o

ASSESSMENT OF GAMMA/DELTA T CELL FUNCTIONALITY FOLLOWING PATHOGENIC HIV/SIV AND NON-PATHOGENIC SIV INFECTIONS

APPROVED BY SUPERVISORY COMMITTEE

_____
Donald Sodora, Ph.D.

_____
Nancy Street, Ph.D.

_____
Lora Hooper, Ph.D.

_____
Michael Gale, Ph.D.

_____
Guido Silvestri, M.D.

DEDICATION

This dissertation is dedicated to my mother, Linda Doris Selman.

I would like to begin by acknowledging and thanking all of the patients who voluntarily enrolled in the studies presented in my dissertation. Their willingness to participate in my project and other studies is greatly appreciated and remains critical to further our understanding of how HIV causes disease. In addition, I acknowledge and appreciate all of the monkeys enrolled in these studies as their contributions were paramount in the successful completion of my dissertation.

I would like to thank the members of my Graduate Committee including Drs. Nancy Street (chairperson), Lora Hooper, Michael Gale, Guido Silvestri, and Donald Sodora (mentor) who have all provided critical insights to aid in the successful completion of my graduate studies. I would also like to thank our collaborators including the veterinary staff at the Yerkes National Primate Research Center and

California National Primate Research Center, as well as the physicians at the University of Texas Southwestern Medical Center AIDS Clinic. In addition, I would like to acknowledge my professors at Texas A&M University – Corpus Christi who encouraged me to pursue a doctoral degree.

I would like to thank the members of the Sodora laboratory, both past and present. They have provided a fantastic, stimulating, and enjoyable work environment and were always willing to provide help and assistance when necessary. I am confident that each will be successful in their future endeavors.

I would like to thank my family for their constant support throughout my life. My mother LInda instilled upon me an independent spirit and manner of thinking which has shaped me into the person I am today. My father David taught me to be open-mind and experience as many things as possible. My sister Karla was always there to provide advice and talk to about anything, which was evident by our memorable and intense scientific debates (that I am sure the rest of the family enjoyed). My step-father Keith always provided kind words of encouragement to help me succeed as well. Finally, I would like to acknowledge my cat Jenny who brings a smile to my face whenever she is near.

ASSESSMENT OF GAMMA/DELTA T CELL FUNCTIONALITY FOLLOWING PATHOGENIC HIV/SIV AND NON-PATHOGENIC SIV INFECTIONS

by

DAVID ALAN KOSUB

DISSERTATION

Presented to the Faculty of the Graduate School of Biomedical Sciences

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

The University of Texas Southwestern Medical Center at Dallas

Dallas, Texas

February, 2007

ASSESSMENT OF GAMMA/DELTA T CELL FUNCTIONALITY FOLLOWING PATHOGENIC HIV/SIV AND NON-PATHOGENIC SIV INFECTIONS

Publication No. _____

David Alan Kosub, Ph.D.

The University of Texas Southwestern Medical Center at Dallas, 2007

Supervising Professor: Donald L. Sodora, Ph.D.

Pathogenic HIV/SIV infection induces high viral loads, aberrant immune activation, and dysfunction in numerous immunologic cells (including gamma/delta ($\gamma\delta$) T cells) leading to opportunistic infections. $\gamma\delta$ T cells bridge the innate and adaptive immune responses primarily via cytokines produced in response to microbial phosphoantigens. $\gamma\delta$ T cells have also been implicated in the control of an SIV challenge infection as evidenced by increased numbers and β-chemokine expression at mucosal sites in vaccinated macaques. The goal of Aim 1 of this thesis was to assess the impact of an acute SIV infection on the levels of $\gamma\delta$ T cells

at mucosal and lymphoid sites in macaques utilizing quantitative PCR. At two days post-infection, a decrease in γδ T cell levels was observed at mucosa sites whereas increased levels were present at regional lymph nodes. Also, an increase in lymphoid homing molecules was observed at these lymph nodes, indicating a mechanism whereby γδ T cells migrate away from mucosal sites towards secondary lymphoid tissues following an acute SIV infection. The redistribution of γδ T cells may be important for the initiation of an anti-viral immune response and control of rapid viral spread.

The goal of Aims 2 and 3 was to assess the ability of γδ T cells in HIV-infected patients to express cytokines and compare these results to analysis of the non-pathogenic SIV infection of sooty mangabeys. Following stimulation with the non-specific activators PMA/Ionomycin or the γδ TCR specific ligand isopentenyl pyrophosphate, a decrease in the percentages of γδ T cells expressing Th1 pro-inflammatory cytokines including TNF-α and IFN-γ was observed in the HIV+ patients (regardless of CD4+ T cell levels). Highly active anti-retroviral therapy (HAART) partially restored the ability of γδ T cells from HIV+ patients to express Th1 cytokines. SIV infection of mangabeys results in high viral replication, low levels of immune activation, and generally no signs of progression to AIDS. Evidence for preserved or increased functionality of γδ T cells from SIV+ mangabeys (regardless of CD4+ T cell levels) was demonstrated by maintained percentages of γδ T cells that expressed Th1 cytokines following *ex vivo* stimulation. These data suggest that in the absence of aberrant immune activation, controlled Th1 responses by γδ T cells

from mangabeys may assist in suppressing damage due to the SIV infection as well as inhibiting the onset of opportunistic infections. These data provide rationale for therapies aimed at increasing γδ T cell functionality in humans, particularly with regard to Th1 cytokine responses to augment protection against opportunistic infections and HIV disease progression.

TABLE OF CONTENTS

PRIOR PUBLICATIONS

1) Milush JM, **Kosub D**, Marthas M, Schmidt K, Scott F, Wozniakowski A, Brown C, Westmoreland S, Sodora DL. Rapid dissemination of SIV following oral inoculation. AIDS. 2004 Dec 3;18(18):2371-80.

2) Milush JM, Reeves JD, Gordon S, Zhou D, Muthukumar A, **Kosub DA**, Chacko E, Giavedoni L, Ibegbu, CC, Cole KS, Miamidian J, Paiardini M, Barry AP, Staprans S, Silvestri G, and Sodora DL. Virally-induced CD4+ T cell depletion is not sufficient to induce AIDS in a natural host. Submitted

3) **Kosub DA**, Lehrman G, Milush JM, Zhou D, Chacko E, Leone AK, Silvestri G, Else J, Kaiser P, Jain MK, Sodora DL. Gamma/Delta T Cell Functional Responses Differ Following Pathogenic HIV and Non-Pathogenic SIV Infections. Submitted.

4) **Kosub DA** and Sodora DL. Decreased Levels of $\gamma\delta$ T cells at Mucosa following an Acute SIV Infection of Rhesus Macaques. In preparation.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AICD	Activation Induced Cell Death
AIDS	Acquired Immunodeficiency Syndrome
APC	Antigen-Presenting Cell
BAL	Bronchoalveolar Lavage
BrHPP	Bromohydrin Pyrophosphate
CCL5/RANTES	C-C Chemokine Ligand 5 / Regulated on Activation Normal T cell Expressed and Secreted
CCL21/6Ckine	C-C Chemokine Ligand 21 / 6Ckine
CCR5	C-C Chemokine Receptor 5
CCR7	C-C Chemokine Receptor 7
CDNA	Complementary DNA
CFC	Cytokine Flow Cytometry
CMV	Cytomegalovirus
DC	Dendritic Cell
DPI	Days Post-Infection
GAPDH	Glyceraldehyde Phosphate Dehydrogenase
HAART	Highly active anti-retroviral therapy
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon-γ
IL-4	Interleukin-4
IV	Intravenous
LN	Lymph Node
LPS	Lipopolysaccharide
M	Media
NK cell	Natural Killer cell
NPRC	National Primate Research Center
OCT	Optimal Cutting Temperature
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PI	PMA / Ionomycin
QRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
PMA	Phorbol-Myristal-Acetate
RB	Rectal Biopsies
RM	Rhesus Macaques
SIV	Simian Immunodeficiency Virus
SM	Sooty Mangabeys
TCR	T cell receptor
TNF-α	Tumor Necrosis Factor-α
Vδ1	Vdelta1
Vδ2	Vdelta2

CHAPTER 1 – General Introduction and Literature Cited

Introduction

On June 5, 1981, the Centers for Disease Control and Prevention released the Morbidity and Mortality Weekly Report describing five patients from Los Angeles, CA who presented with an atypical fungal infection with *Pneumocystis* pneumonia (23). Although the physicians were not aware, these patients were infected with an unknown viral pathogen, later termed the Human Immunodeficiency Virus (HIV), which is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV has had a dramatic human impact during the past 25 years infecting more than sixty-five million persons. Twenty-five million people have already succumbed to HIV/AIDS related diseases. In addition, greater than fourteen million children have been orphaned due to the death of their parents caused by HIV/AIDS. To compound the issue, each year more than five million new cases of HIV infection are reported worldwide primarily in Sub-Saharan Africa, China, India, and Russia (Figure 1-1). In North America, there are about two million people living with HIV (Figure 1-1). Within the United States, Hispanics and African Americans accounted for 70% of all reported HIV cases in 2005 (22). Despite this high percentage, the incidence of infection in these groups is

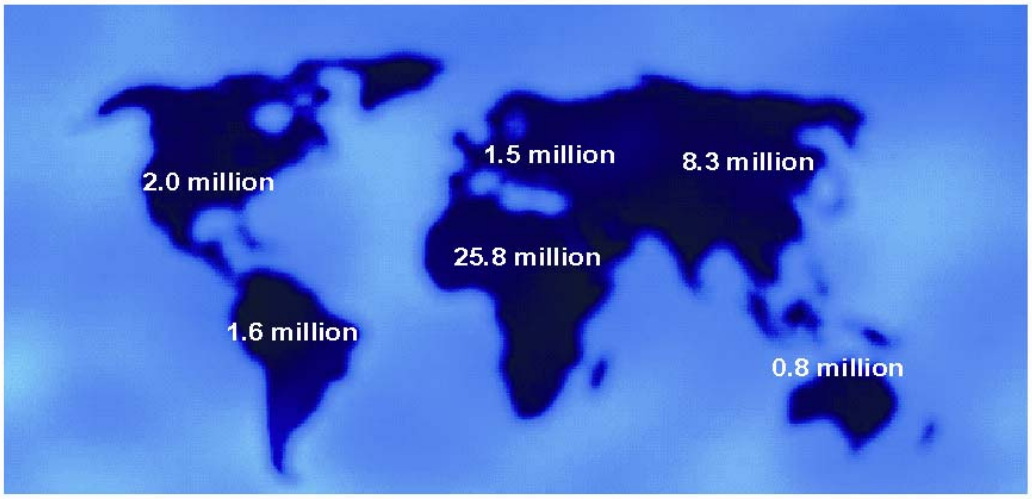

Figure 1-1. Global distribution of Human Immunodeficiency Virus (HIV)-infected individuals – Approximately 40 million people worldwide are infected with HIV as of 2005. The highest concentration of HIV infected persons is within sub-Saharan Africa. HIV is a global epidemic as infected individuals can be found in the Americas, Eurasia, and Oceania.

decreasing in comparison to Caucasians (22). In summary, HIV/AIDS is a global concern affecting every race, creed, gender, and nation in the past 25 years requiring a concerted international effort to solve.

Discovery of HIV as the causative agent of AIDS

In 1983, two independent laboratories led by Drs. Luis Montagnier (Pasteur Institute) and Robert Gallo (National Institutes of Health) published articles in *Science* describing the isolation of a retrovirus, later termed HIV, from an AIDS patient that they deemed the causative agent of AIDS (11, 58). Soon thereafter, *Clavel* and colleagues described a second virus isolated from AIDS patients in Senegal, termed Human Immunodeficiency Virus-2 (HIV-2), which shared some sequence homology with HIV-1 (30). In the past 25 years, disease caused by HIV-2 has remained a localized epidemic focused in West Africa, in contrast to the worldwide pandemic caused by HIV-1 (30, 68, 75). Though both HIV-1 and HIV-2 can cause AIDS, HIV-2 pathogenesis occurs at a slower rate compared to HIV-1 (113). In summary, both HIV-1 and HIV-2 have been identified as the causative agents of AIDS in infected people.

To elucidate the mechanisms by which HIV-1 causes disease, many studies have focused on the origins of the epidemic. More than thirty non-human primate species in Sub-Saharan Africa have been

identified naturally infected with simian immunodeficiency virus (SIV) (68, 76, 95). The current hypothesis for the origin of the HIV-1 pandemic is a zoonotic transmission event of SIV into humans following the butchering and consumption of infected chimpanzee bush meat (Figure 1-2) (68, 75). In fact, sequencing of the SIVcpz genome isolated from infected chimpanzees (*Pan troglodytes*) revealed homology to the HIV-1 genome (60, 68). It is believed that three separate cross-species transmission events from chimpanzees into humans are the origins of HIV-1 groups M, N, and O (68). Similar to the human epidemic, chimpanzees may have acquired SIVcpz through a cross-species transmission event after eating naturally SIV infected red-capped mangabeys and greater spot-nosed monkeys (Figure 1-2) (68).

Sooty mangabeys (*Cercoebus atys*) are a non-human primate species located in West Africa which can be found naturally infected with SIV but do not progress to simian AIDS despite high viral titers (149). The regional HIV-2 epidemic is believed to have arisen from a cross-species transmission event in West Africa following the consumption of sooty mangabey bush meat (Figure 1-2) (68). Separate origins for HIV-1 and HIV-2 is suggested by sequence analysis of the HIV-2 genome which shares homology with SIVsmm found in sooty mangabeys (Figure 1-2) (30, 76). In addition, rhesus macaques (*Macaca mulatta*) housed at the

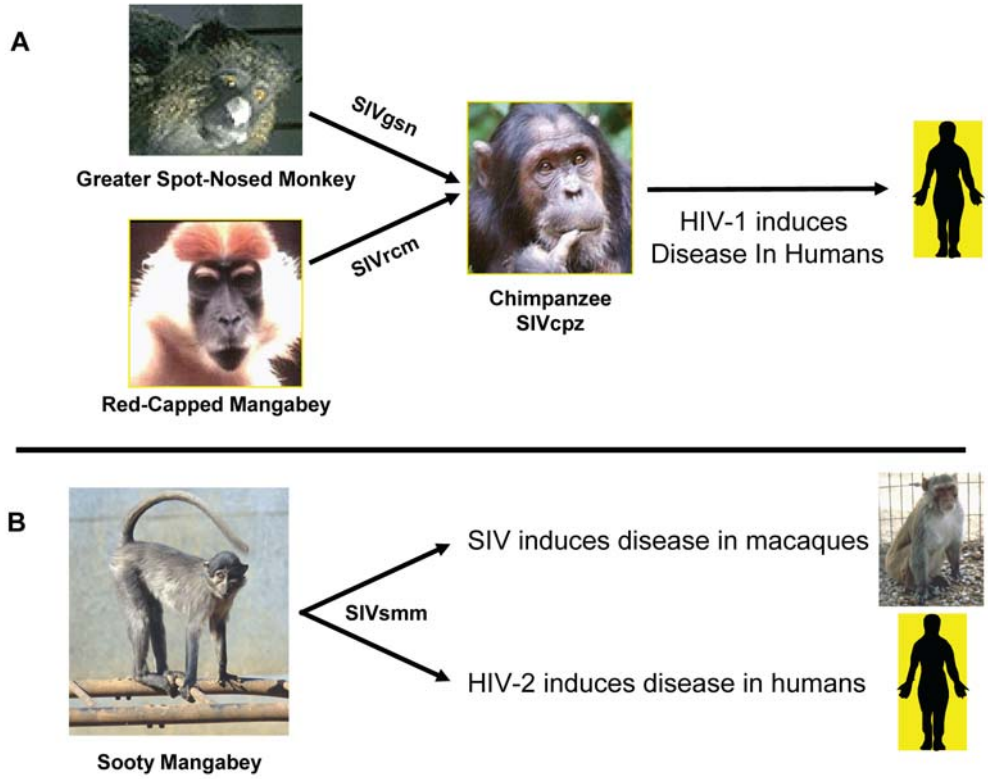

Figure 1-2 – Zoonotic Transmission of SIV from monkeys into humans – (A) The HIV-1 pandemic is believed to have arisen as a result of zoonotic transmission of SIVcpz which infects chimpanzees following the butchering and consumption of infected bush meat. SIVcpz is also believed to have arisen as a result of the predation of greater spot-nosed and red-capped mangabey monkeys (found naturally infected in the wild). (B) The HIV-2 epidemic has its origins from SIVsmm which naturally infects sooty mangabeys following the consumption of infected bush meat. In addition, SIVmac also arose from SIVsmm following co-habitation of mangabeys with macaques in the California National Primate Research Center.

California National Primate Research Center became infected with SIV following a cross species transmission event with sooty mangabeys and experienced AIDS like illnesses. (Figure 1-2) (24, 88). Further analysis of these macaques demonstrated that they were infected with an SIV (termed SIVmac) exhibiting sequence similarity with SIVsmm (88). Therefore, the transmission of SIVsmm from sooty mangabeys is responsible for both HIV-2 and SIVmac which can induce AIDS in their non-natural hosts.

HIV Transmission

HIV enters the body one of two ways, either by traversing a mucosal barrier or through an intravenous (IV) injection such that it goes straight into the blood stream. The IV route for infection has been associated with both intravenous drug users and patients receiving contaminated blood products. Transmission across a mucosal membrane remains the prevalent route for new HIV infections, (12) which generally occurs during sexual transmission across either the rectal or vaginal mucosa. Oral transmission of HIV can also occur via receptive oral intercourse (virus in semen) or vertically from mother-to-child *in utero*, during parturition or breast-feeding (106, 125, 138, 155, 157, 160). The oral cavity shares structural similarity with other mucosal sites including

the vagina as evidenced by the presence of stratified squamous epithelia and a lamina propria layer containing numerous immunologic cells located in the submucosa. Studies in our laboratories and others have demonstrated that viral translocation following an oral exposure can occur at sites proximal to the gastric cardia (above the stomach) including the oral and esophageal mucosa as well as the tonsils (122, 170). Studies have shown that when HIV+ mothers were administered the anti-viral drug zidovudine the rates of perinatal transmission decreased from approximately 25% to 9% (55) (125). This indicates that decreased viral load in the mother can result in a reduced risk of transmission to the infant.

HIV biology

HIV-1 and HIV-2 are lentiviruses belonging to the *Retroviridae* family of RNA viruses (56). The viral genomes are approximately ten kilobases (kb) in length and contain the coding sequences for nine proteins: *gag*, *pol*, *env*, *nef*, *rev*, *vif*, *vpr*, *vpu*, and *tat* (Figure 1-3 and Table 1-1) (56). Each viral particle is composed of an outer phospholipid bilayer studded with host membrane proteins and the viral *env* gp160 trimer (consisting of three heterodimers of gp120 and gp41) surrounding the viral core. The viral core contains two copies of the single-stranded RNA core

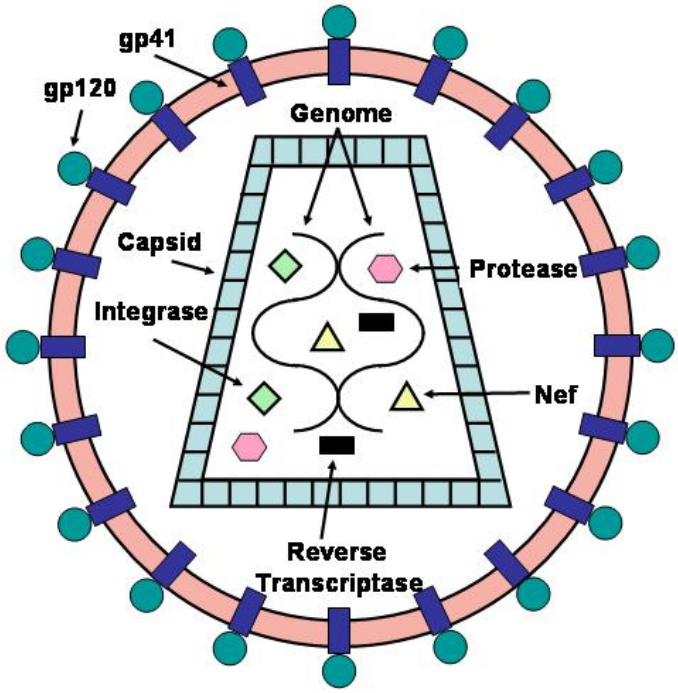

Figure 1-3 – Structure of the HIV virion – Cartoon representation of the structure of the HIV virion. A host-derived phospholipids bilayer studded with viral gp160 (consisting of gp120 and gp41) surrounds the viral core proteins. The viral nucleic acid as well as reverse transcriptase, integrase, protease, and nef proteins are located within the viral core

.

Gene	Full Name	Function
Gag	Group Specific Antigens	Core and matrix proteins
Pol	Polymerase	Reverse transcriptase Protease Integrase RNase H
Env	Envelope	gp120 – Trans-membrane glycoprotein binds CD4, CCR5 or CXCR4 gp41 – Required for viral fusion
Nef	Negative Factor	Enhances viral infectivity Down regulates CD4 and MHC class I Affects T cell activation.
Rev	Regulator of viral expression	Exports un-spliced and singly-spliced viral transcripts from nucleus
Vif	Viral Infectivity Factor	Increases viral infectivity Inhibits cellular APOBEC3G
Vpr	Viral Protein R	Transports viral cDNA to nucleus G2 cell cycle arrest
Vpu	Viral Protein U	Promotes intracellular CD4 degradation Enhances viral release from membrane
Tat	Transactivator	Positive regulator of transcription

Table 1-1 – Characterization of the nine HIV proteins

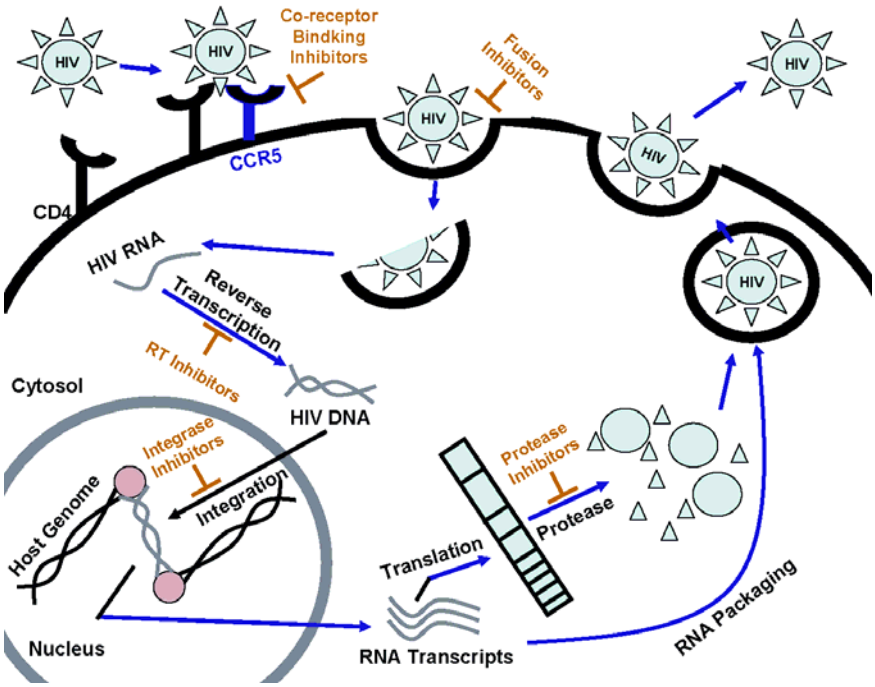

Figure 1-4 – HIV Life Cycle with anti-viral drug Interventions – HIV binds to the cellular surface receptors CD4 and CCR5 and the viral membrane will fuse to the host cell membrane. Then, the capsid will enter the cytosol and release the viral RNA genome. The reverse transcriptase (RT) will generate a complementary DNA strand and this will shuttle into the nucleus to be integrated into the host cell genome via the viral integrase. The pro-viral genome will be transcribed and shuttled out of the nucleus to be either translated into viral proteins or be packaged into the newly formed viral particle. The virus will then bud off from the cell membrane. Current anti-HIV inhibitors are also shown at their appropriate target sites including RT, protease, integrase, and fusion inhibitors.

as well as a variety of proteins including *nef*, *vif*, and protease. For efficient viral infection (Figure 1-4), the viral envelope glycoprotein gp120 binds to the cellular surface protein CD4. HIV has been shown to co-opt different co-receptors to infect cells including the predominate receptors CCR5 and CXCR4 (39, 43, 48, 56, 154). CCR5 is typically expressed on effector T cells located at mucosal sites while CXCR4 is predominantly expressed by naïve and central memory T cells in the peripheral circulation or lymphoid sites (44). In general, CCR5 binding viruses are associated with transmission between hosts while CXCR4 using viruses are associated with expanded co-receptor usage during AIDS disease progression (154). Upon co-receptor binding, the viral and host cell membranes fuse allowing the core to translocate into the cytosol and release of the viral genome. The viral reverse transcriptase (RT) synthesizes a complementary single-strand DNA (cDNA), and host polymerases follow to generate the second strand of the viral cDNA. The viral integrase will shuttle the viral cDNA into the nucleus and randomly integrates it into the host genome. Following transcription, the viral transcripts are escorted to the cytosol allowing for the translation of a large viral poly-protein. The HIV protease cleaves this poly-protein into the necessary structure and non-structural proteins (Table 1-1). Then, the

capsid and envelope proteins assemble with two copies of viral mRNA on the host cell phospholipid membrane and then bud off the cell.

HIV Clinical Treatment

The medications used in clinical practice to treat HIV+ patients are targeted against different viral proteins (Figure 1-4). The first anti-viral drugs suppressed viral replication by inhibiting the viral RT and were classified as either nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) (36, 150, 151). NRTIs including 3'-azido-3'deoxythymidine (AZT; zidovudine) are nucleoside analogs that lack a free 3'-hydroxyl group thereby preventing the synthesis of viral RNA strands (192). In contrast, the classical NNRTI, nevirapine, interacts with amino acids on the viral RT interfering with the ability of the enzyme to add nucleotides (150, 151). The next class of drugs developed was targeted against the viral protease (termed protease inhibitors) (52). Treatment with a single drug class proved insufficient to suppress viral loads long term because drug resistant mutants emerge (20). To combat drug resistance to a single class of inhibitors, highly active anti-retroviral therapy (HAART) combined multiple anti-HIV drugs together in a single "cocktail" consisting of two RT and one protease inhibitor. Current therapy guidelines for treating HIV+ patients state that

HAART should be initiated when CD4+ T cell levels decline to below 300 counts per μl of blood. Other anti-viral compounds which are under development or became recently available serve as an adjunct to the current therapy guidelines. Integrase inhibitors are under development and aimed at preventing the pro-viral genome from integrating into the host cell (73, 74). Along with interfering with viral enzymes, the viral fusion process is also being targeted. Small molecule inhibitors have been identified including T-20 (Trimeris), which bind to and block gp41-co-receptor interactions (42, 99, 127, 146, 180). In summary, novel therapeutic designs attacking multiple stages of the viral life cycle are available that are effective at suppressing HIV replication.

Clinical disease progression following HIV infections

Following an HIV infection, disease progression is generally characterized by the progressive loss of peripheral CD4+ T cells (Figure 1-5). Acute HIV infections are associated with CD4+ T cell decline to two-thirds the level observed pre-infection, which coincides with an increase in plasma viremia. During the acute infection, patients may present with an acute retroviral syndrome characterized by a mononucleosis like illness, including fever, fatigue, lymphadenopathy, and myalgia (84). The chronic stage of infection leads to the establishment of a viral set point which

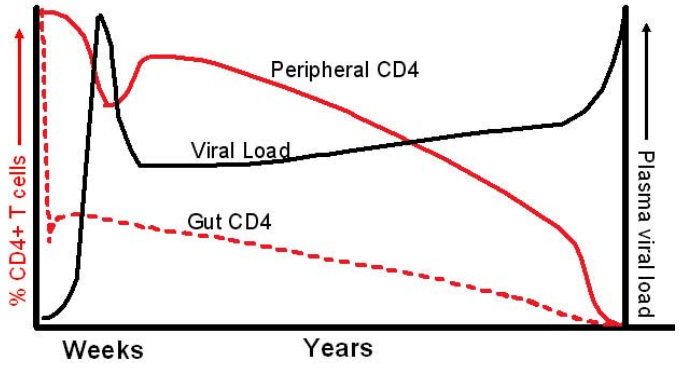

Figure 1-5 – CD4+ T cell levels and viral loads in HIV+ patients – During the acute stage of infection, there is an inverse correlation with rising plasma viremia and decreasing peripheral blood CD4+ T cells. A viral set point is reached during the chronic stages of infection while CD4+ T cells are diminished. During AIDS, the CD4 count decreases to <200 counts/ul blood while plasma viremia increases. In addition to the peripheral circulation, gut mucosal CD4+ T cells are rapidly depleted and not replenished during the acute stage of infection.

generally remains relatively steady until the patient progresses to AIDS. Clinical AIDS is diagnosed when peripheral CD4+ T cell numbers decline to less than 200 cells/ul of blood and is often associated with the onset of opportunistic infections, cancers, or neurological disorders. AIDS patients can present with atypical infections such as *Pneumocystis carnii*, *Candida albicans*, cytomegalovirus, and *Mycobacterium tuberculosis* as well as Kaposi's sarcoma and B cell lymphomas.

Role of Immune activation on HIV/SIV disease progression

Initial research into mechanisms of HIV pathogenesis focused on the role of peripheral CD4+ T cells. In recent years, however, many reports have demonstrated that pathogenesis is a more complex process than peripheral CD4+ T cell decline alone. Additional correlates now known to be related to disease progression include decreased CD4+ T cells at mucosal associated lymphoid tissues (Figures 1-5 and 1-6) (18, 116, 179), increased immune activation (64, 169) and bystander apoptosis of uninfected cells (172). Immune activation is documented by the up-regulation of markers such as CD69 (early activation), HLA-DR (MHC class II), Ki67 (proliferation), CD38, CD95 (Fas), and CD195 (FasL) on multiple immune cells including CD4+ T cells, CD8+ T cells, B cells, and NK cells (53, 63, 64, 158, 169). The events initiating aberrant immune

activation following an HIV infection remain unknown, however a few likely candidate mechanisms have been proposed. Persistent viral replication in CD4+ T cells was hypothesized to be the main contributor to immune activation because these cells express a variety of activation markers following infection. However, multiple immune cells such as B cells and NK cells which are not targets for HIV also become activated following infection, suggesting that viral replication in CD4+ T cells may not be the sole contributor to overall immune activation (44, 94, 101). Alternatively, the immune activation may be driven by the immune response to the HIV antigens, however this seems unlikely due to the few antigen specific cells (29, 89, 134). Another attractive possibility was the selective depletion of regulatory T cells by HIV (1, 46, 90, 175) due to the fact that these cells can express anti-inflammatory cytokines such as IL-10 and TGF-β which may dampen the immune response. In addition, *Douek* and colleagues recently proposed a hypothesis attributing immune activation to the translocation of bacterial components such as lipopolysaccharide (LPS) across mucosal membranes into the peripheral circulation (16, 17). Therefore, HIV disease progression requires more than just depletion of CD4+ T cells and may involve multiple mechanisms including aberrant immune activation over many years of infection.

Natural hosts of SIV

Natural hosts of SIV infection such as sooty mangabeys and African green monkeys (AGMs) can be found SIV infected in the wild. These species have SIV viral loads reaching 10^7 viral RNA copies/ml of blood following infection which is comparable to levels of replication in HIV+ humans (19, 149). Despite high levels of viral replication these monkeys generally have healthy peripheral CD4+ T cell counts throughout infection (93, 95, 149). Healthy CD4 levels in SIV+ mangabeys may be the result of increased expression of the homeostatic cytokine interleukin-7 (IL-7) early after infection (128) as well as the preservation of bone marrow, lymph node, and thymic architecture (25, 166, 168).

Recently our laboratory identified a unique cohort of SIV+ mangabeys which experienced a dramatic depletion of CD4+ T cells (between 50-100 cells/ul blood) in the peripheral circulation yet remained free from simian AIDS (123). This suggests that other host defense mechanisms are keeping these mangabeys from progressing to AIDS. An in-depth analysis of these SIV+ mangabeys identified very low levels of immune activation (HLA-DR, CD69, and Ki67) following chronic infection regardless of CD4+ T cell levels (123, 166). These studies suggest that decreased levels of immune activation may help prevent the progression

towards simian AIDS in mangabeys regardless of the levels of peripheral CD4+ T cells.

Cellular immune responses following chronic HIV infections

Chronic HIV infection is associated with progressive dysfunction in numerous immune cells including CD4+ T cells, CD8+ T cells, dendritic cells, B cells, monocytes/macrophages, natural killer cells, and $\gamma\delta$ T cells (Figure 1-6). The impaired functions observed in these cells may be the result of direct viral cytopathicity or indirect effects including the contribution of increased immune activation.

CD4+ and CD8+ T cells: Assessment of T cells generally focuses on the blood CD4+ T cell depletion in infected patients and correlating their levels with the onset of opportunistic infections when peripheral blood CD4+ T cell levels drop below 200 cells/ul of blood. More recently, studies have focused on other immune sites, such as the mucosa, and the dramatic depletion of CD4+ T cells that occurs at early times post-infection (18, 105, 179). HAART partially restores mucosal CD4+ T cells in HIV+ patients though not to the levels present prior to infection (66, 118). CD4+ T cell levels remain relatively stable in the intestinal mucosa of long-term non-progressing HIV+ patients and may contribute to delayed disease

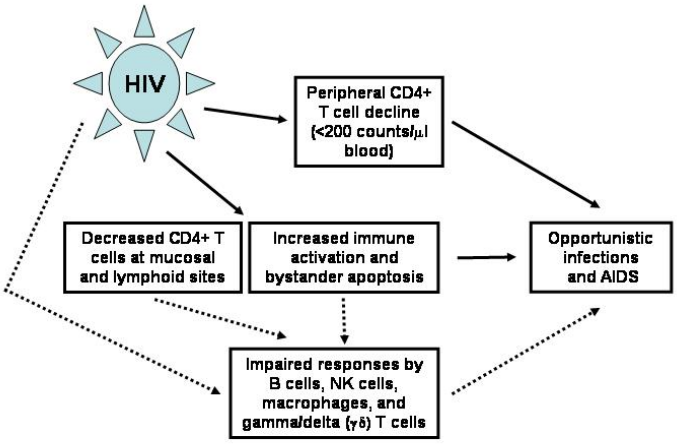

Figure 1-6 – Paradigm following an HIV infection – Following infection, the focus of HIV pathogenesis was on the loss of peripheral CD4+ T cells leading to opportunistic infections and AIDS. However, this paradigm proved more complex as evident by decreased CD4+ T cells at mucosal associated lymphoid tissues as well as increasing immune activation and bystander apoptosis of uninfected cells. In addition, impaired functions in B cells, NK cells, macrophages, and $\gamma\delta$ T cells are observed following an HIV infection.

progression to AIDS in these patients (159). In addition to direct viral cytopathicity, CD4+ T cells can undergo activation-induced cell death (61, 148, 169) as well as become anergized and have express less IFN-γ following *in vivo* stimulation (137). CD8+ T cells also lose their ability to destroy infected cells primarily through the evolution of viral escape mutants as well as the indirect effects of immune activation (6, 135, 173, 174). In addition, HIV+ patients have a skewed repertoire of effector memory, as compared to central memory, CD8+ T cells (104), which also have impaired proliferation (107) and IFN-γ and IL-2 responses following infection (131). In addition, increased numbers of CD8+ T cells expressing activation markers such as CD95 and IFN-γ were present in the brains of SIV-infected macaques suggesting that these cells may also contribute to the neurological immunopathology following an infection (112). Taken together, decreased CD4+ and CD8+ T cell proliferative and cytokine responses throughout an HIV infection may be a result of overall immune activation and contribute to the progression towards AIDS.

B cells: The humoral immune response mediated by B cells is involved in the production of antibodies against extra-cellular bacteria and parasites (184). Recent reports have described a few novel neutralizing antibodies (nAbs) which prevent viral interactions with CD4 and CCR5 (98, 176, 183)

as well as interfering with fusion to the host cell membranes (132, 191). Although the *in vivo* administration of these nAbs can prevent mucosal SIV infection in macaques (9), long-term administration of nAbs leads to the evolution of viral variants where the appropriate *env* epitopes have been disrupted providing a mechanism for escape (98, 183). Further assessment of the humoral immune response during an HIV infection revealed that patients have an increased incidence of B cell lymphomas and hypergammaglobulinemia (37, 100). In addition, B cells have impaired chemokine and complement receptors as well as increased FasL expression, which may contribute to the aberrant bystander apoptosis and immune activation of CD4+ T cells (54, 158, 161). There is also a loss of CD21+ and CD27+ memory B cells in HIV+ patients, which is associated with an increase in activated CD70+ T cells (126, 129, 187). With regards to functionality, B cells from HIV+ patients have an impaired proliferative capacity as well as immunoglobulin expression in response to phorbol esters, CD40L, and anti-IgM *in vitro* stimulation suggesting that they are anergized (37, 126). These reports demonstrate the inability of B cells to proliferate and produce antibodies during an HIV infection which may be due to increased immune activation and contribute to the progression towards AIDS.

Monocytes/Macrophages: Monocyte-derived cells phagocytose and present antigens to both CD4+ and CD8+ T cells, activate NK cells via IL-12 expression, and help to maintain tissue homeostasis (4). Conventionally, macrophages are activated following IFN-γ stimulation leading to up-regulation of antigen-presentation molecules as well as IL-12 expression(4). Macrophages have been shown to be important during HIV pathogenesis as they may serve as one of the earliest targets of infection and may act as long-lived reservoirs (34, 47, 111). In addition, macrophages up-regulate the expression of pro-inflammatory chemokines such as CCL4/MIP-1β and CCL2/MCP-1 upon CCR5 ligation with HIV gp120 (102), which may be a defense to prevent infection of other cells by blocking viral co-receptors. With regards to functionality, the ability of macrophages to phagocytose antigens via complement and Fc mediated processes is impaired following an HIV infection (87), potentially due to decreased actin reorganization (86). Macrophages undergo various phenotypic and functional changes during HIV infection which influence both their susceptibility to infection as well as their ability to perform their phagocytic activities that may be in part due to the increased immune activation present in HIV-infected patients.

Natural Killer cells: Natural killer (NK) cells recognize virally-infected and tumor cells based on decreased expression of MHC class I (13, 130). Numerous studies in HIV-1-infected subjects have demonstrated that significant changes occur within the NK cell compartment during chronic HIV-1 infection (8, 53, 108, 117, 139, 177). Recent studies have demonstrated that NK cells have increased expression of the activation marker HLA-DR following an HIV infection, which inversely correlated with decreased production of perforin and IFN-γ (8, 53). HIV infection induces an expansion of CD56-CD16+ NK cells that do not express cytolytic receptors or Th1 cytokines (IFN-γ and TNF-α) (108, 117). Furthermore, antibody dependent cell-mediated cytotoxicity (ADCC) by NK cells is impaired in SIV-infected macaques when compared to their uninfected counterparts (139, 177). These reports demonstrate that, similar to other immune cells, pathogenic HIV/SIV infection impairs NK cells in a way that can be observed phenotypically as well as functionally and may be due in part to increased levels of immune activation in HIV-infected patients.

Rationale for Studying Gamma/Delta ($\gamma\delta$) T cells

As described above, a study from our laboratory identified a cohort of SIV+ mangabeys which experienced a very dramatic depletion of CD4+ T cells in the peripheral blood as well as in the rectal and pulmonary

mucosa following an SIV infection (123). It is important to note that these CD4-low SIV+ mangabeys have remained free from any signs of clinical AIDS for more than 5 years post-infection. These findings indicated that other immune cells may be functioning in these CD4-low animals to help prevent the progression towards simian AIDS. A comparison of a variety of immune cells identified a significantly increased percentages\ of γδ T cells in the peripheral blood of uninfected mangabeys when compared to humans (to be discussed in Chapter 4 – Figure 4-1). This initial finding led to a further investigation of the function of γδ T cells during HIV/SIV infection. These studies revealed that γδ T cells from SIV+ mangabeys (both CD4-healthy and CD4-low) retained the ability to proliferate in response to *ex vivo* stimulation with the bacterial antigens isopentenyl pyrophosphate (IPP) and lipopolysaccharide (LPS) (123). These results suggested that γδ T cells may retain the ability to respond to opportunistic bacteria thereby providing protection from disease progression (123). Therefore, my thesis project sought to further assess the functionality of γδ T cells throughout the disease course following pathogenic HIV/SIV and non-pathogenic SIV infections. The remainder of this Chapter will discuss the current understanding of γδ T cells following pathogenic HIV/SIV infections.

Introduction to γδ T cells

Though the majority of T cells express rearranged αβ T cell receptors (TCRs), another population exists that express a heterodimeric TCR comprised of rearranged genes from the γ and delta loci (21). The γ and delta loci contain all the germ-line genes necessary for proper TCR rearrangement (21). For example, the delta locus contains 9 variable (V), 3 diversity (D), 4 joining (J) and 1 constant (C) region (Figure 1-7). After a successful TCR rearrangement in the thymus, the γδ T cell can exit to the peripheral circulation or migrate to mucosal sites. In the peripheral circulation of humans, γδ T cells comprise about 1-10% of the circulating T cells though this percentage can rise to as high as 50% in some mucosal sites (21). γδ T cells link the innate and adaptive immune responses (72, 109, 124) by cross-talk with numerous immunologic cells including DCs (33), αβ T cells (15), and B cells (195). Upon recognition of their cognate antigens, γδ T cells proliferate and express either Th1 or Th2 cytokines (49, 124). In summary, γδ T cells play many important immunologic roles by responding to microbial and tumor antigens.

During development in the thymus, T cells will commit to either becoming a member of the αβ or γδ compartment. The development of αβ T cells has been studied more extensively while less is known about the lineage commitment and subsequent development of γδ T cells. Studies

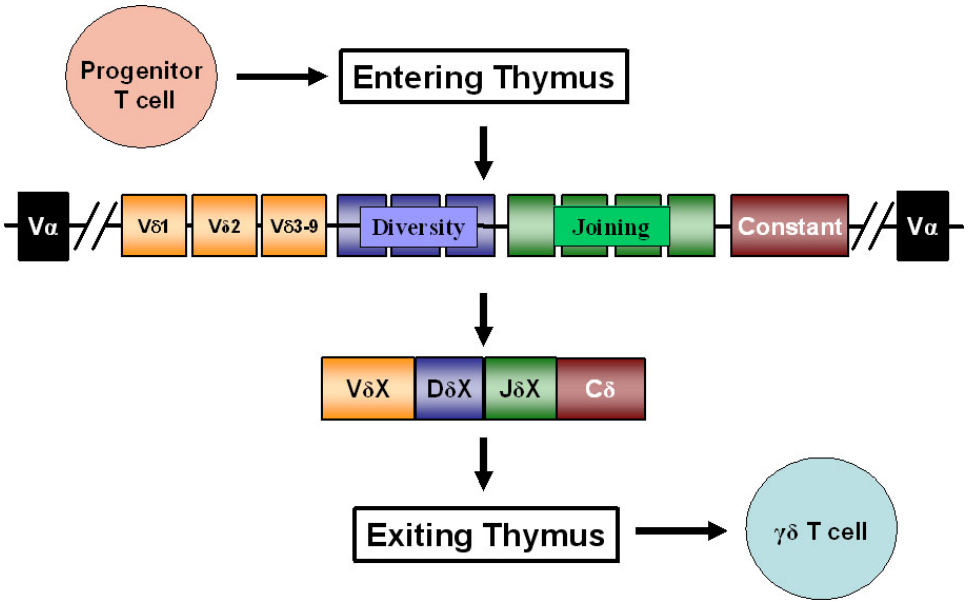

Figure 1-7 – γδ T cell receptor rearrangement – When a progenitor T cell enters the thymus, the cell has the opportunity to rearrange the γ and delta TCR loci. The antigen recognition by γδ T cells is heavily dependent upon the particular delta Variable region chosen. The majority of adult γδ T cells express either the Vdelta1 (Vδ1) or the Vdelta2 (Vδ2) region. Following a successful TCR rearrangement, the cell will exit the thymus and is a functional γδ T cell.

have revealed that similar to pre-T-α receptor expression on maturing αβ T cells, γδ T cells express their TCR during the first CD4/CD8 double negative stage of thymic development (144). Also, in order to undergo proper development, the γδ TCR must be engaged allowing for appropriate signaling to suppress the default developmental αβ T cell pathway (69). Along with appropriate TCR signals, developing γδ T cells need stimulation through CD127 (IL-7 receptor) (81, 110, 133, 144) and LAT (linker of activated T cells) (144). In mice lacking CD127, αβ T cells are present while γδ T cells are absent (110) because IL-7 signaling may be necessary to initiate the transcription of the γ locus (133). IL-7 signals are likely to be necessary for the activation of the RAG genes to induce the rearrangement and subsequent transcription of the receptor but not necessary if a pre-rearranged TCR is already present (133, 193). After TCR rearrangement, γδ T cells leave the thymus to the periphery where they localize to differing tissues based on particular variable delta (Vδ) region and homing molecules expressed.

Antigen Recognition and Reponses by γδ T cells:

Unlike αβ T cells, antigen recognition by γδ T cells is dependent upon the particular variable (V) region of the TCR as opposed to the entire

rearranged TCR (Figure 1-7). The majority of γδ T cells express either the Vdelta1 (Vδ1) or Vdelta2 (Vδ2) γδ TCR (Figure 1-7). Vδ1 γδ T cells respond to the expression of non-classical MHC molecules on the surface of virally-infected or tumor cells and are concentrated at mucosal sites (21, 65, 70, 164). In contrast, Vδ2+ cells are found in the peripheral circulation and respond to non-peptide phosphoantigens including isopentenyl pyrophosphate (IPP) and bromohydrin pyrophosphate (BrHPP) (21, 97, 165). These phosphoantigens are generated during the non-mavelonate and mavelonate pathways utilized by prokaryotic and eukaryotic cells, respectively (50, 152). Vδ2+ γδ T cells have a higher affinity for phosphoantigens derived from the non-mavelonate pathway suggesting that these cells may produce a more robust response to bacterial pathogens (82, 165). In addition to the TCR, γδ T cells can recognize conserved microbial antigens using traditional pattern recognition receptors such as toll like receptors (TLRs). γδ T cells can express toll like receptor-3 (TLR-3) and respond to poly(I:C) stimulation through the production of cytokines (186). In addition, the γδ T cells which reside in the epidermis express TLR-4 and respond to LPS through the production of Th1 cytokines (163). However, the majority of γδ T cells *in vivo* lack TLR-4 expression but may indirectly respond to this antigen via cytokines produced by other immune cells such as APCs (163). γδ T cell recognition

of conserved microbial patterns suggests an innate immune response type function for these cells allowing them to serve as a first response to a microbial challenge. αβ T cells in contrast require days to expand and traffic to the site of infection. This rapid response may prove beneficial in the ability to mount an immune response to common opportunistic pathogens at mucosal sites which may befall HIV+ patients.

In addition to their innate immune cell-like responses, γδ T cells can assist in eliciting an effective adaptive immune response to pathogens. A robust γδ response has been demonstrated for a number of mucosal pathogens including, *Mycobacteria* (10, 162), *Legionella* (96), *Listeria monocytogenes* (83), *Plasmodium falciparum* (156), and West Nile Virus (182). An antigen specific adaptive immune response by γδ T cells is generally assessed by measuring proliferation, cytokine production and expression of activation markers on the cell surface, similar to parameters assessed for αβ T cells. Cellular proliferation is important for immune protection as the mounting of sufficient numbers of cells is important to be able to respond to microbial threats. Proliferation of γδ T cells has been shown to be an important marker of functionality against bacterial infections, most notably *Mycobacteria* (141). Soon after *in vivo* *Mycobacterial* inoculation in rhesus macaques, γδ T cells have been shown to proliferate to high numbers in the pulmonary mucosa (162). γδ T

cells have been shown in two systems to possess a memory function after initial exposure to a viral pathogen. For example, mice lacking γδ T cells that have survived a primary West Nile Virus infection are more susceptible to secondary viral infection in comparison to their wild type counterparts (182). The authors suggested that the production of IFN-γ by γδ T cells was important in controlling the West Nile Virus infection (182). In addition, canary-pox vaccinated patients generated γδ T cells that were able to express IFN-γ following *ex vivo* stimulation with canary pox antigens demonstrating their memory responsiveness (190). They are further able to influence the adaptive immune response indirectly through cytokine production potentially contributing to the appropriateness of the response. Bacterial antigens including LPS can induce γδ T cells from total PBMCs to express both IFN-γ (Th1) and interleukin-4 (IL-4; Th2) (49, 124). The combination of innate and adaptive type immune responses highlights the diverse niches γδ T cells occupy in the immune response.

Alternate Functions of γδ T cells

A controversial γδ T cell function that has yet to be elucidated *in vivo* is their ability to act as antigen presenting cells (APCs).When purified γδ T cells are stimulated *in vitro* via TCR ligation with IPP, Th1 cytokines are expressed which induce the up-regulation of APC markers on co-

cultured DCs (33). γδ T cells also directly interact with B cells as evidenced by the fact that mice deficient in γδ T cells have dysfunctional lymph node germinal center development (185). Furthermore, Vδ2+ γδ T cells isolated from mice up-regulate classical APC markers including HLA-DR, CD80, and CD86 upon 18 hour TCR stimulation with IPP *in vitro* (15). Activated γδ T cells can present toxic-shock syndrome toxin-1 as well as processed *Mycobacterial* peptides, thereby inducing the proliferation of naïve CD4+ and CD8+ T cells (15). Though intriguing, *in vivo* γδ T cells experiments will have to be performed to further evaluate their APC capabilities.

γδ T cells are also known for their ability to recognize non-classical MHC molecules resulting from tumors, wounds, or virally-infected cells. Crystallographic studies indicated that Vδ1+ γδ TCRs recognize non-classical MHC molecules expressed by stressed cells including MICA and T22 (164). In addition, B cell lymphoma Daudi cells are targets for Vδ1+ γδ T cells due to their high surface expression of the stress-induced molecule heat-shock protein-58 (51). Vδ1 γδ T cells also recognize mucosal epithelial cells infected with cytomegalovirus *in vivo* presumably through the expression of stress-induced molecules and then express both TNF-α and IFN-γ (70). Also, mice lacking γδ T cells have slower wound healing following superficial epithelial burns in comparison to their

wild-type counterparts (80). The mechanisms for the impaired wound repair responses were due to keratinocytes not receiving keratinocyte growth factor-1 (KGF-1) at the wounded site (80). Further, epithelial Vδ1+ γδ T cells express KGF-1 following the recognition of stress-induced MHC molecules expressed by keratinocytes (80). Altogether, these reports indicate that γδ T cells can respond to a variety of antigens and modulate the immune environment to protect against tumors and virally infected cells, as well as participate in wound repair.

Protective functions by γδ T cells in response to HIV/SIV

Several reports have demonstrated the importance of γδ T cells during the earliest events following an HIV/SIV infection. γδ T cells can directly inhibit HIV/SIV infection *in vitro* suggesting that these cells may contribute to controlling viral load. When PBMCs were infected *in vitro* with HIV for example, increased numbers of γδ T cells in the cultures inversely correlated with decreased HIV p24 levels suggesting these cells may impair viral replication (140). A potential mechanism to explain the lowered viral replication observed In the PBMC cultures may be due to γδ T cells expressing the CCR5 binding β-chemokines CCL3/MIP-1β and CCL5/RANTES (140). In addition, γδ T cells can induce apoptosis of latently HIV infected U1 cells (14) as well as both HIV- and SIV-infected

primary cells (181) potentially through Fas-FasL interaction (14). In summary, direct inhibition of HIV replication by γδ T cells is a potential mechanism to contribute to viral replication in infected individuals.

To assess whether γδ T cells may have a protective effect during transmission of the virus across a mucosal surface *in vivo* studies have been performed during SIV infection of macaques (103, 171). In these studies the levels of γδ T cells (and DCs) were increased at the viral sites of entry (the tonsils) of SIVmac239Δnef vaccinated macaques following infection with a challenge SIV (171). Furthermore, in a study utilizing an intrarectal inoculation of SIVmac239 gp120 and p27 in macaques, γδ T cells migrated from lymphoid sites to the rectal mucosa in response to a challenge infection and produced CCL3/MIP-1α and CCL5/RANTES (103). The authors attributed the production of these β-chemokines by γδ T cells as a mechanism to prevent the establishment of the challenge viral infection by inhibiting viral gp120-CCR5 co-receptor binding (103). These reports include evidence for a role of γδ T cells in inhibiting viral infection at mucosal sites through an increase in numbers and β-chemokine expression. Thus, augmentation of γδ T cell function could potentially be an effective vaccination strategy to prevent establishment of a mucosal HIV/SIV infection

Dysregulated responses by γδ T cells following chronic HIV/SIV infections

During pathogenic HIV/SIV infection, γδ T cells are susceptible to immune dysfunction reflected in alteration of both their absolute number and functions (5). For example, fewer γδ T cells were present in the intestinal mucosa of macaques by seven days after a rectal inoculation with SIVmacPBj when compared to their uninfected counterparts (26). The lower levels of γδ T cells may be due to the direct infection by HIV/SIV, which has been shown *in vitro* (77). In addition, an assessment of the levels of γδ T cells in the peripheral blood of HIV-infected patients revealed a phenotypic switch in TCR usage where the predominate Vδ2+ γδ T cell population in uninfected donors is replaced with Vδ1 expressing cells (7, 38, 142). This phenotypic switch may be the result of redistribution of Vδ1+ γδ T cells from mucosal sites into the blood (16) as opposed to clonal expansion of Vδ1 γδ T cells (143). Indeed, spectratype analysis of Vδ1+ γδ T cells indicated that there was no expansion of a particular Vδ1 TCR clone in PBMCs or rectal mucosal biopsies from HIV+ patients (143), suggesting that either apoptosis or migration account for the phenotypic switch observed. Therefore, the increased levels of Vδ1 cells in the peripheral blood may be the result of the redistribution of these cells from the mucosa (where they are typically found) (16). Migration

studies in γδ T cell clones from HIV+ patients expressing the chemokine receptor CXCR3 demonstrated a reduced capacity of these cells to migrate in response to the pro-inflammatory chemokines CXCL9/MIG and CXCL10/IP-10 *in vitro* (28). This impaired chemotaxis may result in the failure of γδ T cells to home to appropriate sites thereby contributing to the observed phenotypic switch in the peripheral circulation of HIV+ patients. In summary, these results indicate that an HIV infection leads to altered peripheral γδ T cell populations and may contribute to dysfunctional immune responses including migration.

A further symptom of immune dysfunction in these cells is decreased proliferation in response to antigens following chronic HIV infections. This defect can be attributed to either inherent cellular defects or to indirect effects due to chronic immune activation. For example, γδ T cells from HIV-infected patients had a decreased ability to proliferate in response to *Mycobacterial* lysates (141), which may be the result of many years of immune activation. In addition, HAART was insufficient to restore the ability of γδ T cells to expand *in vitro* when exposed to *Mycobacterial* antigens (71). *Zhou et al* showed the ability of γδ T cells to proliferate was also impaired *in vivo* following an SIV infection in macaques even after a re-exposure to *Mycobacterial* lysates (196). Loss of γδ T cell responsiveness during chronic HIV/SIV infection may contribute to

progression to AIDS by disabling a unique link between the innate and adaptive immune system increasing susceptibility to opportunistic infections.

Cytokine expression is an important function of γδ T cells in their ability to defend against microbial pathogens. For example, γδ T cells from HIV+ patients with over 500 CD4+ T cells/ul blood were able to express IFN-γ in response to bacterial antigenic stimulation (27). However, γδ T cells from AIDS patients (<200 CD4+ T cells/ul blood) showed a decreased ability to express IFN-γ when stimulated with PHA and ionomycin *ex vivo* (41). Interestingly, HAART was able to restore the levels of TNF-α, but not IFN-γ, in the supernatants of PBMCs from HIV+ patients following stimulation with the Vδ2 TCR specific ligand IPP (115). It is important to note that the increased TNF-α levels cannot be solely attributed to γδ T cells as the cytokine detection was performed utilizing ELISA as opposed to cytokine flow cytometry.

Dissertation Summary

Upon beginning this dissertation project, I sought to address some unanswered questions regarding the impact of HIV infections on γδ T cells. These questions included: (1) How early and why are the levels of γδ T cells altered at mucosal sites following a mucosal infection?, (2) How does

HIV disease progression impact the cytokine responses of γδ T cells?, (3) Does a non-pathogenic SIV infection of mangabeys result in a phenotypic switch in the predominate γδ T cell populations as observed in HIV+ humans?, (4) Are γδ T cells present at mucosal sites in SIV-infected mangabeys experiencing low levels of CD4+ T cells?, (5) Do γδ T cells retain their cytokine responses following a non-pathogenic infection? The strength of my thesis project involved the study of γδ T cells during HIV/SIV disease models in order to assess pathogenesis during acute (macaques) and chronic (humans and sooty mangabeys) infections. Chapter 3 (Specific Aim #1) focuses on assessing the levels of γδ T cell subsets at mucosal and lymphoid sites following acute, pathogenic SIV infections of macaques while Chapter 4 (Specific Aims #2 and #3) focuses on a phenotypic and functional comparison of γδ T cells during pathogenic HIV and non-pathogenic SIV disease progression.

CHAPTER 2: Methods and Materials

Human and Animal subjects

Uninfected human blood samples utilized in this study were obtained voluntary from HIV-negative donors in accordance with the University of Texas Southwestern Medical Center at Dallas Institutional Review Board (IRB) guidelines. All HIV+ patient samples were obtained from consenting donors from the UT Southwestern Medical Center AIDS Clinic in accordance with IRB approval. All patients enrolled in this study ranged between 24 to 55 years of age. With regards to the HIV+ patients, viral loads and complete blood counts (CBCs) were performed in order to determine their white blood cell and lymphocyte counts.

All rhesus macaques (*Macaca mulatta*) and sooty mangabeys (*Cercocebus atys*) were cared for in accordance with NIH and local Institutional Animal Care and Use Committee (IACUC) guidelines agreed upon by both UT Southwestern Medical Center, Yerkes National Primate Research Center (YNPRC), and California National Primate Research Center (CNPRC). Rhesus macaques were bred at the CNPRC and ranged between 1-4 years of age. All sooty mangabeys were bred at the YNPRC and ranged from 2-10 years of age when enrolled in the study. Prior to the initiation of the studies, all animals were negative for simian

immunodeficiency virus (SIV), simian T cell leukemia virus (STLV), and simian retrovirus (SRV).

SIV infections of rhesus macaques

Macaques utilized in this study had the following CNPRC designations: 33202 (RM1), 33098 (RM2), 30379 (RM3), 30381, (RM4) 30244 (RM5), 29976 (RM6), 30974 (RM7), 30076 (RM8), 32991 (RM9), 32764 (RM10), 32875 (RM11), and 33463 (RM12). Eight rhesus macaques (RM1-RM8) were anesthetized with 10 mg/kg of ketamine hydrochloride and then orally inoculated with two doses of $1x10^5$ tissue culture infection dose ($TCID_{50}$) of SIVmac251-5/98 in 1 ml each to ensure infection. The viral inoculum was slowly dispensed onto the right cheek pouch using a needless syringe thus allowing for the inoculum to be swallowed. The macaques were euthanized under IACUC standards at 1 (n=2), 2 (n=2), 4 (n=2), 7 (n=1), and 14 (n=1) days post-infection (DPI). The infection of these macaques has been described in a previous publication (122). In addition, four macaques (RM9-RM12) were utilized in this study as uninfected controls. For this study, oral and esophageal mucosal as well as cervical, retropharyngeal, and axillary lymph node tissues were collected and placed into Optimal Cutting Temperature

(OCT) embedding compound (Tissue-Tek/Sakura, McGaw Park, IL) to preserve the nucleic acids and stored at -80^{o}C.

SIV infections of sooty mangabeys

The sooty mangabeys utilized in this study were previously infected either naturally or experimentally (128). The naturally infected mangabeys were identified in the YNPRC colony and ranged between 10-20 years of age. With regards to the experimental infections, plasma from a naturally infected sooty mangabey, FQi, was intravenously (IV) inoculated into six uninfected mangabeys [FFr (SM1), FBr (SM2), FCq (SM3), FCs (SM4), FRu (SM5), and FUq (SM6)]. The mangabeys were followed for CD4+ T cell counts and viral loads (128) and have remained free from simian AIDS for more than six years post-infection.

RNA isolation from frozen macaque tissue

Frozen OCT-embedded tissues were removed from -80^{o}C storage and thawed. For thawing, tissues were placed in a Petri dish and the OCT was removed by submerging the sample in 37^{o}C warmed phosphate buffered saline (PBS) (Sigma, St. Louis, MO). Then, 10 mg of the tissue was cut using a scalpel and transferred into a homogenization tube with sterile resin (Novagen, San Diego, CA) containing 600 ml of TRIzol which

contained phenol (Invitrogen, Carlsbad, CA). A plastic pestle (Fisher, Rockville, MD) was used to disrupt the tissues utilizing a gentle grinding motion. Following homogenization, 200 μl of chloroform (Sigma, St. Louis, MO) was added to the homogenization tube and centrifuged for 15 minutes at 14,000xg at 4°C. After centrifugation, the aqueous phase containing the nucleic acids was separated from the more dense phenol layer and transferred into an RNase/DNase free tube (Sarstedt, Newton, NC) to which equal volumes of 70% ethanol was added. The solution was mixed to precipitate the nucleic acid. The nucleic acid lysate was then added to a RNA isolation spin column (Qiagen, Valencia, CA) and centrifuged to allow for RNA binding to the column. Then, 700 μl of RNA binding buffer (RW1) was used to wash the column twice and 500 μl of column wash buffer (RPE) was added to wash the column once (Qiagen. Valencia, CA). The RNA was eluted from the column with 50 μl of 37°C H_2O. Residual contaminating DNA was removed by treatment with DNase I (Ambion, Austin, TX) for 1 hour at 37°C. The integrity of the RNA was measured using an Agilent 2100 bio-analyzer (Agilent Technologies, Santa Clara, CA), quantified, and stored at -80°C.

Synthesis of complementary DNA

Complementary DNA (cDNA) was synthesized from 1 μg RNA utilizing the Super Script First Strand cDNA synthesis kit (Invitrogen, Carlsbad, CA). The following reagents were added to 1 μg of the RNA: 3 μl of 50 ng/μl random hexamers, 1 μl of 10 mM deoxynucleotides triphosphates (dNTPs), and 1 μl of diethyl-pyrocarbonate (DEPC) treated water and incubated at 60°C for 5 minutes. Next, a master mix was generated with the following reagents: 4 μl of 25 mM magnesium chloride ($MgCl_2$), 2 μl of 10x reverse transcriptase (RT) buffer, 2 μl of 0.1M dithiothreitol (DTT), 1 μl of RNaseOUT, and 1 μl of 50 U SuperScript II RT. The master mix was aliquoted into each sample tube and placed at room temperature for 10 minutes. Then, the samples were incubated at 42°C for 50 minutes (to allow the RT to synthesize the cDNA) followed by 70°C for 15 minutes (to deactivate the enzyme). After heating, 1 μl of RNaseH was added to each sample and incubated at 37°C for 20 minutes. The samples were quantified with a spectrophotometer, diluted to 1 μg of cDNA in 5 μl of DNase/RNase free H_2O, and stored at -20°C.

Nested polymerase chain reaction

Nested polymerase chain reaction (PCR) was performed to amplify rhesus macaque and sooty mangabey Vδ1 and Vδ2 transcripts. The first

round PCR master mix included the following reagents: 5 μl of 10x *Taq* buffer, 1.8 μl of 50 mM $MgCl_2$, 1 μl of 10 mM dNTPs, 0.25 μl of 5 U/ul *Taq* polymerase, 4 μl of 5 μM forward and reverse primers (Table 2-1) and 5 μl of 1 μg/5 μl cDNA. The second round master mix contained the same concentrations of reagents with the exception of 2 μl of the first round products used as the template. The following thermocycler conditions were utilized to amplify the templates: 1 cycle for 5 minutes at 94°C (Pre-denaturation) followed by 40 cycles of 30 seconds at 94°C (Denaturation), 30 seconds for 55°C (Annealing), and 1 minute at 72°C (Extension), and then 1 cycle of 7 minutes at 72°C (Final Extension).

Cloning macaque and mangabey γδ genes

The amplicons generated in the nested PCR were ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) utilizing the following reagents: 2 μl of PCR amplicons, 1 μl of 0.02 μg/ul pCR2.1 vector (Invitrogen, Carlsbad, CA), 1 μl 10x *T4* DNA ligase buffer (New England Biolabs, Ipswich, MA), and 1 μl *T4* DNA ligase (New England Biolabs, Ipswich, MA) and then incubated overnight at 14°C. On the following day, the ligated plasmids were transfected into chemically competent *Top10* bacteria (Invitrogen, Carlsbad, CA) (5 μl of plasmids were added to the bacteria on ice for 30 minutes followed by a heat shock 42°C for 45

Gene	GI #	Primer Location		Nested PCR Sequence
Vdelta1 (Vδ1) TCR	339381	Outside	Forward	5'-ATG CTG TTC TCC AGC CTG CTG TGT-3'
			Reverse	5'-CAG CAT TGT ACT TCC CAC TGG-3'
		Inside	Forward	5'-ATG CTG TTC TCC AGC CTG CTG TGT GTA T-3'
			Reverse	5'-CAG CAT TGT ACT TCC CAC TGG-3'
Vdelta2 (Vδ2) TCR	37314	Outside	Forward	5'-ATG CAG AGG ATC TCC TCC CTC ATC-3'
			Reverse	5'-CAG CAT TGT ACT TCC CAC TGG-3'
		Inside	Forward	5'-ATG CAG AGG ATC TCC TCC CTC ATC CAT C-3'
			Reverse	5'-CAG CAT TGT ACT TCC CAC TGG-3'

Table 2-1 – Forward and reverse primer sets utilized to PCR amplify the Vδ1 and Vδ2 γδ TCRs

seconds and then placed on ice for 2 minutes). Following recovery, the bacteria were incubated at 37^{o}C for 1 hour and plated on Luria Broth (LB)-agar (Sigma, St. Louis, MO) plates containing 50 mg/ml of ampicillin and incubated at 37^{o}C overnight. Single colonies were lifted and cultured in LB broth containing 50 mg/ml of ampicillin. Following over-night culture, plasmids were isolated using the Midi-prep Plasmid DNA kit (Qiagen, Valencia, CA), 5 ml of bacterial cultures were pelleted in a 1.5 ml *Eppendorf* tube to which 300 μl of resuspension buffer (P1) was added (Qiagen, Valencia, CA). Then, 250 μl of homogenization buffer (P2) was added to disrupt the bacteria cell wall and 250 μl of neutralization buffer (N1) was aliquoted to inhibit the homogenization buffer. The solutions were inverted five times and centrifuged for 10 minutes at 14,000xg. The supernatant was transferred to spin columns and centrifuged for 1 minute. The column flow through was discarded and 700 μl of wash buffer (P3) was added. After centrifugation, 50 μl of H_2O was added to the column and centrifuged to elute the plasmids from the column. Following purification, the correct size and sequence of the plasmids were confirmed using 1% agarose gel electrophoresis and DNA sequencing (sequences listed below).

Rhesus macaque Vdelta1 (Vδ1) γδ TCR sequence (5' → 3')

TATGCTGTTCTCCAGCCTGCTGTGTGTATTTGTGGCCTTCAGCTACTC

TGGATCCAGCGTGGCCCAGAAGGTTACTCAAGCCCAGTCATCAGTAT

CCATGCCAGTAGGGAAAGCAGTCACCCTGAACTGCCAGTATGAAACA

AGTTCGTGGTCATATTACCTTTTTTGGTACAAGCAACTTCCCGGCAAA

GAGATGATTTTCCTTATTCGCCAGGGTTCTTCTGAACTGAATGCAAGA

AATGGTCGCTATTCTGTCAACTTCGAGAAAGCAGCTAGCTCCATCGC

CTTAACCATTTCAGCCTTACAGCTAGAAGACTCAGCAACATACTTCTG

TGCTCTCCGGACCACCTTCGTACGTACTGGGGGTCAAGGGGTCACA

GCACAACTCTTCTTTGGAAAGGGAACTCAACTCATCGTGGAACCAGA

AAGACAACCTCATACCAAACCATCCGTTTTCGTCATGAAAAATGGGAC

AAATGTTGCTTGTCTGGTGAAGGACTTCTACCCCAAGGATATAAGAAT

AAATCTCGAGTCATCCAAGAAGATAACAGAGTTTGNC

Rhesus macaque Vdelta2 (Vδ2) γδ TCR sequence (5' → 3')

ATGCAGAGGATCTCCTCCCTCATCCATCTCTCCCTCTTCTGGGCAGG

AGTCATGTCAGCTGTTGAGTTGGTGCCTGAACACCAAACAGTGATTG

TGTCAGTGGGGGACCCTGCCACCCTCAAGTGCTCCATGAAAGGAGA

AGCAATCAGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTA

ACACAATGACTTTCATATACCGAGAAAAGGGCATCTATGGCCCTGGT

TTCAAGGACAATTTCCAAGGTGACATTGATACTGAAGAGAACCAGGC

TGTACTTAAGATCCTTGCACCATCAGAGAGAGATGAAGGGTCTTACT

ACTGTGCCAGTGATAGCGTACTGGGTGGTGGGTTGACAGCACAACT

CTTCTTTGGAAAGGGAACTCAACTCATCGTGGAACCAGAAAGACAAC

CTCATACCAAACCATCCGTTTTCGTCATGAAAAATGGAACAAATGTTG

CTTGTCTGGTGAAGGACTTCTACCCC

Sooty mangabey Vdelta1 (Vδ1) $\gamma\delta$ TCR sequence (5' → 3'): GenBank # 765393

ATGCTGTTCTCCAGCCTGCTGTGTATATTTGTGGCCTTCAGCTACTCT

GGATCCAGTGTGGCCCAGAAGGTTACTCAAACCCAGTCCTCGGTATC

CATGCCAGTGGGGAAAGCAGTCACCCTGAACTGTCAGTATGAAACAA

GTCAGTGGTCATATTACCTTTTTTGGTACAAGCAACCTCCCAGCAAAG

AGATGATTTTCCTTATTCGCCAGGGTTCTTCTGAACAGAATGCAAGAA

ATGGTCGCTATTCTGTCAACTTCGAGAAAGCAGCGAGCTCCATCGCC

TTAACGATTTCAGCCTTACAGCTAGAAGACTCAGCAACATACTTCTGT

GTTCTCCGGGACTTCGGTTTCGTCCGGACTGGGTGGTTTACTGGGG

GGATACCCACGCTTGATAAACTCATCTTTGGAAAAGGAACCCGTGTG

ACTGTGGAACCAAAAAGACAACCTCATACCAAACCATCCGTTTTCGTC

ATGAAAAATGGAACAAATGTTGCTTGTCTGGTGAAGGACTTCTACCC

CAAGGATATAAGAATAAATCTCGAGTCATCCAAGAAGATAACAGAGTT
TGATCCTGCTATTGTCGTCTCTCCCAGTGGGAAGTACAATGCTGA

Sooty mangabey Vdelta2 (Vδ2) γδ TCR sequence (5' → 3'): GenBank # 765371

TATGCAGAGGATCTCCTCCCTCATCCATCTCTCCCTCTTCTGGGCAG
GAGTCATGTCAGCTGTTGAGTTGGTGCCTGAACACCAAACAGTGATT
GTGTCAGTGGGGGACCCTGCCACCCTCAAGTGCTCCATGAAAGGAG
AAGCAATCAGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGT
AACACAATGACTTTCATATACCGAGAAAAGGGCATCTATGGCCCTGG
TTTCAAGGACAATTTCCAAGGTGACATTGATACTGAAGAGAACCAGG
CTGTACTTAAGATCCTTGCACCATCAGAGAGAGATGAAGGGTCTTAC
TACTGTGCCAGTGACGTACTGGGGGGATACGCTTTCACAGCACAACT
CTTCTTTGGAAAGGGAACTCAACTCATCGTGGAACCAGAAAGACAAC
CTCATACCAAACCATCCGTTTTCGTCATGAAAAATGGAACAAATGTTG
CTTGTCTGGTGAAGGACTTCTACCCCAAGGATATAAGAATAAATCTCG
AGTCATCCAAGAAGATAACAGAGTTTGATCCTGCTATTGTCGTCTCTC
CCAGTGGGAAGTANCAATGCTG

Quantitative real-time PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was utilized to assess the impact of an acute SIV infection on gene expression in rhesus macaques. To amplify and quantify Vδ1, Vδ2, and CD4 cDNA, master mixes containing the following reagents were generated: 2.5 μl of 10x *Taq* buffer, 1.75 μl of 50 mM $MgCl_2$, 0.5 μl of 10 mM dNTPs, 2.5 μl of 5 μM forward and reverse primers (Table 2-2), 0.5 μl of 10 μM probe, and 0.125 μl of 5 U/ul *Taq* polymerase. Amplifying the other genes (Table 2-2) involved master mixes containing 10 μl of 2X Taqman buffer with the same concentrations of primers and probes, Each probe contained a 5' FAM (6-carboxyfluorescein) fluorophore, and a 3' TAMRA (6-carboxytetramethylrhodamine) quencher. To amplify the templates, the following thermocycler conditions were utilized: 1 cycle for 10 minutes at 94°C (Pre-denaturation), followed by 40 cycles of 15 seconds at 94°C (Denaturation) and 1 minute at 60°C (Annealing/Extension). Duplicate qRT-PCR reactions were performed on each sample for reproducibility. The qRT-PCR reactions were performed in either a 7900 or 7300 SDS sequence detectors (Applied Biosystems, Foster City, CA).

Determination of gene expression fold change between uninfected and SIV+ macaques

Gene	GI #	Real-Time PCR Sequence	
Vdelta1 (Vδ1) TCR	339381	Forward	5'-TCG CCT TAA CCA TTT TAG CC-3'
		Reverse	5'-AAC GGA TGG TTT GGT ATG AGG T-3'
		Probe	5'-FAM-TAC AGC TAG AAG ACT CAG CAA CAT ACT TCT GTG CTC T-TAMRA-3'
Vdelta2 (Vδ2) TCR	37314	Forward	5'-GAG AAC CAG GCT GTA CTT AAG ATC CTT-3'
		Reverse	5'-TGA CGA AAA CGG ATG GTT TG-3'
		Probe	5'-FAM-AGA GAG AGA TGA AGG GTC TTA CTA CTG TGC CAG TG-TAMRA-3'
CD4	393417	Forward	5'-CCA CTG GAA AAA ACT CCA ACC A-3'
		Reverse	5'-CGA TCG CTC AGC TTG GAT G-3'
		Probe	5'-FAM-AAA GAT TCT GGG AAT TCA GGG CTC CTT CTT AAC TAA-TAMRA-3'
CCL21 / 6Ckine	74136270	Forward	5'-TAC CGG AAG CAG GAA CCA AG-3'
		Reverse	5'-CTG CTC CAT CCC AGC TAT CCT GTT CTT G-3'
		Probe	SYBR Green
CCR7	74136330	Forward	5'-GGG GAA ACC AAT GAA AAG T-3'
		Reverse	5-GTG ACC TCA TCT TGA CAC AGG C-3'
		Probe	Probe # 77 (Roche - Universal Probe Library)
CCL5 / RANTES	74136260	Forward	5'-ACC AGT GGC AAG TGC TCC A-3'
		Reverse	5'-GGT TGG CAC ACA CTT GGC G-3'
		Probe	5'-FAM-CCA GCA GTC GTC TTT GTC ACC CGA AA-TAMRA-3'
CCR5	116812906	Forward	5'-TAC CTG CTC AAC CTG GCC AT-3'
		Reverse	5'-TTC CAA AGT CCC ACT GGG C-3'
		Probe	5'-FAM-CCT GCT TTT CCT TCT TAC TGT CCC CTT CTG-TAMRA-3'
GAPDH	93004459	Forward	5'-GCA CCA CCA ACT GCT TAG CAC-3'
		Reverse	5'-TCT TCT GGG TGG CAG TGA TG-3'
		Probe	5'-FAM-TCG TGG AAG GAC TCA TGA CCA CAG TCC-TAMRA-3'

Table 2-2 – Primer and probe sets utilized to assess gene expression in rhesus macaques

Gene expression from SIV+ macaques was compared to four uninfected macaques to determine the impact of an acute SIV infection. The delta Ct (ΔCt) method was utilized to determine fold change in gene expression of SIV-infected compared to uninfected macaques. To perform this method, the average Ct value of the housekeeping gene (GAPDH for these studies) was subtracted from the average Ct value of the target gene to determine a ΔCt value. In the 4 uninfected macaques an average of the ΔCt values was derived then subtracted from the ΔCt value of the target gene to generate the ΔΔCt value. Fold change was then determined by the following formula: $2^{-\Delta\Delta Ct}$ (3). In the event that the ΔΔCt value was positive indicating the fold change was less than 1 (a negative fold change), the fold change value was calculated using the following formula: $(-1)(2^{\Delta\Delta Ct})$. For example, a ΔΔCt value of 3 would result in a fold change of $2^{-3} = 0.125$. To display this fold change graphically as a negative change, the formula would be $(-1)(2^3)$ or a -8 fold change. An average fold change and standard deviation of the target gene was calculated for the uninfected macaques and used to assess target gene mRNA levels in the infected animals. Changes in mRNA expression of a target gene in an infected macaque was deemed either increased or decreased if its fold change was greater than two standard deviations of the average of the four uninfected controls.

Isolation of peripheral blood mononuclear cells

Approximately 40 ml of whole blood was drawn from both uninfected and HIV+ patients in acid-citrate-dextrose (ACD) anti-coagulant tubes. Whole blood was diluted by half with PBS, layered on top of Ficoll (Amersham Biosciences, Piscataway, NJ), and centrifuged at 2,000 revolutions per minute (rpm) for 20 minutes with the brake off. The white leukocyte layer (located at the interface between the Ficoll and serum layers) was transferred into a separate 50-ml conical tube. The peripheral blood mononuclear cells (PBMCs) were washed with PBS at a final volume of 50 ml and centrifuged at 1,700 rpm for 10 minutes to remove residual Ficoll. Then, thrombocytes were removed utilizing a second PBS wash and subjected to centrifugation at 1,200 rpm for 10 minutes. The supernatant was discarded and the cell pellet was resuspended in 3 ml of Dulbecco's Modified Eagle Medium (DMEM) (Sigma, St. Louis, MO) supplemented with penicillin, streptomycin, glutamine (P/S/G), and 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO). Next, 50 μl of PBMCs were diluted in 4.5 ml PBS (1:100 dilution) and transferred to a hemocytometer for counting. After counting, the cells were resuspended to a final volume of $2x10^6$ cells / 200 μl of DMEM supplemented with P/S/G and 10% FBS.

Phenotypic assessment of γδ T cells

PBMCs were aliquoted into a 96-well plate at a concentration of $2x10^6$ cells/200 μl media (one well per flow cytometry panel to be tested as well as six wells for compensation tubes). Following over-night culture at 37°C with 5% CO_2, the PBMCs were washed with FACS wash buffer (containing PBS+4% FBS) and centrifuged at 1,500 rpm for 7 minutes. Then, the cells were stained with fluorescent antibodies specific for cellular surface antigens (Table 2-3) for 30 minutes at 4°C in the dark. The antibodies were directly conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), PE-Cy7, allophycocyanin (APC), or APC-Cy7 (Table 2-3). Following antibody staining, the PBMCs were washed twice with PBS and then fixed in 1% paraformaldehyde (PFA). Nine-parameter flow cytometric analysis (forward and side scatter, pulse-width, and six fluorophores) was performed on a Cyan flow cytometer (Dako-Cytomation; Fort Collins, CO) and analyzed utilizing FlowJo software (FlowJo, Ashland, OR).

Cytokine flow cytometry

PBMCs were aliquoted into a 96-well plate at a concentration of $2x10^6$ cells/200 μl media (four separate wells per patient to assess the

Fluorophore	Channel	Antigen	Company	Clone
FITC	FL1	Pan-γδ TCR	Endogen	5A6.E9
PE	FL2	Vδ2 γδ TCR	Pharmingen	B6
PerCP	FL4	HLA-DR	Pharmingen	L243
PE-Cy7	FL5	CCR7	Pharmingen	3D12
APC	FL8	CD62L	Pharmingen	MEL14
APC-Cy7	FL9	CD3	Pharmingen	SK7

Table 2-3 – Fluorescent antibody panels utilized to assess expression of lymph node homing receptors and activation markers on γδ T cells

separate stimulation conditions as well as six wells for compensation tubes). Intracellular analysis of cytokines using cytokine flow cytometry (CFC) was performed as previously described with only minor changes (15, 145, 186), PBMC were stimulated over-night at 37°C and 5% CO_2 with media alone (M; negative control) or the bacterial antigens isopentenyl pyrophosphate (IPP; 30 μg/ml) (Sigma, St. Louis, MO) or lipopolysaccharide (LPS; 1 μg/ml) (Sigma, St. Louis, MO). Also, the mitogen phorbol-myristal-acetate (PMA; 25 ng/ml) (Sigma, St. Louis, MO)in combination with the calcium ionophore, ionomycin, (I; 1 μg/ml) (Sigma, St. Louis, MO) were added to a separate well for 1 hour at 37°C and 5% CO_2. After *ex vivo* stimulation, 1 μg/ml Brefeldin A (Sigma, St. Louis, MO) was added to each sample and incubated for 5 hours at 37°C and 5% CO_2 to allow cytokines to accumulate within the cells. The cells were washed with FACS wash buffer and then permeabilized by the addition of 200 μl of *FACSJuice* [100 ml 10x FACSLyse (BD Pharmingen, San Diego, CA) with 400 ml ddH_2O and 250 μl Tween20 (Sigma, St. Louis, MO)] and incubated for 3 minutes at room temperature. The cells were washed twice with FACS wash buffer, centrifuged, and stained with fluorescent antibodies specific for cellular surface antigens as well as cytokines (Table 2-4) for 30 minutes at 4°C in the dark. The antibodies were directly conjugated to FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, or

APC-Cy7 (Table 2-4). Following antibody staining, the PBMCs were washed twice with PBS and then fixed in 1% PFA. Nine-parameter flow cytometric analysis was performed on a Cyan flow cytometer (Dako-Cytomation; Fort Collins, CO) and analyzed utilizing FlowJo software (FlowJo, Ashland, OR).

Isolation of lymphocytes from lymph node biopsies, rectal biopsies and bronchoalveolar lavage

Lymph node (LN) biopsies (axillary or inguinal) and rectal mucosal (RM) biopsies as well as bronchoalveolar lavages (BALs) were performed on two SIV+ CD4-low (SM1 and SM2) and two SIV+ CD4-healthy (SM3 and SM4) mangabeys. RM biopsies were obtained using a sigmoidoscope with forceps and mononuclear cells were isolated by collagenase digestion (two sequential 30 minute incubations at 37ºC in RPMI containing 0.75 mg/ml collagenase). The digested suspension was passed through 70 um cell strainers and then enriched for lymphocytes by Percoll density gradient. Mononuclear cells were collected (present at the interface between the 35 and 60% Percoll layers), washed and resuspended in complete RPMI.

For the BALs, a fiber optic bronchoscope was placed into the trachea after local anesthetic was applied to the larynx. Four 35 ml

Fluorophore	Channel	Antigen	Company	Clone
FITC	FL1	Pan- γδ TCR	Endogen	5A6.E9
PE	FL2	Vδ2 γδ TCR	Pharmingen	B6
PerCP-Cy5.5	FL4	CD3	Pharmingen	SP34-2
PE-Cy7	FL5	IL-4	Pharmingen	8D4-8
APC	FL8	TNF-α	Pharmingen	MAb11
APC-Cy7	FL9	IFN- γ	Pharmingen	4SB3

Table 2-4 – Fluorescent antibody panels utilized to assess cytokine expression by γδ T cells

aliquots of warmed saline were injected into the right primary bronchus and collected by aspiration before a new aliquot was instilled. Following aspiration and collection of the BAL cells, PBMCs were isolated utilizing Ficoll density gradient centrifugation as described earlier. After the cells were counted, they were stained with antibodies recognizing the Pan- $\gamma\delta$ TCR and CD3 then assessed utilizing flow cytometric analysis.

Statistical analysis for human and mangabey studies

Statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). A Mann-Whitney test (non-parametric, two tailed, and unpaired) was performed to determine the differences between uninfected and infected groups; comparison of uninfected humans to HIV+ patients and uninfected mangabeys to SIV+ mangabeys. Statistically significance is identified as *p* values less than 0.05 (95% confidence interval).A Spearman correlation was used to determine the inverse correlation between CD4+ T cell levels and viral loads in HIV+ patients.

CHAPTER 3: Decreased Levels of Gamma/Delta T Cells at Mucosal Sites but Increased at Lymphoid Sites Following Acute SIV Infection of Macaques

Introduction

The goal of this chapter is to assess the levels of gamma/delta (γδ) T cells at mucosal and lymphoid sites during acute SIV infection. γδ T cells bridge the innate and adaptive immune responses in multiple ways including through the expression of Th1 and Th2 cytokines following T cell receptor (TCR) stimulation (49, 109, 124). Assessment of levels and functions of γδ T cells during SIV infection of macaques has been undertaken previously by a number of investigators (26, 103, 171, 196). Following vaccination of macaques with an attenuated virus (SIVmac239Δnef), an increase in the levels of γδ T cells and DCs was observed in the monkeys able to block the infection of a SIV challenge inoculum applied to the tonsils (171). Also, intrarectal inoculation of purified SIVmac239 gp120 resulted in increased numbers of γδ T cells expressing the β-chemokines CCL3/MIP-1α and CCL5/RANTES at the rectal mucosa following challenge with infectious virus (103). The authors suggested that the production of these β-chemokines by infiltrating γδ T cells was preventing viral infection of target cells as these chemokines are

capable of inhibiting viral gp120-CCR5 co-receptor binding (103). Finally, lower levels of γδ T cells were present in the intestinal mucosa 7 days after intrarectal inoculation of macaques with SIVmacPBj (26). Taken together, these reports provide evidence that γδ T cells are affected by SIV infection and may, in a vaccine setting, provide protection against SIV challenge by producing β-chemokines that can block SIV gp120-CCR5 co-receptor interaction.

The antigenic specificity and distribution of γδ T cells are strongly dependent upon the delta variable region selected during TCR rearrangement; the most common being the Vδ1 or Vδ2 delta variable regions (21). Cells expressing the Vδ1 TCR are primarily located at mucosal sites and recognize non-classical MHC expression on virally infected cells (80, 162). T cells expressing the Vδ2 TCR recognize non-peptide phosphoantigens independent of MHC presentation and are primarily distributed in the peripheral blood (21, 97). With regard to functionality, fewer CXCR3-expressing γδ T cell clones derived from chronically HIV-infected patients migrated across an epithelial monolayer in response to the pro-inflammatory chemokines CXCL10/IP-10 or CXCL9/MIG as compared to cells from uninfected donors (142). In addition, *in situ* hybridization has revealed decreased expression of lymph node homing chemokines and receptors (CCL21/6Ckine and CCR7) in the

lymph nodes of SIV-infected macaques (28). Similar to the dysfunction observed in other immune cells (5), these reports indicate that the migratory potential of γδ T cells to lymphoid and inflammatory sites is impaired following pathogenic HIV/SIV infection.

In my studies, the distribution of γδ T cells was assessed at the earliest times (1 – 14 days) following a primary pathogenic infection of macaques inoculated orally with SIVmac251. In particular, these studies identified decreased levels of Vδ1 and Vδ2 γδ T cells at the oral and esophageal mucosa and increased levels at the cervical and retropharyngeal LNs during an SIVmac251 infection. Increased expression of the homeostatic chemokine CCL21/6Ckine, as well as its cognate receptor CCR7, was observed in lymphoid sites concomitant with the higher levels of γδ T cells. These findings suggest a mechanism by which γδ T cells migrate away from mucosal sites towards secondary lymphoid tissues following an acute infection with SIVmac251, possibly to assist in the initiation of an anti-viral immune response and control of rapid viral spread.

Results

Decreased levels of γδ T cells at mucosal sites following an acute SIV infection

A previous study from our laboratory determined that following an oral inoculation of rhesus macaques, SIV DNA could be detected in oral and esophageal mucosal sites as early as 1-2 days after infection, identifying these mucosal sites as likely entry points for SIV/HIV into the host (Table 3-1) (122). SIV infection also correlates with decreased levels of γδ T cells in the intestinal mucosa observed as early as seven days following rectal SIV inoculation (26). The goal of the experiments presented in this Chapter was to assess the levels of γδ T cells at early times post-infection (1 – 14 days) in macaques infected orally with SIV. Due to the small amount of biopsy tissue available and the relatively low levels of γδ T cells present in these tissues, a quantitative real-time PCR based approach was utilized. These studies were initiated with an assessment of γδ T cell receptor (TCR) expression at mucosal (oral and esophageal) and lymphoid (retropharyngeal and cervical lymph nodes) sites from uninfected macaques to determine a two standard deviation range of gene expression. In order to determine if γδ T cell mRNA levels were increased or decreased following an infection, matched tissues from SIV-inoculated macaques were analyzed and compared to the those obtained from the uninfected macaques. The levels of Vδ1 and Vδ2 TCR expression in the esophageal mucosa of two macaques infected for 1 day were within the two standard deviation range as determined from

uninfected macaques, however RM2 demonstrated decreased TCR expression at the oral mucosa (Figures 3-1A and 3-1B). At 2 and 4 days post-infection (DPI), a decrease in Vδ1 and Vδ2 γδ TCR expression was observed at both the oral and esophageal mucosa (Figures 3-1A and 3-1B). Interestingly, the lower levels of Vδ1 and Vδ2 TCR expression at these mucosal sites 2-4 DPI coincided with the detection of SIV DNA (Table 3-1). The mRNA levels of the CD4 gene were assessed at the same mucosal sites during acute SIV infection and was generally within the two standard deviation range of uninfected macaques (Figures 3-1C and 3-1D).This indicates that the decline in γδ T cell levels was relatively specific and not a reflection of changes in all T cell subsets.

Increased γδ TCR mRNA expression in the cervical and retropharyngeal lymph nodes during an acute SIV infection

The cervical and retropharyngeal lymph nodes (LNs) drain the oral cavity where the viral inoculum was administered (122). SIV DNA can be detected in one of these LNs at 1 day post-infection, as well as at both sites by 2 days, suggesting that the spread of SIV from the site of inoculation to regional lymphoid tissues occurs rapidly (Table 3-1) (122). Analysis of the cervical and retropharyngeal LNs determined that the Vδ1 γδ TCR mRNA levels increased at both the cervical and retropharyngeal

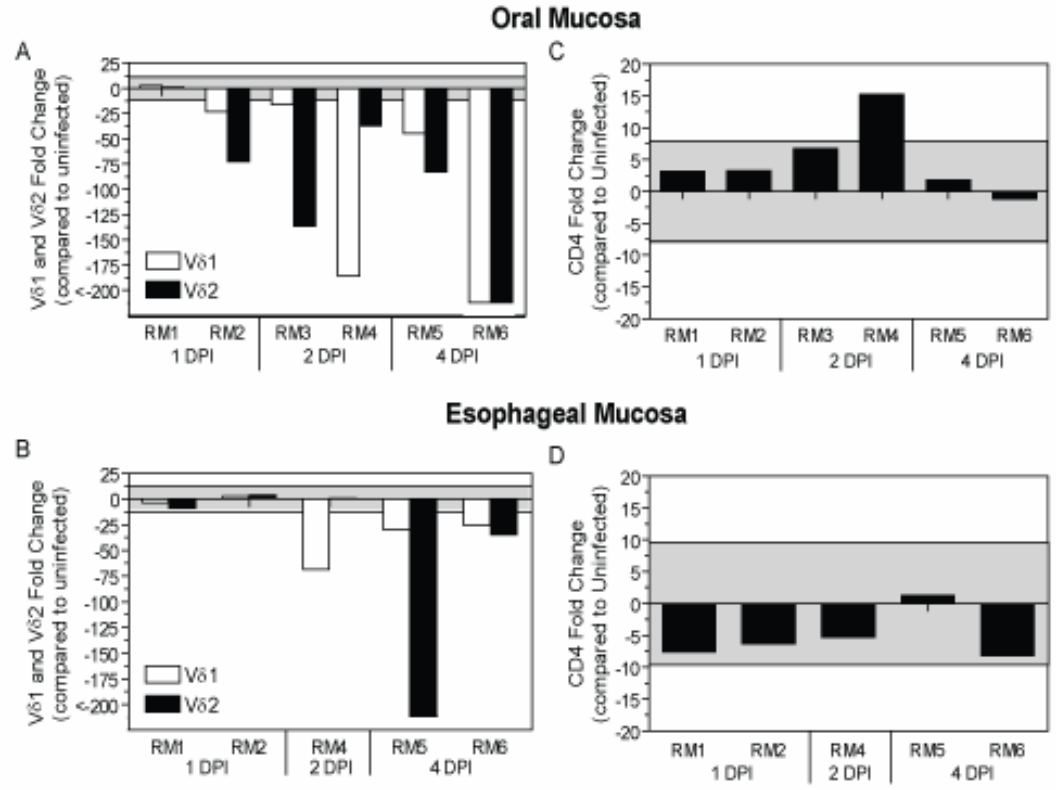

Figure 3-1 – Decreased Vδ1 and Vδ2 γδ TCR expression at the oral and esophageal mucosa during acute SIV infection. The fold change in Vδ1, Vδ2, and CD4 gene expression was assessed in oral (A and C) and esophageal (B and D) mucosal tissues of SIV^{neg} and SIV+ rhesus macaques. Changes in Vδ1 TCR expression are in white bars while changes in Vδ2 TCR expression are in black bars. The mRNA levels shown are reported as fold change with regard to mRNA levels in matched samples from three to four uninfected macaques. The grey shaded area represents a two standard deviation range of the average expression in uninfected macaques. Bars extending beyond the grey shaded area represent samples that are increased or decreased with regard to the uninfected controls. The duplicate Ct values for each sample were generally within 10% of each other.

RM #	Monkey ID	DPI	Mucosa		Lymph Node		
			Oral	Esophageal	Cervical	Retropharyngeal	Axillary
RM1	33098	1	+	-	++	-	-
RM2	33202	1	+	-	-	+	-
RM3	30379	2	++	+	+	+	-
RM4	30381	2	++	++	++	++	++
RM5	29976	4	++	+	+	++	+
RM6	30244	4	++	-	++	+	++
RM7	30974	7	++	++	++	++	++
RM8	30076	14	++	++	++	+	++

Table 3-1 – Detection of SIV DNA in mucosal and lymphoid tissues (122) – All tissues were assessed in carefully controlled nested PCR reactions for SIV gag DNA in 3–10 replicates. DPI – Days Post-Infection; (– Indicates all PCR reactions were negative; (+) indicates that < 50% of PCR reactions were positive; (++) Indicates that >50% of PCR reactions were positive.

LNs 1 day post-infection (Figures 3-2A and 3-2B). At 2 and 4 DPI, Vδ1 TCR expression levels generally continued to remain elevated in both the cervical and retropharyngeal LNs (Figures 3-2A and 3-2B). In contrast, Vδ2 TCR expression levels at 2 and 4 DPI generally remained within the 2 standard deviation range in both the cervical and retropharyngeal LNs (Figures 3-2A and 3-2B). Changes in Vδ1 γδ T cell levels were therefore not a general phenomenon as reflected by the Vδ2 and CD4 mRNA levels remaining generally within the 2 standard deviation range. Assessment of Vδ1 and Vδ2 TCR expression levels in the axillary LNs of these orally-infected macaques was performed to determine if lymphoid tissues distal to the inoculation site showed similar changes in γδ TCR expression levels. Within the first four days post-infection, neither the Vδ1 nor Vδ2 TCR expression levels in the axillary LNs were altered compared to uninfected macaques (Figure 3-2C). The observations here that increased expression of Vδ1 TCR expression occur in the cervical and retropharyngeal lymph nodes as early as 1-2 days post-oral inoculation suggest that Vδ1+ γδ T cells may be responding to the presence of SIV at the mucosa and migrating to regional lymph nodes.

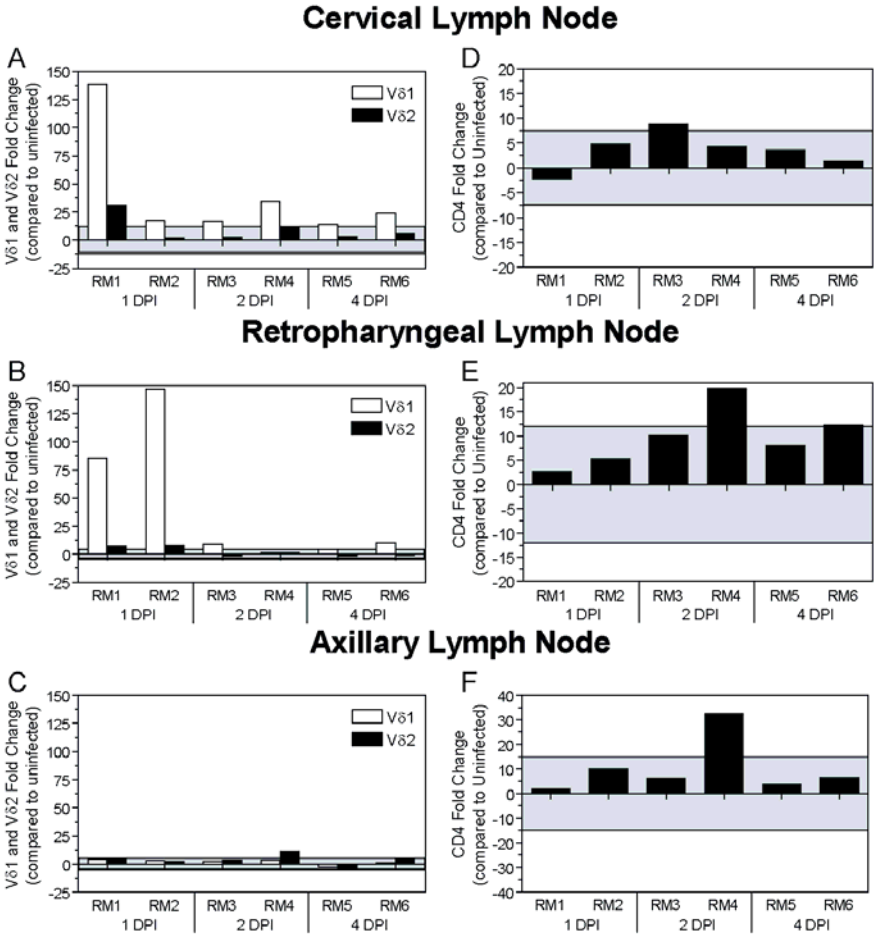

Figure 3-2 – Increased Vδ1 and Vδ2 γδ TCR mRNA expression detected in the cervical and retropharyngeal LNs during acute SIV infection. The fold change in Vδ1, Vδ2, and CD4 gene expression was assessed in the cervical (A and D), retropharyngeal (B and E), and axillary (C and F) lymph nodes of SIV^{neg} and SIV+ rhesus macaques. Changes in Vδ1 TCR expression are in white bars while changes in Vδ2 TCR expression are in black bars. The mRNA levels shown are reported as fold change with regard to mRNA levels in matched samples of four uninfected macaques. The grey shaded area represents a two standard deviation range of the average expression in uninfected macaques. Bars extending beyond the grey shaded area represent samples that are increased or decreased with regard to the uninfected controls. The duplicate Ct values for each sample were generally within 10% of each other.

Decreased γδ T cell levels observed by 7 and 14 days post-SIV infection

By 7 and 14 days post-oral inoculation, we have previously found that SIV DNA can be detected in virtually all mucosal and lymphoid tissues assessed (122). Therefore, I was interested in determining how γδ T cell levels were impacted at this later stage of acute infection. At 7 DPI, Vδ1 and Vδ2 TCR mRNA expression were decreased at the oral mucosa (Figure 3-3A). In addition, Vδ1 and Vδ2 TCR mRNA expression at the regional cervical and retropharyngeal LNs was increased at 7 days post infection (Figure 3-3A). An analysis at 14-days post infection revealed decreased Vδ1 and Vδ2 TCR expression levels at the oral and esophageal mucosal tissues compared to uninfected animals (Figure 3-3B). However, the γδ TCR expression in the cervical and retropharyngeal LNs at this time point was within the two-standard deviation range (Figure 3-3B). CD4 gene expression was also analyzed at these mucosal and lymphoid sites at 7 and 14 days following oral inoculation demonstrating a decreased CD4 gene expression at the esophageal mucosa, as well as the cervical and retropharyngeal LNs by 14 days post-infection (Figure 3-3D). In summary, macaques infected for 7 and 14 days revealed decreased Vδ1 and Vδ2 γδ TCR expression levels at the oral and esophageal mucosa,

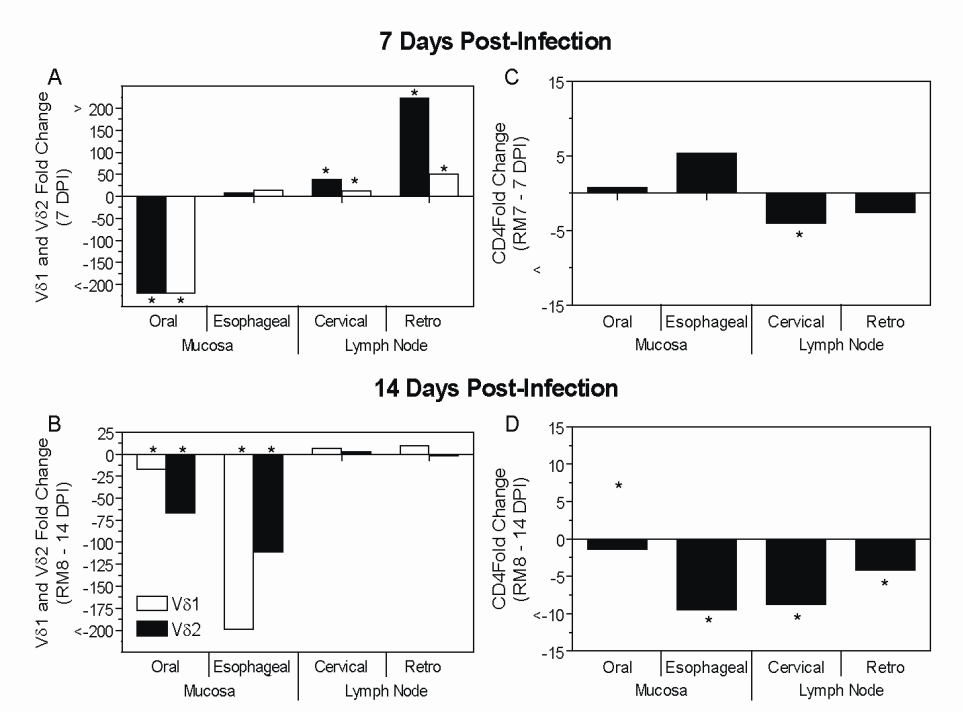

Figure 3-3 – Decreased Vδ1 and Vδ2 γδ TCR expression at the oral and esophageal mucosa following 14 days post-oral SIV infection. The fold change in Vδ1, Vδ2, and CD4 gene expression was assessed in oral and esophageal mucosa as well as the cervical and retropharyngeal lymph nodes at 7 (A and C) and 14 (B and D) days post-infection. Changes in Vδ1 TCR expression are in white while changes in Vδ2 TCR expression are in black. The mRNA levels shown are reported as fold change with regard to mRNA levels in matched samples of four uninfected macaques. The asterisks (*) represent gene expression that was outside of the two standard deviation range determined from uninfected macaques. The duplicate Ct values for each sample were generally within 10% of each other.

while CD4 depletion was observed at both mucosal and lymphoid sites by 14 days post-oral SIV infection.

Increased CCL21 and CCR7 expression at lymphoid sites following an acute SIV infection

Decreased levels of γδ T cells at mucosal sites during an acute SIV infection could be explained by three potential hypotheses: (1) direct viral cytopathicity in which SIV infects and kills γδ T cells (Figure 3-4B), (2) activation-induced cell death (AICD) of γδ T cells (Figure 3-4C), or (3) γδ T cell migration away from the mucosa towards the secondary lymph nodes in response to chemokines (Figure 3-4D). The third hypothesis (Figure 3-4D) suggests a redistribution (or migration) of γδ T cells from the mucosa to the regional lymphoid organs. In support of this hypothesis, I have observed decreased levels of Vδ1 and Vδ2 TCR expression levels in the oral and esophageal mucosa (Figures 3-1A, 3-1B, 3-3A, and 3-3B) and increased levels of particularly Vδ1 TCR expression in draining lymph nodes (Figures 3-2 A, 3-3B, and 3-3C). Therefore, to address this hypothesis, gene expression of the lymph node homing chemokine CCL21/6Ckine, as well as its cognate receptor CCR7, were assessed in the cervical and retropharyngeal LNs of macaques infected for 1, 2, or 4

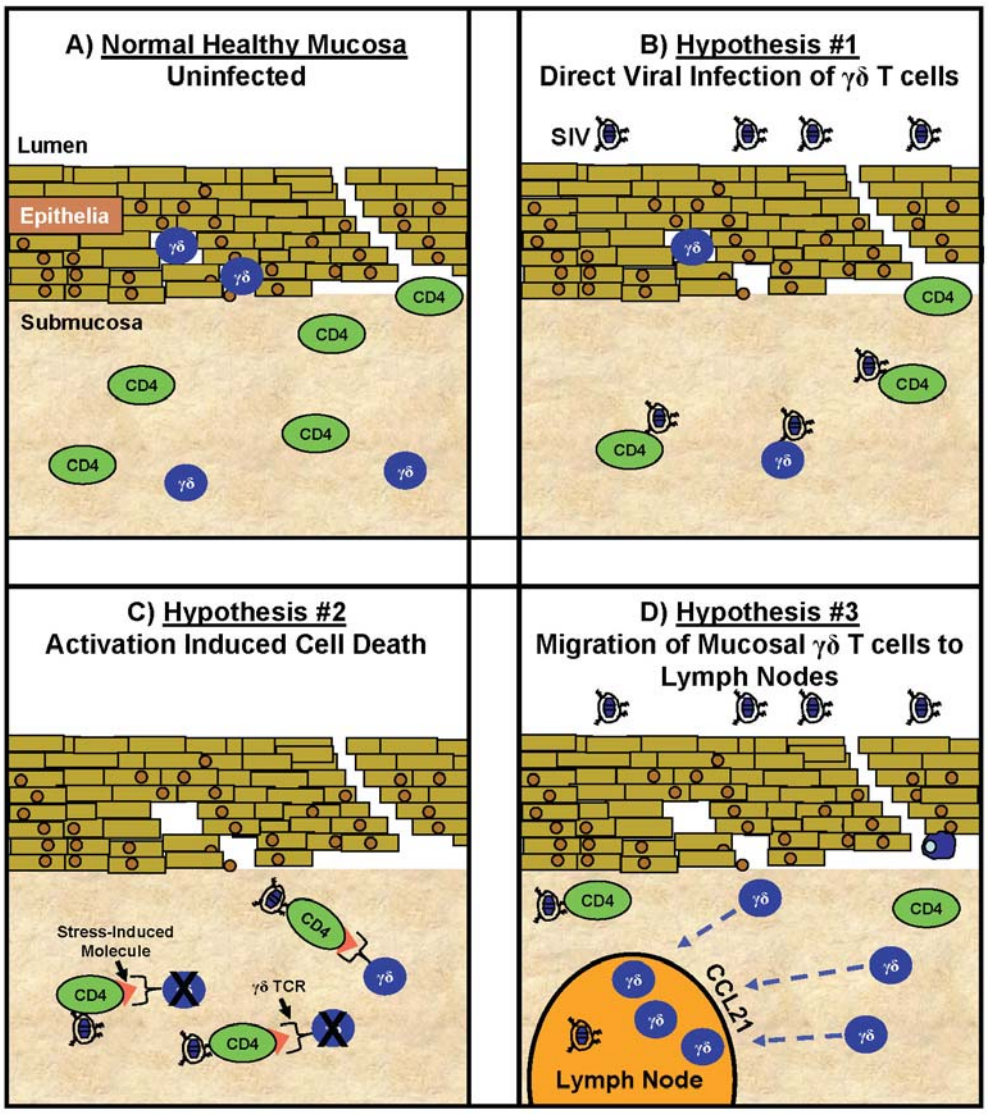

Figure 3-4 – Potential mechanisms for the decreased levels of Vδ1 and Vδ2 **γδ *TCR expression at mucosal sites.*** The results presented here indicate decreased expression of Vδ1 and Vδ2 γδ TCR expression at mucosal sites but increased at secondary lymphoid organs during acute SIV infection. These results led to three potential hypotheses to describe these findings: direct viral cytopathicity in which γδ T cells are infected with SIV and killed (Hypothesis #1), activation induced cell death as indicated by the "X" (Hypothesis #2), or cellular migration in response to CCL21/6Ckine (Hypothesis #3).

days when the increased γδ T cell levels were observed. As in previous experiments, real-time PCR analyses assessed the mRNA levels of the mixed population of cells in this biopsy sample. The 1 day infected macaque, RM1, exhibited increased CCL21/6Ckine mRNA expression in both the retropharyngeal and cervical LNs, while RM2 experienced increased CCL21/6Ckine only in the retropharyngeal LN (Figure 3-5A). Furthermore, at 2 DPI, CCL21/6Ckine expression remained elevated in both the cervical and retropharyngeal LNs, although lower in comparison to 1 DPI (Figure 3-5A). Expression of CCL21/6Ckine mRNA at 4 DPI indicated that the range generally was within the levels observed in uninfected macaques (Figure 3-5A). mRNA expression of the CCL21/6Ckine receptor, CCR7, was also up-regulated in at least one LN from each of the macaques at 1 DPI and a more substantial increase in expression by 2 days in the retropharyngeal LNs (Figure 3-5B), suggesting that immune cells may be migrating towards these lymphoid sites in response to the up-regulation of CCL21/6Ckine. To determine if these results were specific for CCL21/6Ckine and CCR7, the expression of the pro-inflammatory chemokine CCL5/RANTES and its receptor CCR5 were assessed. During the first four days of infection, the levels of CCL5/RANTES and CCR5 were generally within the range observed in uninfected macaques (Figures 3-5C and 3-5D) suggesting that the results

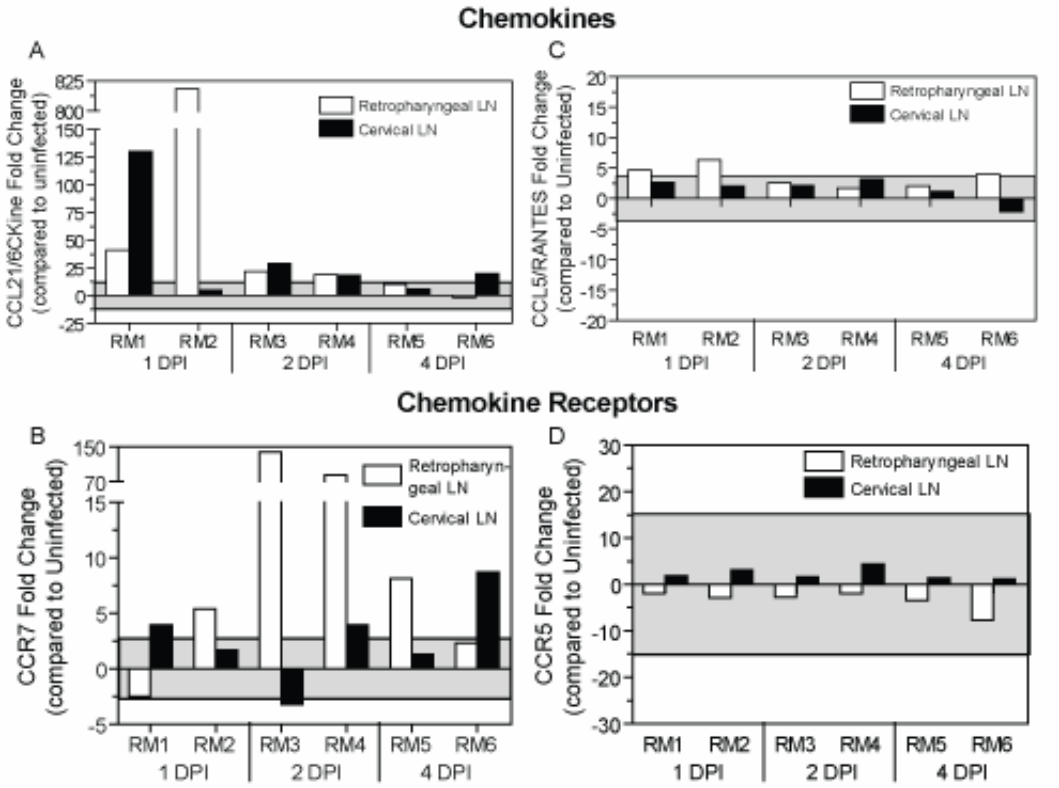

Figure 3-5 – Increased CCL21 and CCR7 mRNA expression at the cervical and retropharyngeal LNs following an acute SIV infection. Quantitative real-time PCR was utilized to assess the mRNA levels of (A) CCL21/6Ckine, (B) CCL5/RANTES, (C) CCR7 and (D) CCR5 in the retropharyngeal (white bars) and cervical (black bars) LNs. The mRNA levels shown are reported as fold change with regard to mRNA levels in matched samples of four uninfected macaques. The grey shaded area represents a two standard deviation range of the average expression from uninfected macaques. Bars extending beyond the grey shaded area represent samples that are increased or decreased with regard to the uninfected controls. The duplicate Ct values for each sample were generally within 10% of each other.

observed for CCL21/6Ckine and CCR7 were not a general phenomenon. Elevated CCL21/6Ckine and CCR7 expression in LNs draining the oral cavity provide evidence to support hypothesis #3, suggesting the loss of mucosal γδ T cells may be the result of migration to the draining LNs in response to an acute SIV infection.

Decreased CCR7 and CD62L expression on γδ T cells following HIV infection

An assessment of HIV-infected patients was also performed in this study to determine if HIV-disease progression would impact LN homing receptor expression on γδ T cells. Peripheral blood was obtained through a collaborative effort with physicians at the UT Southwestern AIDS Clinic. Peripheral blood mononuclear cells from uninfected and HIV+ donors were stained with fluorescent antibodies to determine the expression of CCR7 on γδ T cells utilizing flow cytometry. A representative gating strategy utilized to identify CCR7 expressing γδ T cells from an uninfected patient is shown in Figures 3-6A – 3-6E. An assessment of CCR7 revealed significantly decreased percentages of γδ T cells expressing this receptor in HIV+ patients compared to uninfected donors (Figure 3-6F). By dividing the γδ T cell populations into Vδ2+ and $V\delta2^{neg}$ (Vδ1) subsets, I observed that HIV+ patients had a significantly decreased percentage of

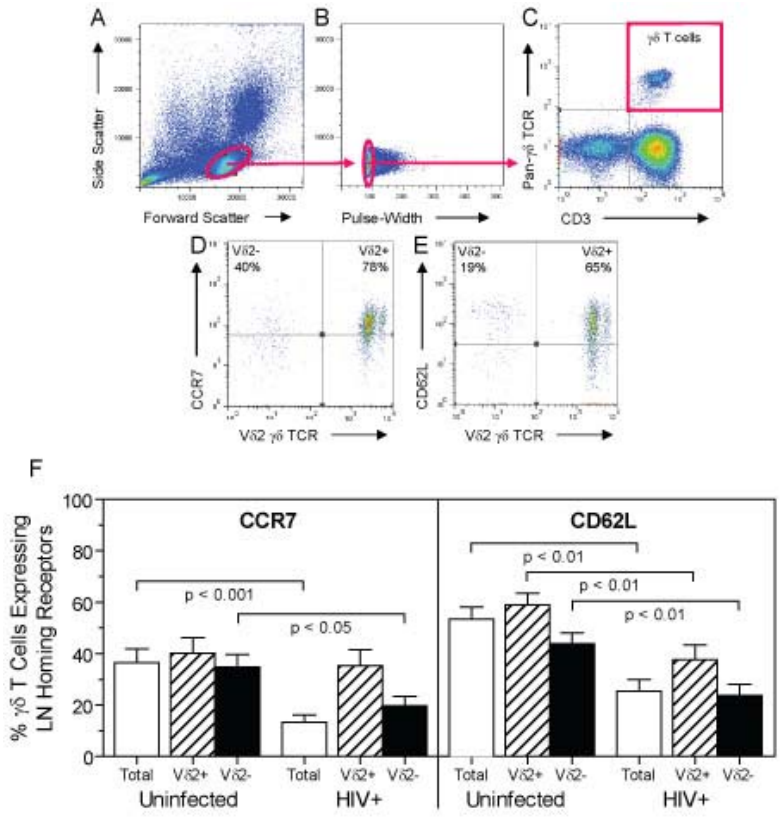

Figure 3-6 – Decreased CCR7 and CD62L expression on γδ T cells from HIV+ patients. PBMCs from uninfected (n=13) and HIV+ (n=14) patients were stained with fluorescently labeled antibodies recognizing the Pan- γδ TCR, Vδ2 γδ TCR, CD3, CCR7, and CD62L and assessed using flow cytometry. A representative gating strategy from an uninfected donor is shown indicating the (A) lymphocytes, (B) pulse-width to remove doublets, (C) total CD3+ γδ T cells, and the expression of (D) CCR7 or (E) CD62L on CD3+ γδ T cells. (F) The percentage of total γδ T cells (white bars), Vδ2+ (striped bars), or $V\delta2^{neg}$ (black bars) cells expressing either CCR7 or CD62L are shown. CCR7 expression (left panel) was significantly decreased on total and $V\delta2^{neg}$ γδ T cells in HIV+ patients compared to uninfected controls. CD62L expression (right panel) was significantly decreased on all three groups of γδ T cells in HIV+ patients compared to uninfected controls. A Mann-Whitney *t* test at a 95% confidence interval was utilized to determine statistical significance between the uninfected and HIV+ groups. Total = Percentage of total γδ T cells; Vδ2+ = Percentage of Vδ2+ γδ T cells; Vδ2- = Percentage of $V\delta2^{neg}$ (Vδ1) γδ T cells

CCR7+ Vδ2^{neg} cells in relation to their uninfected counterparts (Figure 3-6F). Furthermore, to determine if these results were specific for CCR7, another lymphoid homing receptor, CD62L (L-selectin), was assessed in the HIV+ patients. Here again, HIV+ donors showed significantly decreased percentages of γδ T cells that expressed CD62L when compared to uninfected individuals (Figure 3-6F). These results demonstrate that HIV infection reduces the percentages of CCR7+ and CD62L+ γδ T cells in the peripheral blood, which may inhibit the ability of γδ T cells to migrate to secondary lymphoid organs.

Discussion

Disease progression following an HIV infection is generally characterized by the depletion of peripheral CD4+ T cells. However, progression to AIDS is more complex as evidenced by recent reports describing the dramatic loss of CD4+ T cells at rectal and intestinal mucosa as early as two weeks post infection (18, 105, 116, 179). The primary goal of the study presented in this Chapter was to assess the levels of γδ T cells at mucosal and lymphoid sites within the first four days after an SIVmac251 infection. The *Tenner-Racz* report, which identified increased numbers of γδ T cells at the tonsils of vaccinated macaques (171), led to my initial hypothesize that the numbers of γδ T cells would

also be increased following an oral SIV infection at the likely sites of viral entry, the oral and esophageal mucosa. However, decreased Vδ1 and Vδ2 γδ T cell levels were observed at these mucosal sites as early as two days following a primary SIV infection (Figures 3-1A, 3-1B, 3-3A, and 3-3B). The results presented herein were the first to identify the loss of γδ T cells prior to CD4+ T cell depletion at mucosal sites during the first four days after oral SIV inoculation. These data were in contrast to my initial hypothesis. In addition, the loss of oral and esophageal mucosal γδ T cells by 7 days post-oral infection agree with a previous report by *Chen et al* describing a similar loss of γδ T cells in the intestine one week after an intrarectal SIV inoculation (26). In contrast to the loss of mucosal γδ T cells, these findings identified an increase in the levels of γδ T cells at the regional cervical and retropharyngeal LNs (Figure 3-2). These findings suggest a mechanism by which γδ T cells migrate away from mucosal sites towards secondary lymphoid tissues following an acute infection with SIVmac251, possibly to assist in the initiation of an anti-viral immune response and control of rapid viral spread.

The loss of mucosal γδ T cells could be explained by three potential hypotheses (Figure 3-4): (1) direct viral killing of γδ T cells, (2) activation induced cell death (AICD), or (3) migration of γδ T cells from the mucosa to lymphoid sites in response to chemokines. One study reported that

purified γδ T cells from the blood of HIV-1 infected donors have detectable pro-viral DNA and can be infected *in vitro* if they express the appropriate surface receptors (CD4 with either CCR5 or CXCR4) (77). However, this report fails to determine if infected γδ T cells are rapidly killed or even if they express the appropriate receptors necessary for viral infection at mucosal sites. Therefore, it is unlikely that the depletion of γδ T cells within the first four days as observed in my study could be due solely to direct viral cytopathicity (Figure 3-4B). The second hypothesis (Figure 3-4C) suggests that the depletion of γδ T cells may be the result of AICD. γδ T cells have been shown to recognize and destroy virally-infected CD4+ T cells *in vitro* in an attempt to control viral replication (14, 140, 181). This anti-viral function may be mediated through an interaction between the γδ TCR and stress-induced molecules expressed on the surface of the infected cells. This mechanism has been previously described with regard to the ability of γδ T cells to recognize and destroy cytomegalovirus-infected epithelial cells (70). Furthermore, other reports have described that extensive γδ TCR stimulation may lead to AICD *in vitro* (59, 91). However, the number of infected CD4+ T cells at mucosal sites remains relatively low during the earliest times post-infection (105, 120) suggesting that γδ T cells may not be subject to repeated TCR stimulation and thus are unlikely to undergo AICD within the first four days post-infection. The

final hypothesis (Figure 3-4D) suggests that γδ T cells may migrate away from the mucosa and travel to lymphoid sites. Lymphoid homing receptors such as CCR7 and CD62L are important for proper LN migration and are up-regulated by γδ T cells upon TCR stimulation (15, 45). In addition, γδ T cells can migrate in response to the LN homing chemokine CCL21/6Ckine (189) as well as present antigens inducing the proliferation of naïve CD4+ and CD8+ T cells *in vitro* (15). Therefore, the third hypothesis provides an explanation addressing the decreased levels of γδ T cells at mucosal sites with an increase in secondary lymphoid organs suggesting a redistribution of γδ T cells at these immunologic sites during an acute SIV infection. In my study, an up-regulation of the homeostatic lymphoid chemokine CCL21/6Ckine was observed by 1 DPI in the cervical and retropharyngeal LNs of SIV-inoculated macaques (Figure 3-4A). Though CCL21/6Ckine is constitutively expressed by stromal cells in LNs, higher mRNA levels detected in this study may be the result of the recognition of viral antigens in the secondary lymphoid organs (114, 136). These results were supported by the finding that CCR7 expression was also elevated at these same lymphoid sites at 2 days post infection, (Figure 3-4B) suggesting that γδ T cells and other immune cells may have migrated to these sites soon after the up-regulation of CCL21/6Ckine. In addition, both CCL5/RANTES and CCR5 levels in general were not

altered following the infection further indicating that migration may be a response to lymphoid homing as opposed to pro-inflammatory chemokines. Though this lymphoid homing may seem rapid, other reports have demonstrated that dendritic cells have the ability to migrate quickly toward secondary LNs from the vaginal mucosa within 24-hours after an inoculation with SIV (121, 153). Taken together, the up-regulation of CCL21/6Ckine early after an SIV infection further supports the mechanisms proposed in Hypothesis #3 (Figure 3-4D) whereby γδ T cells (and probably other immune cells) migrate to secondary LNs.

An assessment of the impact of HIV disease progression on the expression of LN homing receptors on γδ T cells was also undertaken as a means of comparing the SIV and HIV infections Our access to HIV+ patients through the UT Southwestern AIDS Clinic provided an opportunity to directly assess the impact of HIV disease on CCR7 expression by γδ T cells from patient blood. This study identified significantly reduced percentages of γδ T cells expressing the lymph node homing receptors CCR7 and CD62L in peripheral blood from HIV+ patients (Figure 3-6). Due to this finding, I speculate that γδ T cells from HIV+ patients may have a reduced ability to migrate toward secondary LNs, further contributing to overall immune dysfunction. This speculation is supported by a report describing the decreased mRNA expression of both CCL21/6Ckine and

CCR7 in the LNs of SIV+ macaques which have progressed to simian AIDS (28). The authors suggested that the inability of immune cells to migrate to these secondary lymphoid organs and mount an adaptive immune response was associated with progression to simian AIDS (28). The ability of γδ T cell clones from HIV+ patients to migrate across an epithelial cell layer was also decreased in response to pro-inflammatory chemokines including CXCL9/MIG and CXCL10/IP-10 (142). These results suggest that decreased levels of CCR7+ γδ T cells may represent immune dysfunction following HIV infection, which could impact the number of cells that could migrate toward lymphoid sites and participate in an anti-viral adaptive immune response.

The results in this Chapter reveal the loss of γδ T cells at mucosal sites within the first four days after a pathogenic SIV infection prior to the depletion of CD4+ T cells. This Chapter also provides a mechanism proposing the migration of γδ T cells from the mucosa towards secondary lymphoid organs in response to CCL21/6Ckine, which may be an attempt to initiate an anti-viral immune response. I speculate that this anti-viral response may be the result of γδ T cells taking up and processing viral antigens at the mucosal surface and then migrating towards the regional LNs to present these antigens to naïve CD4+ and CD8+ T cells. In addition, the γδ T cells may home to these regional lymphoid sites in an

attempt to control the rapid spread of the virus to multiple tissues, possibly through the expression of β-chemokines to interfere with viral co-receptor binding to target cells (103). However, this anti-viral response from γδ T cell is not always successful as the rapid viral spread to multiple tissues makes it difficult to effectively prevent the infection (122, 170). If however γδ T cells were primed at mucosal surfaces with attenuated viruses (171) or purified viral proteins (103), then these cells could potentially have a more protective role in preventing a secondary challenge HIV/SIV infection at mucosal surfaces. Therefore, the levels and migratory capacity of γδ T cells at mucosal sites should be considered during the development of novel mucosal vaccination strategies to prevent HIV infections.

CHAPTER 4 – Gamma/Delta T Cell Functional Responses Differ Following Pathogenic HIV and Non-Pathogenic SIV Infections

Introduction

Although HIV/SIV infections are generally associated with progression to AIDS, natural host primate species in Africa can be infected with SIV, but remain free of any clinical disease signs (68, 93, 149, 166). Sooty mangabeys (*Cercocebus atys*) are of particular interest as cross-species transmission of SIVsmm from mangabeys to humans appears to have initiated the HIV-2 epidemic in West Africa (68, 76). SIV replicates to high levels in mangabeys (149) suggesting that protection from AIDS is not due to inherent anti-viral mechanisms of a more robust adaptive immune response including the development of SIV-specific cytotoxic T cells (85) or SIV specific antibodies (57). Studies to date indicate that the ability of mangabeys to prevent progression to AIDS is due in part to the reduced levels of immune activation and apoptosis when compared to pathogenic infections (25, 123, 166). The mechanism by which mangabeys are able to maintain high levels of viral replication and relatively low levels of immune activation remains unknown. Our laboratory recently identified a cohort of SIV+ mangabeys that experienced a dramatic and sustained decline in their CD4+ T cell counts to levels indicative of AIDS in HIV+ patients (123). This indicated that low CD4+ T cell levels were not sufficient to induce disease in these natural hosts of SIV (123). In our assessment of

additional immune cells, we observed that γδ T cells from both SIV+ CD4-healthy and CD4-low mangabeys maintained their ability to proliferate in response to the bacterial antigens isopentenyl pyrophosphate (IPP) and lipopolysaccharide (LPS) (123). These reports suggest that γδ T cells and other immune cells may remain functional following chronic SIV infection. In humans, γδ T cells have critical roles in the recognition of microbial pathogens and can influence adaptive immune responses by the production of both Th1 and Th2 cytokines (49). γδ T cells can be influenced by HIV infection as evident by a phenotypic switch from predominately Vδ2 to Vδ1 γδ T cells within the peripheral blood of HIV+ patients (7, 38). In addition, a decrease in the number of effector (CD27-CD45RA-) γδ T cells were observed in immunocompromised patients (62) and SIV infected rhesus macaques (196), which may be due to the induction of anergy in these cells of infected hosts (141). Pathogenic HIV infection also reduces the ability of γδ T cells to migrate in response to pro-inflammatory chemokines including CXCL10/IP-10 and CXCL9/MIG (142) as well as their cytotoxic capacity (181). Taken together, these data suggest that γδ T cells lose the ability to respond to invading opportunistic pathogens during chronic HIV/SIV infection, likely contributing to the generalized immune dysfunction associated with the progression to AIDS.

This Chapter presents a phenotypic and functional comparison of γδ T cells during pathogenic HIV infection of humans and non-pathogenic SIV infection of mangabeys. Our initial findings from a cross-sectional analysis of uninfected mangabeys and humans revealed that mangabeys had significantly

elevated levels of γδ T cells in their peripheral circulation when compared to humans. Having shown increased levels of γδ T cells, we then addressed the question of whether these cells were functional during HIV/SIV infections. Evidence for preserved or increased functionality of γδ T cells from SIV+ mangabeys (regardless of CD4+ T cell levels) was demonstrated by maintained percentages of γδ T cells that expressed Th1 cytokines following *ex vivo* stimulation. These data suggest that in the absence of aberrant immune activation, controlled Th1 responses by γδ T cells from mangabeys may assist in suppressing damage due to the SIV infection as well as inhibiting the onset of opportunistic infections.

Results

Comparative phenotypic assessment of peripheral γδ T cell levels in humans, macaques and mangabeys

Rhesus macaques and sooty mangabeys are non-human primates used to study pathogenic and non-pathogenic models of HIV infection respectively (122, 166, 171, 196). Peripheral blood mononuclear cells (PBMCs) from these monkeys, as well as humans, were assessed to identify changes within the gamma/delta (γδ) T cells following SIV/HIV infection. A representative gating strategy to assess the levels of γδ T

cells in peripheral blood is depicted (Figures 4-1A – 4-1C). The frequency of γδ T cells is presented as the percentage of CD3+ T cells expressing the γδ T cell receptor within the different species and with regard to infection status (Figures 4-1D – 4-1E). A significantly higher frequency of γδ T cells was observed in uninfected mangabeys (12.5%) compared to macaques (6.1%) and humans (3.8%) (Figure 4-1D). A cross sectional analysis of HIV+ patients enrolled at the University of Texas Southwestern Medical Center AIDS Clinic (Table 4-1), was performed to examine the relationship between peripheral blood γδ T cell frequencies and disease progression. Patients were divided into three groups as follows: (1) HIV+ CD4-healthy (>200 CD4+ T cells/μl blood), (2) HIV+ CD4-low (<200 CD4+ T cell counts/μl blood), and (3) HIV+ on HAART (350 – 2100 CD4+ T cell counts/μl blood). An analysis of γδ T cells in these groups indicated that both CD4-healthy and CD4-low HIV+ patients had significantly elevated frequencies of γδ T cells compared to uninfected donors (Figure 4-1E). HAART treatment resulted in γδ T cell frequencies that were intermediate between HIV+ and uninfected donors (Figure 4-1E). In addition, an analysis of peripheral γδ T cell frequencies was also undertaken in both CD4-healthy and CD4-low SIV+ mangabeys (Table 4-2). In contrast to HIV infected patients, the frequency of γδ T cells significantly decreased in the CD4-healthy SIV+ mangabeys in comparison to their uninfected

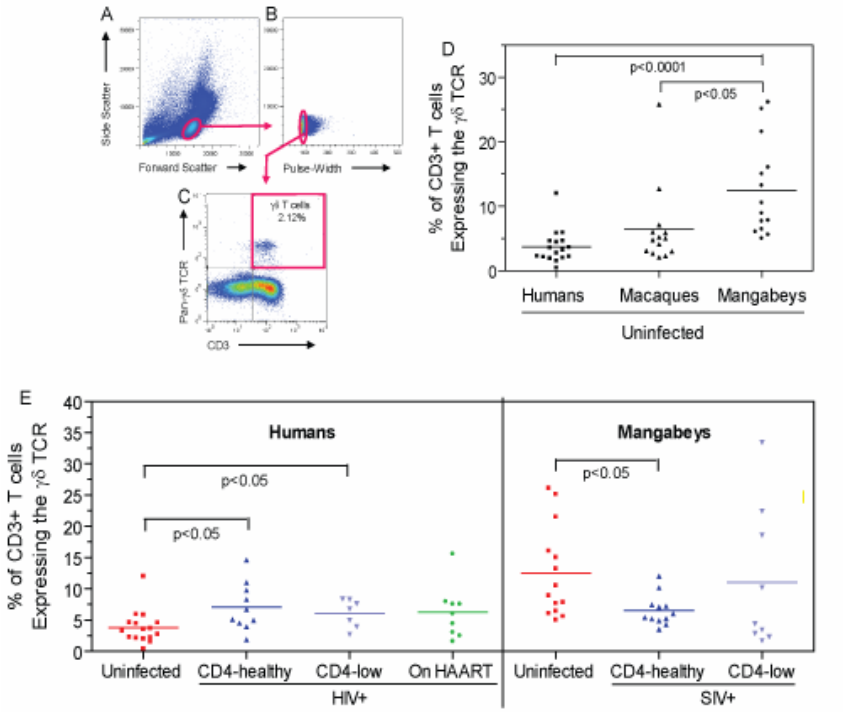

Figure 4-1 – Mangabeys have significantly higher percentages of γδ T cells in their peripheral blood circulation compared to macaques and humans. Peripheral blood mononuclear cells (PBMCs) from humans and mangabeys were stained with fluorescently labeled cross-reactive antibodies against the Pan-γδ TCR and CD3 and assessed using flow cytometric analysis. A representative gating strategy from an uninfected donor is shown indicating the (A) forward and side scatter to gate on lymphocytes, (B) pulse-width to gate on single cells, and (C) CD3 and Pan-γδ TCR to gate on γδ T cells. (D) Graphical representation of the percentage of CD3+ T cells expressing the Pan-γδ TCR from uninfected humans (■), macaques (▲), and mangabeys (●). The percentage of γδ T cells in uninfected mangabeys was significantly higher when compared to both humans and macaques. (E) Assessment of the levels of γδ T cells was performed in the CD4-healthy (dark blue ▲) and CD4-low (light blue ▼) HIV+ patients and SIV+ mangabeys, as well as HIV+ patients on HAART (green ●) and compared to uninfected donors (red ■). There were increased percentages of γδ T cells in the peripheral circulation of HIV+ patients when compared to uninfected donors. The SIV+ CD4-healthy mangabeys had decreased percentages of peripheral blood γδ T cells when compared to their uninfected counterparts, whereas on average, the SIV+ CD4-low mangabeys demonstrated similar levels as uninfected mangabeys.

Table 1 - HIV+ Patients										
Groups	#	Sex	Age	WBC	Lymphocyte	CD4+	γδ	Vδ2+	Vδ2neg	Viral Load
				# cells (x10^3) / μl blood		# cells / μl blood				vRNA / ml plasma
HIV+ CD4-Healthy Patients	1	F	50	3.94	1.84	592	93	22	71	9,400
	2	M	30	8.69	1.53	440	71	11	60	901
	3	M	35	6.36	2.05	387	38	8	30	2,190
	4	M	45	4.22	1.95	409	190	9	181	136,000
	5	M	42	9.80	1.59	681	175	12	163	>750,000
	6	M	36	4.09	1.29	221	64	14	50	>750,000
	7	M	24	4.60	1.58	210	106	17	89	231,000
	8	M	39	6.10	2.02	481	167	2	165	236,000
	9	M	49	5.77	3.39	485	119	2	117	302,000
	10	M	50	3.63	1.06	237	45	39	6	442,000
HIV+ CD4-Low Patients	11	M	47	4.48	0.62	2	31	5	26	>750,000
	12	M	40	3.59	2.07	12	173	20	153	347,000
	13	F	27	6.66	0.91	18	25	1	24	>750,000
	14	M	37	3.62	0.71	45	54	1	53	>750,000
	15	M	53	4.30	0.70	76	45	5	40	318,000
	16	F	31	4.50	0.71	11	30	2	28	590,000
	17	M	41	2.72	1.01	68	25	2	23	130,000
HIV+ Patients on HAART	18	M	47	5.30	1.24	387	55	1	54	<400
	19	F	31	6.22	4.57	2088	345	95	250	<400
	20	M	39	5.33	2.22	593	39	8	31	<400
	21	M	49	6.13	2.21	482	133	33	100	<400
	22	F	48	4.12	2.01	1023	19	6	13	<400
	23	M	36	5.03	2.75	888	44	32	12	<400
	24	M	45	5.06	1.92	361	237	2	235	<400
	25	F	34	4.70	1.93	409	108	12	96	<400
	26	M	45	3.97	1.31	544	30	23	7	<400

Table 4-1 – White blood cell counts and viral loads in HIV+ humans. Complete blood counts and viral loads are shown for each HIV+ patient enrolled in this study. Each HIV+ patient was placed into one of three groups: (1) HIV+ CD4-healthy (>200 CD4+ T cells / μl blood), (2) HIV+ CD4-low (<200 CD4+ T cells / μl blood), and (3) HIV+ on HAART (highly active anti-retroviral therapy). WBC – White blood cell; vRNA – Viral RNA; M – male; F – female

Table 2 - Sooty Mangabeys						
Groups	#	ID	Sex	WBC	Lymphocyte	γδ T Cell
				# cells ($x10^6$) / µl blood		# cells / µl blood
Uninfected Sooty Mangabeys	1	FFu	F	6.30	4.03	164
	2	FFZ	M	6.50	3.64	329
	3	FYy	M	7.90	3.48	515
	4	FZy	M	9.60	3.26	425
	5	FGv	F	12.00	5.28	485
	6	FJz	F	11.20	4.14	181
	7	FOv	F	7.20	4.10	165
	8	FSu	F	14.20	4.40	530
SIV+ CD4-healthy Sooty Mangabeys	9	FWw	F	11.70	5.62	135
	10	FAq	M	6.90	4.21	306
	11	FKr	F	12.10	4.36	175
	12	FOq	F	8.90	3.56	345
	13	FVw	F	11.60	4.87	155
	14	FTq	M	4.80	3.60	152
	15	FWr	M	8.40	5.88	338
SIV+ CD4- Low Sooty Mangabeys	16	FBr	F	5.60	2.46	107
	17	Fzo	M	8.50	6.89	113
	18	FFr	F	6.30	2.71	476
	19	FAn	F	12.30	9.23	194
	20	FJy	M	4.70	2.40	305
	21	FPv	M	7.20	3.86	707
	22	FTv	M	8.20	3.12	108

***Table 4-2* – White blood cell counts in SIV^{neg} and SIV+ mangabeys.** Complete blood counts were performed on each SIV+ mangabeys enrolled in this study. Each SIV+ mangabeys was placed into one of three groups: (1) Uninfected, (2) SIV+ CD4-healthy (>200 CD4+ T cells / µl blood), (3) SIV+ CD4-low (<200 CD4+ T cells / µl blood). ID – Monkey Identification; WBC – White blood cell; M – male; F – female

counterparts (Figure 4-1E). Some of the SIV+ CD4-low mangabeys exhibited very low γδ T cell frequencies, although others exhibited frequencies comparable to the uninfected mangabeys (Figure 4-1E).

Assessment of Vδ1 and Vδ2 γδ T cell levels in humans and mangabeys

The ability of the γδ TCR to recognize antigens is determined predominantly by the particular delta variable region expressed (21, 65, 97). γδ T cells in the peripheral blood primarily express the Vδ2 γδ TCR and, to a lesser extent, the Vδ1 γδ TCR. Indeed, in this study, γδ T cells from uninfected individuals demonstrated a predominance of Vδ2 + cells in the peripheral circulation as indicated by a negative $V\delta2^{neg}$/Vδ2+ ratio (Figures 4-2A and 4-2B). However, the HIV+ patient cohort had a predominately $V\delta2^{neg}$ (Vδ1) γδ T cell population in the peripheral blood (Figure 4-2B) in agreement with previous studies (7, 38, 142). In order to assess the γδ TCR usage in mangabeys, a quantitative real-time PCR approach was utilized due to the lack of a cross-reacting anti-human Vδ2 antibody. Prior to infection, mangabey peripheral blood γδ T cells predominantly expressed the Vδ1, rather than Vδ2, γδ TCR (Figure 4-2C). Following SIV infection, the Vδ1 TCR continued to be the predominate receptor expressed at 12 weeks and 100 weeks post-infection (Figure 4-

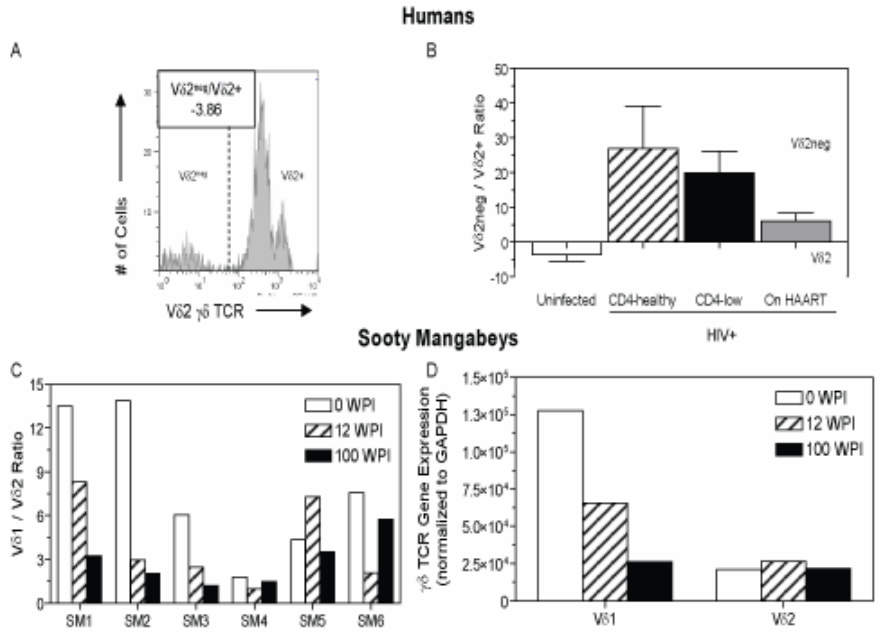

Figure 4-2 – γδ T cells from HIV+ Patients and SIV+ Mangabeys predominately express the Vδ1 γδ TCR. PBMCs from humans were stained with fluorescently labeled cross-reactive antibodies recognizing the Pan-γδ TCR, Vδ2 γδ TCR, and CD3 and the ratio of $V\delta2^{neg}$ to Vδ2+ γδ T cells is reported. (A) A representative histogram shows the $V\delta2^{neg}$/Vδ2+ ratio from an uninfected donor. The ratio is determined by dividing the percentage of $V\delta2^{neg}$ by the Vδ2+ cells. (B) Graphical representation of the $V\delta2^{neg}$/Vδ2+ γδ T cell ratio from uninfected (white bars), HIV+ CD4-healthy (striped bars), HIV+ CD4-low (black bars), and HIV+ on HAART (gray bars) patients. HIV+ patients had an increased $V\delta2^{neg}$/Vδ2+ ratio when compared to their uninfected counterparts. (B) To assess γδ T cell ratios in sooty mangabeys a real-time PCR approach was utilized to quantify the levels of Vδ1 and Vδ2 mRNA. An assessment of the Vδ1/Vδ2 ratio was determined in mangabeys prior to (white bars) as well as 12 weeks (striped bars) and 100 weeks (black bars) post-infection. The Vδ1 TCR was predominately expressed by γδ T cells both prior to and after SIV infection in mangabey PBMCs. (C) Determination of the levels of Vδ1 and Vδ2 in 1x10^6 equivalents of GAPDH mRNA is depicted for the representative mangabeys SM3. These data indicated that the decrease in the Vδ1/Vδ2 ratio as observed following an SIV infection is due to a decrease in Vδ1 γδ TCR expression levels, not an increase in the Vδ2 expression levels.

2C). The declining Vδ1/Vδ2 ratio observed throughout infection was the result of a decrease in the absolute number of Vδ1 TCR transcripts rather than an increase in Vδ2 transcripts (Figure 4-2D). In summary, the Vδ2 to Vδ1 phenotypic switch observed in the peripheral blood γδ T cells of HIV+ patients did not occur in the SIV+ mangabeys as they maintained elevated levels of Vδ1 γδ T cells both before and following SIV infection.

γδ T cells are present at the rectal mucosa during chronic SIV infection of mangabeys

Many opportunistic infections associated with AIDS disease progression including *Mycobacteria* and others occur at mucosal sites. γδ T cells have been documented at mucosal sites where they likely participate in the defense against opportunistic pathogens (21). Currently, there are no previous studies examining the levels of γδ T cells at mucosal sites in SIV-infected mangabeys. Assessment of γδ T cells at two mucosal (the rectal and pulmonary mucosa) and lymphoid sites was undertaken in both CD4-healthy and CD4-low SIV+ mangabeys. A representative flow cytometric gating strategy of the cells obtained from the rectal mucosa biopsies from an SIV+ mangabey is depicted in Figures 4-3A and 4-3B. The percentage of CD3+ T cells expressing the γδ TCR in the rectal biopsies from the SIV+ CD4-low and CD4-healthy mangabeys ranged

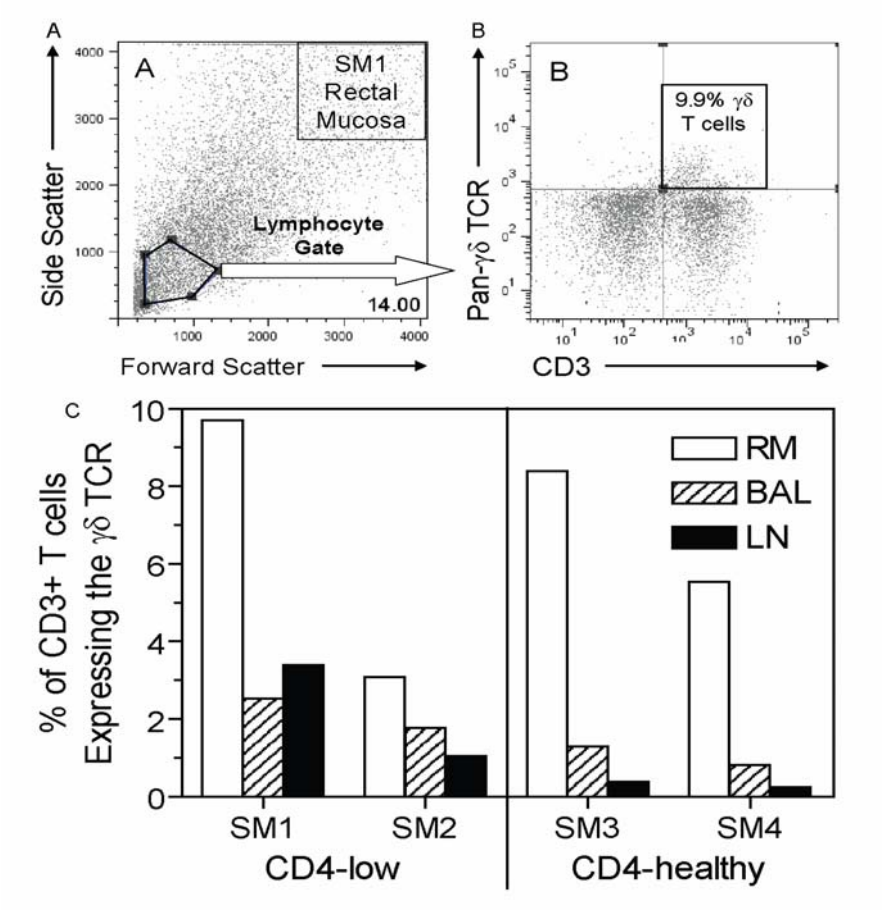

Figure 4-3 – γδ T cells at the rectal mucosa of SIV+ CD4-low mangabeys. Cells were obtained from lymph node (LN). rectal mucosal (RM) biopsies and bronchoalveolar lavages (BALs) and analyzed for the expression of CD3 and the Pan-γδ TCR. Flow cytometric analysis depicting (A) forward and side scatter and (B) CD3 and Pan-γδ TCR expression are represented following RM biopsy of SM1 (SIV+ CD4-healthy). (D) Graphical representation of the percentage of CD3+ T cells expressing the Pan-γδ TCR from the RM (open bar), BAL (striped), and LN (shaded). The RM biopsies exhibited the highest percentage of γδ T cells within the CD3+ T cell population, in comparison to the BALs and LN in all the mangabeys.

from approximately 3.0 – 9.8% with no discernable trend associated with CD4 depletion (Figure 4-3C). In contrast, cells recovered from bronchoalveolar lavage (BAL) demonstrated a trend toward increased percentages of γδ T cells in the pulmonary mucosa from the SIV+ CD4-low, as compared to the CD4-healthy, mangabeys (Figure 4-3C). Similar to the BAL, increased percentages of γδ T cells in the LN of the two SIV+ CD4-low mangabeys was observed, compared to the CD4-healthy mangabeys (Figure 4-3C). Taken together, these data indicate that γδ T cells are present at mucosal and lymphoid sites during chronic SIV infections of mangabeys irrespective of CD4+ T cell levels, thus possibly serving a role in protection against mucosal opportunistic pathogens.

Th1 cytokine responses are maintained or increased by γδ T cells from SIV+ mangabeys but not HIV+ patients

γδ T cells demonstrate a variety of functions which include the production of cytokines to augment the adaptive immune response at the sites of infection or tumors (49, 109, 124). Here, the cytokine expression of γδ T cells was assessed utilizing cytokine flow cytometry on peripheral blood γδ T cells from both HIV+ patients and SIV+ mangabeys. To induce cytokine production *ex vivo* the peripheral blood cells were incubated with media alone (M; negative control), PMA/Ionomycin (PI; global stimulation),

the γδ T cell specific bacterial ligand isopentenyl pyrophosphate (IPP; specific for Vδ2 γδ TCR) or lipopolysaccharide (LPS; TLR-4 ligand). To determine the impact of non-pathogenic SIV infection and CD4+ T cell levels, the percentage of γδ T cells expressing IFN-γ (Figure 4-4 and 4-5) or TNF-α (Figure 4-4 and 4-6) from both CD4-healthy or CD4-low SIV+ mangabeys was compared to uninfected mangabeys. In general, neither media alone nor LPS induced Th1 cytokine secretion by peripheral γδ T cells from either uninfected or SIV+ mangabeys (Figures 4-4). However, γδ T cells from both CD4-healthy and CD4-low SIV+ mangabeys stimulated with PI or IPP had a maintained or slightly increased ability to express IFN-γ (Figure 4-4C) and TNF-α (Figure 4-4D). Unlike SIV+ mangabeys, an assessment of γδ T cells from HIV+ patients revealed significantly decreased percentages of γδ T cells expressing these Th1 cytokines (IFN-γ and TNF-α) following stimulation with PI and IPP (Figures 4-5B and 4-6B). γδ T cells from HIV+ patients on HAART had a partially restored ability to express these Th1 cytokines following stimulation, though not to the extent that were produced by γδ T cells from uninfected patients (Figure 4-5B and 4-6B). The availability of an anti-human Vδ2 antibody permitted us to more specifically assess the cytokine responses of Vδ2+ and $V\delta2^{neg}$ (Vδ1) γδ T cells in HIV+ patients. When exposed to PI, the majority of Vδ2+ γδ T cells from uninfected human donors expressed

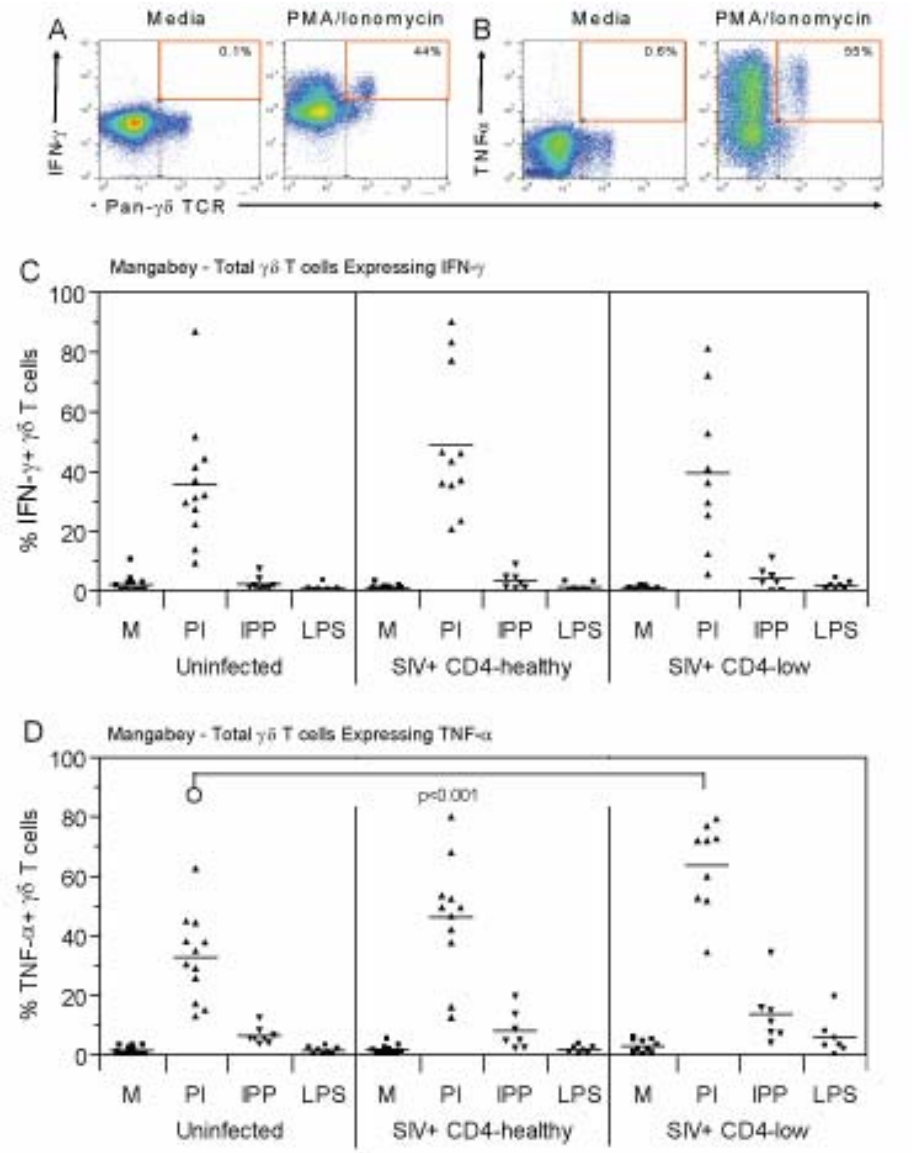

Figure 4-4 – Increased Th1 cytokine expression by γδ T cells from SIV+ mangabeys with low levels of CD4+ T cells. PBMCs from uninfected and HIV+ patients were stimulated *ex vivo* with media alone (■), PI (▲), IPP (▼), or LPS (●) and the expression of (A) IFN-γ and (B) TNF-α by γδ T cells was assessed using flow cytometry. There was an increase in the percentage of γδ T cells expressing TNF-α in response to global activation with PI as well as the Vδ2 γδ TCR specific ligand IPP. With regards to IFN-γ, increased percentages of γδ T cells from SIV+ mangabeys expressed this cytokine following *ex vivo* stimulation with PI.

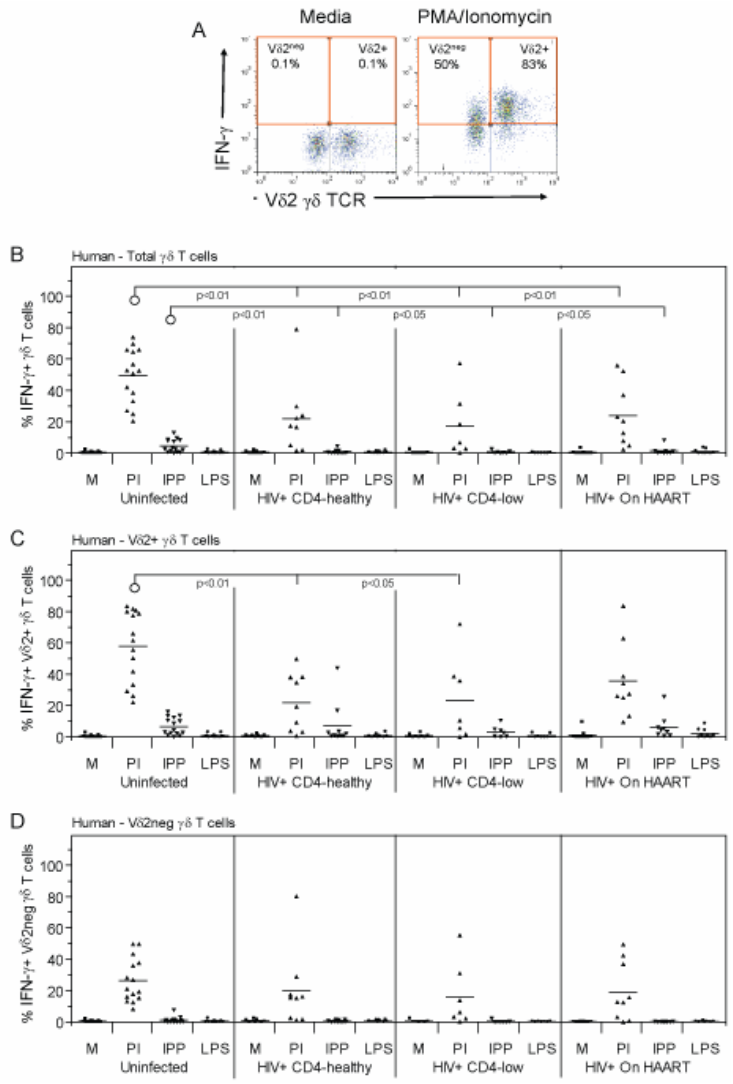

Figure 4-5 – Impaired IFN-γ expression by γδ T cells from HIV+ patients with low levels of CD4+ T cells. (A) Representative gating strategy for γδ T cells expressing IFN-γ following media and PI stimulation. PBMCs from uninfected and HIV+ patients were stimulated *ex vivo* with media alone (■), PI (▲), IPP (▼), or LPS (●) and the expression of IFN-γ by (A) total (C) Vδ2+ and (C) $V\delta2^{neg}$ γδ T cells was assessed using flow cytometry. With regards to PI stimulation, decreased percentages of both Vδ2+ and $V\delta2^{neg}$ γδ T cells from HIV+ patients expressed IFN-γ when compared to uninfected donors. In addition, fewer Vδ2+ γδ T cells in both the CD4-healthy and CD4-low HIV+ groups expressed IFN-γ following IPP stimulation.

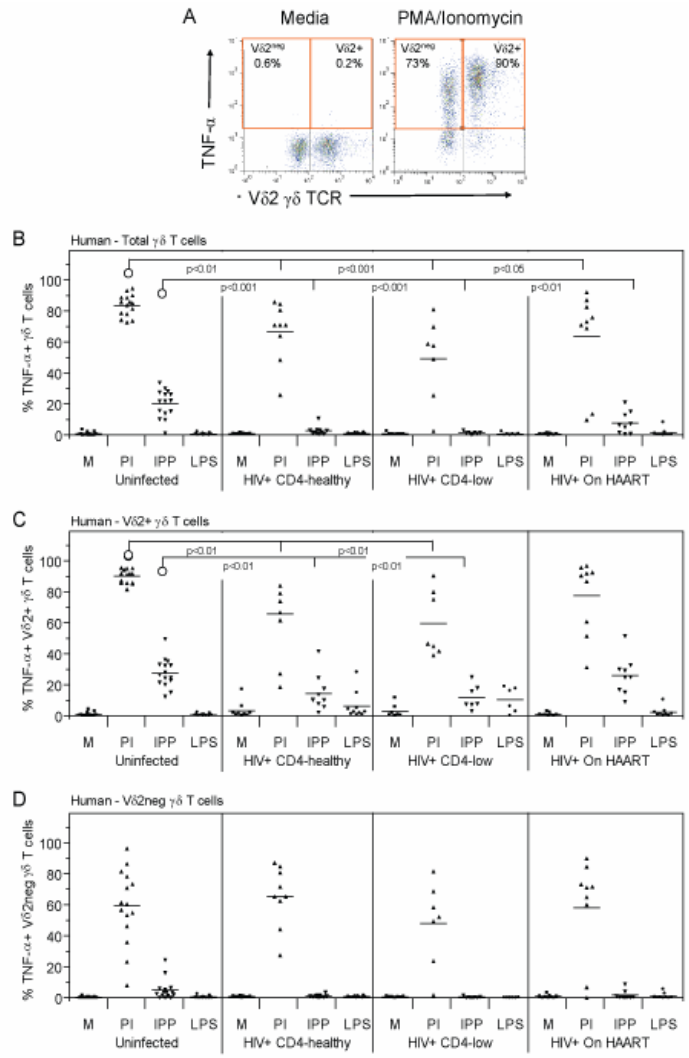

Figure 4-6 – Impaired TNF-α expression by γδ T cells from HIV+ patients with low levels of CD4+ T cells. (A) Representative gating strategy for γδ T cells expressing TNF-α following media and PI stimulation.PBMCs from uninfected and HIV+ patients were stimulated *ex vivo* with media alone (■), PI (▲), IPP (▼), or LPS (●) and the expression of TNF-α by (A) total (B) Vδ2+ and (C) $V\delta2^{neg}$ γδ T cells was assessed using flow cytometry. Decreased percentages of Vδ2+ γδ T cells from HIV+ CD4-healthy and CD4-low patients expressed TNF-α when compared to uninfected donors. In addition, fewer Vδ2+ γδ T cells in both the CD4-healthy and CD4-low HIV+ groups expressed IFN-γ following IPP stimulation.

IFN-γ or TNF-α (Figures 4-5C and 4-6C). However, the percentages of Vδ2+ γδ T cells expressing these Th1 cytokines following PI stimulation significantly declined in both the CD4-healthy and CD4-low HIV+ patients (Figures 4-5C and 4-6C). Similarly, the specific Vδ2 TCR agonist IPP stimulated fewer γδ T cells to produce Th1 cytokines in the HIV+ compared to uninfected patients (Figures 4-5C and 4-6C). HAART treatment partially restored the ability of Vδ2+ γδ T cells to express Th1 cytokines following PI and IPP treatment although not to the extent observed in uninfected patients (Figures 4-5C and 4-6C). $V\delta2^{neg}$ cells were responsive to PI stimulation, but did not produce Th1 cytokines when stimulated with IPP or LPS (Figures 4-5D and 4-6D). Therefore, the majority of the decline in responsiveness in the HIV+ patients could be attributed to decreased cytokine production by the Vδ2+ γδ T cells.

Although the stimulations of the γδ T cells tended to produce both IFN-γ and TNF-α in most situations, the levels of the production of the two cytokines did vary. Indeed, a higher percentage of γδ T cells expressed TNF-α, compared to IFN-γ, after PI and IPP stimulation in the majority of conditions assessed. For example, when Vδ2+ γδ T cells from uninfected donors were stimulated with PI, nearly 90% of the cells expressed TNF-α, compared to 60% expressing IFN-γ (Figures 4-5C and 4-6C). Moreover,

TNF-α was almost exclusively expressed by human Vδ2+ γδ T cells following stimulation with IPP (Figures 4-5 and 4-6). The increased expression of TNF-α suggests that γδ T cells may preferentially express this cytokine for the potential killing of HIV/SIV infected cells or modulating the immune system in response to opportunistic pathogens (14, 49, 70, 109, 181).

Th2 cytokine expression by γδ T cells is not altered in HIV+ patients or SIV+ mangabeys

Along with pro-inflammatory cytokines, γδ T cells can express Th2 pro-humoral/anti-inflammatory cytokines in response to antigenic stimulation (49, 124). An assessment of IL-4 expression by γδ T cells was performed here to determine if either non-pathogenic SIV or pathogenic HIV infections impacts Th2 cytokine responses by these cells. With regards to mangabeys, similar percentages of γδ T cells from uninfected and SIV+ monkeys expressed IL-4 following *ex vivo* stimulation with the global activator PI (Figure 4-7A). In general, mangabey γδ T cells did not express IL-4 following stimulation with media alone or the bacterial antigens IPP and LPS (Figure 4-7A). A similar assessment of peripheral γδ T cells revealed that these cells generally expressed low levels of IL-4

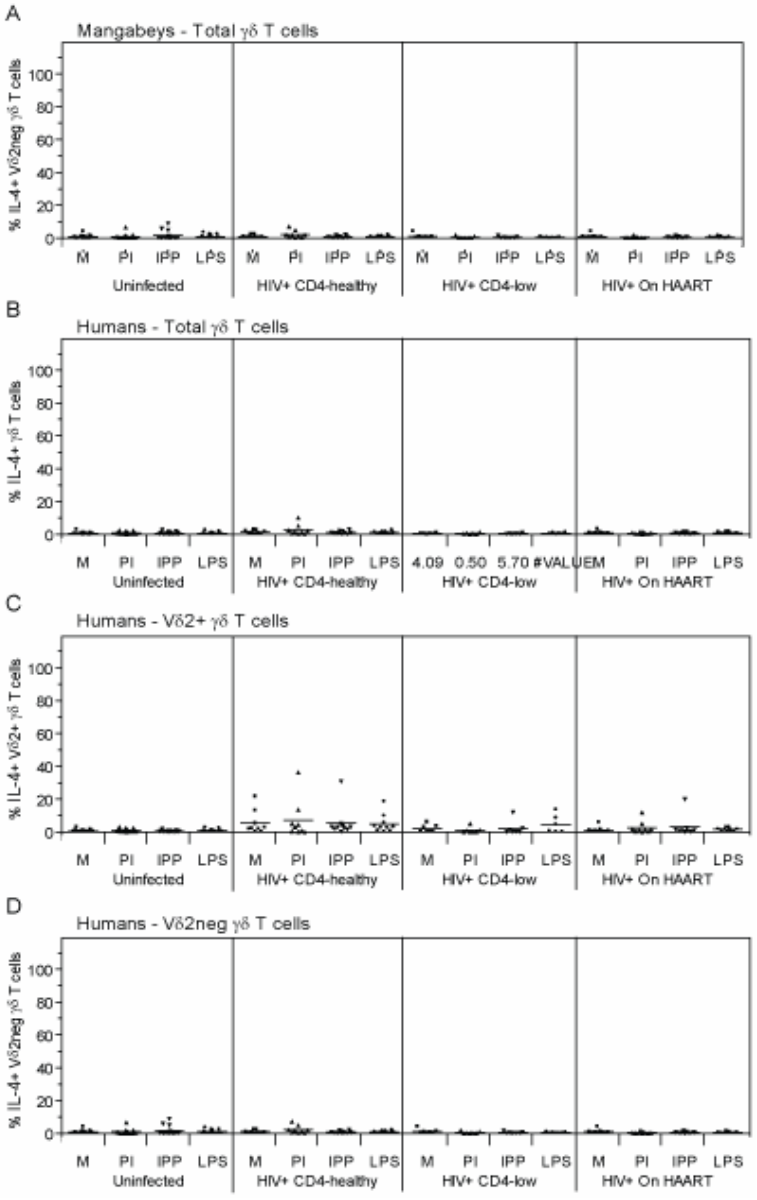

Figure 4-7 – IL-4 cytokine expression by γδ T cells was not altered following an HIV infection. PBMCs from uninfected and HIV+ patients were stimulated *ex vivo* with media alone (■), PI (▲), IPP (▼), or LPS (●) and the expression of IL-4 by (A) total γδ T cells in mangabeys as well as (B) Vδ2+ and (C) $V\delta2^{neg}$ γδ T cells from humans was assessed using flow cytometry. In general, IL-4 expression by γδ T cells in SIV+ mangabeys or HIV+ humans was not altered following *ex vivo* stimulation in comparison to their uninfected counterparts.

following *ex vivo* stimulation of both uninfected and HIV+ patients (Figures 4-7B, 4-7C, AND 4-7D). In summary, these results indicate that unlike Th1 cytokines, IL-4 production by γδ T cells is not impacted following either pathogenic HIV of humans or non-pathogenic SIV infections in mangabeys.

Assessment of Th1 responses in γδ T cells from SIV+ mangabeys and HIV+ humans

To clearly compare the HIV+ humans and SIV+ mangabeys, the γδ T cell Th1 cytokine responses following either PI or IPP stimulation is depicted in Figure 5. SIV infection of mangabeys resulted in γδ T cells with maintained IFN-γ and increased TNF-α response following *ex vivo* stimulation with PI and IPP (Figure 4-8). The level of CD4+ T cells did not appear to impact Th1 cytokine production as the maintained or increased were observed in both CD4-healthy and CD4-low SIV+ mangabeys. In contrast, the ability of γδ T cells from HIV+ patients, both CD4-healthy and CD4-low, to produce IFN-γ and TNF-α decreased significantly (Figure 4-8). The increased percentages of γδ T cells expressing Th1 cytokines in the HIV+ HAART treated patients indicated some recovery of IFN-γ and TNF-α production, but not to the levels observed in the uninfected patients

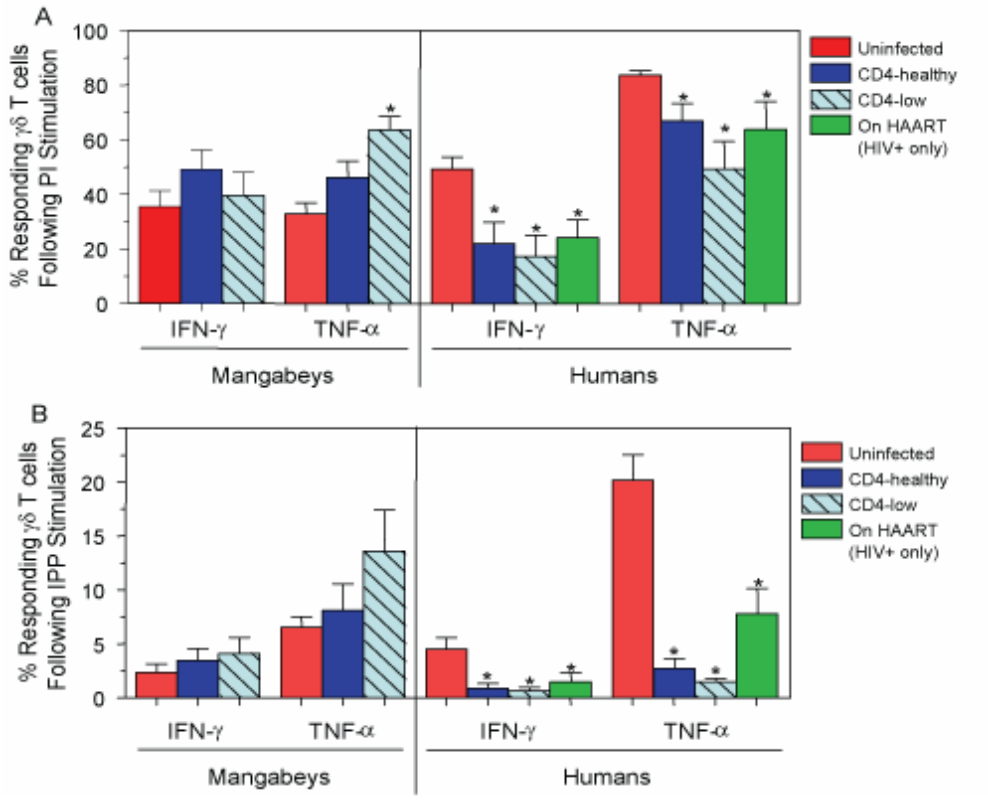

Figure 4-8 – Comparative assessment of γδ T cell Th1 cytokine responses between SIV+ mangabeys and HIV+ humans. Graphical representation of the percentage of total γδ T cells from uninfected (red bars), CD4-healthy (dark blue bars), or CD4-low (light blue bars with stripes) in mangabeys and humans is presented. Also included are HIV+ patients that were on HAART therapy (green bars). The ability of the γδ T cells to express the Th1 cytokines IFN-γ and TNF-α in response to (A) PMA/ionomycin (PI) or (B) isopentenyl pyrophosphate (IPP) is presented such that comparisons can be easily made between species and infection status. γδ T cells from CD4-healthy and CD4-low SIV+ mangabeys had maintained IFN-γ and increased TNF-α responses to *ex vivo* PI and IPP stimulation in comparison to their uninfected counterparts. In contrast, HIV+ patients showed decreased percentages of γδ T cells expressing these Th1 cytokines when compared to healthy donors. γδ T cells from HIV+ patients on HAART had a partially restored ability to express these Th1 cytokines following stimulation, though not to the extent that were produced by γδ T cells from uninfected patients.

(Figure 4-8). These results indicate that γδ T cells maintain their functionality in SIV+ mangabeys, however, γδ T cells from HIV+ patients have an impaired functional response that is only partially restored with HAART treatment.

Discussion

The finding that dramatic and sustained CD4+ T cell depletion in SIV+ mangabeys was not sufficient to induce clinical signs of simian AIDS indicated that along with low levels of immune activation, other immune cells, such as γδ T cells, may be important in preventing AIDS disease progression in this species (123). γδ T cells have important roles in bridging the innate and adaptive immune responses (109, 124) primarily by responding to bacterial antigens such as isopentenyl pyrophosphate or the recognition of stress-induced non-classical MHC molecules expressed on virally-infected cells (65, 70). The role of γδ T cells during HIV/SIV infection is not clear, although there is evidence that they may participate in defense against acute SIV infection of vaccinated macaques following oral (171) or rectal (103) challenge and are activated (express cytokines) following in vitro stimulation with HIV-infected cells (70, 140). However, the functional responses of γδ T cells are impaired following chronic

pathogenic HIV/SIV infections as evident by a decreased ability to proliferate in response to the opportunistic pathogen *Mycobacteria* (141, 196) and a decreased capacity to express IFN-γ following *in vitro* stimulation with phytohemagglutin (41). The data presented in this study demonstrated a switch in the predominate Vδ2+ peripheral γδ T cell population to a $V\delta2^{neg}$ subset following HIV infection (Figure 4-2) in agreement with other reports (7, 38). In addition, the γδ T cells from CD4-low and CD4-healthy HIV+ patients showed an impaired ability to express IFN-γ and TNF-α following bacterial antigen stimulation (Figures 4-4, 4-5, and 4-6). These studies support previous reports whereby alterations in both the phenotypic and functional responses of γδ T cells occur following chronic HIV infection.

Prior to HIV infection, CD4+ T cells express predominately Th1 cytokines in response to *ex vivo* stimulation with phorbol esters, but this response is switched to predominantly Th2 cytokines during chronic HIV infection (31, 32, 92, 119). The mechanism for the observed Th1 to Th2 cytokine skewing remains unknown. In agreement with the majority of published reports in the αβ T cell literature (31, 32, 92, 119), decreased Th1 cytokine expression by γδ T cells in HIV+ patients was observed (following *ex vivo* IPP and PI stimulation) (Figures 4-4, 4-5, and 4-6). The fact that Th1 responses are impaired in both αβ and γδ T cell subsets

suggests that this phenomenon may be due in part to immune dysfunction as opposed to direct viral cytopathicity. Through an analysis of the Vδ2+ and Vδ2^{neg} γδ T cell subsets these findings indicate that the dysfunction is primarily localized to the Vδ2+ subset. Therefore, not only is the percentage of this subset declining in the blood throughout infection but the ability of Vδ2+ γδ T cells to produce Th1 cytokines is also compromised. Although γδ T cells can express Th2 pro-humoral/anti-inflammatory cytokines, such as IL-4, in response to antigenic stimulation (49, 124) there was no evidence for a Th1 to Th2 shift in any subset of γδ T cells as IL-4 expression remained generally low in HIV+ patients and SIV+ mangabeys (data not shown). Therefore, these data support a model in which Th1 cytokine response by γδ T cells from SIV+ mangabeys function in the maintenance of immunologic health by preventing opportunistic infections and SIV disease progression in this species (Figure 4-9).

Dysfunctional responses by γδ T cells were historically attributed to the loss of CD4+ T cells during pathogenic HIV/SIV infections (27, 41, 167, 196). The studies presented here addressed the dependence of γδ T cells on CD4+ T cell help for proper function by comparing the γδ T cell responses of CD4-healthy and CD4-low cohorts (Figures 4-4, 4-5, and 4-6). Our findings during pathogenic HIV infection in humans identified

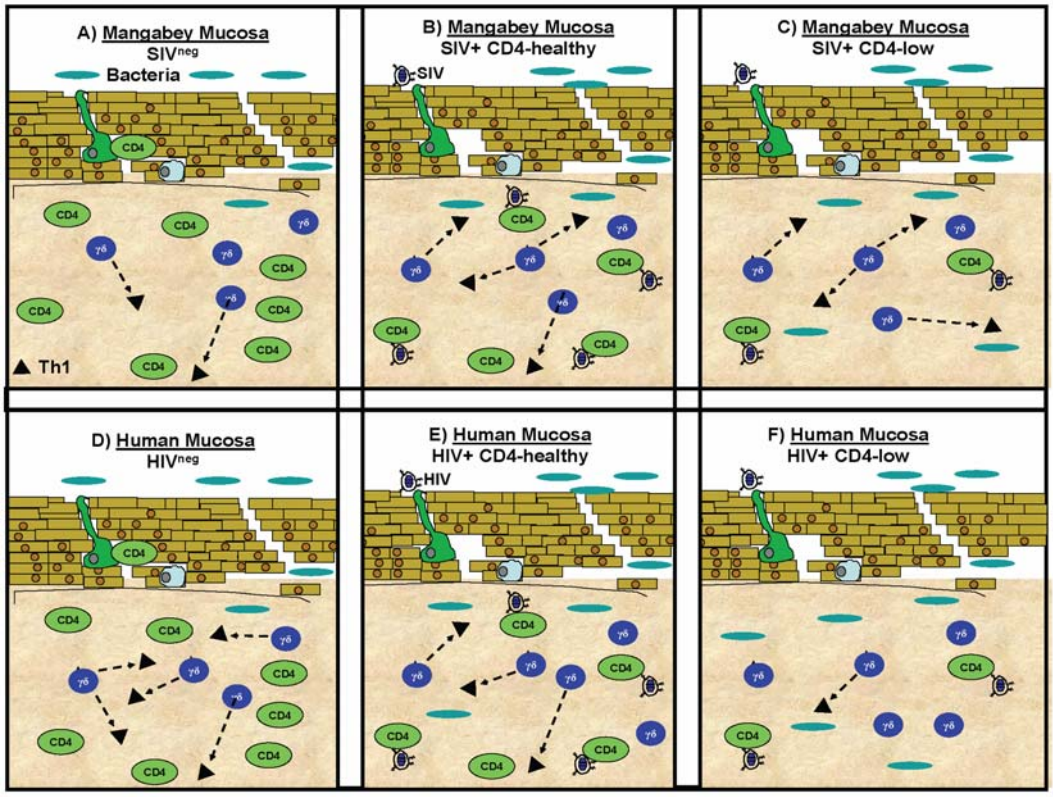

Figure 4-9 – Proposed role for mucosal γδ T cells during non-pathogenic SIV infections of mangabeys and pathogenic HIV infections of humans. A representative mucosal environment is shown for (A-C) mangabeys and (D-E) humans identifying the γδ and CD4+ T cells in the sub-mucosal layer. γδ T cells are demonstrated expressing Th1 cytokines (▲) in response to the presence of opportunistic bacterial pathogens which have translocated from the lumen into the sub-mucosa. (A-C) γδ T cells have a maintained or increased ability to express Th1 cytokines in mangabeys even as CD4+ T cells are being depleted. (D-E) However, γδ T cells show impaired Th1 responses at human mucosal surfaces in response to the bacterial pathogens as CD4+ T cells are being lost.

impaired Th1 cytokine responses of γδ T cells, however depletion of CD4+ T cells in the CD4-low patients did not further abrogate the ability of the γδ T cells to make Th1 cytokines (Figures 4-4, 4-5, and 4-6). In our non-pathogenic sooty mangabey model, despite CD4+ T cell depletion, γδ T cells maintained the ability to proliferate (123) and express Th1 cytokines when stimulated with bacterial antigens in an environment where immune activation remained low (Figures 4-4, 4-5, and 4-6). These data suggest that the impairment of Th1 cytokine expression by γδ T cells in the HIV+ humans may not be due solely to the loss of CD4+ T cells, but rather other indirect HIV-induced immunologic changes (Figures 4-8 and 4-9). Persistent immune activation observed in pathogenic HIV infection may be a key factor in the alteration of the γδ T cell function (169). Therefore, low levels of immune activation in chronically SIV+ mangabeys, as opposed to CD4+ T cell levels, may contribute to the preservation of Th1 cytokine expression by γδ T cells

The presence of γδ T cells at mucosal sites suggests that these cells may contribute to the protection against pathogenic mucosal microorganisms. During the CD4+ T cell depletion in the mucosa of SIV and HIV infections (18, 105, 116), the ability of γδ T cells to respond to microbes at mucosal surfaces may be important to prevent disease progression following infection. We propose that mangabey γδ T cells

may prevent opportunistic bacterial pathogens from establishing infections which otherwise might contribute to persistent immune activation (Figure 4-9). Therefore, augmenting Th1 responsiveness by human γδ T cells may reduce the rate of AIDS disease progression by enhancing innate cellular immune defenses against opportunistic infections (Figure 4-9). Clinical augmentation of γδ T cell functions has been demonstrated previously whereby the anti-tumor properties of γδ T cells can be effectively increased in melanoma patients through the administration of bisphosphates (40, 188), which are a class of compounds related to IPP. However, the timing, administrative route and dosages of any drugs designed to increase γδ T cell function would require careful assessment during pathogenic SIV-macaque infections prior to administration in HIV+ patients. In this regard, the findings depicted here assessing the non-pathogenic SIV-mangabey infections can be utilized as a benchmark indicating the optimum γδ T cell responses that we may hope to elicit with any γδ T cell enhancing treatment administered to HIV+ patients.

CHAPTER 5 – Final Discussion and Future Directions

Final Discussion

This thesis assesses the levels and functions of γδ T cells throughout pathogenic HIV/SIV and non-pathogenic SIV disease course. The studies described for Chapter 3 (Specific Aim #1) focused on the levels of γδ T cells during acute SIV infection of macaques, at both mucosal and lymphoid sites. A major finding in this study was evidence for a redistribution of mucosal γδ T cells towards secondary lymphoid organs during the earliest times post-oral SIV infection. Chapter 4 (Specific Aims #2 and #3) involved a comparative analysis of γδ T cell levels and functions during pathogenic HIV and non-pathogenic SIV disease progression. These studies were the first to demonstrate the maintenance of γδ T cell Th1 pro-inflammatory cytokine responses during non-pathogenic SIV infection of mangabeys. Also, these studies demonstrated that the lack of CD4+ T cells was not the sole contributor to impaired γδ T cell functionality in HIV+ humans as patients with low levels of CD4+ T cells had γδ T cells with impaired responses similar to patients with healthy CD4+ T cell levels. Taken together, these studies indicate that migratory and Th1 pro-inflammatory cytokine responses by γδ T cells may have important roles throughout the course of an HIV infection.

The experiments presented in Chapter 3 were designed to assess the levels of γδ T cells in orally SIV-infected macaques at the viral sites of entry (the oral and esophageal mucosa) as well as regional lymphoid sites (the cervical and retropharyngeal LNs). The hypothesis prior to initiating this study was that the levels of γδ T cells would increase at the oral and esophageal mucosa soon after an oral inoculation in response to the SIV infection. A rationale for this hypothesis was based on a study where increased numbers of γδ T cells were observed at the tonsils of macaques vaccinated with an attenuated virus thus prevented the establishment of an oral-tonsillar challenge, pathogenic SIV infection (171). However, the studies presented in Chapter 3 identified a loss of γδ T cells at the oral and esophageal mucosa as early as two days post-oral SIV inoculation in macaques (Figure 3-1). The loss of γδ T cells also appears to occur prior to the loss of CD4+ cells at these mucosal sites (Figure 3-1). These studies therefore had contrasting findings likely due to the primary pathogenic SIV infection used to orally infected macaques. The oral vaccination of macaques with an attenuated virus (171) may prime the tonsillar γδ T cells, which may allow for an expansion of these cells upon a subsequent oral-tonsillar SIV challenge. Therefore, my results suggest that in unvaccinated macaques, the loss of oral and esophageal mucosal

γδ T cells (either by direct infection, AICD, or migration from the mucosa) may occur as a result of a primary, acute, pathogenic SIV infection.

Chen and colleagues first reported a depletion of γδ T cells at the intestinal mucosa following 7 days after an intrarectal SIVmacPBj inoculation which may have contributed to the establishment of the viral infection (26). My study differed from the *Chen* report (26) because it involved an oral inoculation and utilized a viral quasi-species SIVmac251 instead of the SIVmacPBj cloned virus. In addition, my study allowed for an assessment of γδ T cells at additional tissues sites including the cervical and retropharyngeal LNs because the macaques enrolled were necropsied at various days soon after infection (1, 2, and 4 days) (Table 3-1). An increase in the levels of γδ T cells was observed at the regional cervical and retropharyngeal LNs during an acute infection which suggested a redistribution of these cells from the mucosa to the secondary LNs (Hypothesis #3 – Figure 3-4D). A mechanism to explain the redistribution of γδ T cells was provided when increased expression of the LN homing chemokine CCL21/6Ckine and its receptor CCR7 was observed (Hypothesis #3 – Figure 3-4D). I speculate that at these lymphoid sites, γδ T cells may participate in the development of an anti-viral immune response in an attempt to prevent the rapid spread of the virus. This could be achieved through antigen processing as the mucosal

γδ T cells could potentially take up SIV antigens and then migrate to the regional LNs to present these antigens to naïve CD4+ or CD8+ T cells, in a similar fashion as has been shown for *Mycobacterial* antigens *in vitro* (15, 124).

Along with levels, the functionality of γδ T cells is also impacted by pathogenic HIV/SIV infections. This includes their ability to proliferate in response to bacterial derived antigens associated with AIDS disease progression including *Mycobacteria* (141, 196). The results in Chapter 4 revealed γδ T cells from HIV+ patients (both CD4-healthy and CD4-low) had an impaired ability to express both the Th1 cytokines IFN-γ and TNF-α following *ex vivo* stimulation with the global activator PI and the bacterial antigen IPP (Figure 4-8). This suggested that γδ T cells from HIV-infected patients have an impaired functional response and therefore may be unable to respond to opportunistic infections appropriately. A similar analysis with regards to non-pathogenic infections demonstrated that γδ T cells from SIV+ mangabeys (CD4-healthy and CD4-low) had a maintained (or in some cases increased) capacity to express Th1 cytokines when compared to their uninfected counterparts (Figure 4-8). These results suggest that in the absence of aberrant immune activation (123), the ability of mangabey γδ T cells to express Th1 cytokines in response to antigenic stimulation can occur regardless of CD4+ T cell levels and may

participate in the prevention of opportunistic infections. Reports have suggested that the expression of pro-inflammatory cytokines during an HIV/SIV infection may contribute to a cytokine storm and immune activation (2, 147, 178). However, the fact that γδ T cells from HIV+ patients have impaired pro-inflammatory cytokine responses following *ex vivo* stimulation indicates that γδ T cell-derived Th1 cytokines may not contribute heavily to the overall immune activation. Therefore, the maintenance of Th1 cytokines by γδ T cells from SIV+ mangabeys may contribute to the health of these animals as well as help prevent AIDS disease progression irrespective of CD4+ T cell levels.

Recently, a mechanism was proposed attributing HIV-induced immune activation to the translocation of bacterial components such as LPS across mucosal barriers into the systemic circulation (16, 17). These bacterial components may be present in the peripheral circulation because of decreased mucosal integrity and the loss of both mucosal CD4+ T cells (16, 17) and epithelial cells (105). In contrast, SIV+ mangabeys had low levels of detectable LPS in the peripheral circulation (17) which may be the result of maintained mucosal integrity. Interestingly, SIV+ mangabeys also experienced low levels of immune activation even following gut mucosal CD4+ T cell depletion (123). This finding suggested that SIV+ mangabeys may also have low levels of detectable LPS in the peripheral

circulation. Therefore, other immune components aside from CD4+ T cells may be contributing to the mucosal integrity and general immune health of the SIV-infected mangabeys, including γδ T cells which were present at the rectal mucosa in CD4-healthy and CD4-low SIV+ mangabeys (Figure 4-3). In conjunction with the functional data, I speculate that mucosal γδ T cells from SIV+ mangabeys have a controlled Th1 cytokine response to microbial pathogens (Figure 4-9). The ability of mucosal γδ T cells to maintain their cytokine responses following an SIV infection may be important in controlling opportunistic pathogens (35, 67, 79, 162, 194), to induce apoptosis of infected cells (70), or control activation in other immune cells (78). The maintained functionality by γδ T cells in the absence of CD4+ T cells may help prevent immune activation in mangabeys by controlling opportunistic pathogens at the mucosal surface.

This thesis has assessed the responses of γδ T cells (lymphoid homing and pro-inflammatory cytokine expression) at mucosal and peripheral tissues during pathogenic and non-pathogenic infections. This work demonstrates that γδ T cells play important roles from the earliest stages of infection to the latest stages of disease progression and AIDS. Proper γδ T cell functionality with regards to migration and Th1 cytokine expression may be useful clinically for HIV-infected patients. A precedent

for the clinical augmentation of γδ T cell functions already exists with regards to the treatment of melanoma (40, 188). The Dieli *et al* and Wilhelm *et al* studies were the first clinical study to demonstrate the use of a stimulating compound to modulate γδ T cell anti-tumor effects establishing the foundation for the use of these cells clinically with regards to other diseases. In addition, novel vaccine strategies should consider the impact of their inoculum on the functions of γδ T cells at mucosal sites. In conclusion, this thesis provides further insights into the important immunologic roles of γδ T cells and gives a rationale to continue assessing the impact of these cells in HIV+ patients.

Future Directions

The results presented in this thesis provide novel insights into the role of γδ T cells including the maintenance of γδ T cell functions which may protect from opportunistic infections and progression towards AIDS. This thesis provides a rationale to address the functions of γδ T cells further *in vivo* following SIV infection in macaques and mangabeys. For example, what impact would the depletion of γδ T cells in monkeys have on SIV disease progression? In addition, would the augmentation of γδ T cell functions in monkeys influence SIV disease progression? With regards to the first question, there are no antibodies available at this time

which can deplete γδ T cells in monkeys. Our laboratory has been in contact with Dr. Keith Reimann at the Harvard School of Medicine to begin the development and testing of a γδ T cell depleting antibody to address this issue. This depleting antibody would have to be administered multiple times throughout the course of disease to determine if the lack of γδ T cells impacts the progression to simian AIDS. Unfortunately, we are not sure if the proposed depleting antibody will be able to remove γδ T cells from all sites or if the host will generate a response against the foreign antibody following multiple infusions. Therefore, the γδ T cell depletion from SIV+ monkeys is one possible avenue of future research although the success for these experiments in fully depleting γδ T cells and answering these questions is not clear.

The augmentation of γδ T cell functions *in vivo* during an SIV infection may also provide insights into the roles of these cells on disease progression and may lead to novel clinical therapeutics for HIV+ patients. For example, increased numbers and Th1 cytokine responses by γδ T cells was achieved *in vivo* following the administration of bromohydrin pyrophosphate (BrHPP) in uninfected cynomolgus macaques (165). In addition, this report identified the dosage and timing for the use of BrHPP in non-human primates (165) and provided a rationale to assess the impact of BrHPP administration in SIV+ monkeys. The goal of these

proposed studies are to determine if specific activation of a single cell subset, γδ T cells, has on the viral and immune components associated with HIV/SIV disease progression. The hypothesis for these studies is that BrHPP activation will result in increased γδ T cell functional responses in SIV+ monkeys. It is possible that activating one immune cell subset such as γδ T cells and subsequent expression of pro-inflammatory cytokines may result in a cascade event whereby other immune cells become activated thus leading to systemic immune activation. In order to address these issues, the activation of γδ T cells would be assessed during pathogenic SIV infections of macaques as well as non-pathogenic SIV infections of mangabeys (both CD4-healthy and CD4-low).

The initial studies would begin with an assessment of the impact of BrHPP administration on γδ T cells during pathogenic SIV disease progression in rhesus macaques. Though the *Sicard et al* BrHPP study was undertaken in cynomolgus macaques, there is a precedent to use the same dosage and administration routes for the rhesus macaques because *in vitro* evidence has shown that rhesus macaque γδ T cells can also proliferate in response to BrHPP as well (165). The experimental design for these proposed studies would utilize two groups of rhesus macaques (n=6) including (1) an uninfected group and (2) a second group which would be inoculated with SIV 5 weeks (day 0) prior to the first BrHPP

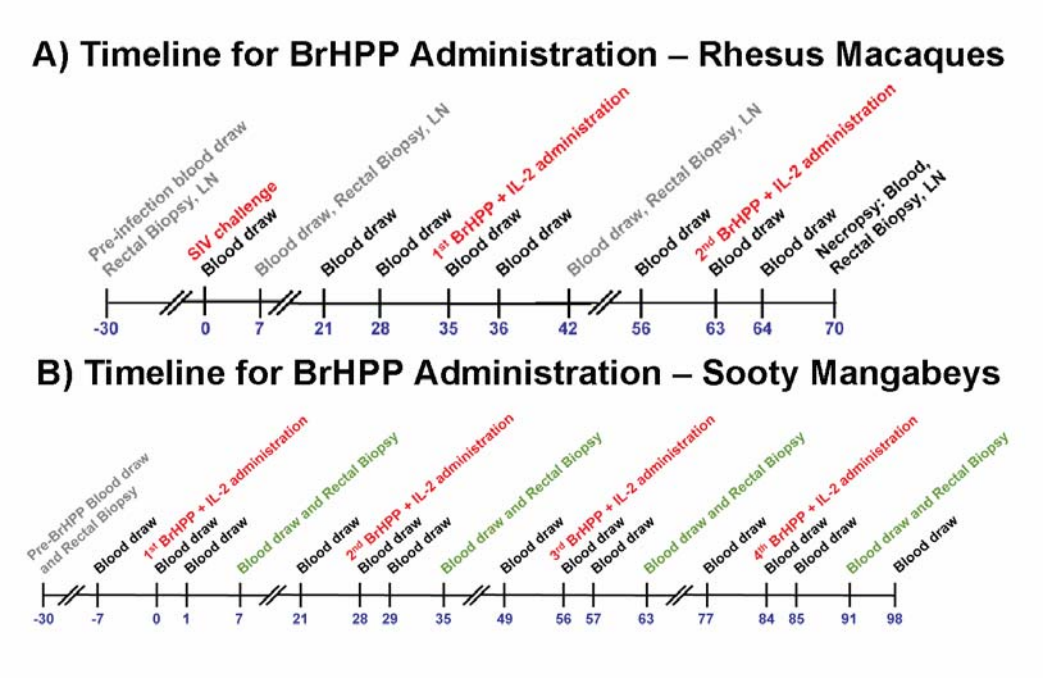

Figure 5-1 – Timeline for BrHPP administration in rhesus macaques and sooty mangabeys. Timeline of BrHPP administration in (A) macaques and (B) mangabeys. Blue numbers denote days pre- or post-SIV challenge. Red lettering represents SIV challenge or BrHPP administration; gray lettering represents biopsy procedures, including rectal and lymph node (LN) biopsies, and blood draw; black lettering represents blood draw only or necropsy.

administration (Figure 5-1A). To achieve an effect *in vivo*, the BrHPP would be administered subcutaneously (day 35) following five consecutive days of IL-2 (0.3 million Units). The BrHPP would be provided via a 30 minute infusion in 50 ml of saline similar to previous descriptions (165). A second dose of BrHPP and IL-2 would be administered 4 weeks after the first dose (day 63) and the macaques necropsied 1 week later (day 70) such that the impact of the BrHPP administration on numerous tissue compartments could be determined. Blood draws and rectal mucosal biopsies would be performed throughout the time-course (Figure 5-1A) and flow cytometry would be utilized to assess γδ T cells including phenotypes, activation status, and ability to express cytokines in response to *ex vivo* stimulation. In addition to macaques, similar infusion protocols will be given to the SIV+ mangabeys (CD4-healthy and CD4-low) to assess the impact of BrHPP and IL-2 administrations on γδ T cell functionality over a 14 week period (Figure 5-1B). The initial BrHPP infusion will begin on day 0 and the subsequent three administrations will occur every four weeks (Figure 5-1B). Blood draws and rectal mucosal biopsies will be performed such that the effect of BrHPP administration could be assessed on γδ T cells throughout the time-course. Mangabeys would continue to be monitored throughout the year for the long-term impact of the BrHPP administrations. One possible outcome for these

experiments is that exogenous BrHPP stimulation might induce persistent γδ T cell Th1 responses leading to a cascade of activation in numerous immunologic cells which may contribute to AIDS disease progression. Alternatively, γδ T cell functions could be augmented *in vivo* providing enhanced protection from opportunistic pathogens and would not express pro-inflammatory cytokines following the suppression of these pathogens. Regardless of the outcome, utilizing SIV+ mangabeys for these experiments provides an invaluable opportunity to assess the impact of γδ T cell augmentation in an environment of low immune activation.

In summary, these proposed studies might provide further insights into the impact of selective activation of a single immune cell population (γδ T cells) on pathogenic and non-pathogenic SIV infections. Utilizing these monkey models for HIV-infection provides invaluable resources to provide insights into the timing, dosage, and administration routes most useful to augment γδ T cell functions *in vivo*. Aminobisphosphates and other related γδ T cell stimulating molecules may be useful to help to augment the defense against opportunistic pathogens and when used in conjunction with current anti-viral medications to control viral replication, they may prove beneficial to HIV+ patients. Clinical augmentation of γδ T cells has been performed previously with patients suffering from multiple myeloma and the administration of aminobisphosphates proved

efficacious at controlling some of the patients symptoms (40, 188). These proposed studies may prove useful in the development of novel therapeutics aimed at modulating the levels and functions of γδ T cells in HIV+ patients with the goal of preventing AIDS disease progression.

BIBLIOGRAPHY

1. **Aandahl, E. M., J. Michaelsson, W. J. Moretto, F. M. Hecht, and D. F. Nixon.** 2004. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol **78:**2454-9.
2. **Abel, K., B. Pahar, K. K. Van Rompay, L. Fritts, C. Sin, K. Schmidt, R. Colon, M. McChesney, and M. L. Marthas.** 2006. Rapid virus dissemination in infant macaques after oral simian immunodeficiency virus exposure in the presence of local innate immune responses. J Virol **80:**6357-67.
3. **ABI.** 1998. Applied Biosystems - User Bulletin No.2, ABI Prism 7700 Sequence Detection System.
4. **Aderem, A., and D. M. Underhill.** 1999. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol **17:**593-623.
5. **Alfano, M., and G. Poli.** 2005. Role of cytokines and chemokines in the regulation of innate immunity and HIV infection. Mol Immunol **42:**161-82.
6. **Allen, T. M., D. H. O'Connor, P. Jing, J. L. Dzuris, B. R. Mothe, T. U. Vogel, E. Dunphy, M. E. Liebl, C. Emerson, N. Wilson, K. J. Kunstman, X. Wang, D. B. Allison, A. L. Hughes, R. C. Desrosiers, J. D. Altman, S. M. Wolinsky, A. Sette, and D. I. Watkins.** 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature **407:**386-90.
7. **Autran, B., F. Triebel, C. Katlama, W. Rozenbaum, T. Hercend, and P. Debre.** 1989. T cell receptor gamma/delta+ lymphocyte subsets during HIV infection. Clin Exp Immunol **75:**206-10.
8. **Azzoni, L., E. Papasavvas, J. Chehimi, J. R. Kostman, K. Mounzer, J. Ondercin, B. Perussia, and L. J. Montaner.** 2002. Sustained impairment of IFN-gamma secretion in suppressed HIV-infected patients despite mature NK cell recovery: evidence for a defective reconstitution of innate immunity. J Immunol **168:**5764-70.
9. **Baba, T. W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L. A. Cavacini, M. R. Posner, H. Katinger, G. Stiegler, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, Y. Lu, J. E. Wright, T. C. Chou, and R. M. Ruprecht.** 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med **6:**200-6.

10. **Balbi, B., M. T. Valle, S. Oddera, D. Giunti, F. Manca, G. A. Rossi, and L. Allegra.** 1993. T-lymphocytes with gamma delta+ V delta 2+ antigen receptors are present in increased proportions in a fraction of patients with tuberculosis or with sarcoidosis. Am Rev Respir Dis **148:**1685-90.
11. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220:**868-71.
12. **Bergmeier, L. A., Y. Wang, and T. Lehner.** 2002. The role of immunity in protection from mucosal SIV infection in macaques. Oral Dis **8 Suppl 2:**63-8.
13. **Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather.** 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol **17:**189-220.
14. **Biswas, P., M. Ferrarini, B. Mantelli, C. Fortis, G. Poli, A. Lazzarin, and A. A. Manfredi.** 2003. Double-edged effect of Vgamma9/Vdelta2 T lymphocytes on viral expression in an in vitro model of HIV-1/mycobacteria co-infection. Eur J Immunol **33:**252-63.
15. **Brandes, M., K. Willimann, and B. Moser.** 2005. Professional antigen-presentation function by human gammadelta T Cells. Science **309:**264-8.
16. **Brenchley, J. M., D. A. Price, and D. C. Douek.** 2006. HIV disease: fallout from a mucosal catastrophe? Nat Immunol **7:**235-9.
17. **Brenchley, J. M., D. A. Price, T. W. Schacker, T. E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B. R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J. N. Martin, F. M. Hecht, L. J. Picker, M. M. Lederman, S. G. Deeks, and D. C. Douek.** 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **12:**1365-71.
18. **Brenchley, J. M., T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek.** 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med **200:**749-59.

19. **Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan.** 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. J Virol **75:**2262-75.
20. **Brown, A. J., and D. D. Richman.** 1997. HIV-1: gambling on the evolution of drug resistance? Nat Med **3:**268-71.
21. **Carding, S. R., and P. J. Egan.** 2002. Gammadelta T cells: functional plasticity and heterogeneity. Nat Rev Immunol **2:**336-45.
22. **CDC.** 2005. Centers For Disease Control and Prevention - HIV/AIDS Surveillance Report: Cases of HIV Infection and AIDS in the United States and Dependent Areas. **17:**1-54.
23. **CDC.** 1981. Pneumocystis pneumonia--Los Angeles. MMWR Morb Mortal Wkly Rep **30:**250-2.
24. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature **328:**543-7.
25. **Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx.** 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. J Virol **74:**1209-23.
26. **Chen, Z. W., Y. Shen, I. C. Davis, L. Shen, N. L. Letvin, and P. N. Fultz.** 2000. Down-regulation of macaque gammadelta + T cells in lymphoid compartments after rectal infection with SIVsmmPBj14. J Med Primatol **29:**143-7.
27. **Chervenak, K. A., M. M. Lederman, and W. H. Boom.** 1997. Bacterial antigen activation of Vdelta1 and Vdelta2 gammadelta T cells of persons infected with human immunodeficiency virus type 1. J Infect Dis **175:**429-33.
28. **Choi, Y. K., B. A. Fallert, M. A. Murphey-Corb, and T. A. Reinhart.** 2003. Simian immunodeficiency virus dramatically alters expression of homeostatic chemokines and dendritic cell markers during infection in vivo. Blood **101:**1684-91.
29. **Chun, T. W., J. S. Justement, C. Sanford, C. W. Hallahan, M. A. Planta, M. Loutfy, S. Kottilil, S. Moir, C. Kovacs, and A. S. Fauci.** 2004. Relationship between the frequency of HIV-specific CD8+ T cells and the level of CD38+CD8+ T cells in untreated HIV-infected individuals. Proc Natl Acad Sci U S A **101:**2464-9.
30. **Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M. A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Dauguet, C. Katlama,**

C. Rouzioux, and et al. 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science **233:**343-6.

31. **Clerici, M., F. T. Hakim, D. J. Venzon, S. Blatt, C. W. Hendrix, T. A. Wynn, and G. M. Shearer.** 1993. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. J Clin Invest **91:**759-65.
32. **Clerici, M., and G. M. Shearer.** 1993. A TH1-->TH2 switch is a critical step in the etiology of HIV infection. Immunol Today **14:**107-11.
33. **Conti, L., R. Casetti, M. Cardone, B. Varano, A. Martino, F. Belardelli, F. Poccia, and S. Gessani.** 2005. Reciprocal activating interaction between dendritic cells and pamidronate-stimulated gammadelta T cells: role of CD86 and inflammatory cytokines. J Immunol **174:**252-60.
34. **Crowe, S. M., N. J. Vardaxis, S. J. Kent, A. L. Maerz, M. J. Hewish, M. S. McGrath, and J. Mills.** 1994. HIV infection of monocyte-derived macrophages in vitro reduces phagocytosis of Candida albicans. J Leukoc Biol **56:**318-27.
35. **Davies, A., S. Lopez-Briones, H. Ong, C. O'Neil-Marshall, F. A. Lemonnier, K. Nagaraju, E. S. Metcalf, and M. J. Soloski.** 2004. Infection-induced expansion of a MHC Class Ib-dependent intestinal intraepithelial gammadelta T cell subset. J Immunol **172:**6828-37.
36. **De Clercq, E.** 1992. HIV inhibitors targeted at the reverse transcriptase. AIDS Res Hum Retroviruses **8:**119-34.
37. **De Milito, A.** 2004. B lymphocyte dysfunctions in HIV infection. Curr HIV Res **2:**11-21.
38. **De Paoli, P., D. Gennari, P. Martelli, G. Basaglia, M. Crovatto, S. Battistin, and G. Santini.** 1991. A subset of gamma delta lymphocytes is increased during HIV-1 infection. Clin Exp Immunol **83:**187-91.
39. **Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature **381:**661-6.
40. **Dieli, F., N. Gebbia, F. Poccia, N. Caccamo, C. Montesano, F. Fulfaro, C. Arcara, M. R. Valerio, S. Meraviglia, C. Di Sano, G. Sireci, and A. Salerno.** 2003. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood **102:**2310-1.

41. **Dobmeyer, T. S., R. Dobmeyer, D. Wesch, E. B. Helm, D. Hoelzer, and D. Kabelitz.** 2002. Reciprocal alterations of Th1/Th2 function in gammadelta T-cell subsets of human immunodeficiency virus-1-infected patients. Br J Haematol **118:**282-8.
42. **Doms, R. W.** 2004. Viral entry denied. N Engl J Med **351:**743-4.
43. **Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell **85:**1149-58.
44. **Douek, D. C., L. J. Picker, and R. A. Koup.** 2003. T cell dynamics in HIV-1 infection. Annu Rev Immunol **21:**265-304.
45. **Ebert, L. M., S. Meuter, and B. Moser.** 2006. Homing and function of human skin gammadelta T cells and NK cells: relevance for tumor surveillance. J Immunol **176:**4331-6.
46. **Eggena, M. P., B. Barugahare, N. Jones, M. Okello, S. Mutalya, C. Kityo, P. Mugyenyi, and H. Cao.** 2005. Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol **174:**4407-14.
47. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature **362:**359-62.
48. **Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger.** 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science **272:**872-7.
49. **Ferrick, D. A., M. D. Schrenzel, T. Mulvania, B. Hsieh, W. G. Ferlin, and H. Lepper.** 1995. Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. Nature **373:**255-7.
50. **Feurle, J., E. Espinosa, S. Eckstein, F. Pont, V. Kunzmann, J. J. Fournie, M. Herderich, and M. Wilhelm.** 2002. Escherichia coli produces phosphoantigens activating human gamma delta T cells. J Biol Chem **277:**148-54.
51. **Fisch, P., M. Malkovsky, S. Kovats, E. Sturm, E. Braakman, B. S. Klein, S. D. Voss, L. W. Morrissey, R. DeMars, W. J. Welch, and et al.** 1990. Recognition by human V gamma 9/V delta 2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. Science **250:**1269-73.
52. **Flexner, C.** 1998. HIV-protease inhibitors. N Engl J Med **338:**1281-92.

53. **Fogli, M., P. Costa, G. Murdaca, M. Setti, M. C. Mingari, L. Moretta, A. Moretta, and A. De Maria.** 2004. Significant NK cell activation associated with decreased cytolytic function in peripheral blood of HIV-1-infected patients. Eur J Immunol **34:**2313-21.
54. **Forster, R., T. Emrich, E. Kremmer, and M. Lipp.** 1994. Expression of the G-protein--coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. Blood **84:**830-40.
55. **Fowler, M. G., and L. Mofenson.** 1997. Progress in prevention of perinatal HIV-1. Acta Paediatr Suppl **421:**97-103.
56. **Frankel, A. D., and J. A. Young.** 1998. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem **67:**1-25.
57. **Fultz, P. N., T. P. Gordon, D. C. Anderson, and H. M. McClure.** 1990. Prevalence of natural infection with simian immunodeficiency virus and simian T-cell leukemia virus type I in a breeding colony of sooty mangabey monkeys. Aids **4:**619-25.
58. **Gallo, R. C., P. S. Sarin, E. P. Gelmann, M. Robert-Guroff, E. Richardson, V. S. Kalyanaraman, D. Mann, G. D. Sidhu, R. E. Stahl, S. Zolla-Pazner, J. Leibowitch, and M. Popovic.** 1983. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science **220:**865-7.
59. **Gan, Y. H., S. S. Lui, and M. Malkovsky.** 2001. Differential susceptibility of naive and activated human gammadelta T cells to activation-induced cell death by T-cell receptor cross-linking. Mol Med **7:**636-43.
60. **Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn.** 1999. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature **397:**436-41.
61. **Gascon, R. L., A. B. Narvaez, R. Zhang, J. O. Kahn, F. M. Hecht, B. G. Herndier, and M. S. McGrath.** 2002. Increased HLA-DR expression on peripheral blood monocytes in subsets of subjects with primary HIV infection is associated with elevated CD4 T-cell apoptosis and CD4 T-cell depletion. J Acquir Immune Defic Syndr **30:**146-53.
62. **Gioia, C., C. Agrati, R. Casetti, C. Cairo, G. Borsellino, L. Battistini, G. Mancino, D. Goletti, V. Colizzi, L. P. Pucillo, and F. Poccia.** 2002. Lack of CD27-CD45RA-V gamma 9V delta 2+ T cell effectors in immunocompromised hosts and during active pulmonary tuberculosis. J Immunol **168:**1484-9.

63. **Giorgi, J. V., L. E. Hultin, J. A. McKeating, T. D. Johnson, B. Owens, L. P. Jacobson, R. Shih, J. Lewis, D. J. Wiley, J. P. Phair, S. M. Wolinsky, and R. Detels.** 1999. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis **179:**859-70.

64. **Giorgi, J. V., Z. Liu, L. E. Hultin, W. G. Cumberland, K. Hennessey, and R. Detels.** 1993. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr **6:**904-12.

65. **Groh, V., A. Steinle, S. Bauer, and T. Spies.** 1998. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science **279:**1737-40.

66. **Guadalupe, M., E. Reay, S. Sankaran, T. Prindiville, J. Flamm, A. McNeil, and S. Dandekar.** 2003. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol **77:**11708-17.

67. **Guk, S. M., T. S. Yong, and J. Y. Chai.** 2003. Role of murine intestinal intraepithelial lymphocytes and lamina propria lymphocytes against primary and challenge infections with Cryptosporidium parvum. J Parasitol **89:**270-5.

68. **Hahn, B. H., G. M. Shaw, K. M. De Cock, and P. M. Sharp.** 2000. AIDS as a zoonosis: scientific and public health implications. Science **287:**607-14.

69. **Haks, M. C., J. M. Lefebvre, J. P. Lauritsen, M. Carleton, M. Rhodes, T. Miyazaki, D. J. Kappes, and D. L. Wiest.** 2005. Attenuation of gammadeltaTCR signaling efficiently diverts thymocytes to the alphabeta lineage. Immunity **22:**595-606.

70. **Halary, F., V. Pitard, D. Dlubek, R. Krzysiek, H. de la Salle, P. Merville, C. Dromer, D. Emilie, J. F. Moreau, and J. Dechanet-Merville.** 2005. Shared reactivity of V{delta}2(neg) {gamma}{delta} T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells. J Exp Med **201:**1567-78.

71. **Havlir, D. V., R. D. Schrier, F. J. Torriani, K. Chervenak, J. Y. Hwang, and W. H. Boom.** 2000. Effect of potent antiretroviral therapy on immune responses to Mycobacterium avium in human immunodeficiency virus-infected subjects. J Infect Dis **182:**1658-63.

72. **Hayday, A. C.** 2000. [gamma][delta] cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol **18:**975-1026.
73. **Hazuda, D. J., P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J. A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau, and M. D. Miller.** 2000. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science **287:**646-50.
74. **Hazuda, D. J., S. D. Young, J. P. Guare, N. J. Anthony, R. P. Gomez, J. S. Wai, J. P. Vacca, L. Handt, S. L. Motzel, H. J. Klein, G. Dornadula, R. M. Danovich, M. V. Witmer, K. A. Wilson, L. Tussey, W. A. Schleif, L. S. Gabryelski, L. Jin, M. D. Miller, D. R. Casimiro, E. A. Emini, and J. W. Shiver.** 2004. Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. Science **305:**528-32.
75. **Heeney, J. L., A. G. Dalgleish, and R. A. Weiss.** 2006. Origins of HIV and the evolution of resistance to AIDS. Science **313:**462-6.
76. **Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson.** 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. Nature **339:**389-92.
77. **Imlach, S., C. Leen, J. E. Bell, and P. Simmonds.** 2003. Phenotypic analysis of peripheral blood gammadelta T lymphocytes and their targeting by human immunodeficiency virus type 1 in vivo. Virology **305:**415-27.
78. **Inagaki-Ohara, K., T. Chinen, G. Matsuzaki, A. Sasaki, Y. Sakamoto, K. Hiromatsu, F. Nakamura-Uchiyama, Y. Nawa, and A. Yoshimura.** 2004. Mucosal T cells bearing TCRgammadelta play a protective role in intestinal inflammation. J Immunol **173:**1390-8.
79. **Islam, D., and B. Christensson.** 2000. Disease-dependent changes in T-cell populations in patients with shigellosis. Apmis **108:**251-60.
80. **Jameson, J., K. Ugarte, N. Chen, P. Yachi, E. Fuchs, R. Boismenu, and W. L. Havran.** 2002. A role for skin gammadelta T cells in wound repair. Science **296:**747-9.
81. **Jiang, Q., J. Huang, W. Q. Li, T. Cavinato, J. R. Keller, and S. K. Durum.** 2007. Role of the Intracellular Domain of IL-7 Receptor in T Cell Development. J Immunol **178:**228-34.
82. **Jomaa, H., J. Feurle, K. Luhs, V. Kunzmann, H. P. Tony, M. Herderich, and M. Wilhelm.** 1999. Vgamma9/Vdelta2 T cell activation induced by bacterial low molecular mass compounds

depends on the 1-deoxy-D-xylulose 5-phosphate pathway of isoprenoid biosynthesis. FEMS Immunol Med Microbiol **25:**371-8.

83. **Jouen-Beades, F., E. Paris, C. Dieulois, J. F. Lemeland, V. Barre-Dezelus, S. Marret, G. Humbert, J. Leroy, and F. Tron.** 1997. In vivo and in vitro activation and expansion of gammadelta T cells during Listeria monocytogenes infection in humans. Infect Immun **65:**4267-72.
84. **Kahn, J. O., and B. D. Walker.** 1998. Acute human immunodeficiency virus type 1 infection. N Engl J Med **339:**33-9.
85. **Kaur, A., J. Yang, D. Hempel, L. Gritz, G. P. Mazzara, H. McClure, and R. P. Johnson.** 2000. Identification of multiple simian immunodeficiency virus (SIV)-specific CTL epitopes in sooty mangabeys with natural and experimentally acquired SIV infection. J Immunol **164:**934-43.
86. **Kedzierska, K., A. Maerz, T. Warby, A. Jaworowski, H. Chan, J. Mak, S. Sonza, A. Lopez, and S. Crowe.** 2000. Granulocyte-macrophage colony-stimulating factor inhibits HIV-1 replication in monocyte-derived macrophages. Aids **14:**1739-48.
87. **Kedzierska, K., J. Mak, A. Jaworowski, A. Greenway, A. Violo, H. T. Chan, J. Hocking, D. Purcell, J. S. Sullivan, J. Mills, and S. Crowe.** 2001. nef-deleted HIV-1 inhibits phagocytosis by monocyte-derived macrophages in vitro but not by peripheral blood monocytes in vivo. Aids **15:**945-55.
88. **Kestler, H. W., 3rd, Y. Li, Y. M. Naidu, C. V. Butler, M. F. Ochs, G. Jaenel, N. W. King, M. D. Daniel, and R. C. Desrosiers.** 1988. Comparison of simian immunodeficiency virus isolates. Nature **331:**619-22.
89. **Kim, J. J., L. K. Nottingham, J. I. Sin, A. Tsai, L. Morrison, J. Oh, K. Dang, Y. Hu, K. Kazahaya, M. Bennett, T. Dentchev, D. M. Wilson, A. A. Chalian, J. D. Boyer, M. G. Agadjanyan, and D. B. Weiner.** 1998. CD8 positive T cells influence antigen-specific immune responses through the expression of chemokines. J Clin Invest **102:**1112-24.
90. **Kinter, A. L., M. Hennessey, A. Bell, S. Kern, Y. Lin, M. Daucher, M. Planta, M. McGlaughlin, R. Jackson, S. F. Ziegler, and A. S. Fauci.** 2004. CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med **200:**331-43.

91. **Kirkham, P. A., H. H. Takamatsu, E. W. Lam, and R. M. Parkhouse.** 2000. Ligation of the WC1 receptor induces gamma delta T cell growth arrest through fumonisin B1-sensitive increases in cellular ceramide. J Immunol **165:**3564-70.
92. **Klein, S. A., J. M. Dobmeyer, T. S. Dobmeyer, M. Pape, O. G. Ottmann, E. B. Helm, D. Hoelzer, and R. Rossol.** 1997. Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. Aids **11:**1111-8.
93. **Kornfeld, C., M. J. Ploquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin.** 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. J Clin Invest **115:**1082-91.
94. **Kourtis, A. P., S. R. Nesheim, D. Thea, C. Ibegbu, A. J. Nahmias, and F. K. Lee.** 2000. Correlation of virus load and soluble L-selectin, a marker of immune activation, in pediatric HIV-1 infection. Aids **14:**2429-36.
95. **Kraus, G., A. Werner, M. Baier, D. Binniger, F. J. Ferdinand, S. Norley, and R. Kurth.** 1989. Isolation of human immunodeficiency virus-related simian immunodeficiency viruses from African green monkeys. Proc Natl Acad Sci U S A **86:**2892-6.
96. **Kroca, M., A. Johansson, A. Sjostedt, and A. Tarnvik.** 2001. V gamma 9V delta 2 T cells in human legionellosis. Clin Diagn Lab Immunol **8:**949-54.
97. **Kunzmann, V., E. Bauer, J. Feurle, F. Weissinger, H. P. Tony, and M. Wilhelm.** 2000. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood **96:**384-92.
98. **Kwong, P. D., R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, and W. A. Hendrickson.** 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature **393:**648-59.
99. **Lalezari, J. P., K. Henry, M. O'Hearn, J. S. Montaner, P. J. Piliero, B. Trottier, S. Walmsley, C. Cohen, D. R. Kuritzkes, J. J. Eron, Jr., J. Chung, R. DeMasi, L. Donatacci, C. Drobnes, J. Delehanty, and M. Salgo.** 2003. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med **348:**2175-85.

100. **Lane, H. C., H. Masur, L. C. Edgar, G. Whalen, A. H. Rook, and A. S. Fauci.** 1983. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med **309:**453-8.
101. **Lederman, M. M., L. A. Kalish, D. Asmuth, E. Fiebig, M. Mileno, and M. P. Busch.** 2000. 'Modeling' relationships among HIV-1 replication, immune activation and CD4+ T-cell losses using adjusted correlative analyses. Aids **14:**951-8.
102. **Lee, C., Q. H. Liu, B. Tomkowicz, Y. Yi, B. D. Freedman, and R. G. Collman.** 2003. Macrophage activation through CCR5- and CXCR4-mediated gp120-elicited signaling pathways. J Leukoc Biol **74:**676-82.
103. **Lehner, T., E. Mitchell, L. Bergmeier, M. Singh, R. Spallek, M. Cranage, G. Hall, M. Dennis, F. Villinger, and Y. Wang.** 2000. The role of gammadelta T cells in generating antiviral factors and beta-chemokines in protection against mucosal simian immunodeficiency virus infection. Eur J Immunol **30:**2245-56.
104. **Lewis, D. E., D. S. Tang, A. Adu-Oppong, W. Schober, and J. R. Rodgers.** 1994. Anergy and apoptosis in CD8+ T cells from HIV-infected persons. J Immunol **153:**412-20.
105. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature **434:**1148-52.
106. **Liuzzi, G., P. Bagnarelli, A. Chirianni, M. Clementi, and M. Piazza.** 1998. Quantitative techniques and HIV-1 load in plasma and semen. Aids **12:**673.
107. **Lloyd, T. E., L. Yang, D. N. Tang, T. Bennett, W. Schober, and D. E. Lewis.** 1997. Regulation of CD28 costimulation in human CD8+ T cells. J Immunol **158:**1551-8.
108. **Lucia, B., C. Jennings, R. Cauda, L. Ortona, and A. L. Landay.** 1995. Evidence of a selective depletion of a CD16+ CD56+ CD8+ natural killer cell subset during HIV infection. Cytometry **22:**10-5.
109. **Mak, T. W., and D. A. Ferrick.** 1998. The gammadelta T-cell bridge: linking innate and acquired immunity. Nat Med **4:**764-5.
110. **Maki, K., S. Sunaga, Y. Komagata, Y. Kodaira, A. Mabuchi, H. Karasuyama, K. Yokomuro, J. I. Miyazaki, and K. Ikuta.** 1996. Interleukin 7 receptor-deficient mice lack gammadelta T cells. Proc Natl Acad Sci U S A **93:**7172-7.

111. **Mann, D. L., S. Gartner, F. Le Sane, H. Buchow, and M. Popovic.** 1990. HIV-1 transmission and function of virus-infected monocytes/macrophages. J Immunol **144:**2152-8.
112. **Marcondes, M. C., E. M. Burudi, S. Huitron-Resendiz, M. Sanchez-Alavez, D. Watry, M. Zandonatti, S. J. Henriksen, and H. S. Fox.** 2001. Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. J Immunol **167:**5429-38.
113. **Marlink, R.** 1996. Lessons from the second AIDS virus, HIV-2. Aids **10:**689-99.
114. **Marsland, B. J., P. Battig, M. Bauer, C. Ruedl, U. Lassing, R. R. Beerli, K. Dietmeier, L. Ivanova, T. Pfister, L. Vogt, H. Nakano, C. Nembrini, P. Saudan, M. Kopf, and M. F. Bachmann.** 2005. CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. Immunity **22:**493-505.
115. **Martini, F., R. Urso, C. Gioia, A. De Felici, P. Narciso, A. Amendola, M. G. Paglia, V. Colizzi, and F. Poccia.** 2000. gammadelta T-cell anergy in human immunodeficiency virus-infected persons with opportunistic infections and recovery after highly active antiretroviral therapy. Immunology **100:**481-6.
116. **Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer.** 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature **434:**1093-7.
117. **Mavilio, D., G. Lombardo, J. Benjamin, D. Kim, D. Follman, E. Marcenaro, M. A. O'Shea, A. Kinter, C. Kovacs, A. Moretta, and A. S. Fauci.** 2005. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proc Natl Acad Sci U S A **102:**2886-91.
118. **Mehandru, S., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, and M. Markowitz.** 2004. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med **200:**761-70.
119. **Meyaard, L., E. Hovenkamp, I. P. Keet, B. Hooibrink, I. H. de Jong, S. A. Otto, and F. Miedema.** 1996. Single cell analysis of IL-4 and IFN-gamma production by T cells from HIV-infected individuals: decreased IFN-gamma in the presence of preserved IL-4 production. J Immunol **157:**2712-8.
120. **Miller, C. J., Q. Li, K. Abel, E. Y. Kim, Z. M. Ma, S. Wietgrefe, L. La Franco-Scheuch, L. Compton, L. Duan, M. D. Shore, M.**

Zupancic, M. Busch, J. Carlis, S. Wolinsky, and A. T. Haase. 2005. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. J Virol **79:**9217-27.

121. **Miller, C. J., and R. J. Shattock.** 2003. Target cells in vaginal HIV transmission. Microbes Infect **5:**59-67.
122. **Milush, J. M., D. Kosub, M. Marthas, K. Schmidt, F. Scott, A. Wozniakowski, C. Brown, S. Westmoreland, and D. L. Sodora.** 2004. Rapid dissemination of SIV following oral inoculation. Aids **18:**2371-80.
123. **Milush, J. M., J. D. Reeves, S. Gordon, D. Zhou, A. Muthukumar, D. A. Kosub, E. Chacko, L. Giavedoni, C. C. Ibegbu, K. S. Cole, J. Miamidian, M. Paiardini, A. P. Barry, S. Staprans, G. Silvestri, and D. L. Sodora.** Submitted. Virally-induced CD4+ T cell depletion is not sufficient to induce AIDS in a natural host.
124. **Modlin, R. L., and P. A. Sieling.** 2005. Immunology. Now presenting: gammadelta T cells. Science **309:**252-3.
125. **Mofenson, L. M.** 1997. Mother-child HIV-1 transmission: Timing and determinants. Obstet Gynecol Clin North Am **24:**759-84.
126. **Moir, S., A. Malaspina, K. M. Ogwaro, E. T. Donoghue, C. W. Hallahan, L. A. Ehler, S. Liu, J. Adelsberger, R. Lapointe, P. Hwu, M. Baseler, J. M. Orenstein, T. W. Chun, J. A. Mican, and A. S. Fauci.** 2001. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. Proc Natl Acad Sci U S A **98:**10362-7.
127. **Moore, J. P.** 2005. Topical microbicides become topical. N Engl J Med **352:**298-300.
128. **Muthukumar, A., D. Zhou, M. Paiardini, A. P. Barry, K. S. Cole, H. M. McClure, S. I. Staprans, G. Silvestri, and D. L. Sodora.** 2005. Timely triggering of homeostatic mechanisms involved in the regulation of T-cell levels in SIVsm-infected sooty mangabeys. Blood **106:**3839-45.
129. **Nagase, H., K. Agematsu, K. Kitano, M. Takamoto, Y. Okubo, A. Komiyama, and K. Sugane.** 2001. Mechanism of hypergammaglobulinemia by HIV infection: circulating memory B-cell reduction with plasmacytosis. Clin Immunol **100:**250-9.
130. **Natarajan, K., N. Dimasi, J. Wang, R. A. Mariuzza, and D. H. Margulies.** 2002. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. Annu Rev Immunol **20:**853-85.

131. **Nicastri, E., L. Sarmati, L. Ercoli, G. Mancino, E. D'Ambrosio, G. d'Ettorre, C. M. Mastroianni, V. Vullo, and M. Andreoni.** 1999. Reduction of IFN-gamma and IL-2 production by peripheral lymphocytes of HIV-exposed seronegative subjects. Aids **13:**1333-6.
132. **Pantophlet, R., and D. R. Burton.** 2006. GP120: target for neutralizing HIV-1 antibodies. Annu Rev Immunol **24:**739-69.
133. **Perumal, N. B., T. W. Kenniston, Jr., D. J. Tweardy, K. F. Dyer, R. Hoffman, J. Peschon, and P. M. Appasamy.** 1997. TCR-gamma genes are rearranged but not transcribed in IL-7R alpha-deficient mice. J Immunol **158:**5744-50.
134. **Petrovas, C., Y. M. Mueller, and P. D. Katsikis.** 2004. HIV-specific CD8+ T cells: serial killers condemned to die? Curr HIV Res **2:**153-62.
135. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. Bangham, C. R. Rizza, and et al.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature **354:**453-9.
136. **Piqueras, B., J. Connolly, H. Freitas, A. K. Palucka, and J. Banchereau.** 2006. Upon viral exposure, myeloid and plasmacytoid dendritic cells produce 3 waves of distinct chemokines to recruit immune effectors. Blood **107:**2613-8.
137. **Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino, and L. J. Picker.** 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat Med **5:**518-25.
138. **Pizzo, P. A., and K. M. Butler.** 1991. In the vertical transmission of HIV, timing may be everything. N Engl J Med **325:**652-4.
139. **Poaty-Mavoungou, V., F. S. Toure, C. Tevi-Benissan, and E. Mavoungou.** 2002. Enhancement of natural killer cell activation and antibody-dependent cellular cytotoxicity by interferon-alpha and interleukin-12 in vaginal mucosae Sivmac251-infected Macaca fascicularis. Viral Immunol **15:**197-212.
140. **Poccia, F., L. Battistini, B. Cipriani, G. Mancino, F. Martini, M. L. Gougeon, and V. Colizzi.** 1999. Phosphoantigen-reactive Vgamma9Vdelta2 T lymphocytes suppress in vitro human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. J Infect Dis **180:**858-61.
141. **Poccia, F., S. Boullier, H. Lecoeur, M. Cochet, Y. Poquet, V. Colizzi, J. J. Fournie, and M. L. Gougeon.** 1996. Peripheral V

gamma 9/V delta 2 T cell deletion and anergy to nonpeptidic mycobacterial antigens in asymptomatic HIV-1-infected persons. J Immunol **157:**449-61.

142. **Poggi, A., R. Carosio, D. Fenoglio, S. Brenci, G. Murdaca, M. Setti, F. Indiveri, S. Scabini, E. Ferrero, and M. R. Zocchi.** 2004. Migration of V delta 1 and V delta 2 T cells in response to CXCR3 and CXCR4 ligands in healthy donors and HIV-1-infected patients: competition by HIV-1 Tat. Blood **103:**2205-13.
143. **Poles, M. A., S. Barsoum, W. Yu, J. Yu, P. Sun, J. Daly, T. He, S. Mehandru, A. Talal, M. Markowitz, A. Hurley, D. Ho, and L. Zhang.** 2003. Human immunodeficiency virus type 1 induces persistent changes in mucosal and blood gammadelta T cells despite suppressive therapy. J Virol **77:**10456-67.
144. **Prinz, I., A. Sansoni, A. Kissenpfennig, L. Ardouin, M. Malissen, and B. Malissen.** 2006. Visualization of the earliest steps of gammadelta T cell development in the adult thymus. Nat Immunol **7:**995-1003.
145. **Re, F., F. Poccia, A. Donnini, B. Bartozzi, G. Bernardini, and M. Provinciali.** 2005. Skewed representation of functionally distinct populations of Vgamma9Vdelta2 T lymphocytes in aging. Exp Gerontol **40:**59-66.
146. **Reeves, J. D., S. A. Gallo, N. Ahmad, J. L. Miamidian, P. E. Harvey, M. Sharron, S. Pohlmann, J. N. Sfakianos, C. A. Derdeyn, R. Blumenthal, E. Hunter, and R. W. Doms.** 2002. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc Natl Acad Sci U S A **99:**16249-54.
147. **Relucio, K. I., H. T. Beernink, D. Chen, D. M. Israelski, R. Kim, and M. Holodniy.** 2005. Proteomic analysis of serum cytokine levels in response to highly active antiretroviral therapy (HAART). J Proteome Res **4:**227-31.
148. **Resino, S., E. Seoane, M. D. Gutierrez, J. A. Leon, and M. A. Munoz-Fernandez.** 2006. CD4(+) T-cell immunodeficiency is more dependent on immune activation than viral load in HIV-infected children on highly active antiretroviral therapy. J Acquir Immune Defic Syndr **42:**269-76.
149. **Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti.** 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. J Virol **72:**3872-86.

150. **Richman, D., A. S. Rosenthal, M. Skoog, R. J. Eckner, T. C. Chou, J. P. Sabo, and V. J. Merluzzi.** 1991. BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. Antimicrob Agents Chemother **35:**305-8.
151. **Richman, D., C. K. Shih, I. Lowy, J. Rose, P. Prodanovich, S. Goff, and J. Griffin.** 1991. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc Natl Acad Sci U S A **88:**11241-5.
152. **Rohmer, M., M. Knani, P. Simonin, B. Sutter, and H. Sahm.** 1993. Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem J **295 (Pt 2):**517-24.
153. **Romani, N., G. Ratzinger, K. Pfaller, W. Salvenmoser, H. Stossel, F. Koch, and P. Stoitzner.** 2001. Migration of dendritic cells into lymphatics-the Langerhans cell example: routes, regulation, and relevance. Int Rev Cytol **207:**237-70.
154. **Rossi, D., and A. Zlotnik.** 2000. The biology of chemokines and their receptors. Annu Rev Immunol **18:**217-42.
155. **Rothenberg, R. B., M. Scarlett, C. del Rio, D. Reznik, and C. O'Daniels.** 1998. Oral transmission of HIV. Aids **12:**2095-105.
156. **Roussilhon, C., M. Agrapart, P. Guglielmi, A. Bensussan, P. Brasseur, and J. J. Ballet.** 1994. Human TcR gamma delta+ lymphocyte response on primary exposure to Plasmodium falciparum. Clin Exp Immunol **95:**91-7.
157. **Ruprecht, R. M., T. W. Baba, V. Liska, S. Ayehunie, J. Andersen, D. C. Montefiori, A. Trichel, M. Murphey-Corb, L. Martin, T. A. Rizvi, B. J. Bernacky, S. J. Buchl, and M. Keeling.** 1998. Oral SIV, SHIV, and HIV type 1 infection. AIDS Res Hum Retroviruses **14 Suppl 1:**S97-103.
158. **Samuelsson, A., A. Sonnerborg, N. Heuts, J. Coster, and F. Chiodi.** 1997. Progressive B cell apoptosis and expression of Fas ligand during human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses **13:**1031-8.
159. **Sankaran, S., M. Guadalupe, E. Reay, M. D. George, J. Flamm, T. Prindiville, and S. Dandekar.** 2005. Gut mucosal T cell responses and gene expression correlate with protection against disease in long-term HIV-1-infected nonprogressors. Proc Natl Acad Sci U S A **102:**9860-5.

160. **Schacker, T., A. C. Collier, J. Hughes, T. Shea, and L. Corey.** 1996. Clinical and epidemiologic features of primary HIV infection. Ann Intern Med **125:**257-64.
161. **Scott, M. E., A. L. Landay, T. F. Lint, and G. T. Spear.** 1993. In vivo decrease in the expression of complement receptor 2 on B-cells in HIV infection. Aids **7:**37-41.
162. **Shen, X., and R. F. Siliciano.** 2000. Preventing AIDS but not HIV-1 infection with a DNA vaccine. Science **290:**463-5.
163. **Shimura, H., A. Nitahara, A. Ito, K. Tomiyama, M. Ito, and K. Kawai.** 2005. Up-regulation of cell surface Toll-like receptor 4-MD2 expression on dendritic epidermal T cells after the emigration from epidermis during cutaneous inflammation. J Dermatol Sci **37:**101-10.
164. **Shin, S., R. El-Diwany, S. Schaffert, E. J. Adams, K. C. Garcia, P. Pereira, and Y. H. Chien.** 2005. Antigen recognition determinants of gammadelta T cell receptors. Science **308:**252-5.
165. **Sicard, H., S. Ingoure, B. Luciani, C. Serraz, J. J. Fournie, M. Bonneville, J. Tiollier, and F. Romagne.** 2005. In vivo immunomanipulation of V gamma 9V delta 2 T cells with a synthetic phosphoantigen in a preclinical nonhuman primate model. J Immunol **175:**5471-80.
166. **Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg.** 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity **18:**441-52.
167. **Sindhu, S. T., R. Ahmad, R. Morisset, A. Ahmad, and J. Menezes.** 2003. Peripheral blood cytotoxic gammadelta T lymphocytes from patients with human immunodeficiency virus type 1 infection and AIDS lyse uninfected CD4+ T cells, and their cytocidal potential correlates with viral load. J Virol **77:**1848-55.
168. **Sodora, D. L., D. C. Douek, G. Silvestri, L. Montgomery, M. Rosenzweig, T. Igarashi, B. Bernacky, R. P. Johnson, M. B. Feinberg, M. A. Martin, and R. A. Koup.** 2000. Quantification of thymic function by measuring T cell receptor excision circles within peripheral blood and lymphoid tissues in monkeys. Eur J Immunol **30:**1145-53.
169. **Sousa, A. E., J. Carneiro, M. Meier-Schellersheim, Z. Grossman, and R. M. Victorino.** 2002. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1

and HIV-2 but only indirectly to the viral load. J Immunol **169:**3400-6.

170. **Stahl-Hennig, C., R. M. Steinman, K. Tenner-Racz, M. Pope, N. Stolte, K. Matz-Rensing, G. Grobschupff, B. Raschdorff, G. Hunsmann, and P. Racz.** 1999. Rapid infection of oral mucosal-associated lymphoid tissue with simian immunodeficiency virus. Science **285:**1261-5.
171. **Tenner-Racz, K., C. Stahl Hennig, K. Uberla, H. Stoiber, R. Ignatius, J. Heeney, R. M. Steinman, and P. Racz.** 2004. Early protection against pathogenic virus infection at a mucosal challenge site after vaccination with attenuated simian immunodeficiency virus. Proc Natl Acad Sci U S A **101:**3017-22.
172. **Trager, R. S.** 2003. Microbicides. Raising new barriers against HIV infection. Science **299:**39.
173. **Trimble, L. A., and J. Lieberman.** 1998. Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 zeta, the signaling chain of the T-cell receptor complex. Blood **91:**585-94.
174. **Tsomides, T. J., A. Aldovini, R. P. Johnson, B. D. Walker, R. A. Young, and H. N. Eisen.** 1994. Naturally processed viral peptides recognized by cytotoxic T lymphocytes on cells chronically infected by human immunodeficiency virus type 1. J Exp Med **180:**1283-93.
175. **Tsunemi, S., T. Iwasaki, T. Imado, S. Higasa, E. Kakishita, T. Shirasaka, and H. Sano.** 2005. Relationship of CD4+CD25+ regulatory T cells to immune status in HIV-infected patients. Aids **19:**879-86.
176. **Tugarinov, V., A. Zvi, R. Levy, Y. Hayek, S. Matsushita, and J. Anglister.** 2000. NMR structure of an anti-gp120 antibody complex with a V3 peptide reveals a surface important for co-receptor binding. Structure **8:**385-95.
177. **Tyler, D. S., S. D. Stanley, C. A. Nastala, A. A. Austin, J. A. Bartlett, K. C. Stine, H. K. Lyerly, D. P. Bolognesi, and K. J. Weinhold.** 1990. Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. J Immunol **144:**3375-84.
178. **Vahey, M. T., M. E. Nau, M. Taubman, J. Yalley-Ogunro, P. Silvera, and M. G. Lewis.** 2003. Patterns of gene expression in peripheral blood mononuclear cells of rhesus macaques infected with SIVmac251 and exhibiting differential rates of disease progression. AIDS Res Hum Retroviruses **19:**369-87.

179. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **280:**427-31.
180. **Veazey, R. S., P. J. Klasse, S. M. Schader, Q. Hu, T. J. Ketas, M. Lu, P. A. Marx, J. Dufour, R. J. Colonno, R. J. Shattock, M. S. Springer, and J. P. Moore.** 2005. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. Nature **438:**99-102.
181. **Wallace, M., S. R. Bartz, W. L. Chang, D. A. Mackenzie, C. D. Pauza, and M. Malkovsky.** 1996. Gamma delta T lymphocyte responses to HIV. Clin Exp Immunol **103:**177-84.
182. **Wang, T., E. Scully, Z. Yin, J. H. Kim, S. Wang, J. Yan, M. Mamula, J. F. Anderson, J. Craft, and E. Fikrig.** 2003. IFN-gamma-producing gamma delta T cells help control murine West Nile virus infection. J Immunol **171:**2524-31.
183. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. Nature **422:**307-12.
184. **Welsh, R. M., L. K. Selin, and E. Szomolanyi-Tsuda.** 2004. Immunological memory to viral infections. Annu Rev Immunol **22:**711-43.
185. **Wen, L., and A. C. Hayday.** 1997. Gamma delta T-cell help in responses to pathogens and in the development of systemic autoimmunity. Immunol Res **16:**229-41.
186. **Wesch, D., S. Beetz, H. H. Oberg, M. Marget, K. Krengel, and D. Kabelitz.** 2006. Direct costimulatory effect of TLR3 ligand poly(I:C) on human gamma delta T lymphocytes. J Immunol **176:**1348-54.
187. **Widney, D., G. Gundapp, J. W. Said, M. van der Meijden, B. Bonavida, A. Demidem, C. Trevisan, J. Taylor, R. Detels, and O. Martinez-Maza.** 1999. Aberrant expression of CD27 and soluble CD27 (sCD27) in HIV infection and in AIDS-associated lymphoma. Clin Immunol **93:**114-23.
188. **Wilhelm, M., V. Kunzmann, S. Eckstein, P. Reimer, F. Weissinger, T. Ruediger, and H. P. Tony.** 2003. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. Blood **102:**200-6.

189. **Wilson, E., J. F. Hedges, E. C. Butcher, M. Briskin, and M. A. Jutila.** 2002. Bovine gamma delta T cell subsets express distinct patterns of chemokine responsiveness and adhesion molecules: a mechanism for tissue-specific gamma delta T cell subset accumulation. J Immunol **169:**4970-5.
190. **Worku, S., G. J. Gorse, R. B. Belshe, and D. F. Hoft.** 2001. Canarypox vaccines induce antigen-specific human gammadelta T cells capable of interferon-gamma production. J Infect Dis **184:**525-32.
191. **Wyatt, R., and J. Sodroski.** 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science **280:**1884-8.
192. **Yarchoan, R., and S. Broder.** 1987. Development of antiretroviral therapy for the acquired immunodeficiency syndrome and related disorders. A progress report. N Engl J Med **316:**557-64.
193. **Ye, S. K., K. Maki, H. C. Lee, A. Ito, K. Kawai, H. Suzuki, T. W. Mak, Y. Chien, T. Honjo, and K. Ikuta.** 2001. Differential roles of cytokine receptors in the development of epidermal gamma delta T cells. J Immunol **167:**1929-34.
194. **Yu, Q., C. Tang, S. Xun, T. Yajima, K. Takeda, and Y. Yoshikai.** 2006. MyD88-dependent signaling for IL-15 production plays an important role in maintenance of CD8 alpha alpha TCR alpha beta and TCR gamma delta intestinal intraepithelial lymphocytes. J Immunol **176:**6180-5.
195. **Zheng, B., E. Marinova, J. Han, T. H. Tan, and S. Han.** 2003. Cutting edge: gamma delta T cells provide help to B cells with altered clonotypes and are capable of inducing Ig gene hypermutation. J Immunol **171:**4979-83.
196. **Zhou, D., X. Lai, Y. Shen, P. Sehgal, L. Shen, M. Simon, L. Qiu, D. Huang, G. Z. Du, Q. Wang, N. L. Letvin, and Z. W. Chen.** 2003. Inhibition of adaptive Vgamma2Vdelta2+ T-cell responses during active mycobacterial coinfection of simian immunodeficiency virus SIVmac-infected monkeys. J Virol **77:**2998-3006.

VITAE

David Alan Kosub, Jr. was born in San Antonio, Texas, on August 16, 1980, the son of Linda Doris Selman and David Alan Kosub, Sr.. After completing his work at Health Careers High School, San Antonio, Texas in 1998, he entered Texas A&M University – Corpus Christi at Corpus Christi, Texas. During the summer of 2000, he was awarded a research internship with Dr. Ronald Goldfarb at the University of North Texas Health Science Center at Fort Worth, TX. He later received the degree of Bachelor of Science with a double major in Biology and Chemistry from Texas A&M University – Corpus Christi in May 2002 and graduated Magna cum Laude. In June 2002, he entered the Graduate School of Biomedical Sciences at the University of Texas Southwestern Medical Center at Dallas. He joined the laboratory of Dr. Donald Sodora in the Department of Internal Medicine as well as the Immunology Graduate Program. While in Dr. Sodora's laboratory, he was appointed to the Integrative Immunology Training Grant and was awarded a grant supplement from the National Institutes of Allergy and Infectious Disease. During this time, he became the President of the Graduate Student Organization and was awarded the 2006 University of Texas Southwestern Martin Luther King, Jr. Scholarship for Community Service.

Permanent Address: 13054 Enchanted Vista
Willis, TX 77318