat the pathogen (*4*).

Similar to innate immunity, the adaptive response is composed of humoral and cellular components. The main cellular components in adaptive immunity are B and T lymphocytes. B cells will generate humoral immunity through their ability to produce circulating antibodies. T cells provide cellular immunity and elicit cell based effector functions. B cells and T cells are able to recognize foreign antigen with exquisite specificity based on the somatic recombination of their B cell receptor (BCR) or T cell receptor (TCR), respectively. The genes that encode these receptors undergo somatic recombination of the variable (V), diversity (D) and joining (J) segments, allowing for the formation of a unique gene within each lymphocyte encoding their respective BCR or TCR. Consequently, these uniquely encoded BCRs and TCRs can recognize distinctive antigens. This allows for a diverse collection of BCRs and TCRs in the body, each expressed by their own B or T cell, respectively, that facilitate the recognition of a multitude of antigens (*5*). The B cell BCR can exist as a membrane-bound cell signaling antibody (Ab) or can be secreted in the form of an effector antibody. Antibodies have two major components. The antigen-binding region of the antibody is known as the F'ab region, and the Fc region is the effector portion that binds to cellular Fc-receptors.

1.2 Conventional and unconventional T cell subsets and recognition of their cognate antigens

1.2.1 Conventional, adaptive CD8 T cells

The TCR of a T cell is only found in its membrane-bound form and is used for antigen recognition and signal transduction (*1*). The TCR occurs as the more abundant αβ heterodimer, representing upwards of 90% of total T cells, or the rarer γδ heterodimer. Conventional, polymorphic T cells can be divided into CD4 expressing helper T cells, or CD8 expressing cytotoxic T cells. CD8 and CD4 T cells recognize peptide antigens presented in the context of MHC-I or MHC-II, respectively (**Figure 1**). One of the aims of this thesis focuses on an innate-like adaptive CD8 T cell subset. Therefore, for now I will only focus on the biology of conventional, adaptive CD8 T cells.

The fundamental paradigm that cytotoxic T cells recognize foreign antigen in the context of a self-antigen presenting molecule was first discovered in 1974 using a mouse model of LCMV infection (*6*). Since then it has been appreciated that CD8 T cells (CD3+) have high antigenic specificity towards peptide antigen presented in the context of MHC-I (**Figure 1**) (*7*). The CD8 TCR recognizes antigenic peptides that are 8-10 amino acids in length. Antigenic stimulation of a naïve, or antigen-inexperienced, CD8 T cell also requires costimulation of the activating receptor CD28, which binds with CD80 or CD86 expressed by an antigen presenting cell (APC), and a pro-inflammatory signal such as IL-12 or type-1 interferon (*8*). An effector CD8 T cell response against the presented antigen is mounted over the course of 1-2 weeks after initial TCR engagement. These antigen-specific effector CD8 T cells elicit cytotoxic effector functions on target cells expressing its cognate antigen. This is done through the delivery of cytoplasmic granular molecules into the target cell, a process known as degranulation. Within the granular molecules are perforin, a pore-forming molecule that makes pores in the plasma membrane of the target cell (*9*). Serine proteases, known as granzymes, are also included in these granular molecules, and facilitate cleavage of caspases and the initiation of apoptosis within the target cell (*10*).

T cells that share the same TCR are known as clonotypes, and it is estimated that there are approximately 10^{10} unique clonotypes within the human body (*11, 12*). Antigen recognition by the TCR causes the antigenic-specific clonotypes to undergo massive proliferation, transcriptional alteration, and gain tissue homing capability. This event is known as clonal expansion, where an antigen-specific CD8 T cell expands from a low frequency of naïve cells to millions of effector cells (*13-15*). After antigenic clearance, presumably due to pathogen clearance, the specific CD8 T cell population undergoes a contraction phase wherein the majority of the clonally expanded cells die via apoptosis. There are, however, a subset of antigen-specific cells that enter long-term quiescence and become

memory CD8 T cells. Memory CD8 T cells can respond with rapid effector function upon restimulation with antigen, and that immunological memory may last the lifetime of the host (**Figure 3**) (*16*). These long-term memory effector cells are a mechanism to clear previously-eliminated pathogens upon re-exposure. The transition of a CD8 T cell from antigen-naïve to a memory T cell comes with it a changing phenotype, function and location in the body, with varying expression of surface markers such as CD28, CD45RO, CD45RA, CD62L, CD127 (IL-7Rα), CD27, and CCR7, summarized in **Table 1** (*17-23*).

Table 1. Descriptive characteristics of the maturation of adaptive CD8 T cells.

CD8 T cell subset	Phenotype								Degranulation (CD107a) and cytotoxic molecule expression	IFNγ production	Potential to proliferate	Location in body
	CD45RA	CD45RO	CCR7	CD62L	CD28	CD27	IL-7Rα	CD57				
Naïve	+	-	+	+	+	+	+	-	-	-	+++	Secondary lymphoid tissue
Central memory	-	+	+	+	+	+	+	-	++	+++	++	Secondary lymphoid tissue
Effector memory	-	+	-	-	+/-	+/-	+/-	+/-	+++	+++	+	Blood and inflammed tissue
Terminal effector	+	-	-	-	-	-	-	+	+++	+++	-/+	Blood and inflammed tissue

Information gathered from (23, 24).

There are instances where antigens can persist, such as in chronic inflammation and chronic viral infections, such as HIV-1 (HIV for the remainder of this thesis) infection (*25*). It is in these instances where CD8 T cells can become "exhausted," with altered phenotypic profiles, effector responses, and transcriptional regulation. An exhausted CD8 T cell population can lead to incomplete pathogenic clearance and dysfunctional immune response (*25, 26*). In particular, persistent TCR cell stimulation leads to exhausted antigen-specific T cells, and this impaired functionality of CD8 T cells is often described in HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) infection, as well as cancer in humans (*27*).

In instances of chronic TCR stimulation, such as a persistent viral infection, the ability of CD8 T cells to produce cytokines is lost in a step-wise manner, with IL-2 and TNF production lost first. This can be followed by a decreased ability to produce IFNγ and cytotoxic molecules (*28-30*). TCR-mediated exhaustion of a CD8 T cell can induce altered transcriptional expression of nuclear factor of activated T cells (*NFAT*) and sprouty homologue 2 (*SPRY2*), which is consistent with a role

for ongoing TCR stimulation (*31, 32*). Along with this, an exhausted CD8 T cell upregulates surface expression of a number of inhibitory molecules, a phenotype also observed in antigen-specific T cell populations in chronic viral infections. Examples of such molecules are the checkpoint inhibitor programmed cell death protein 1 (PD1), lymphocyte activation gene 3 protein (LAG3), 2B4, CD160, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) (*33-35*). This exhausted phenotype is accompanied by decreased proliferative capacity, and loss of the ability to self-renew in an IL-7 and IL-15 dependent fashion (*34*). Expression of Eomes and T-bet, transcription factors responsible for maintaining the homeostasis of memory T cell function through the modulation of checkpoint inhibitor expression, can further delineate exhausted CD8 T cells (*36, 37*). The coexpression of inhibitory molecules PD-1, CD160, and 2B4, the expression of which are elevated in chronic HIV infection (CHI), correlates with a T-bet^{dim} Eomes^{hi} transcription factor profile amongst HIV-specific CD8 T cells (*38*). Virus-specific Eomes^{hi}PD1^{hi} exhausted CD8 T cells have been identified in chronic HCV and HIV infection and are likely driven by chronic antigen exposure (*38, 39*). Despite the potential for exhaustion in these states, the response of adaptive, conventional CD8 T cell is critical for controlling pathogens in the host.

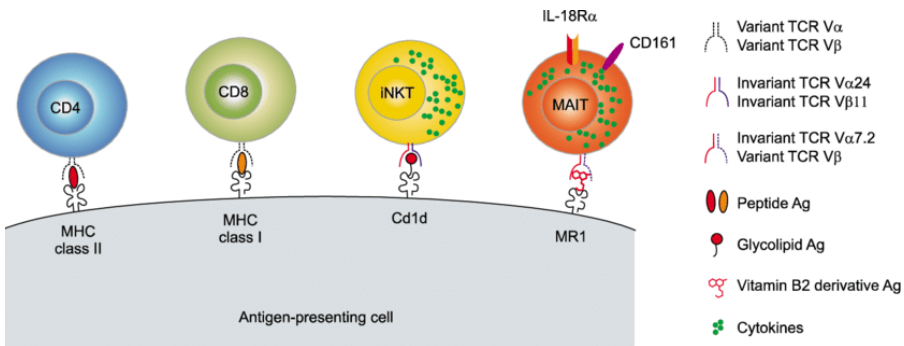

Figure 1. Conventional and unconventional T cells and recognition of their cognate antigens. *Unlike conventional CD4 and CD8 T cells that have variable TCR chains that recognize peptide antigen, MAIT cells and iNKT cells have invariant TCR chains that recognize antigen presented in conserved MHC-I-like molecules that present riboflavin metabolites or glycolipids, respectively. Reprinted with permission from (40).*

1.2.2 Mucosal associated invariant T (MAIT) cells

In between the conventional, adaptive T cells and innate effector cells such as natural killer (NK) cells, there is a family of unconventional T cells that recognize non-polymorphic MHC-I-like molecules that present nonpeptide antigens (**Figure 1**). Mucosal associated invariant T (MAIT) and invariant natural killer T

(iNKT) cells are amongst these unconventional T cells and respond to their cognate antigen in a rapid innate-like fashion. Unlike conventional T cells that need many days to weeks to mount an antigen-specific response and gain memory after antigenic exposure, the unconventional T cells MAIT and iNKT cells can be activated and respond within hours of exposure to foreign pathogens, and exhibit an effector memory phenotype prior to antigen exposure in the periphery (**Table 1**) (*41*). This allows for a buffer of time for conventional T cells to mount their adaptive, antigen-specific response (**Figure 2**). Upon reactivation with their cognate antigen, conventional adaptive T cells that have attained memory can respond more rapidly than their naïve counterparts, in a manner almost in tune with unconventional T cells (**Figure 2**). While most T cells have highly variable TCRs the MAIT and iNKT cells express semi-invariant or invariant TCRs, respectively, that have undergone somatic rearrangement (*42*). This thesis aims to further elucidate the role of iNKT and MAIT cells in HIV infection.

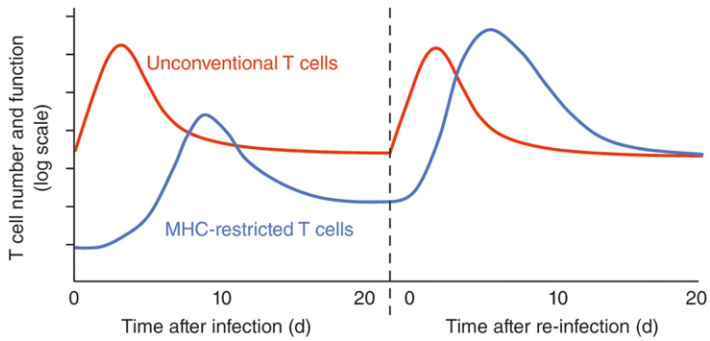

Figure 2. The temporal dynamics of unconventional and conventional T cell responses to their cognate antigen. *Unconventional T cells, including iNKT and MAIT cells, respond to stimulation within hours of their activation. Conventional T cells will take days to weeks to mount their response and at a weaker functional magnitude compared to conventional T cells. However, upon generation of immunological memory, conventional T cells can respond to stimulation at a faster pace and stronger magnitude, akin to unconventional T cells, which already poses an effector memory phenotype prior to antigen recognition in the periphery.* ***Material from:*** *Godfrey et al., Nature Immunology, 2015, Nature Publishing (43)*

MAIT cells in particular exist in large numbers in healthy humans, up to 10% of the circulating T cell pool, and thus form a formidable part of the immune defense (*44-47*). MAIT cells can be found throughout the body, including tissues such as the gastrointestinal or "gut" mucosa (3-5% of total gut associated T cells), and the liver where they are most abundantly observed (10-35% of total T cells) (*44, 48-51*). There are relatively few MAIT cells in the thymus, but their numbers expand in the periphery (*52*). Functionally competent MAIT cells have been identified in the cord blood and human fetal tissue, albeit with a naïve phenotype including surface

expression of CD45RA (**Table 1**). The development of a central memory phenotype as is found in adults is thought to occur within the first year of life, possibly stimulated by the establishment of the commensal flora (*51, 53, 54*). The frequency of MAIT cells in the blood is lower in children, peaks in young adulthood, and declines in the elderly (*55*).

In humans, MAIT cells express the invariant Vα7.2 TCR chain, limited Jα segments (Jα12, Jα20, and Jα33) and have a limited β-chain repertoire (*43, 56-60*). Their TCR recognizes microbially derived pyrimidine intermediates from the riboflavin biosynthesis pathway that are presented in the context of major histocompability complex class Ib-like molecule 1 (MR1) (*45, 47, 61-63*). MR1 is ubiquitously expressed at the gene level in the majority of human lymphocytes and APCs in the blood, and is highly evolutionarily conserved (*45, 47, 61-63*). Thymic MAIT cells require engagement with MR1 and an unknown antigen in order to develop and egress into the periphery (*52*). The ability to metabolize riboflavin is found in many bacteria and fungi, implicating MAIT cells in immunity against a vast range of microbial infections (*62*).

Great advances have been made to identify MAIT cells, which express high levels of the C-type lectin CD161 (*KLRB1*), a marker commonly associated with NK cells and innate-like functionality. CD161 can be found on CD8 T cells as either $CD161^{dim}$ or $CD161^{bright}$, with the majority of the latter being MAIT cells (*64*). The role of CD161 on MAIT cells has yet to be determined, but MAIT cells have classically been identified in flow cytometry through the coexpression of $CD161^{bright}$ and the TCR Vα7.2 amongst CD3 T cells (*65, 66*). With the identification of a main MAIT activating antigen, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), MR1 tetramer can also be used to identify MAIT cells by flow cytometry, and encompass the vast majority of $CD161^{bright}$ Vα7.2+ T cells (*67*). In addition, MAIT cells express high levels of IL-18Rα and IL-12R, allowing them to be activated in a TCR-independent manner through innate cytokine stimulation (**Figure 3**). They acquire expression of the transcription factor promyelocytic leukemia zinc finger (PLZF) (*ZBTB16*) in the thymus. This transcription factor is also expressed in iNKT cells and is believed to play a role in innateness and the pro-apoptotic nature of these cells (*49, 68, 69*). MAIT cells can express CD8 and are primarily CD8αα (>90%) but can be express the CD8αβ heterodimer (*70*). While greater than 70% of MAIT cells express CD8 in humans, a smaller proportion of MAIT cells lack expression of both CD4 and CD8 and are deemed double negative (DN) (14%). An even smaller subset expresses CD4 (6%) (*49, 58, 68, 70*). Their CD8 expression reflects their cytotoxic potential, and ligation of their TCR with antigen-loaded MR1 elicits rapid and broad cytokine production in both the blood and mucosal sites. MAIT cells can production of T_H1 cytokines such as interferon (IFN) γ, tumor necrosis factor (TNF), and cytotoxic molecules such as granzyme (Grz) to deliver to target cells and induce cell death (**Figure 3**)

(*62, 70, 71*). *Ex vivo* studies demonstrate that mucosal MAIT cells can produce IL-17 and IL-22 and may contribute to mucosal immunity (*42, 46-48)*. Peripheral blood MAIT cells can express these cytokines at lower levels, but predominantly produce granzymes and T_H1 cytokines but not T_H2 cytokines *in vitro* (*58, 70, 72, 73*). Recently, it was determined that the education of the T_H17 MAIT cell subset occurs in the thymus upon establishment of commensal microbiome (*44, 48-50, 74*).

Their ability to produce a wide range of cytokines in response to microbially-derived antigen in both the peripheral blood and mucosal sites implicates MAIT cells in combating bacterial and fungal pathogens. MR1 knockout mice lacking MAIT cells due to the lack of MR1 education in the thymus, have higher bacterial loads upon challenge with of *Francisella tularensis*, *Klebsiella pneumoniae,* and *Mycobacterium bovis* (*75-77*). Humans with cystitis have MAIT cells present in their urine, and patients with active tuberculosis (TB) have an increase in MAIT cells in the lung. These findings support a notion that MAIT cells assist with bacterial control at the site of infection and are recruited to places of bacterial infection (*78, 79*). Furthermore, MR1 knockout mice have lowered gut integrity and an infiltration of bacterial DNA in the peripheral blood compared to mice that have MAIT cells, suggesting a role for MAIT cells in protecting gut homeostasis (*80*). Studies in mice also demonstrate MAIT cells acquire a tissue-repair gene signature after the resolution of a bacterial infection. This finding points towards a role for MAIT cells in wound repair. It may be that damage to the mucosa results in engagement of the MAIT cell TCR with either commensal or pathogenic bacteria to not only induce a proinflammatory response to clear the bacterial invasion, but also begin assisting in the healing process of the damaged tissue (*81*).

Peripheral blood MAIT cells in adults display an effector memory (CD45RO+ CD28+CD62L-CCR7-) phenotype and express an array of surface receptors associated with tissue homing, such as α4β7, CCR2, CCR5, CCR6, CXCR6, and CCR9 (*44, 68, 82, 83*). MAIT cells are distinct from conventional T cells in that they co-express the transcriptional factors Eomes, T-bet, RORγτ, and Helios (*84*). Despite their relatively homogenous phenotype compared to conventional CD8 T cells, there are subsets of MAIT cells that can be divided based on CD8/CD4 expression, CD56 expression, and also some level of antigen discrimination based on TCR β-chain composition (*83*). For example, the abundant Vβ13.2+ subset of MAIT cells are hyper-responsive to *C. albicans* stimulation, while MAIT cells expressing Vβ8, Vβ13.1, and Vβ13.6 are hypo-responsive to *E. coli* stimulation (*83*). The DN and CD8+ MAIT cell subsets may be functionally distinct, and the DN subset may derive from CD8+ MAIT cells. Furthermore, DN MAIT cells have a pro-apoptotic gene signature and higher propensity towards activation-induced cell death (*85*). Finally, a subset of MAIT cells express high levels of the NK-cell associated molecule CD56 also express more IL-12R and IL-18R on their surface, and in turn produce more IFNγ in response to these innate cytokines *in vitro* (*86*).

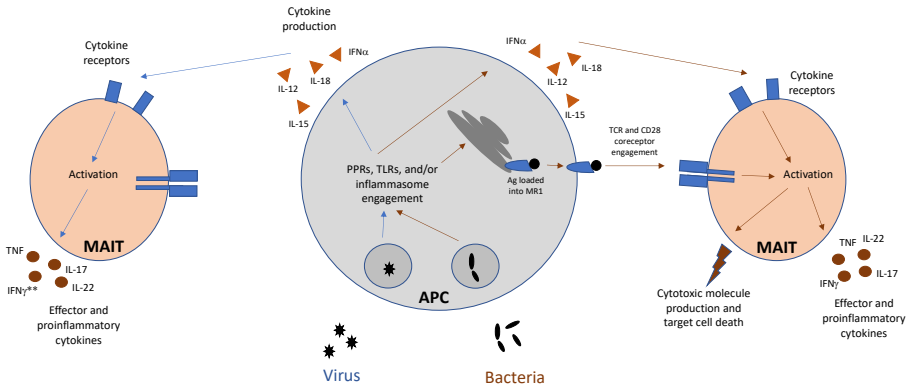

Figure 3. MAIT cell response to bacterial compared to viral infections. *APCs can present riboflavin metabolites in the context of MR1 to the MAIT cell TCR, along with CD28 coreceptor engagement. This can elicit the production of cytolytic effector molecules such as GrzB and perforin which can be delivered to target cells. Additionally, TLR, PRR, or inflammasome signaling during either bacterial or viral infections can trigger the production of inflammatory cytokines such as IL-12 and IL-18 and engage relevant receptors on the MAIT cell surface. Engagement with IL-12 and IL-18 receptors on the MAIT cell elicits production of effector cytokines, predominantly IFNγ. Figure generated with information from (87, 88).*

1.2.2.1 MAIT cells in viral infections

There is no known viral ligand recognized by MAIT cells, however given that MAIT cells can produce the antiviral cytokine IFNγ and are altered during several viral infections, their role in viral infections warrants exploring (reviewed in *88*). There are phenotypic and functional characteristics of MAIT cells that suggest a potential role in combating viral infection as well. MAIT cells uniformly express IL-18R, and as with NK and other T cell subsets, IL-18 can synergize with IL-12R ligation to produce IFNγ (**Figure 3**) (*89, 90*). These innate cytokines can be elicited by APCs in the example of a viral infection, through TLR8 binding of viral RNA, leading to IL-12 and IL-18 production and supporting a role for MAIT cells in antiviral immunity (*51, 91*). Furthermore, the subset of MAIT cells that express CD56 and respond more readily to these innate-like signaling induced by viral infections, support a role for MAIT cells in contributing to antiviral immunity (*83*). MAIT cells can also be activated by type-I interferons, molecules produced by a wide variety of immune cells and tissue types during viral infections. Stimulation with IFNα or IFNβ in combination with IL-18 results in IFNγ production by MAIT cells (*92*). Type-I interferons can also act as a costimulatory molecule upon MAIT TCR engagement, along with other innate signals shown to do the same, including TNF and IL-1β, together with IL-23 (*93*).

The impact of MAIT cells in viral infections is emphasized by their changing characteristics during acute and chronic viral infections. For example, MAIT frequency and phenotype is altered in humans infected by flaviviruses. Decreased MAIT cell frequencies are observed in acute Dengue virus infection, and MAIT cells have increased expression of activation markers HLA-DR and CD38 in natural dengue infection. MAIT cells will also produce IFNγ following *in vitro* stimulation of total PBMC with Zika virus. (*94*). Several studies have observed MAIT cell perturbations in chronic HCV infection, where MAIT cells are depleted in both the blood and liver. In chronic HCV infection MAIT cells in the blood and liver have increased expression of activation and exhaustion markers, with hepatic MAIT cells becoming dysfunctional (*95*). In HIV and HCV co-infection, MAIT cells are depleted from the peripheral blood, and therapy is unable to restore their loss. However, intrahepatic MAIT cells are restored with therapy in HCV monoinfection (*96, 97*). While MAIT cells are not depleted in HBV, they have an increase in activation marker expression (*98*). Human T-lymphotropic virus (HTLV) -1 infection causes depletion and dysfunction in peripheral blood MAIT cells as well, and MAIT cell loss is independent of viral load and they do not appear to be targets of HTLV-1 infection (*99*). MAIT cells can be activated and produce IFNγ through stimulation *in vitro* with macrophages or dendritic cells infected with dengue virus, Zika virus, influenza, or HCV (*92, 94*). Activation of MAIT cells in this manner depends on IL-12 and IL-18 together, or IL-18 alone. In mouse models, MAIT cells also contribute to protection against lethal influenza A challenge in a TCR-independent manner (*100*).

MAIT cell alterations in chronic HIV monoinfection has been explored in cross-sectional studies compared to healthy controls, and is reviewed later in this thesis and is the focus of **Paper II**. Altogether, *in vitro* work, coupled with observable alterations *in vivo* in viral infection, suggest MAIT cells respond to viral infections.

1.2.3 Invariant natural killer T (iNKT) cells

There are three types of cells commonly referred to as "NKT" cells; Type I, Type II, and NK-like T cells. NK-like T cells are a relatively heterogenous and large (5-20%) population of T cells that are identified generally through the expression of the NK-associated markers CD161 or CD56 (*101*). They are conventional, adaptive T cells that recognize antigen using classical MHC molecules (*102*). True NKT cells are an unconventional T cell subset subdivided into Type I and Type II, that can recognize and rapidly respond to non-peptide antigens presented in the context of the MHC-I-like molecule, CD1d (*103*). CD1d presents a variety of synthetic, exogenous and endogenous glycolipid antigens to the NKT TCR. All CD1 (CD1a, b, c and d) molecules present lipid antigen to T cells and are expressed by DCs. But only CD1d is expressed by additional cell types including epithelial cells, B cells, and macrophages (*104*). Type I and Type II NKT cell discovery was based

on the observation of an unusual phenotypically mature subset of CD4 and CD8 double negative T cell within the mouse thymus that also expressed the NK-related marker NK1.1, and produce the cytokines IFNγ and IL-4 *in vitro* (*105-107*). These cell types also had biased expression of Vβ8 TCR chain. Type II NKT cells have diverse αβ TCRs, and are outside of the scope of this thesis (reviewed in ref *108*). Type I NKT cells, however, have an invariant αβ TCR and are often called invariant NKT (iNKT) cells, and are a focus of **Paper III** in this thesis. Engagement of the iNKT cell TCR triggers the production of a wide breadth of both T_H1 and T_H2 cytokines, giving them an immunomodulatory role thus far not described in MAIT cells. All iNKT cells express the innate-like transcription factor PLZF (*ZBTB16*), a trait they share in common with MAIT cells (*109, 110*). Like MAIT cells, iNKT cells are evolutionarily conserved within mammals, and in humans invariably express TRAV10-TRAJ18 paired with TRBV25 as their TCR α and β chains (translating to Vα24 and Vβ11 at the protein level) (*111, 112*). The iNKT cells can be identified in flow cytometry using monoclonal antibodies against their Vα24 and Vβ11 TCR components amongst CD3 T cells in humans. The synthetic marine sponge-derived lipid α-galactosylceramide (αGalCer) antigen, when loaded into the CD1d molecule, can stimulate iNKT cells. This complex also allows for their identification via flow cytometry and further description (*113, 114*). The frequency of iNKT cells in human blood is usually very low (0.1-1% of total T cells), is variable in the liver (0-3%), and can be somewhat enriched (1-3% of T cells) in the gut (*115, 116*). This low frequency of iNKT cells in the blood in humans declines 2 to 10 fold lower in old age (*117*). Mice have differing frequencies compared to humans in the blood and liver, representing between 0.2-0.5% of total blood T cells, but 30-70% of T cells in the mouse liver (*118*). Mouse studies have revealed that iNKT cells undergo positive selection by endogenous self-ligand presented by CD1d in the thymus (*119*).

Phenotypically, iNKT cells in the blood can express NK-associated markers CD161 and sometimes CD56 and have an effector memory phenotype with expression of CD45RO, CCR7, and CD28 (*120*). Upon activation, iNKT cells can express classical markers of T cell activation including HLA-DR and CD38, but they also upregulate inhibitory molecules such as PD-1, Tim-3 and LAG-3 (*121*). iNKT cells can be subdivided into CD4+ or CD4- subsets, with distinct phenotypes and functional profiles (*122*). The CD4+ iNKT cells represent 12-36% of iNKT cells. They are found enriched in the gut mucosa and thymus, and are functionally distinct from CD4- iNKT cells, with a bias towards T_H2 cytokine production. Considered immunomodulatory, CD4+ iNKT can produce IL-4, IL-6, and IL-10, and IL-13 and are implicated in suppressing the immune response in some mouse disease models including diabetes (*123*). Phenotypically, CD4+ iNKT cells can express CD62L giving them lymph node homing capabilities, but can also express tissue-homing receptors α4β7, CCR4, CXCR4 and CCR5. The CD4- iNKT subset can be

divided into DN (12-36% of iNKT cells) or CD8+ (1-5% of iNKT cells) (*124*). The CD4- subset expresses a wider variety of chemokine receptors including CCR1, CCR6, CXCR6 and again, high levels of CXCR4 and CCR5 (*122*). Amongst the CD4- iNKT cells, some express the T cell regulatory transcription factor T-bet and produce T_H1 cytokines like IFNγ and TNF, along with cytotoxic molecules like perforin and granzyme (*125-127*). The production of these molecules can occur within minutes to hours upon *in vitro* stimulation (*125-127*). Others in the CD4- iNKT cell subset express the RORγτ transcription factor and have a T_H17 functional profile with the ability to produce IL-17, IL-21 and IL-22 *in vitro*. This subset has suggested roles in supporting mucosal immunity (*127, 128*). Additionally, iNKT cells can provide B cell help with a T_{FH} (follicular helper) functional profile and the expression of follicular homing molecule CXCR5, aiding in the formation of early germinal centers in a Bcl-6 dependent manner (*129*). These T_{FH} iNKT cells are predominantly CD4+. In germinal centers, B cells undergo somatic hypermutation and the selection of B cell populations that express high-affinity BCRs to antigen, giving rise to mature memory B cells. Finally, all iNKT cells can facilitate direct cell to cell killing via FAS/FASL interactions (*130*).

The antigens loaded into the CD1d molecule that engage the iNKT cell TCR can be either endogenously or exogenously derived. The endogenous antigens that have been identified include isoglobotrihexosylceramide (iGb3) and ganglioside GD3, and there is evidence mammals can produce a-linked glycosylceramides (*131-134*). Exogenous antigens are microbially derived, including a-glucouronosylceramide from *Bacteroides fragilis*, a common inhabitant of the human gut microbiome, and bacterial pathogens such as *Borrelia burgdoferi, Sphingomonas,* and mycobacteria (*135-138*). Despite their small numbers, iNKT cells likely play a role in defending against microbial pathogens, as they recognize these lipid-derived antigens derived from a variety of microbes. However, particular attention has been made to TCR-independent activation of iNKT cells through engagement of PAMPs like LPS by PRRs expressed by APCs. In response, the APC will produce the innate cytokines IL-12 and IL-18 which engage the IL-12R and IL-18R expressed on the surface of iNKT cells (*139, 140*). In return, iNKT cells will produce IFNγ to elicit antimicrobial effects against bacterial infections, even if the bacteria cannot produce the cognate glycolipid antigen recognized by iNKT cells, such as in the case of *Salmonella typhimurium (141)*. Additional research has implicated the same mechanism driving iNKT cell activation by fungi, viruses, and protozoa, with IL-18 being the dominant cytokine driving this activation, and DCs being the dominant APC (*142*). Interestingly, despite the inability of a pathogen to generate cognate lipid antigen to engage the iNKT cell TCR, blocking with CD1d antibodies reduces IFNγ production *in vitro*, suggesting a role for TCR engagement in this type cytokine-mediated activation. This may depend on the endogenous antigen presented by APCs (*143*). *In vivo*, infection with microbes in mouse models has

elucidated changes iNKT cell phenotype, function and frequency induced by microbial infections. iNKT cells have been found enriched in tissues, such as the lung in *Cryptococcus neoformans* infection, and in the liver of mice infected with plasmodium (*144, 145*). Chronic exposure to microbes in mouse models induces an exhausted phenotype in iNKT cells, with an upregulation of PD-1 and hyporesponsive functionality, followed by apoptosis and sometimes contraction of the iNKT cell compartment (*146, 147*). This suggests that while iNKT cells can be rapidly activated to help combat microbial infections, chronic exposure to antigen can lead to an exhausted phenotype, similar to MAIT cells and conventional, adaptive T cells.

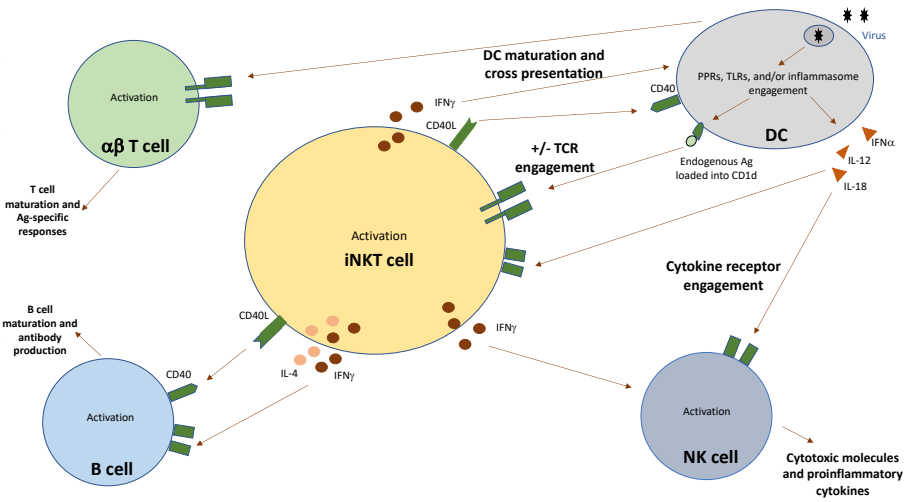

Figure 4. Proposed role of iNKT cell help during viral infections. *iNKT cells can be activated by an APC such as a DC that activates iNKT cells through engagement with cytokine receptors on the surface of iNKT cells with or without the TCR engagement with an endogenous antigen. IFNγ production by the activated T cell, along with CD40L engagement can facilitate DC maturation, including the upregulation of CD80/CD86 as well as MHC-II on the surface of DC cells. DC maturation can aid in αβ T cell activation and maturation towards virus-specific responses from αβ T cells. Upregulation of CD40L on iNKT cells can also provide B cell help, along with IFNγ and IL-4 production, and allow for B cell activation and maturation, thereby eliciting effector antibody responses in leu of CD4 helper T cells (150). IFNγ production by iNKT cells can also activate NK cells, thereby eliciting their effector responses. Figure generated with information from (122, 151).*

1.2.3.1 iNKT cells in viral infections

To date iNKT cells have been studied in mice in the context of several viral infections including Lymphocytic choriomeningitis virus (LCMV) and influenza virus. LCMV infection causes long-term depletion of iNKT cells from the blood, spleen and liver in mice. The mechanism behind this loss may be activation-induced

apoptosis, as remaining iNKT cells have higher expression of caspase 3 (*152*). In influenza A virus models, CD1d knockout mice that lack iNKT cells were more susceptible to death from influenza challenge (*153*). Furthermore, bronchioalveolar lavage fluid these mice measured significantly less IFNγ than in WT mice, coupled with less NK-cell activity and antigen-specific CD8+ T cell responses. The influenza A viral infection mouse models suggest the IFNγ produced by iNKT cells helps CD8 T and NK cells combat viral infection.

The iNKT cell dynamics have also been studied in natural viral infections in humans, with studies performed in HIV (reviewed later in this thesis), HCV, and influenza virus infected patients. In HCV infection, there are divergent findings on iNKT cells. Some studies have shown that iNKT cells are depleted from the blood in individuals with detectable viremia compared to healthy controls, and iNKT cells found in the liver have an activated phenotype in these individuals (*154-156*). Others have observed similar frequencies of blood iNKT cells in HCV infection compared to controls (*157*). iNKT cells in chronic HCV infection may have enhanced ability to produce the T_H2 cytokine IL-13 *ex vivo* compared to healthy controls, suggesting iNKT cells could be activated to provide B cell help (*158*). *In vitro* work with influenza A virus suggests iNKT cells may counter the actions of the immunosuppressive cell type myeloid-derived suppressor cells (MDSCs), which in the absence of activated iNKT cells suppress the function of influenza-specific CD4 and CD8 T cells (*159*). The important role of iNKT cells in viral infections is supported by the concept that certain viruses can downregulate and prevent the recycling of the CD1d molecule from the surface of APCs. This has been demonstrated in HSV and HIV infection (*160, 161*), and is a suggested mechanism for the evasion of iNKT cell responses in HCV infection (*157*).

1.3 The immunodynamics of the mucosal barrier

Mucosal barriers play a major role in innate immunity and act as not only physical but also immunological barriers. They contain immune cells and tight junctions between lining epithelial cells that protect the host against a vast array of microbes that inhabit these locations. Of particular relevance to this thesis is the mucosal barrier of the gastrointestinal tract or "gut," which is populated by a commensal microbiota in the healthy state (reviewed extensively elsewhere *162*). The commensal microbiome can be of benefit to the host, for example the generation of vitamin K and vitamin B, which humans require for nutrition but cannot be metabolize themselves. While maintaining the presence of these beneficial commensal microbiota, the gut mucosal barrier must also prevent the invasion of these bacteria into the blood and prevent the over proliferation of pathogenic or inflammatory bacteria.

The gastrointestinal mucosal barrier has several mechanisms to maintain this balance. The first is the structure of the tissue itself, with an outer mucous layer produced by goblet cells in the intestine. This is underlaid by a border of epithelial cells, followed by the tissue underneath the epithelial layer known as the lamina propria. Some lymphocytes reside in the epithelial layer itself. These are predominantly CD8 T cells and the unconventional T cell subset γδ T cells, which are enriched here compared to the blood (*163*). The lamina propria underlying the epithelial layer that lines the gastrointestinal lumen is populated with large amounts of immune cells. These immune cells include innate and adaptive populations such as T cells, B cells, and macrophages. These function to maintain the mucosal barrier through cytokine production and the prevention of microbes from entering the underlying blood stream. Of relevance to this thesis, it is important to note which immune cells participate in maintaining the gut barrier and play a role in HIV infection. Unconventional T cells are found in this tissue, including iNKT and MAIT cells which play unique roles in the control of invading pathogens. CCR5 expressing CD4 T cells are also found in abundance in the gut mucosa and display an T_{EM} and T_{TM} phenotype and function (*164*). IL-17 and IL-22 producing cells, such as CD4 T cells and MAIT cells, can also be found in the lamina propria and produce the cytokines that assist in maintaining the gut barrier (*163*). IL-17 can recruit neutrophils, promote the production of antibacterial molecules known as defensins, and induce the proliferation of enterocytes, the epithelial cells lining the gastrointestinal lumen (*165*). IL-22 also promotes enterocytes proliferation, and can stimulate the production of mucous by goblet cells (*166*). It is the maintenance of this single layer of epithelial cells and the tight junctions between them that prevent the gastrointestinal microbiota from passing into the lamina propria and entering the blood stream. Gut homeostasis can be altered, such as when immune cells are depleted or altered at this site as a consequence of a viral infection like HIV. Such alterations can allow microbes and microbial by products to cross the epithelial layer and enter the blood stream to be distributed throughout the body. This occurrence is a potential cause of systemic inflammation.

1.4 The immune reponse to HIV infection

From the identification of the virus that causes acquired immunodeficiency syndrome (AIDS) in the 1980s, to the introduction of the first antiviral therapy only few years later, the scientific community has made monumental advancements in patient treatment and life expectancy in those that become infected in a very short time (*167*). However, HIV continues to challenge the minds of researchers and clinicians alike (*168*). The epidemic still rages on in sub-Saharan Africa with smaller epicenters in locations like India, Russia and the United States, yet an effective preventative vaccine remains elusive (*169, 170*). Additionally, because of the need for complex medical infrastructure, significant financial investment,

and the complex societal stigmas that surround effectively treating HIV, a significant percentage of those at risk for acquiring or already infected with HIV do not have access to treatment (*171*). Current therapies do not eliminate the latent HIV reservoir, wherein circulating viral loads are reduced or undetectable. HIV persists in a subset of infected CD4 T cells with limited or undetectable viral replication. To add to this, those successfully on treatment continue to be susceptible to serious non-AIDS events such as cardiovascular disease, malignancies, bacterial and fungal co-infections, and sustained immune activation and inflammation (*172*). Furthermore, the ability to reduce the HIV reservoir could signal the beginning of an era of HIV cure. These notions drive the need for a cost-effective and efficacious vaccine, and improved therapies for HIV infection. These goals may be achievable with a greater understanding of the complex immunological events in both the acute and chronic stages of HIV infection (*170*).

1.4.1 The immunopathology and T cell response to HIV infection

HIV can be transmitted from an HIV infected individual through semen, vaginal secretions, blood, and breast milk. Upon infection, HIV disease progression can be divided into three phases: acute HIV infection (AHI), chronic infection (CHI), and acquired immunodeficiency syndrome (AIDS). AHI infection occurs in the first days to weeks post-HIV transmission. There is first an eclipse phase lasting 10-12 days post-transmission where HIV RNA is undetectable in the blood with current diagnostic testing (*173*). This is followed by a phase marked by high levels of HIV plasma viremia as the virus replicates, with peak viremia occurring on average 13 days post infection (*174, 175*). Infected individuals can present with acute retrovirus syndrome during this time period, with symptoms such as fever, malaise, and lymphadenopathy (*175*). The primary target of viral infection are the CD4 T cells, wherein HIV enters through the binding of the viral envelope protein gp120 to the CD4 receptor as well as the coreceptors CCR5 or CXCR4 (*176-180*). The HIV retrovirus enters a host cell as two, single-stranded RNA molecules, capable of integrating into the host genome. It does this by first by reverse transcribing its own viral genome into DNA using the reverse transcriptase enzyme released from the infectious virion. The viral cDNA can integrate into the host DNA, preferentially at transcriptionally active sites. Once integrated, HIV then uses the host cell transcriptional and translational machinery to generate new infectious virions, a process known as productive infection (*181*). It is widely accepted that HIV-specific CD4 T cells are the main source of productive infection in HIV, and their activation and proliferation provide a transcriptionally active state wherein new virions can be produced efficiently (*182*). Both infected and uninfected mucosal CD4 T cells are rapidly reduced in frequency during AHI. An activated CD4 T cell that is productively infected may die from the induction of the apoptotic pathway facilitated by caspase 3 (*183*). A resting, abortively infected CD4 T cell likely dies

via the inflammatory cell death process, pyroptosis, and may be the fate of the majority (>95%) of infected CD4 T cells (*183*). In this scenario, the accumulation of incomplete reverse transcripts and sensing of viral DNA through IFI16 induces formation of the inflammasome and cell death via caspase 1. CD4 T cells can also die through the immune response to a virally infected cell or through "bystander" induced apoptosis (*184-186*). However, a percentage of infected CD4 T cells will enter the resting phase of the cell cycle and become resting memory CD4 T cells. These resting and relatively transcriptionally dormant CD4 T are then considered latently infected and are a part of the viral reservoir that escapes both the host immune response and antiretroviral therapy (ART) (*187-189*). Early events in AHI define subsequent disease progression, with higher peak viral loads in AHI associated with higher viral set point into CHI, establishment of a larger viral reservoir, and faster progression to AIDS (*190, 191*). AHI is likely an ideal time for initiation of ART to reduce the size of the viral reservoir, limit inflammation and immune activation, and better preserve immunity (*191-193*).

Productively infected CD4 T cells disseminate HIV throughout the body. The virus initially replicates intensely until peak viral load is observed in the plasma, after which it recedes to a set point level approximately 30 days post infection (*175*). In the mucosa, within four days post infection mucosal epithelial cells exposed to HIV recruit plasmacytoid dendritic cells (pDCs) to the site of infection. These cell initiate the mounting of a cytokine storm. The earliest of these cytokines found in the blood in AHI are interferons such as IFNα, inflammatory cytokines such as IL-15, and beta chemokines like MIP-1α and MIP-1β (*194, 195*). This cytokine milieu recruits other immune cells to the site of infection, such as macrophages and T cells, and facilitates a target rich environment of activated CD4 T cells that are susceptible to infection and necessary for viral propagation. Subsequently, over half of the memory CD4 T cell compartment is depleted, and the HIV-specific cytotoxic CD8 T cells expand in response to the infection (**Figure 5**) (*195-197*). HIV-specific cytotoxic CD8 T cells will control the virus to some extent and contribute to establishing viral set point and the beginning of CHI. In most cases, HIV escapes away from the host immune response by mutating away from the adaptive HIV-specific response, establishing a persistent chronic infection (*197, 198*). Due to the persisting virus, CD4 T cell levels continue to decline, and CD8 T cells are exposed to chronic antigenic stimulation leading to their functional exhaustion into CHI (*33, 199*). The depletion of the immune system overtime in CHI may ultimately lead to the last stage of HIV infection, AIDS. AIDS can be defined clinically as a CD4 T cell count below 200 cells/mm, and depletion of immune system to this point allows for a number of opportunistic infections. In fact, it is the depletion of these essential CD4 helper T cells that leads ultimately to immune failure (*200, 201*). However, with the advancement of effective of therapies, individuals on ART may never reach this stage and the associated severe immunodeficiency (*202*).

In the earliest days of HIV infection, there are dynamic changes within the T cell compartment, that continue into CHI and AIDS. Amongst these other T cell subsets impacted conventional adaptive CD8 T, MAIT, and iNKT cells, the dynamics of which during HIV infection are the focus of this thesis.

1.4.2 The adaptive CD8 T cell response to HIV infection

The exact mechanism of how CD8 T cells control HIV infection is incompletely understood, though there is evidence of their contribution to viral control. In most who are infected with HIV, the HIV-specific CD8 T cell clonotypes expand in coincidence with declining viremia, implicating their role in fighting the virus and reducing the viral burden to set point (**Figure 5**) (*197*). Viral mutants are able to escape the adaptive CD8 T cell response within the first weeks of HIV infection, and persist into CHI. This supports the notion that immune pressure from CD8 T cells is being exerted against the virus (*203*). Functionally, CD8 T cells may be controlling virus through either cytotoxic or soluble factor-mediated mechanisms, or a combination of both. HIV-specific CD8 T cells ability to produce perforin and Grz directly *ex vivo* is higher in individuals that are able to spontaneously control virus, supporting a role for CD8 T cell cytotoxic functionality in controlling HIV replication (*204*). HIV control may also be established by CD8 T cells through the ability to produce soluble factors, including the beta-chemokines RANTES, MIP-1α, and MIP-1β (*205*). These are molecules that prevent the entry of R5-tropic HIV into a target cell. The production of beta chemokines from CD8 T cells can limit viral replication *in vitro* and also correlates with individuals able to spontaneous control virus (*206, 207*).

The CD8 T cells that persist in HIV infection exhibit an exhausted phenotype, with upregulation of markers of activation such as HLA-DR and CD38 as well as exhaustion markers PD-1 and TIM-3 (*208, 209*). The expression of these markers in CHI is accompanied by impairment of CD8 T cytotoxic capacity and cytokine production, and reduced ability to proliferate (*33, 210*). The cause of this phenotype and function likely has several contributing mechanisms. One possible cause is chronic antigenic stimulation for HIV-specific CD8 T cells, driven by persistent viral replication and the absence of help from the depleted CD4 T cell compartment (*211*). As many as 6% of circulating CD8 T cells are HIV-specific in untreated individuals, contributing to a large pool of CD8 T cell that may become exhausted in this manner (*212*). Bystander activation may also drive exhaustion and functional decline in adaptive CD8 T cells during HIV infection. This TCR-independent stimulation is facilitated through the engagement of chemokine and cytokine receptors expressed by CD8 T cells (*213*). While the exact mechanism of TCR-independent CD8 T cell activation and exhaustion in HIV infection has yet to be determined, peak CD8 T cell activation correlates with peak type-I interferon

levels in the plasma during the course of infection, suggesting a role for type-I interferons as a contributor of CD8 T cell activation (*214*). Non-HIV specific CD8 T cells may be activated in this manner in CHI. Elevated expression of HLA-DR and CD38 is observed on CD8 T cells specific for Epstein bar virus, cytomegalovirus, and influenza A virus from HIV infected individuals (*213*). In chronic untreated and treated HIV infection the CD4:CD8 ratio remains low (**Figure 5**), suggesting there are elevated levels of CD8 T cells beyond those of HIV-specific clonotypes. A third possible cause of adaptive CD8 T cell altered phenotype and functionality in CHI is chronic immune activation, propelled by the decline in gut integrity and introduction of microbial products into the peripheral blood (*24, 197, 215, 216*). The presence of LPS and other markers of microbial translocation in the blood can activate APCs to produce activating cytokines that lead to CD8 T cell activation. The role of microbial translocation in HIV infection is reviewed in more detail later in this thesis.

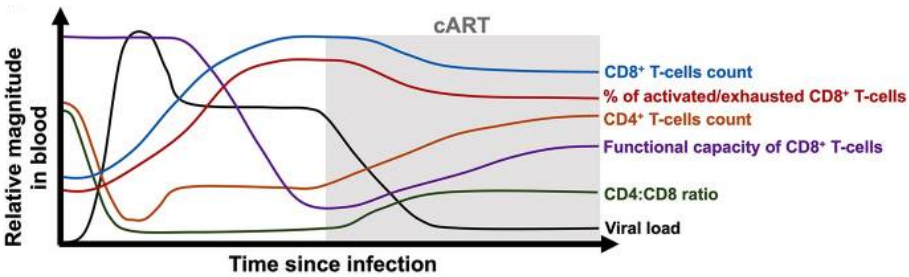

Figure 5. Numerical, phenotypic, and functional changes in conventional CD8 T cells in acute and into chronic, treated HIV infection. *Dynamics of conventional CD8 and CD4 T cells in HIV infection, with rapid decline of CD4 T cells in acute infection and their reconstitution upon ART initiation. CD8 T cells expand in response to the viral burden, become activated, and maintain that level of activation to some extent even with ART initiation. ART initiation also restores CD8 functionality to some extent, albeit not the same levels as pre-infection.* ***Material from:*** *Perdomo-Celis, Taborda and Rugeles, CD8⁺ T-Cell Response to HIV Infection in the Era of Antiretroviral Therapy, Frontiers in Immunology, 2019, Frontiers Journal Series.*

In most cases HIV is unable to be completely controlled by the adaptive CD8 T cell response as the infection progresses, leading to a rise of inefficient and exhausted CD8 T cells and advancement to AIDS (*33, 199, 203, 217, 218*). The exceptions where individuals can spontaneously control HIV are referred to as "HIV controllers." These individuals were initially shown to have enrichment of an MHC-I encoding allele, *HLA-B*57* (*219)*. It is thought that this allele, similar to other protective alleles like *HLA-B*27* and *HLA-B*14*, presents viral epitopes that mount a more effective HIV-specific CD8 T cell response (*219, 220*). CD8 T cells in controllers maintain higher levels of cytolytic effector molecules, have a great ability to proliferate, and demonstrate higher polyfunctionality, or the

ability to produce multiple cytokines in response to *in vitro* stimulation, than non-controllers (*221-225*). The antigenic target of the HIV-specific CD8 T cells also appears to play a role in spontaneous viral control. Individuals with early adaptive CD8 T cell responses directed towards the HIV Gag protein are more likely to be controllers (*226, 227*). Several *in vitro* studies also suggest that in controllers, the HIV-specific CD8 T cells are more cross-reactive, meaning they can recognize a broader array of antigens compared to non-controllers (*228*). Despite the potential for CD8 T cell control, the overwhelming majority of the time, HIV is able to escape the adaptive immune pressure from CD8 T cells and persist in the host.

In another chronic viral infection where CD8 T cells become exhausted, HCV infection, many individuals are unable to clear the virus, thought in part due to impaired CD8 T cell function and viral escape away from the HCV-specific CD8 T cell response (*229, 230*). An anomalous CD8 T cell phenotype was observed in chronic HCV infection with a significantly higher proportion of αβ CD8 T cell expressing FcγRIIIA (CD16). FcγRIIIA is a receptor that binds the Fc portion of antibodies and is associated with antibody-dependent cellular cytotoxicity (ADCC). FcγRIIIA is a low-affinity Fc receptor, meaning that binding will occur only if there is a large amount of antibody present, such as the opsonization of a cell (*231*). When an effector cell expressing FcγRIIIA engages the exposed Fc portion of a bound antibody on a target cell, FcγRIIIA activates the effector cell to deliver cytotoxic molecules like granzymes to kill the target cell (*232*). This function and receptor expression are usually attributed to NK cells, but can be expressed in other cell types including neutrophils, monocytes, and γδ T cells (*233-236*). FcγRIIIA expressing CD8 T cells in chronic HCV infection have a terminal effector phenotype, and functionally respond in a manner similar to that of NK cells upon CD16 stimulation, with robust degranulation after *in vitro* stimulation (*237*). This suggests that CD16+ CD8 T cells may contribute to the immune control of the virus by ADCC through an unexpected mechanism. ADCC is a critical function in HIV infection, as ADCC responses in adults are associated with slower disease progression (*238, 239*). Furthermore, HIV negative infants born to HIV positive mothers have evidence of passive acquisition of HIV-specific, ADCC-mediating antibodies. This same study showed that in infants that do become infected, higher levels of HIV-specific ADCC mediating antibodies correlated with reduced infant mortality (*240*). Importantly, in the RV144 HIV vaccine trial ADCC-mediating IgG was associated with decreased acquisition of HIV (*241*). However, the role of the expression of ADCC-mediating FcγRIIIA on CD8 T cells has yet to be elucidated in CHI. Therefore, a cohort of chronically infected, untreated individuals from the Rakai District of Uganda was used to survey for the expression of FcγRIIIA on CD8 T cells in **Paper IV** of this thesis (**Figure 8**). The ability of FcγRIIIA expressing CD8 T cells to mediate ADCC, their phenotype, and their transcriptional signature was also examined.

1.4.3 The MAIT cell response to HIV infection

Although it has not been shown that MAIT cells can recognize HIV antigen or be infected with HIV, perturbations in MAIT cells in cross sectional studies show that MAIT cells are altered during HIV infection. MAIT cells are lost permanently in the periphery and remaining MAIT cells are dysfunctional in CHI (*73, 242*). Studies of SIV infection in nonhuman primates (NHP) show similar findings of numerical decline in the blood. These studies also show fewer MAIT cell in the lymph nodes and broncho-alveolar lavage fluid compared to uninfected NHPs (*243*). This loss may contribute to weakened mucosal immunity and the susceptibility of chronically infected individuals to fungal and bacterial co-infections such as tuberculosis, non-typhoidal *Salmonella*, and *Streptococcus pneumoniae* infections (*244-247*). As TB is a common and sometimes deadly coinfection of the lung in those infected with HIV, studying the implication of declining MAIT cells to the susceptibility to TB is of great interest as MAIT cells can be found in the lung mucosa. MAIT cells in chronic HIV/TB co-infection are severely affected as in HIV monoinfection, but MAIT depletion is not seen in the periphery in TB mono-infection, suggesting HIV as the driver of MAIT cell decline (*248*). Furthermore, MAIT cells are enriched in bronchio-alveolar lavage fluizd in individuals with active tuberculosis (*301*). Several cross-sectional studies of CHI have observed activation and dysfunctionality in MAIT cells in tissues other than the peripheral blood, such as the lymph nodes and gut mucosa, compared to healthy controls (*73, 242, 249*). It was initially thought that the MAIT cell loss observed in CHI was due to the downregulation of CD161 on the MAIT cell surface, but use of the 5-OP-RU loaded MR1 tetramer confirmed the numerical loss of MAIT cells in the blood (*242, 250*). Still, loss of CD161 expression is a contributing factor to this pattern in patients with late stage chronic HIV infection (*66*). The residual MAIT cell population circulating in chronic untreated HIV infection express markers of activation , HLA-DR, CD69, CD38, and PD-1 (*73, 84, 242*). They also express higher levels of tissue-homing markers compared to uninfected controls such as CCR5, CCR6, CCR9, and CXCR6, suggesting MAIT cells may be recruited to sites of inflammation during HIV infection. Work with NHP models further elucidated that peripheral blood MAIT cells upregulate the gut-homing marker α4β7 within 3 weeks post SIV/SHIV infection. This, coupled with increased expression of proliferation marker Ki-67 and the expanded frequency of MAIT cells in the gut, suggested that loss of MAIT cells in the blood may be in part due to trafficking to the gut (*251*). Because mucosal barrier homeostasis and integrity is markedly disrupted during HIV infection, MAIT cells may be recruited to sites of bacterial infiltration in the mucosa in order to combat the rising microbial burdens. This prolonged activation of MAIT cells at these sites may ultimately cause their demise due to activation-induced apoptosis (*73, 252*).

Functionally, MAIT cells from chronically HIV infection individuals have reduced ability to produce IFNγ, TNF, and IL-17a after mildly fixed *E. coli* stimulation, *in vitro* (*253*). This functional decline is not observed in early HIV infection when MAIT cells are stimulated *in vitro* with the strong MAIT cell antigen 5-OP-RU (four months post-infection) (*250*). However, several years of ART partially restores the ability of peripheral MAIT cells to produce IL-17a and IFNγ, but was unable to diminish the expression of PD-1 (*242*). Recently, single-cell transcriptomic work showed a large upregulation of IFN-induced genes in MAIT cells in HIV infected individuals. The same study demonstrated that sustained IFNα signaling induced IL-10 production in monocytes, which in turn reduced IL-12 signaling, a powerful costimulatory molecule for MAIT cell functionality. This ultimately inhibited the MAIT cell response to *E. coli* stimulation *in vitro*, suggesting a mechanism contributing to diminished MAIT cell function in chronic HIV infection (*254*). The cytokine IL-7, which is thought to be necessary for T cell memory, may contribute to enhanced MAIT cell function. Short stimulation with IL-7 induces cytolytic "arming" of MAIT cells *in vitro* (84*)*. IL-7 is reduced in the blood in CHI, and administration as an HIV treatment may reduce the HIV reservoir (*84, 255*). In a small pilot study of HIV infected individuals on ART, administration of IL-7 was able to partially restore circulating frequencies of MAIT cells, suggesting IL-7 treatment may alleviate circulating MAIT cell loss (*256*). However, the effect of IL-7 administration systemically, especially in mucosal sites where MAIT cells may have their most important role, has not been determined. The exact cause and temporal dynamics of the loss, activation, and ultimate functional exhaustion of MAIT cells in HIV infection is unknown.

Because of the proposed role that MAIT cells play in combatting bacterial coinfections, and because they are irreversibly lost in the blood and to some extent the gut in HIV infection, this thesis aims in part to understand the temporal dynamics of MAIT cells in AHI (**Paper II**). Using a unique, prospective cohort from the U.S. Military HIV Research Program (MHRP) with cryopreserved blood samples from donor-matched, pre- and post-HIV infection time points captured within the first days of infection (**Figure 6**), the temporal dynamics of MAIT cells in AHI can be examined (*175*).

1.4.4 The iNKT cell response to HIV infection

As with MAIT cells, iNKT are reduced in frequency in the blood in HIV infection. In fact, CD4 expressing iNKT cells and are preferentially depleted from the blood compared to conventional CD4 T cells (*257-259*). However, the mechanism of decline for iNKT cells is likely different than that of MAIT cells. In two landmark studies by Sandberg et al. and Motsinger et al. in 2002, iNKT cells were shown to be preferential targets of HIV infection *in vitro* (*257, 258*). Given that iNKT cells express high levels of the HIV entry coreceptors CCR5 and CXCR4 alongside

the CD4 molecule, and are preferentially infected over conventional CD4 T cells, iNKT cells may play a significant role in viral seeding, propagation of the founder virus, and establishment of latent HIV reservoirs.

The CD4+ subset of iNKT cells is depleted faster than their CD4 negative counterparts during HIV infection, suggesting that they may be depleted by different mechanisms. HIV viral load measurements correlate inversely with the CD4+ iNKT cell counts and percentages, while the CD4 negative subset does not (*257, 258*). In NHPs, it was not only shown that iNKT cells decline in frequency and can be infected with SIV, but in a monkey species that lacks CD4+ iNKT cells, there is no iNKT cell depletion in SIV infection compared to healthy controls (*257, 260*). In the gut mucosa, CD4+ iNKT cells are more prevalent compared to the blood, but are depleted in individuals with CHI compared to healthy controls (*261*). Loss of gut, but not peripheral blood, CD4+ iNKT cells correlates with T cell activation and viral load, suggesting a link to disease progression. Furthermore, in individuals on therapy and thus undetectable viremia, CD4+ iNKT cells were found to be depleted in the blood but not in the gut mucosa (*262*). This is an interesting finding, and it may be that initiation of ART begins the trafficking of CD4+ iNKT cell from the blood into the gut, allowing for replenishment of their numbers in this tissue and not the peripheral blood (*251*). These authors also discovered that elite controllers had higher frequencies of iNKT cells in their gut mucosa that non-controllers, including those on therapy. This may suggest that natural control of HIV viremia prevents the loss of CD4+ iNKT cells in the gut. As the majority of iNKT cells in the gut express CD4 and produce T_H2 cytokines such as IL-4 and IL-10, the authors propose that the immunoregulatory role of these persisting iNKT cells suppress the immune activation and microbial translocation normally observed in HIV. Supporting this, the ability of gut iNKT cells from HIV infected people to produce IL-4 and IL-10 after PMA/ionomycin stimulation *in vitro* correlated inversely with CD4 and CD8 T cell activation. Along these lines, amount of IL-4 produced by iNKT cells *in vitro* correlated inversely with levels of marker of microbial translocation, LPS-binding protein, in the plasma.

Another case motivating further understanding iNKT cell dynamics in HIV infection involves the HIV proteins Nef and Vpu. Nef and Vpu downregulate a variety of host cell surface molecules, including MHC-I, in order to evade the immune response to viral antigen presentation. HIV can downregulate CD1d on the surface of APCs by interfering with its recycling from endosomal compartments (*161*). Thus, HIV has a mechanism to directly evade iNKT cell responses. Furthermore, iNKT cells can recognize HIV-infected DCs through a TCR-mediated mechanism (*263*). It has yet to be determined if iNKT cells are infected with HIV *in vivo*, which is one of the goals of **Paper III** of this thesis. In **Paper III** the temporal dynamics of iNKT cell depletion in the blood and gut mucosa in untreated and early treated HIV infection is explored, as well as the effects of AHI on iNKT cell phenotype, function, and gene expression profile in the blood. These analyses

were performed with the same cryopreserved blood samples from unique cohort of natural HIV infection as in **Paper II**, as well as an additional cohort of patients where ART was initiated in AHI from Thai donors using cryopreserved blood and fresh gut biopsy samples (**Figures 6** and **7**).

1.4.5 Disruption of the gut mucosal barrier in HIV infection and associated microbial translocation

Studies in NHPs illuminate how rapid depletion of gut-associated CD4 T cells in acute SIV infection through direct infection with SIV, or bystander cell death by the apoptosis of nearby cells that are either infected or uninfected, leads to a permanently altered balance of the gut barrier (*196, 264*). It is thought that the loss of IL-17 producing CD4 T cells is particularly devastating to the epithelial layer, as this cytokine and thus the cells that produce it are critical for enterocyte proliferation and maintenance. IL-17 producing CD4 T cells are also slow to recover after ART initiation (*265*). HIV infection also causes dysregulation of the gut microbiome, with declining representation of commensal bacteria and increasing frequency in more inflammatory bacterial species, even upon ART initiation (*266*). Finally, there is disruption of tight junctions between epithelial cells in the gut in HIV infection, and HIV proteins themselves may facilitate this disruption. The HIV proteins Tat and gp120 can be released from infected CD4 T cells and have been implicated in the disruption of tight junctions in the gastrointestinal tract (*267, 268*). This is facilitated through the aberrant internalization and sometimes proteasomal degradation of surface proteins involved in tight junction formation after induction of the mitogen-activated protein kinase (MAPK) pathway in epithelial cells (*269*). Additionally, proinflammatory cytokines released from epithelial cells and gut associated immune cells, including TNF, can stimulate NF-κB signaling, inducing actin cytoskeleton contraction and downregulate expression of proteins involved in tight junction formation between epithelial cells, as well (*270*). Observations of damaged and inflamed gut epithelia and lamina propria are observed even upon ART initiation in the acute stages of HIV infection, albeit the inflammation is somewhat ameliorated after treatment initiation. Furthermore, ART initiation in acute HIV has been shown to preserve IL-17 producing CD4 T cells in the gut (*286*). However even upon ART initiation in AHI, a biomarker of enterocyte turnover, intestinal fatty-acid binding protein (I-FABP), and marker of monocyte activation and microbial translocation, soluble CD14 (sCD14), remains elevated above healthy control levels (*271*). With or without ART, damage to this critical component of the immune system occurs during HIV infection and facilitates microbial translocation, or the translocation of microbial products from the lumen of the intestine into the bloodstream and driving systemic immune activation (*272*).

Systemic immune activation in HIV is marked by sustained levels of inflammatory soluble factors in the blood such as IFNα and TGF-β, polyclonal B cell activation, T cell activation, and a high turnover of CD4 and CD8 T cells (*273-276*). This leads

to both T cell exhaustion and the generation of new targets for HIV infection. The presence of microbial products in the blood stream is systemic and is observed even in ART treated individuals with durably suppressed viral loads (*272, 277*). When gastrointestinal microflora translocate into the lamina propria of the gut in a healthy state, they are rapidly phagocytosed by the host immune cells (*278*). However, in HIV infection there is dysregulation of the immune cell homeostasis in the lamina propria, and thus translocated microbes and microbial by products make their way into the blood and can even persist within the body at extraintestinal sites (*279*). LPS is a component of the cell wall in gram-negative bacteria and a TLR4 agonist as previously mentioned, and is most well studied in dendritic cell and macrophage activation (*280*). Initial studies of microbial translocation in HIV reported LPS levels elevated in the blood in CHI compared to uninfected controls (*272*). LPS levels correlated with soluble markers of innate immune activation, including IFNα. *In vitro* work in this study showed the induction of sCD14 expression from LPS-stimulated monocytes. In a somewhat disputed conclusion, the authors argue that sCD14 can act as a surrogate biomarker of microbial translocation. LPS can bind CD14 when bound to LPS-binding protein (LBP), another surrogate biomarker for microbial translocation produced in the liver. The LPS-LBP complex binds to CD14-expressing Kupffer cells, or liver-resident macrophages, which in turn shed their CD14 molecules (*281*). This hypothesis that sCD14 is exclusively a marker of microbial translocation is disputed by the notion that monocytes can be activated and produce sCD14 through innate cytokine stimulation, such as with IFNα, which is also elevated in the blood during chronic HIV infection (*282*). Whether or not sCD14 is a true marker of microbial translocation, its elevated levels in chronic HIV infection coupled with elevated levels of LBP, EndoCAb (endotoxin-specific IgG), LPS, and I-FABP in circulation strongly suggests that microbial translocation is occurring and influencing the immune response during chronic HIV infection (*283*). Additionally, Brenchley *et al.* found that LPS levels in the blood in CHI correlate with CD8 T cell activation measured as the coexpression of CD38 and HLA-DR, suggesting microbial translocation-induced immune activation can be a driver of the sustained CD8 T cell activation observed in HIV infection. Another marker of immune activation is C-reactive protein (CRP), and is a reliable marker of disease progression (*284*). It is produced is mainly from the liver, although IL-6 produced from monocytes can also cause the vascular endothelium to release this soluble factor. IFNγ inducible protein 10 (IP-10) is also a soluble marker of immune activation associated with HIV infection, and is a chemokine released from a variety of cell types including lymphocytes in response to IFNγ signaling (*285*). Sustained immune activation is thought to a leading cause of non-AIDS related co-morbidities like HIV-associated neurocognitive disease (HAND), cardiovascular disease, and liver and renal disease (*172*). Additionally, sustained immune activation and the increased microbial presence in the periphery may be a driver of the MAIT and iNKT cell depletion and activation observed in chronic HIV infection studies, the temporal dynamics of which are aims in **Paper II** and **III** of this thesis.

2 AIMS

Initially, the goal of this thesis was to gain an understanding of the unknown immunodynamics of MAIT cells in AHI. To do this, an optimized flow cytometry panel was required to identify and sort for MAIT cells. The iNKT cell markers were also included in this flow cytometry panel to enhance the use of a precious cohort of natural HIV infection. Exploration of these unconventional and innate-like T cells in HIV infection added motivation to complete an unfinished story about an innate-like conventional CD8 T cell subset, also in the context of HIV. Therefore the aims of this these are as follows:

1. Develop a flow cytometry panel to identify conventional and unconventional T cells and markers of activation on their surface to maximize use of a precious longitudinal cohort in HIV infection.
2. Determine the phenotypic, functional, and transcriptional changes in peripheral MAIT cells during AHI and define drivers of any observed changes such as correlations to viral load or markers of microbial translocation.
3. Determine the phenotypic, functional, and transcriptional changes in peripheral and colonic iNKT cells during AHI and upon ART initiation during AHI.
4. Examine for the presence of a unique, innate-like CD8 T cells in chronic, untreated HIV infection and define their functional relevance in HIV.

3 METHODS

3.1 Ethics

All subjects in studies RV217/WRAIR#1373, RV254/SEARCH 010/ WRAIR#1494, and RV304/SEARCH 013/WRAIR#1751, were adults and provided written informed consent. For subjects that were unable to read, the consent document was read to them with an impartial witness present; the volunteer, the witness and the study staff obtaining consent signed the affidavit with a signature or mark. Studies were reviewed and approved by the human subject ethics and safety committees in each country, as well as by the Walter Reed Army Institute of Research (WRAIR) (Silver Spring, MD, USA), in compliance with relevant federal guidelines and institutional policies. RV304: The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University; and the WRAIR Institutional Review Board. RV217: Institutional Review Board Royal Thai Army Medical Department; Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU); Uganda National HIV/AIDS Research Committee (NARC); Mbeya Medical Research and Ethics Committee (MMREC) and the National Health Research Ethics Committee (NatHREC); and the WRAIR Institutional Review Board. RV254: The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University; and the WRAIR Institutional Review Board.

The RV228 Rakai District cohort was approved by the following institutional review boards in the United States and Uganda: the institutional Review Boards of Uganda's National Council for Science and Technology and the National AIDS Research Committee, as well as Division of Human Subjects Protection at the Walter Reed Army Institute of Research. All participants gave written informed consent. For samples from the Couples Observational Study (COS) in Kampala, Uganda, all participants gave written-informed consent, and ethical approvals for the study were obtained from Uganda's National Council for Science and Technology and the National AIDS Research Committee and the University of Washington.

3.2 Subjects

3.2.1 The RV217 Early Capture HIV Cohort study (ECHO)

To investigate MAIT and iNKT cell dynamics in AHI, longitudinal samples from 29 individuals were available for the study of MAIT cells (**Paper II**), and 23 overlapping individuals for the study of iNKT cells (**Paper III**) in AHI. These individuals were enrolled in a unique U.S. Military HIV Research Program (MHRP) cohort, as previously described (*175*). Briefly, consenting, HIV uninfected adults at high-risk for acquiring HIV were enrolled in this prospective trial to study HIV incidences rates and viral load dynamics in AHI in three countries in East Africa and Thailand (**Figure 6)**. HIV uninfected participants had lifestyle qualifications

for enrollment, with women exchanging goods for sex as the predominant enrollees in East African countries, and men who have sex with men (MSM) as the predominant enrollees in Thailand. Enrollees were educated on HIV prevention and given access to providers for infection treatment, based on national guidelines. Participants were screened bi-weekly for the presence of HIV RNA in the plasma using the Real-Time HIV Assay (m2000 Real Time System, Abbott Molecular), and if HIV was detected, were entered in a second phase of study where follow up visits collected larger blood volumes in order to study the immunodynamics of AHI. Cyropreserved PBMC were used with donor-matched pre-infection and at least 3 post-HIV infection time points, with time points ranging from 1–1,040 days post first positive test for HIV RNA. The majority of the data about iNKT and MAIT cell dynamics in AHI in this thesis come from time points coinciding with peak viral load (VL) (median 16 days, range 14–22, since first positive test for HIV RNA), set point VL (median 43 days, range 31–50, since first positive test for HIV RNA), and early chronic infection (median 85 days, range 60–126, since first positive test for HIV RNA (**Figure 6**)).

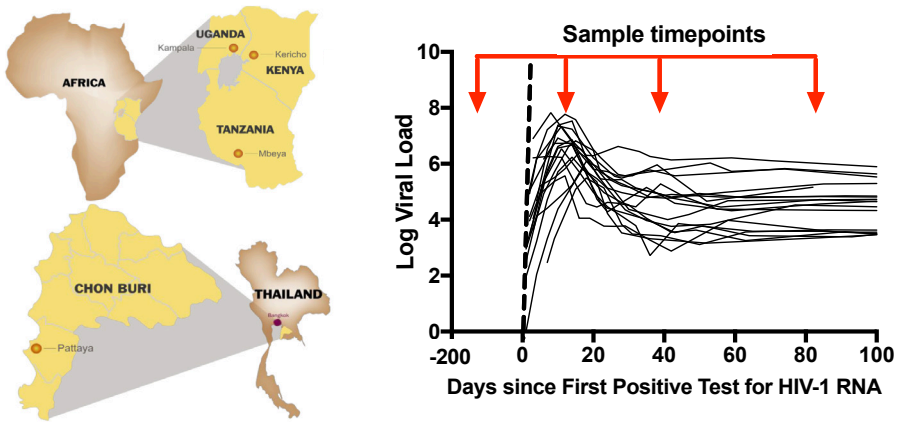

Figure 6. The RV217 Early Capture HIV Cohort (ECHO). *Samples from the RV217 cohort were used to study MAIT and iNKT cell dynamics in acute HIV infection. This study enrolled high-risk, consenting adults at four clinical research sites: Walter Reed Project, Kericho, Kenya; Makerere University Walter Reed Project, Kampala, Uganda; Mbeya Medical Research Center, Mbeya, Tanzania; and Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.*

3.2.2 The RV254/SEARCH 010 early ART initiation, and the RV304 uninfected control cohorts

RV254 is a currently ongoing study in Bangkok, Thailand that enrolls acutely HIV infected individuals in order to study the effects of ART initiation in the acute stages of HIV infection, wherein ART is initiated a median of 4 days from cohort enrollment (**Figure 7**). For **Paper III**, cryopreserved PBMC were used to study iNKT

cell frequency, phenotype, and functionality in the blood, with samples from 40 donors after ART initiated during AHI. Rectosigmoid colon biopsies collected by sigmoidoscopy from RV254 participants were used to study MAIT and iNKT cell frequency in the colon in acute, untreated HIV infection. In total, 26 donors from RV254 participating in elective rectosigmoid biopsies at any time during diagnosis, and from 20 donors followed up after 24 months of treatment, were collected to study iNKT cell phenotype in AHI and after treatment initiation in the colon (**Paper III)**. Of the acutely HIV infected donors from the RV254 cohort, samples from 7 were used to study MAIT cell frequencies in the colon before ART initiation (**Paper II)**. Rectosigmoid colon biopsies from 17 uninfected age- sex- and risk- matched uninfected controls from the RV304/SEARCH 013 study were used to compare MAIT cell frequency in HIV uninfected controls (**Paper II**), whereas samples from 28 donors were used to study colonic iNKT cell frequency and phenotype as uninfected controls.

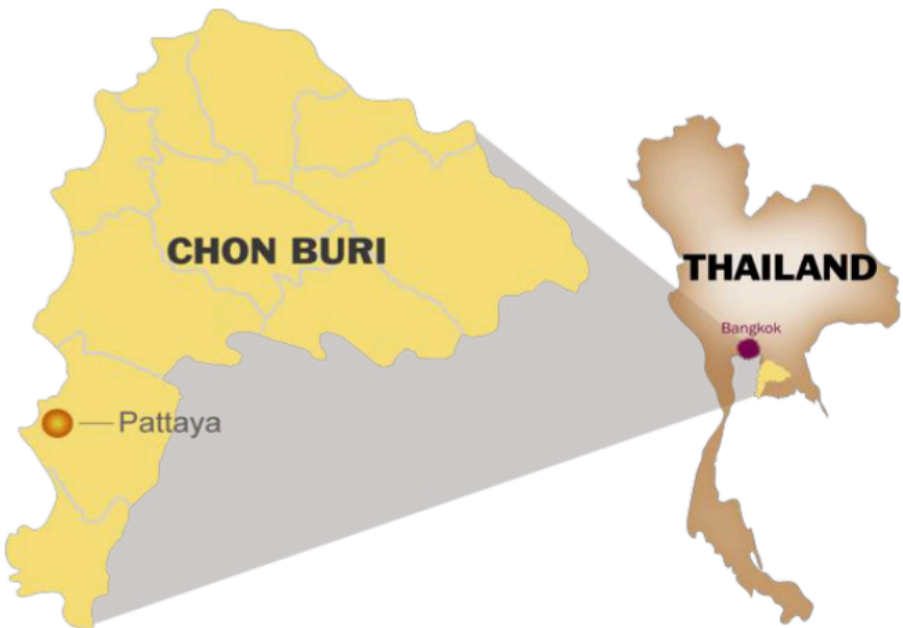

Figure 7. The RV254 early ART initiation cohort. *Donors with acute HIV infection from Thailand were used to study the immunodynamics of ART initiated in the acute stages of HIV infection. Cryopreserved PBMC and rectosigmoid colon biopsies from the RV254 cohort were used to study the effect of treatment initiation on iNKT cells in acute HIV infection before and after treatment. Rectosigmoid colon biopsies were used to study MAIT cell frequencies in untreated, acute HIV infection.*

3.2.2.1 Biopsy processing and calculation of absolute number of colonic T-cell subset

RV254 or RV304 subjects underwent a routine sigmoidoscopy procedure under moderate conscious sedation. Approximately 30 endoscopic biopsies were randomly collected from the sigmoid colon using Radial Jaw 3 biopsy forceps (Boston Scientific, Natick, MA, USA), with 20–25 processed for flow cytometry analysis within 30 minutes of collection, as previously described (*286*). Cell counts were performed manually by Trypan Blue exclusion, which allows exclusion of epithelial cells due to morphological differences compared to lymphocytes. Absolute numbers of conventional CD4 T, iNKT, and MAIT cells per gram of gut tissue were calculated by multiplying the total viable lymphocyte count by frequencies of cell subsets obtained from flow cytometric analysis. The total lymphocyte count per gram of tissue was calculated by dividing the viable lymphocyte count by tissue weight. This proportion was then multiplied by the percent of cells in the live lymphocyte gate and that number was subsequently multiplied by the percent of T cells. The absolute number of colonic T cells was used in conjunction with the subset percentages to determine the absolute number of each T cell subset per gram of biopsy tissue.

3.2.3 The RV228 chronic untreated HIV infection cohort

Donors with chronic, untreated HIV infection and community-matched uninfected controls were used to both optimize performance of OMIP-046 (**Paper I**) and examine the prevalence of FcγRIIIA+ CD8 T cells in CHI (**Paper IV**). Study participants aged 15–49 years old were enrolled in a prospective community-based cohort to assess the prevalence and incidence of HIV infection in Rakai District of Uganda, from 1998 to 2004 (**Figure 8**) (41–43). The Rakai District borders Lake Victoria and is largely a fishing district with a high incidence of HIV infection, predominantly in women who exchange goods for sex (*287-289*). Infected and enrolled subjects had continued annual follow up through 2008. Blood samples from 103 randomly selected HIV seropositive individuals and 40 community-matched seronegative controls were obtained. PBMCs were isolated and cryopreserved as described previously (*290*). None of the patients had received ART. The HIV–infected study participants initiating ART were from the Couples Observation Study (COS) in Kampala Uganda as previously described (*291*). 32 HIV-infected partners in each HIV–serodiscordant couple was followed up after the initiation of ART. Samples were collected; CD4 T cell counts determined and viral load assessments made at baseline, 6 and 12 months after initiation of ART.

Figure 8. The RV228 chronic untreated HIV infection cohort to study the prevalence of FcγRIIIA expressing CD8 T cells. *Cryopreserved PBMC from the Rakai District, Uganda were used to determine the frequency, phenotype, function, and transcriptional signature of FcγRIIIA-expressing CD8 T cells in chronic, untreated HIV infection. Samples from the Kayunga District were used to determine the frequency of FcγRIIIA-expressing CD8 T cells pre- and post-antiretroviral treatment.*

3.3 Polychromatic flow cytometry

Polychromatic flow cytometry panels were used to measure MAIT, iNKT, and conventional CD8 T cell phenotype, function, and for cell sorting for downstream transcriptomics. The panels can be found in detail in **Paper I, II, III** and **IV.** Briefly, cryopreserved samples were thawed in pre-warmed media, washed, stained with LIVE/DEAD Fixable Aqua Dead Cell dye (ThermoFisher), blocked for Fc receptors using normal mouse serum (ThermoFisher), and surface stained with antibody cocktail. Samples were surface stained at room temperature for 30 minutes, and some were intracellularly stained using BD Perm/Wash Buffer (BD Biosciences) at room temperature for 30 minutes. For assessment of transcription factors, cells were washed, permeabilized and fixed using an optimized kit (FOXP3 transcription factor staining buffer set) before intranuclear stain. Some samples were fixed in 2% paraformaldehyde or BD FIX/PERM Buffer (BD Biosciences) before acquisition on a 5 laser, 16-parameter BD LSRII SORP flow cytometer (BD Biosciences) within 12 hours of staining. Other samples used for sorting for downstream transcriptomics were resuspended in sorting buffer (PBS containing 1% BSA) and sorted for either RNA-Seq, targeted multiplexed RT-qPCR with Fluidigm Biomark, or the functional PanToxiLux Assay. Data were analyzed with FlowJo v.9.9.4 (TreeStar). Flow cytometry data were acquired with a BD LSR II instrument or a BD FACSCanto II instrument (BD Biosciences). Sorting was performed on a 5-laser BD FACSAria II SORP (BD Biosciences) contained in a

biosafety cabinet. Clinical lymphocyte immunophenotyping was performed using the FACS MultiSET System and run on a FACSCalibur using the single-platform Multitest four-color reagent in combination with Trucount tubes (BD Biosciences).

3.3.1 Multiplexed targeted quantitative gene expression analysis (RT-qPCR)

To study gene expression changes in MAIT cells in AHI, CD161^{bright} Vα7.2+ MAIT cells from 20 individuals from the RV217 acute capture cohort from one pre-infection and three post-infection time points were bulk sorted (100 cells/well) directly into reaction mixture (SuperScript III Reverse Transcriptase/Platinum Taq Mix, Cells Direct 2X Reaction Mix, Invitrogen) into a 96 well plate (**Paper II**). The same was done for three sorted T cell populations and CD56^{dim} CD3- NK cells from 8 donors from the RV228 Rakai District chronic HIV infection cohort to study gene expression in FcγRIIIA expressing CD8 T cells (500-1,000 cells/well) (**Paper IV**). RNA from bulk sorted Vβ11+Vα24+ iNKT cells from one pre- and two post-infection time points was used from 10 donors from the RV217 cohort to study gene expression in iNKT cells in AHI (**Paper III**). Targeted genes evaluated for each cell type can be found in detail in **Paper II** , **III**, and **IV**. Briefly, reverse transcription and specific transcript amplification were performed using a thermocycler (Applied Biosystems Gene Amp PCR System 9700) with the following parameters: 50 °C for 15 minutes, 95 °C for 2 minutes, and 95 °C for 15 seconds; and then 60 °C for 30 seconds for 18 cycles. Amplified cDNA was then loaded into a Biomark 96.96 Dynamic Array chip using the Nanoflex IFC controller (Fluidigm). This microfluidic platform was then used to conduct quantitative PCR (qPCR). Threshold cycle (CT), as a measurement of relative fluorescence intensity, was extracted from the BioMark Real-Time PCR Analysis software.

3.3.2 RNA-seq analysis

In **Paper II**, peripheral blood MAIT cells identified as CD161^{bright} Vα7.2+ were purified by sorting (range of 1911–64,011 cells) using a FACS Aria SORP (BD Biosciences), pelleted, and overlaid with 250 µl of RNAlater (ThermoFisher) and frozen at −20 °C. Sequencing was performed at SUNY Molecular Analysis Core. RNA was extracted using the RNeasy Mini Kit (Qiagen), and RNA quality and concentration were assessed with the Agilent 2100 Bioanalyzer Pico Chip. RNA-Seq libraries were prepared using the SMART-Seq v4 Ultra Low Input RNA Kit (Clontech) according to the manufacturer's instructions. Amplified material was purified using Agencourt AMPure XP beads (Beckman). cDNA quantity was assessed on a Qubit 3.0 (ThermoFisher) and fragment size was evaluated on a 2100 BioAnalyzer (Agilent). The PCR products were next indexed using the Nextera XT DNA Library Prep Kit (Illumina) according to the manufacturer's instructions. Briefly, products were tagmented using the Amplicon tagment mix containing Tn5

transposase and indexed using Nextera index 1 (i7) and index 2 (i5) primers. The libraries were again cleaned-up with Agencourt AMPure XP beads, quantified, pooled, and sequenced across 75 base pairs (bp) using a single-end strategy with a 75-cycle high output flow cell on a NextSeq 500 (Illumina). Nine biological replicates were sequenced, with four donor-matched time points corresponding to one pre-infection and three post HIV-infection time points at peak viral load, set point viral load, and early chronic infection. Median reads per sample was 22.9 million. The Unix based program Spliced Transcripts Alignment to a Reference (STAR) v.2.6.1 with human genome hg38 was used for alignment, and transcription mapping was performed using RNA-seq by Expectation Maximization (RSEM) v.1.3.155 (*292, 293*). The feature Counts program was used for counting mapped reads. RUVSeq v1.12 was used to remove unwanted variation and differentially expressed gene list was generated by edgeR v3.20. R package (*294, 295*). The GSEA method as used for finding statistically significant pathways with 5,917 gene sets of GO in Molecular Signatures Database (MSigDB) issued by Broad Institute (*296*).

3.4 Functional assays

3.4.1 iNKT and MAIT cell functionality measured in vitro

MAIT and iNKT cell functionality were assessed using one of three stimulation techniques (**Paper II** and **III**). PBMC were stimulated for 24 hours with briefly fixed *E. coli* (D21) together with anti-CD28, or for 24 hours with rhIL-12 and rhIL-18, or for 6 hours with PMA/ionomycin as the manufacturer described (eBioscience™ Cell Stimulation Cocktail (500X), ThermoFisher). All conditions included BFA and monensin for the last 6 hours of stimulation and were stained for flowy cytometry for markers of degranulation and intracellular cytokines.

3.4.2 PanToxiLux Assay to measure ADCC

In **Paper IV**, measurement of ADCC was performed using the PanToxiLux assay (OncoImmunin, Gaithersburg, MD) similar to previously described (*297*). rHIV BaL gp120 (catalog no. 4961; obtained through the National Institutes of Health AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health) were used to coat target CEM. NKR_{CCR5} cells, which are resistant to spontaneous NK cell lysis. After coating CEM. NKR_{CCR5} target cells with gp120 in 0.5% FBS–RPMI media, cells were labeled with a fluorescent target cell marker, TFL4 (OncoImmunin), for 15 minutes at 37°C and 5% CO_2. Cells were washed twice with PBS and stained with LIVE/DEAD Fixable Aqua Dead Cell Stain (Life Technologies) for viability for 30 minutes at RT. After washing in 0.5% FBS–RPMI media, cells were resuspended to a concentration of 8.0×10^5 cells/ml. Sorted effector cell populations (NK cells,

CD45RA+CD57+ CD8 T cells, and CD45RA-CD57- CD8 T cells) were washed in 0.5% FBS–RPMI media and resuspended to a final concentration of 24 x 10^6 cells/ml for an E:T ratio of 30:1. In a 96-well polypropylene plate, 25 µl of both target and effector cell suspensions were both added to wells along with 75 µl of the granzyme B substrate (OncoImmunin). After incubation for 5 minutes at room temperature, 25 µl of HIV-Ig (North American Biologicals, Miami, FL) at a 0.5 µg/ml was added, and the plate was incubated for another 15 minutes at room temperature. The plate was then spun at 300xg for 1 minutes and incubated at 37°C and 5% CO_2 for 1 hour. After washing cells were acquired on an LSR II SORP (BD Biosciences) on the same day. Fluorophores were detected using a 488-nm 50-mW laser with 515/20 filters to detect the granzyme B substrate, a 406-nm 100-mW laser with 525/50 filters to detect Aqua LIVE/DEAD stain, and a 640-nm 40-mW laser with 670/30 filters to detect TFL4 target cell stain. Data were analyzed by using FlowJo 9.7.5 (Ashland, OR).

3.5 Soluble cytokine analysis

Luminex® bead based detection assays were used to measure plasma levels of CRP and IL-6 (EMD Millipore, Billerica MA) per manufacturer's instructions. Samples were mixed with a cocktail of MagPlex® magnetic microspheres bound to capture antibody specific to proteins of interest. After incubation with the sample overnight at 4 °C, excess sample was washed away using a magnetic plate washer (BioTek Instruments, Winooski VT). Biotinylated detection antibody cocktail was added for 1 hour at RT. Streptavidin-phycoerythrin was added for 30 minutes before a final wash and resuspension in sheath fluid. Data was collected on a FlexMap 3D® system. Levels of sCD14 and I-FABP were measured by chemiluminescent detection ELISA (R&D Systems, Minneapolis MN) per manufacturer's instructions and read on a VersaMax® reader (Molecular Devices, Sunnyvale CA). Data were analyzed in Prism version 6.0 for Mac OS X (GraphPad, La Jolla CA) using a 4-parameter fit standard curve.

3.6 Statistical analyses

The Wilcoxon Signed Rank test was used for comparison of non-parametrically distributed paired data sets. Unpaired data was analyzed using the Mann–Whitney test, and the Spearman rank correlation test was used to compare correlation between two parameters. For matched longitudinal analysis a nonparametric Friedman test was performed with the Dunn's multiple comparison test. Statistical analyses were performed with GraphPad Prism v.6.0c (GraphPad Software). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

4 RESULTS

4.1 An optimized multiparameter flow cytometry panel (OMIP-046) to identify conventional and unconventional T cells and surface expression of markers of activation and exhaustion

Because of the unique nature of the cohorts used in this thesis, it was essential that the methods used were optimized to maximize use of precious cohorts with longitudinal sampling before and after untreated, AHI. In particular, the nature of the cohort is invaluable given that current protocol of HIV treatment now recommends beginning ART upon diagnosis. Being able to study the immunodynamics of natural, untreated HIV infection is a gift and therefore every precaution was taken to ensure that no samples were wasted or under-utilized. The dynamics of unconventional T cells in acute, untreated HIV infection has yet to be explored in this setting, therefore the first step was to design a flow cytometric panel to measure the frequency and phenotype of conventional and unconventional T cells. This panel was designed to be compatible with FACS sorting, with the goal of sorting several cell subsets for downstream gene expression analysis. Thus, in **Paper I**, a 14-color flow cytometric panel was designed to identify conventional, adaptive CD8 and CD4 T cells, as well as iNKT and MAIT cells (**Figure 9a** and **b**). Cell surface expression of T cell activation markers HLA-DR and CD38, and markers of T cell exhaustion PD-1 and TIGIT were measured on each of these four subsets, with particular attention paid to the unconventional T cell subsets (**Figure 9c**). Although the initial goal of this thesis was to determine MAIT cell dynamics in AHI as in **Paper II**, the ability to have similar data for iNKT cells was beneficial and primed additional studies, resulting in **Paper III**.

Using OMIP-046, the relative baseline frequencies of MAIT cells and iNKT cells in HIV uninfected individuals could be determined (**Figure 10**). As MAIT cells and iNKT cells can represent anywhere from 1-10% or 0.1-1% of peripheral T cells, respectively, establishing baseline frequencies of these variable T cell subsets between geographic locations outside of HIV infection was important. This information is essential to interpret subtle changes in frequencies in donors from different regions post HIV-infection.

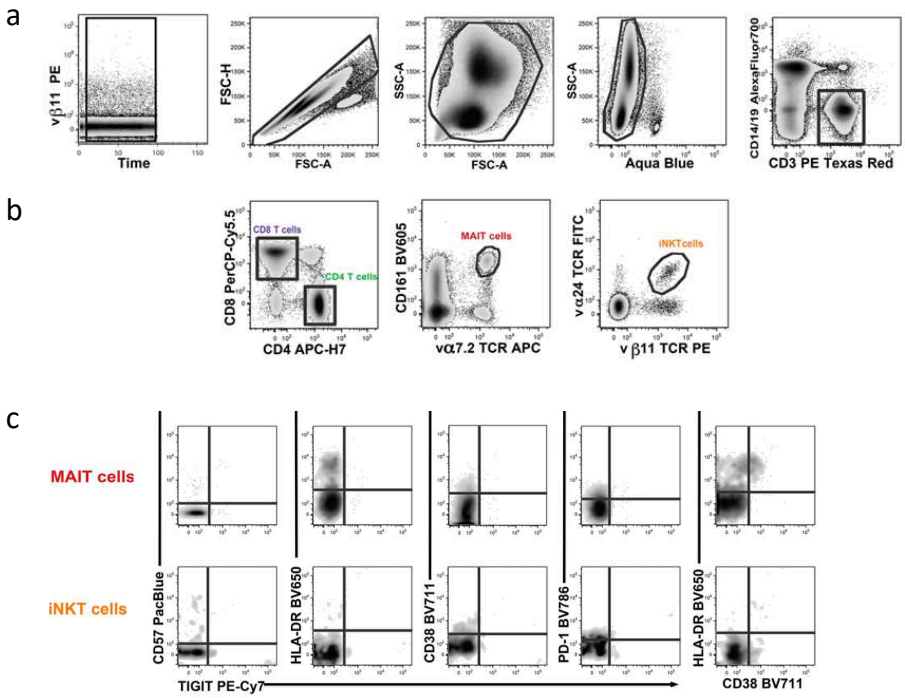

Figure 9. Unconventional and conventional T cells and the expression of markers of T cell activation and exhaustion can be identified using OMIP-046. a) *Example gating strategy to identify T cells from cryopreserved PBMC from a chronically infected, HIV positive donor.* ***b)*** *Identification of the four T cell populations of interest, convention CD4 T cells (**green**), conventional CD8 T cells (**purple**), MAIT cells (**red**) and iNKT cells (**orange**).* ***c)*** *Example staining of markers of T cell activation HLA-DR and CD38 markers of exhaustion PD-1 and TIGIT on the surface of the unconventional T cell subsets. Reproduced with permission from (298).*

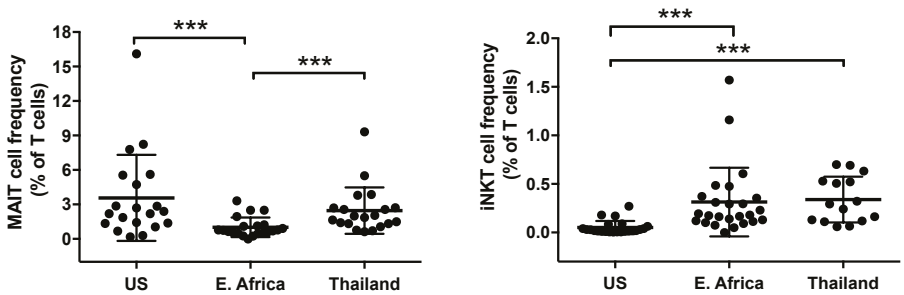

Figure 10. OMIP-046 can identify the relative frequency of unconventional T cell subsets in HIV uninfected donors in the U.S., and in East African and Thai donors from the RV217 cohort. *Unconventional T cell subsets were identified in cryopreserved PBMC from uninfected U.S. based donors and compared to the frequencies of pre-HIV infection time points in donors from East Africa and Thailand in the RV217 cohort.*

Interestingly, compared to HIV uninfected U.S. donors, HIV uninfected individuals from East Africa had lower frequencies of MAIT cells but higher frequencies of iNKT cells in the peripheral blood. Uninfected Thai donors had comparable levels of MAIT cells compared to the U.S. donors, but higher levels of iNKT cells. The reasons underlying the differences between unconventional T cell frequencies between geographical locations is beyond the scope of this thesis, however genetic and environmental factors could play a role in determining unconventional T cell frequencies in the blood.

4.2 MAIT cells expand in the blood and the gut in acute HIV infection

After optimizing OMIP-046, the panel was used to study MAIT cell frequency and phenotype in AHI (**Figure 11a**). This was done using cryopreserved PBMC from the RV217 cohort, with longitudinal samples of natural HIV infection from pre- and post-HIV infection time points in AHI from donors from East Africa and Thailand (**Paper II, Supp. Table 1**) (**Figure 6**). In analysis of one pre-infection time point and donor matched post- HIV infection time points ranging from a median of 14 days post first test for HIV RNA out to 3 years post-infection, it was determined that the MAIT cell frequency is relatively unchanged compared to pre-infection within the acute infection stage (**Figure 11b**). This was surprising, given that previous research using cross-sectional studies would suggest that the MAIT cell loss observed in CHI may occur within the acute infection time frame. Data generated from the RV217 cohort suggests that the loss occurs later than these studies hypothesized. However, the absolute count of MAIT cells revealed dynamic changes within the MAIT cell compartment in the blood in AHI. A brief period of MAIT cell expansion was observed at the time point corresponding to a median of 43 days post-HIV infection, around VL set point, with return to pre-infection levels later in infection (**Figure 11c** and **Figure 19**). Using an additional cohort, RV254, in which rectosigmoid colon biopsies from acutely infected individuals were collected (**Paper II**, **Supp. Table 2**), an expansion of the absolute cell count of MAIT cells was also observed in the colon (**Figure 11d**). As detailed earlier in this thesis, the T cell compartment is incredibly dynamic during AHI. Therefore, normalized values of T cell abundance are critical measurements for understanding their perturbations during this time period. Taken together, the overall MAIT cell count expands in both the blood and colonic gut mucosa in AHI. In donors where follow up samples were available out to 3 years post-infection, overall MAIT cell frequency began to decline, consistent with observations of MAIT cell decline observed in cross sectional studies of chronic HIV infection (**Figure 11b**).

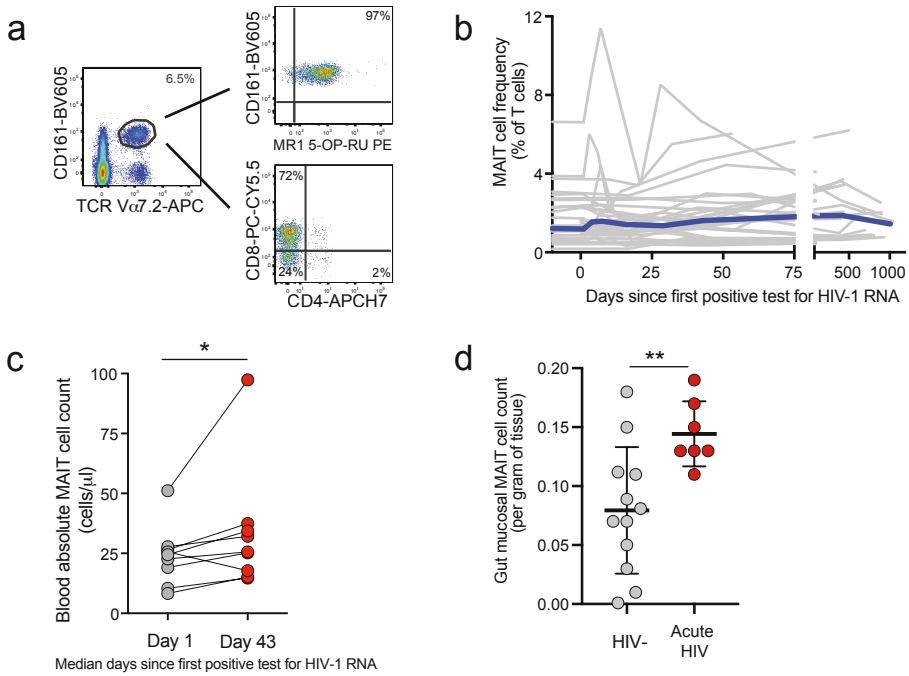

Figure 11. MAIT cells expand in the blood and gut in acute infection HIV infection. a*) MAIT cells were identified as CD161^{bright} Vα7.2+ amongst T cells in cryopreserved PBMC using OMIP-046 on donors from the RV217 cohort.* ***b****) The frequency of MAIT cells was determined in longitudinal samples from pre-infection and into AHI.* ***c****) Absolute counts of MAIT cells comparing 1 day post first positive test for HIV RNA and 43 days post first positive test for HIV RNA, the time point corresponding to set point VL in the RV217 cohort.* ***d****) The normalized MAIT cell count per gram of tissue in recto-sigmoid biopsies in uninfected control group compared to HIV infected donors in acute infection.* ***Material from:*** *Lal et al., Dynamic MAIT cell response with progressively enhanced innateness during acute HIV-1 infection, Nature Communications, 2019, Nature Research.*

4.3 MAIT cells have an activated phenotype and gene expression profile, and enhanced functional capacity in acute HIV infection

The data set generated with OMIP-046 was also interrogated for the expression of markers of activation and exhaustion measured on the surface of MAIT cells within AHI from the RV217 cohort. At the first measured time point post-infection, corresponding to peak VL, MAIT cells express significantly higher levels of T cell activation markers HLA-DR and CD38. They also express higher levels of markers of T cell exhaustion, the check point inhibitors PD-1 and TIGIT (**Paper II**, **Figure 2a**). In MAIT cells sorted based on the OMIP-046 panel for targeted multiplexed gene expression analysis (RT-qPCR), markers of activation and exhaustion were also elevated at the transcript level (**Paper II, Figure 2b**). Expression of these markers

correlated with the gene encoding Ki-67 (*MKI67*) at the post-infection time point corresponding to peak VL (**Paper II, Figure 2d**), suggesting activation induced cell proliferation. Induction of MAIT cell proliferation due to activation at peak VL may indicate why there is an increase in MAIT cell levels a few weeks later at the time of set point viral load (**Figure 19**). Increased expression of markers of activation and exhaustion is observed in MAIT cells throughout AHI, and out to 3 months post- infection.

MAIT cells in the blood are dysfunctional in chronic HIV infection compared to healthy controls, but the timing of the onset of this dysfunction is unknown. Therefore, MAIT cell functionality was explored through *in vitro* stimulation of RV217 samples. Total PBMC were stimulated with either mildly fixed *E. coli* or PMA/ionomycin, and expression of markers of cytotoxicity (CD107a and GrzB) or cytokine (IFNγ and TNF) produced by MAIT cells was analyzed (**Paper II, Figure 6a-c**). MAIT cells displayed a brief boost in functional capacity at the time point corresponding to set point VL, driven by enhanced CD107a and GrzB production after *E. coli* stimulation (**Paper II, Figure 6d**). However, at the time point corresponding to early, chronic infection (3 months post-infection) MAIT cells showed declining functional capacity compared to pre-infection, with lowered ability to produce IFNγ and TNF after PMA/ionomycin stimulation, possibly marking the beginning of the dysfunctionality observed in MAIT cells in chronic HIV infection (**Paper II, Figure 6d**).

Taken all together, MAIT cells become activated as early as peak VL in AHI, and this may lead to activation-induced cell proliferation, the consequence of which is measured a few weeks later with an increase in MAIT cell counts in the blood and colon. MAIT cell activation may in turn induce a boost in functional capacity, measured at set point VL. MAIT cells may be exhausted as a consequence of activation and functional boost early on, given that their decline in functional capacity is observed by 3 months post-infection.

4.4 MAIT cell activation and function correlate with markers of microbial translocation and innate immune activation

To further understand what drives MAIT cell activation and functional changes in AHI, MAIT cell activation and function were correlated with soluble factors in the plasma that correspond to innate immune activation and markers of microbial translocation. As MAIT cells have a proposed protective role at the gut mucosa, and their loss in chronic HIV infection may contribute to sustained immune activation and microbial translocation, MAIT cells could be associated with markers of these events in AHI. Measurements of soluble factors in the plasma in AHI show peak levels of marker of innate immune activation CRP at peak VL, with gradual and moderately elevated levels of sCD14 and I-FABP within the acute infection stage (**Paper II, Figure 7a**). IL-6 had gradually rising levels, with the

highest observed at set point VL. The peak of MAIT cell activation, observed at peak VL (median days post first positive test for HIV RNA = 14), correlated with sCD14 levels at the same time point (**Paper II, Figure 7b**). Likewise, the peak of MAIT cell function, observed at viral set point (median days since first positive test for HIV RNA = 43), correlated with levels of sCD14, also at the same time point (**Paper II, Figure 7b**). Further analysis revealed that CRP levels at peak VL (median day since first positive test for HIV RNA = 16), where CRP levels are highest, can predict MAIT cell functionality at later time points in acute HIV, correlating positively with TNF and IFNγ expression by MAIT cells at set point VL (median day since first positive test for HIV RNA= 43) and early chronic infection (median days since first positive test for HIV RNA = 85) (**Paper II**, **Figure 7c**). These data suggest that systemic immune activation and microbial translocation may be contributing to perturbations in MAIT cell function and phenotype in AHI.

4.5 In acute HIV infection MAIT cells become more innate-like phenotypically, functionally, and transcriptionally

The altered gene expression in MAIT cells in AHI measured by targeted multiplexed RT-qPCR motivated a deep dive into changing gene expression patterns induced by AHI in MAIT cells. Therefore, RNA-seq was performed on bulk sorted $CD161^{bright}$ Vα7.2+ MAIT cells from nine donors from 1 pre-infection time point and 3 post-infection infection time points in AHI. RNA-seq revealed a large upregulation of genes at the post-infection time point compared to pre-infection, corresponding to peak VL, with a trend towards normalized gene expression profile by the time point corresponding to early chronic infection (**Figure 12a**). When these differentially expressed genes in AHI were subjected to Gene Set Enrichment Analysis (GSEA), a pathway entitled Natural Killer cell mediated immunity was found to be upregulated in MAIT cells (**Figure 12b**). Further analysis from the targeted multiplexed gene expression data on MAIT cells also revealed the upregulation of a large number of genes normally attributed to NK cells, such as *NCAM1*, *KLRD1*, *NKG7*, *IL12RB1*, and *IL18R1* (**Figure 12c**). Taken together, MAIT cells in AHI are skewed towards an innate transcriptional profile compared to pre-infection. An innate-like MAIT cell phenotype was next explored, motivated by previous research that identified a subset of MAIT cells that expresses CD56 (NCAM-1), a cell surface protein that is also often associated with NK cells (*83*). CD56+ MAIT cells express elevated levels of IL-12R and IL-18R on their surface and produce more IFNγ in response to stimulation with the innate cytokines IL-12 and IL-18 *in vitro* compared to their CD56- counterparts. This pattern was confirmed in RV217 uninfected controls (**Figure 12e**). In AHI, MAIT cells expressing CD56 expanded overtime, with the largest increase observed at the last available time point out to 3 years post infection (**Figure 12d**). Stimulation of pre- and post-HIV infection time points *in vitro* revealed a maintenance of the ability of both CD56- and

CD56+ MAIT cells to respond to IL-12 and IL-18 stimulation *in vitro* post-HIV infection. This is counter to what is observed with other means of MAIT cell stimulation, where MAIT cells have declining functional responses at the same time point into early, chronic infection in response to PMA/ionomycin or *E. coli* stimulation (median days since first positive test for HIV RNA=85) (**Paper II, Figure 6d**). These data suggest an enhancement of innate-like gene expression, phenotypic, and functional profile in MAIT cells in AHI.

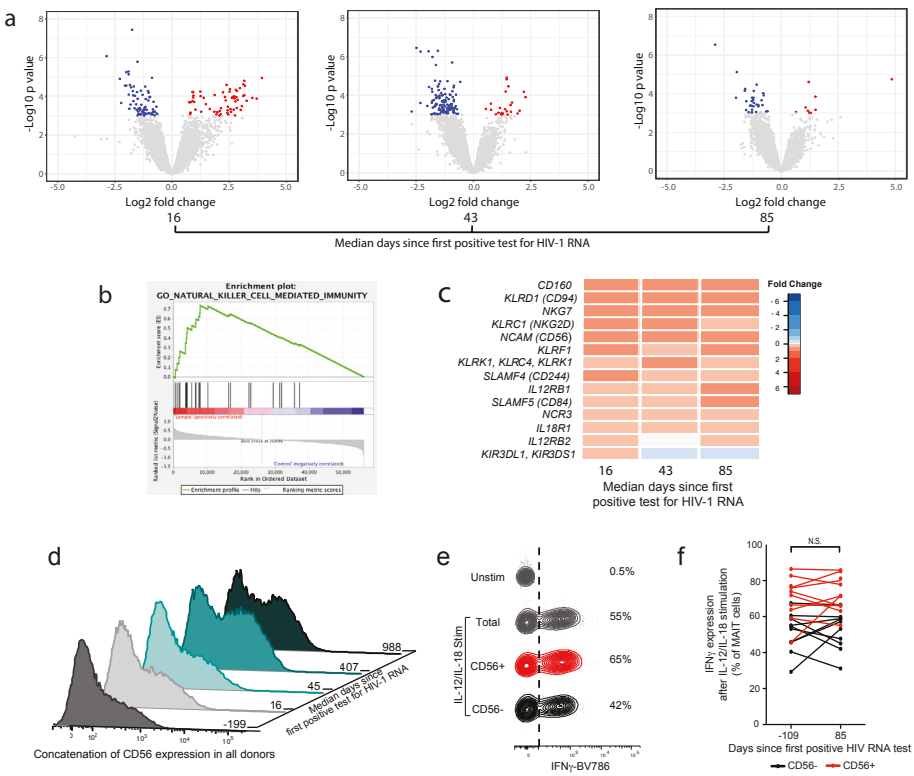

Figure 12. MAIT cells have an enhanced innate-like transcriptional signature, phenotype, and functionality in acute HIV infection. ***a)*** *The upregulated* ***(red)*** *and downregulated* ***(blue)*** *genes in three post-infection time points in AHI are compared to pre-infection in bulk sorted CD161^{bright} Vα7.2+ MAIT cells subjected to RNA-seq.* ***b)*** *Enrichment plot of an upregulated gene signature after GSEA of bulk sorted MAIT cell RNA-seq data.* ***c)*** *Differential expression of genes associated with innate immunity post-HIV infection compared to pre-infection after targeted, multiplexed gene expression analysis of sorted MAIT cells.* ***d)*** *Concatenation of protein expression of CD56 in MAIT cells from all samples and time points pre and post-HIV infection.* ***e)*** *Example staining of IFNγ expression in MAIT cells and CD56 MAIT cell subsets after IL-12 and IL-18 stimulation in vitro, and* ***(f)*** *the amount of IFNγ produced by MAIT cells in acute HIV infection in both CD56- and CD56+ MAIT cells.* ***Material from:*** *Lal et al., Dynamic MAIT cell response with progressively enhanced innateness during acute HIV-1 infection, Nature Communications, 2019, Nature Research.*

4.6 Rapid and preferential activation, dysfunction, and decline of CD4+ iNKT cells in the acute stages of HIV infection

The other unconventional T cell subset whose identifying markers were included in the flow cytometry panel included in the OMIP-046, the iNKT cells, was examined next (**Figure 13a**). Using some of the same overlapping donors in RV217 where MAIT cell dynamics was measured, the temporal dynamics of iNKT frequency, phenotype, function and gene expression was explored (**Paper III, Table 1**). There was a significant decline in the frequency (**Figure 13b**) and absolute counts (**Figure 19**) of blood iNKT cells at the first post-infection time point corresponding to peak VL in AHI. The relative depletion of CD4+ iNKT cells at this time point was more substantial than that of conventional CD4 T cells (**Figure 19**). As it is thought that the CD4- and CD4+ iNKT cell subsets differ in several respects in the healthy host and in their susceptibility to death in HIV infection. Therefore, determining factors driving both CD4- and CD4+ iNKT cells loss is important. The CD4+ subset declined at a faster rate and more robustly than CD4- iNKT cells, supporting differing causes of their loss in the blood (**Figure 13 c** and **d**). In fact, CD4+ iNKT cells may be directly infected early and play a role in establishing viral replication in the acute infection stage, as pre-infection levels of iNKT cells, and more strikingly CD4+ iNKT cells, correlate with peak VL post-infection in AHI (**Paper III, Figure 3c-e**).

The expression of markers of T cell activation and exhaustion were also measured on iNKT cells in AHI. iNKT cells expressed elevated levels of the activation makers CD38 and HLA-DR, but not of markers of exhaustion PD-1 and TIGIT, at the time point corresponding to peak VL (**Figure 13e**). Additionally, iNKT cell functionality was measured after PBMC from RV217 were stimulated with PMA/ionomycin. Here, it was elucidated that iNKT cell dysfunction observed in CHI may begin in AHI, with declining ability of iNKT cells to produce TNF and IFNγ *in vitro* by approximately 3 months post-infection (**Figure 13g**). Bulk sorted iNKT cells from AHI also showed altered gene expression profiles compared to pre-infection as determined by targeted multiplexed RT-qPCR. iNKT cells post-infection upregulated genes marking T cell activation such as the chemokines *CCL4* and *CCL3*, and *LAG3* which encodes an inhibitor protein in T cells (**Paper III, Figure 2**). Additionally *IFI16*, a marker of pyroptosis, the pathway thought to drive HIV-infected conventional CD4 T cell death, was upregulated in iNKT cells at peak VL (**Paper III, Figure 2**).

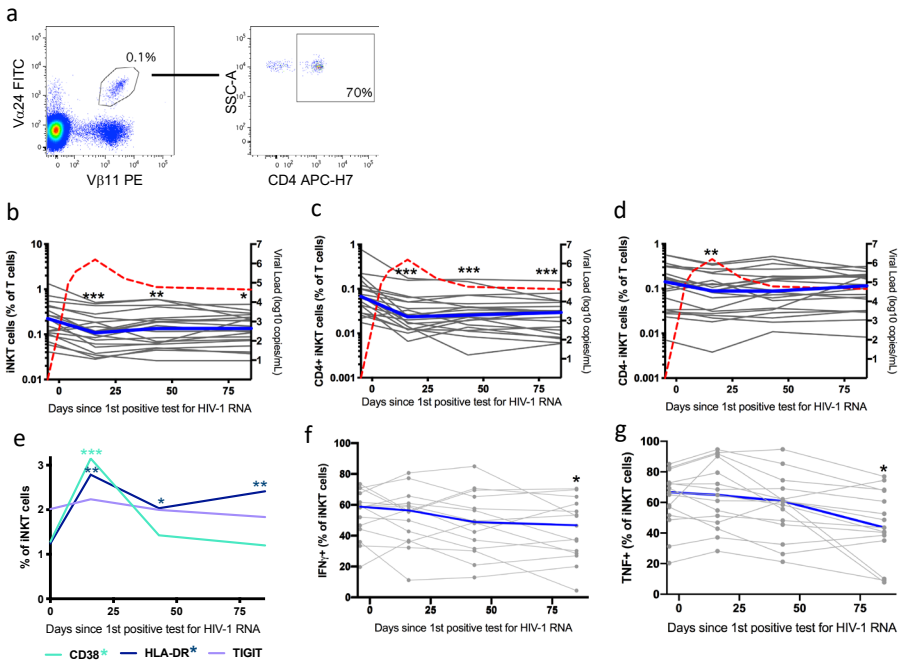

Figure 13. iNKT cells decline in frequency, become activated and dysfunctional in acute HIV infection. ***a****) Representative staining of iNKT cells and their expression of CD4 amongst living T cells.* ***b****) Total iNKT cells, (****c****) CD4+ iNKT cell subset and (****d****) CD4- iNKT cell subset frequencies in acute HIV infection amongst T cells, the VL is shown in* ***red*** *and the median overtime is shown in* ***blue****.* ***e****) The median expression overtime of CD38, HLA-DR, and TIGIT on iNKT cells in acute HIV infection, and the (****f*** *and* ***g****) expression of cytokines by iNKT cells after stimulation in vitro with PMA/ionomycin.*

4.7 ART initiation in acute HIV infection prevents iNKT cell loss in the blood, but not in the gut

The RV217 acute capture cohort demonstrated that iNKT cells rapidly decline in acute untreated HIV infection, and previous research has shown this loss is not fully recovered upon ART initiation in chronic HIV infection. The impact of initiating ART in AHI on iNKT cell dynamics, at a time when most individuals are unaware they are infected with HIV, is unknown. To address this, samples from RV254, a cohort where ART is initiated in AHI, were utilized and compared to community-matched controls (**Paper III**, **Table 2**). Donors were categorized based on Fiebig stages (I-III). Unlike untreated HIV infection, ART initiation during the acute stage prevented iNKT cell decline in the blood 24 months post-ART, even in the highly susceptible CD4+ iNKT cells (**Figure 14a**). There was also no detectable change in the expression of markers of activation HLA-DR, CD38, or TIGIT in iNKT cells compared to uninfected controls 24 months post

ART initiation (**Figure 14b**). Finally, the amount of TNF and IFNγ produced by blood iNKT cells *in vitro* after PMA/ionomycin stimulation was similar to that of uninfected controls (**Figure 14c**). This promising result gives incentives for early treatment initiation, as peripheral blood iNKT cells in acute, treated HIV infection are preserved.

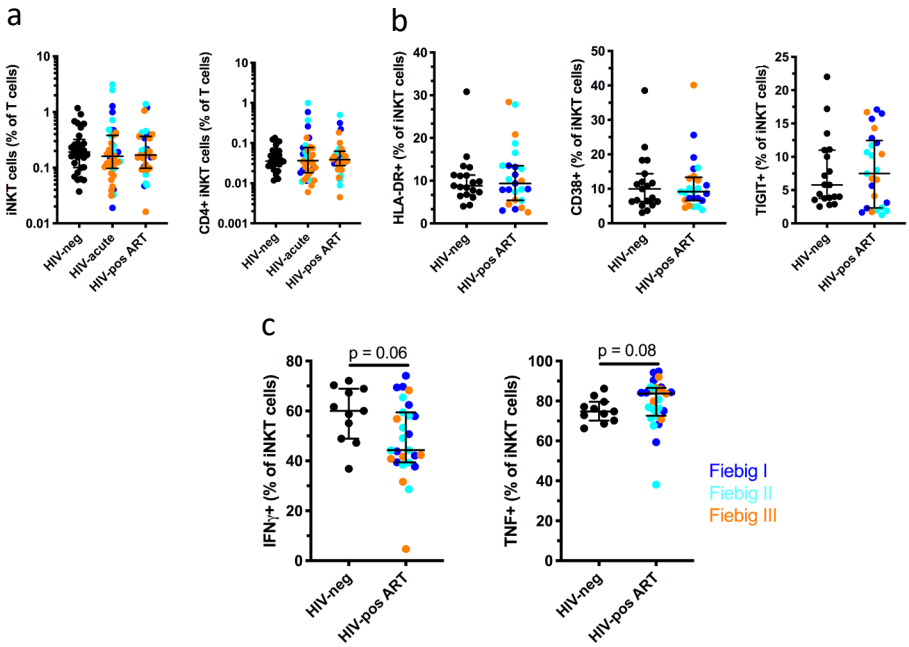

Figure 14. ART initiation in acute HIV infection prevents iNKT cell loss, activation, and dysfunction in the blood. ***a)*** *Total iNKT cell frequency or the CD4+ iNKT subset in HIV negative donor-matched subjects compared to acutely infected, HIV+ untreated donors and 24 months post-ART initiation.* ***b)*** *Expression of HLA-DR, CD38 and TIGIT in HIV uninfected controls compared to 24 months after ART initiated in acute HIV infection.* ***c)*** *Cytokine expression in iNKT cells in HIV uninfected controls compared to 24 months after ART initiated in acute HIV infection after stimulation with PMA/ionomycin in vitro.*

Despite the breadth of knowledge that can be gained using peripheral blood sampling, the real battle in HIV infection occurs in the gut mucosa. iNKT cells are inhabitants of the gut, and possible targets of HIV infection. Therefore, their dynamics in this location are essential to understanding their role in HIV seeding, pathogenesis, and the progression to AIDS. Sigmoid colon biopsies from the RV254 acute treatment initiation cohort and uninfected matched controls from the RV304 cohort were used to study iNKT cells in the colon. Prior to treatment in AHI, donors already had significantly lower counts of iNKT cells per gram of tissue in the colon compared to uninfected controls (**Figure 15a**). This reduction

was most pronounced in the CD4+ iNKT cells subset (**Figure 15b** and **c**). Donors that received 24 months of ART initiated in AHI still had lower levels of colonic iNKT cells compared to healthy controls. Prior to treatment in acute HIV infection, the plasma VL correlated negatively to the number of CD4+, but not CD4-, iNKT cells in the colon (**Figure 15d** and **e**). Depletion of colonic CD4+ iNKT cells was also more severe than that of conventional CD4+ T cells at this site, with more pronounced relative depletion compared to healthy controls before (**Figure 15f**) and after treatment (**Figure 15g**). Levels of CD4+ iNKT cells in the gut also correlated negatively with levels of soluble factor and marker of immune activation IP-10 in the plasma. This suggests that the depletion of the immunomodulatory CD4+ iNKT cell subset from the colon may be a partial contributor to systemic immune activation. (**Paper III**, **Figure 8a**).

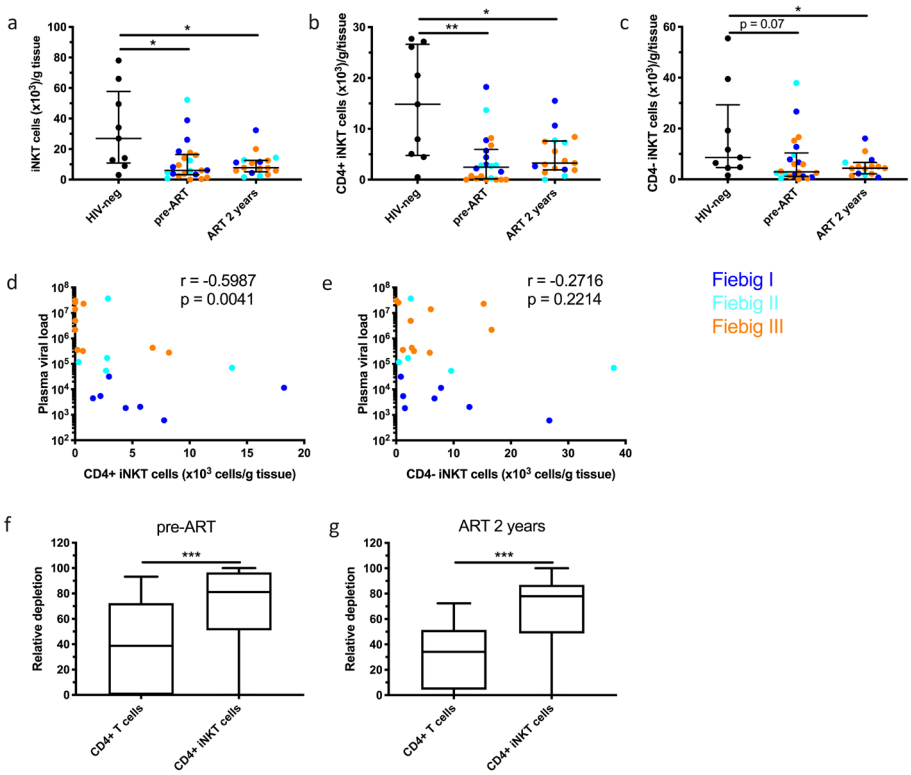

Figure 15. Early ART initiation does not prevent the rapid and preferential depletion of iNKT cells in the colon. ***a****) Total iNKT cells, (****b****) the CD4+ iNKT cell and (****c****) CD4- iNKT cell subsets per gram of tissue in recto-sigmoid colon biopsies in uninfected controls compared to untreated, HIV infected donors in acute infection and 24-month post ART initiation in acute infection. The plasma viral load at time of biopsy sampling in untreated, acutely infected individuals correlated with the (****d****) CD4+ iNKT cell subset, and (****e****) CD4- iNKT cell subset. The relative fold change of the depletion of conventional CD4+ T cells and the CD4+ iNKT cell subset (****f****) pre-ART initiation, and (****g****) after 24 months on ART initiated in acute HIV infection.*

4.8 Chronic untreated HIV infection leads to the emergence of a terminal effector, innate-like CD8 T cell subset that expresses FcγRIIIA

After exploring innate-like, unconventional T cells in AHI, it seemed like a plausible hypothesis that in CHI, chronic activation of conventional adaptive CD8 T cells through persistent antigen exposure and systemic inflammation could lead to an altered, possibly innate-like properties in conventional T cells. Toward that end, cryopreserved PBMC from a cohort of chronically infected, untreated HIV+ donors from the Rakai District of Uganda were used to explore the prevalence of FcγRIIIA+ (CD16) expression in conventional CD8 T cells. This population has previously been observed in chronic HCV infection (*237*). The function of CD16 is often studied in the NK cells and can facilitate ADCC. In this cohort there was a significant increase in FcγRIIIA expressing CD8 T cells in chronic HIV infection compared to uninfected controls (**Figure 16a** and **b**). This expanded subset expressed higher levels of the marker of T cell activation, CD38, but not the immune checkpoint inhibitor PD-1, suggesting they may not be exhausted compared their CD16-negative counterparts, and retain some functional capacity (**Figure 16a, c,** and **d**). This was supported by elevated levels of the cytolytic effector molecule perforin in CD16+ CD8 T cells compared to CD16- (**Paper IV, Figure 2c**). Further exploration of their phenotype revealed that CD8 T cells expressing FcγRIIIA in CHI express CD45RA and CD57, but not CCR7 or CD27, a phenotype consistent with terminally differentiated effector memory T cells re-expressing CD45RA (TEMRA) (**Table 1**) (**Paper IV, Figure 2a**, **b**, and **c)**. Expression of the activating and inhibitor receptors killer Ig-like receptors (KIRs) was also evaluated on CD16+ CD8 T cells and compared to their CD16- counterparts and NK cells in HIV uninfected and infected individuals. CD16+ CD8 T cells from donors with CHI had higher expression levels and a more diverse array of KIRs on their surface, akin to KIR expression on NK cells, compared to CD16+ CD8 T cells in uninfected controls. CD16+ CD8 T cells express more KIRs in both disease states than the CD16- counterparts (**Paper IV**, **Figure 2d**). As KIR expression is generally attributed to NK cells, similar to CD16, this suggests that FcγRIIIA expressing CD8 T cells adopt an NK cell-like phenotype in CHI. Using a second cohort of longitudinally sampled donors from the Kayunga District in Uganda who initiated ART at chronic stages of HIV infection, the persistence of CD16+ CD8 T cells and their phenotype was measured 6- and 12-months post treatment initiation. Even 12 months post-treatment, there was no decline in the prevalence of FcγRIIIA expressing CD8 T cells, but there was a detectable decline in the expression CD38 on their surface (**Figure 16e** and **f).**

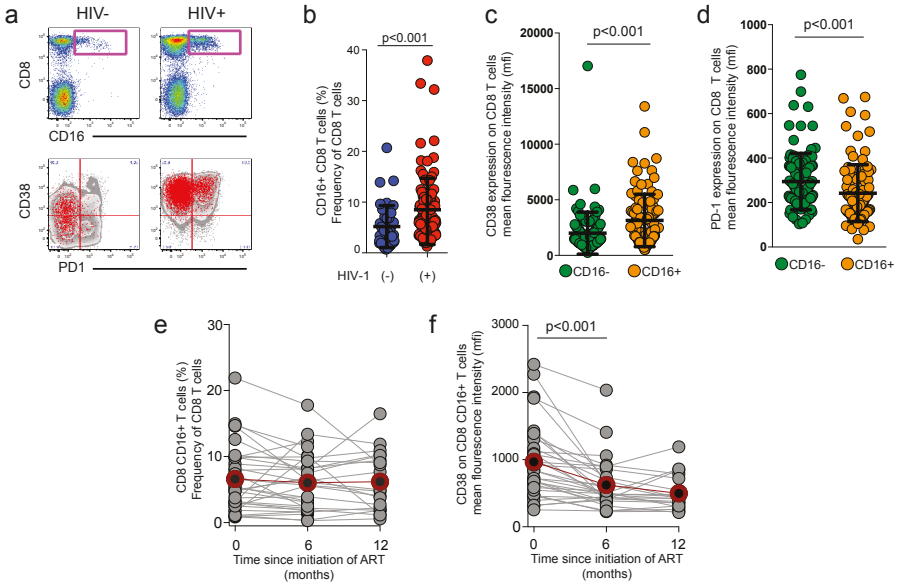

Figure 16. FcγRIIIA+ CD8 T cells expand in chronic untreated HIV infection, have an activated, terminally differentiated effector memory phenotype, and persist upon treatment initiation. a) *Example staining of FcγRIIIA (CD16) in CD8+ T cells amongst CD3+ cells in an HIV negative control and a chronically HIV infected donor, and the expression of CD38 and PD-1 on CD16+ CD8 T cells* ***(red)*** *compared to the CD16- CD8 T cells* ***(gray)****.* ***b)*** *The frequency of CD16+ CD8 T cells and the expression of* ***(c)*** *CD38 and* ***(d)*** *PD-1 on CD16+ compared to CD16- CD8 T cells in HIV positive donors compared to HIV uninfected controls.* ***e)*** *The relative frequency of CD16+ CD8 T cells, or* ***(f)*** *the expression of CD38 on their surface before and after 6 or 12 months of ART in chronic HIV infection. Reprinted with permission from (299).*

4.9 The FcγRIIIA+ CD8 T cell subset has an innate-like transcriptional signature

After observing the unique phenotype of CD8 T cells that express FcγRIIIA, gene expression profiles of these cells were next compared to the FcγRIIIA- counterparts, central memory CD8 T cells, and NK cells. These four cell populations were sorted for targeted, multiplexed RT-qPCR to measure the expression of 96 genes. 74 of these 96 gene assays passed quality control, and were subjected to the dimensionality reduction tool, Principal Component analysis (PCA). PCA revealed that that CD16+ CD8 T cells have a transcriptional profile that falls in between NK cells and CD16- CD8 T cells, and share no overlap with the central memory CD8 T cell population (**Figure 17a**). Principal component 1 contributed 26% to the variability of the dataset, with expression of genes *GZMB*, *LAIR1*, *GZMK*, *PRF1*, and *CD244* contributing the most to this component. Principal component 2 contributed 14.7% of the variability in the dataset, with *GZMK*, *IL-6ST*, *TGFB1*, *CD38*, and *CD160* contributing the most to this principal component.

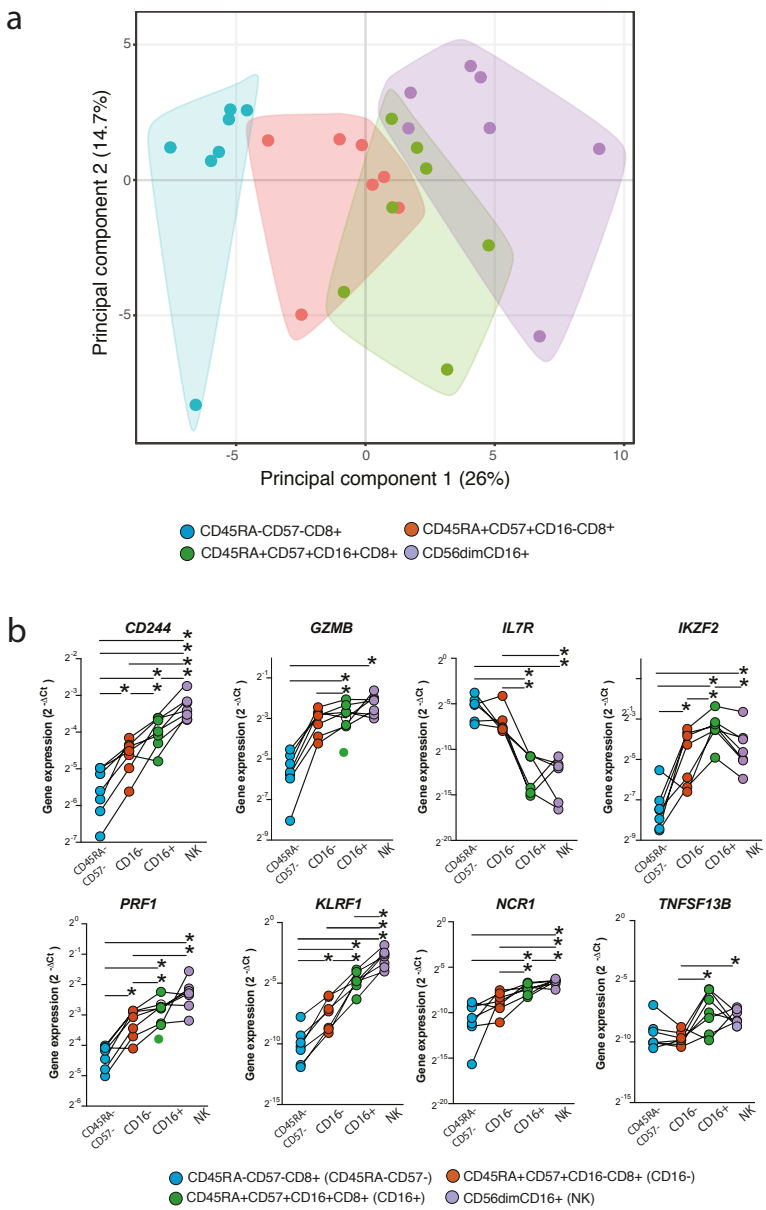

Figure 17. FcγRIIIA+ CD8 T cells express high levels of Helios and low IL7R, characteristics generally associated with NK cells. a) *Principal component analysis of 74 genes measured using targeted multiplexed RT-qPCR analysis in four sorted cell populations; CD8+CD45RA-CD57- T cells (**blue**), CD8+CD45RA+CD57+CD16- T cells (**red**), CD8+CD45RA+CD57+CD16+ T cells (**green**) and CD56^{dim} NK cells (**purple**) in 8 donors with chronic HIV infection. (**b**) Individual gene expression data from targeted multiplexed RT-qPCR, shown as $2^{-\Delta Ct}$, in four sorted cell populations in eight donors with chronic HIV infection. Reprinted with permission from (299).*

Looking at the individual gene level, it became evident that CD16+ CD8 T cells express higher levels of innate-like genes compared to CD16- or central memory CD8 T cells (**Figure 17b**). CD16+ CD8 T cells expressed higher levels of *IKZF2*, the gene that encodes the transcription factor Helios, than the other three cell populations. Additionally, *IL7R* was expressed at lower levels, similar to NK cells, at both the gene expression (**Figure 17b**) and protein level as measured through IL-7R expression by flow cytometry (**Paper IV**, **Figure 4c**). CD16+ CD8 T cells also expressed higher levels of genes associated with cytoxicity, such as *GRZMB* (GrzB) and *PRF1* (perforin), than the other CD8 T cell populations. *NCR1*, *KLRF1*, *TNFSF13B*, and *CD244*, genes also normally associated with NK cells and innate like function, were highly expressed by CD16+ CD8 T cells compared to the other CD8 T cells analyzed.

High levels of the transcription factor Helios within this population inspired analysis of other transcription factors that influence T cell differentiation, including Eomes and T-bet, at the protein level by flow cytometry. In chronically HIV infected donors, CD8 T cells expressing FcγRIIIA not only expressed high levels of Helios, but also co-expressed Eomes and T-bet at levels higher than CD8 T cells that do not express FcγRIIIA (**Paper IV, Figure 3a** and **b**). While CD16- CD8 T cells have variable expression of T-bet and Eomes, a median of 60% of CD16+ CD8 T cells expressed a T-bet^{high} and Eomes^{high} phenotype similar to that seen in NK cells (**Paper IV, Figure 3b** and **c**). Examination of the gene expression and the transcription factor profile of CD16+ CD8 T cells, their CD16- counterparts, and NK cells suggests CD16+ CD8 T cells inhabit a space in between conventional CD8 T cells and NK cells.

4.10 FcγRIIIA+ CD8 T cells perform ADCC, a function generally attributed to NK cells

The next step was to determine if FcγRIIIA expressing CD8 T cell could execute ADCC, a function generally attributed to FcγRIIIA expressing NK cells. ADCC may be an important effector function in controlling HIV replication, as non-neutralizing IgG antibodies are a correlate of protection in HIV vaccines (*300*). To do this, cells were FACS sorted and evaluated using the PanToxiLux assay, a flow cytometry based assay used to measure direct cytotoxicity of an effector cell against fluorescently labeled target cells through ADCC. Successful delivery of a lethal hit from a cytotoxic effector cell is measured through cleavage of a cell permeable fluorogenic substrate by granzyme and upstream caspase activity within the target cell.

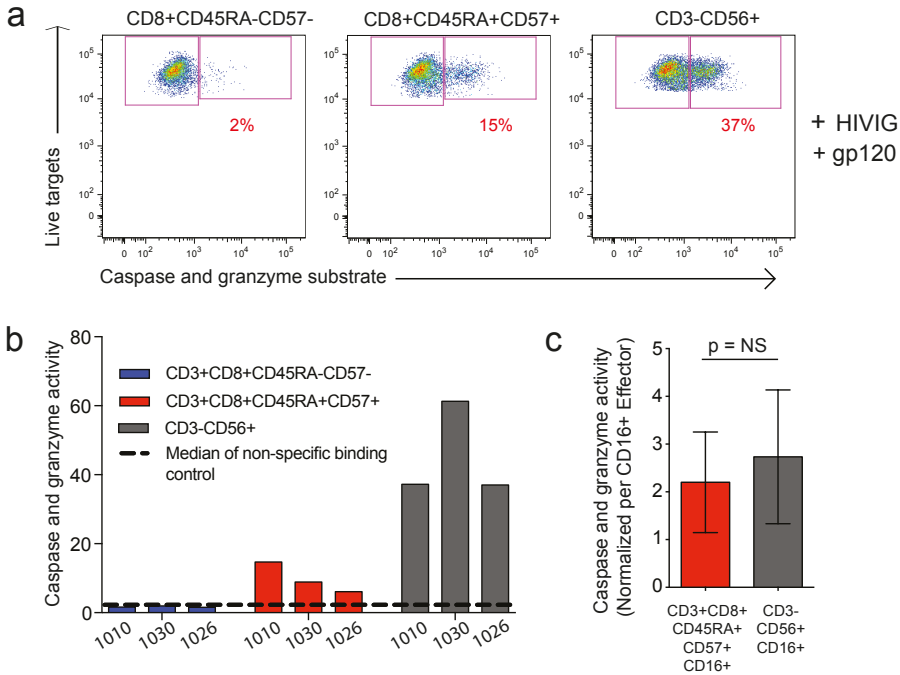

Figure 18. FcγRIIIA+ CD8 T cells can facilitate ADCC. a*) Sorted cell populations CD8+ CD45RA- CD57- T cells, CD8+ CD45RA+CD57+ T cells (enriched for CD16+ CD8 T cells), or NK cells were used in the PanToxiLux assay to measure the delivery of a cytotoxic hit to fluorescently labeled target cells (CEM.NKR$_{CCR5}$ cell line) coated with gp120 in the presence of polyclonal HIV-Ig as an antibody source.* ***b****) Measurement of granzyme delivered to target cells in the PanToxiLux assay by each respective sorted cell type, and* ***(c)*** *normalization on a per-CD16 expressing cell basis the granzyme delivered to target cells. Reprinted with permission from (299)****.***

Since the monoclonal antibody against CD16 may block or trigger its downregulation, CD16+ CD8 T cells were enriched for by sorting CD45RA+CD57+ CD8 T cells, and FcγRIIIA+ NK cells were enriched for by sorting CD56^{dim} CD3- NK cells (**Figure 18a**). The CD45RA-CD57- CD8 T cells, which include very few CD16+ cells, were used as a negative control. HIV BaL gp120 coated NKR. CEM$_{CCR5}$ cells, which express CD4 and CCR5 and are resistant to spontaneous lysis by NK cells, were used as targets and co-incubated with one of the sorted effector cell populations in the presence of HIV-Ig as an antibody source (**Figure 18a**). CD56^{dim} NK cells were able to facilitate ADCC at levels higher than the sorted T cell populations (**Figure 18b**). However when the killing activity was re-calculated based on the percentage of CD16+ cells present in the CD45RA+ CD57+ CD8 T cells (9-21%) and CD56^{dim} NK cells (69-96%), it was evident that the CD16+ CD8 T cells facilitated ADCC at a similar level as the NK cells on a per cell basis (**Figure 18c**). These findings indicate that CD8 T cells expressing FcγRIIIA can facilitate HIV-specific ADCC at levels similar to NK cells.

5 DISCUSSION AND FUTURE DIRECTIONS

HIV infection is a severe and persistent assault on the immune system, even in the presence of the effective therapies that are available today. The target of HIV is the immune system itself, creating a negative feedback cycle wherein the immune system is progressively depleted, further weakening the host while the virus continues to propagate into chronic infection and the progression to AIDS. The study of HIV immunopathogenesis will continue to be important in the absence of a preventative vaccine, the persistence of comorbidities even upon therapy initiation, and no cure. This thesis hopes to further our understanding of the changes occurring in innate-like T cell subsets, a group of immune cells often overlooked as they are not the classically identified targets of HIV infection nor often considered players in controlling the virus itself. Exploring a unique, prospective cohort of individuals where pre- and post- infection time points are available in natural HIV infection (RV217), unconventional T cell dynamics were studied in the earliest days of HIV infection. This is a time period that sets the stage for disease progression into chronic infection. CD4+ iNKT cells, which are preferentially infected with HIV *in vitro*, decline significantly and more profoundly than conventional CD4 T cells in the acute infection stage (**Figure 19**). The quantity of these cells pre-infection correlates positively with peak VL post HIV infection. These findings suggest iNKT cells may play a significant role in propagating HIV virus in acute infection, and contribute to the establishment of the elusive viral reservoir where HIV persists despite therapy. CD4- iNKT cells, however, decline at a slower rate than CD4+ iNKT cells, suggesting their loss is caused by other factors beyond direct viral infection *per se*. It is possible that iNKT cells may die because of chronic antigen exposure, or they may be lost from the blood due to recruitment to mucosal sites, as hypothesized for MAIT cell loss.

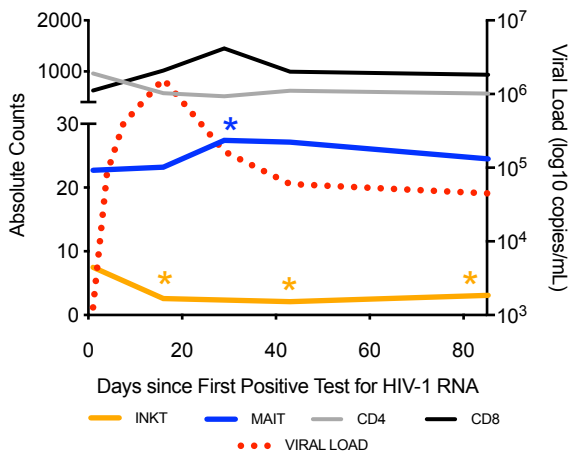

Figure 19. iNKT cells are lost rapidly and persistently lost, while MAIT cells briefly expand in acute HIV infection.

The iNKT cells and MAIT cells share many characteristics, such as the ability to recognize microbial antigen presented by evolutionarily conserved MHC class I-like molecules, and rapid innate-like effector function. However, this thesis supports the notions that these two unconventional cells subtypes differ in the temporal dynamics of their loss, phenotypic changes, and functional alterations in AHI. Therefore, they likely have differing roles in HIV immunopathogenesis. For example, there is a brief but significant expansion of MAIT cells (**Figure 19**), along with a boost in functionality post- peak VL, and this is not observed in iNKT cells. This expansion and cytotoxic functionality boost, along with increased expression of activation and exhaustion markers, may be driven by increasing microbial burden because of lowered gut integrity found in AHI, and engagement of the MAIT cell TCR. This is supported by findings that peak MAIT cell function and activation correlate with levels of the proposed marker of microbial translocation sCD14 (**Paper II, Figure 7b)**. The loss of the important immunomodulatory CD4+ iNKT cells in the gut during AHI may be a significant contributing factor behind the increase in microbial translocation at this early time point, driving immune activation (**Paper III, Figure 8**). Both iNKT and MAIT cells experience a loss of functional capacity as chronic HIV infection begins, but the drivers of functional impairment may be different as well. MAIT cells upregulate check point inhibitory receptors already at this time point, while iNKT cells do not. It can be hypothesized that chronic TCR stimulation of MAIT cells drives their exhaustion and functional decline, as is the case with conventional CD8 T cells which share a similar exhausted phenotype and functional potential in chronic untreated HIV infection due to chronic antigen exposure.

The immune system is elegantly collaborative. Its components have unique responsibilities, but at the same time there is redundancy, in order to create an umbrella of protection in the host against whatever pathogen, and for however long the pathogen may endure. It is therefore not surprising that T cells have mechanisms of activation beyond TCR-dependent function to be used in instances where antigen-specific response are exhausted. The expansion and maintenance of an enhanced innate-like phenotype, function, and transcriptional signature in the MAIT cell compartment in HIV infection, characterized by expression of the NK-marker CD56, may benefit the failing immune system in a chronic viral infection. With an ability to rapidly produce the antiviral cytokine IFNγ upon engagement of IL-12R and IL18R on their surface, the maintenance of this directly innate responding subset of MAIT cells could prove to help fight the virus itself, as has been shown in other viral infections. However, the exact role of CD56 expressing MAIT cells in HIV infection has yet to be determined.

Given that harnessing the innate-like functions of T cells may be a way of repurposing T cells towards alternate functions, it stands to reason that an adaptive CD8 T cell subset expressing a marker commonly found on NK cells, FcγRIIIA

(CD16), would expand in CHI. It is unknown if FcγRIIIA expressing CD8 T cells are, in fact, HIV-specific CD8 T cells. However, it is interesting to speculate that the massive expansion of HIV-specific CD8 T cell clonotypes that exhaust their ability to be activated via TCR-mediated triggering would upregulate other effector mechanisms to help combat viral infection. HIV-targeted ADCC, the functionality facilitated by FcγRIIIA engagement, is a correlate of protection in HIV vaccine efficacy and important for control of viral infections. Therefore, the repurposing of another cell type, beyond the normally utilized NK cell, to perform ADCC may benefit the host in an attempt to control viral replication.

Further studies need to be performed to determine if enhanced innate functionality in conventional CD8 T cells or MAIT cells is beneficial to the host in HIV infection, or simply a consequence of a chronic viral infection. It has yet to be determined if iNKT cells also utilize an innate-like effector capacity in chronic HIV infection, however innate cytokine stimulation of iNKT cells contribute to control in other viral infections. Together, the findings of this thesis suggest innate-like T cells may have an important role in HIV immunopathogenesis, or at the very least, are engaged in HIV infection.

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