



Publicly Accessible Penn Dissertations


1-1-2012

Impact of HAART on T Cell activation in HIV-Infected Adolescents

Danielle Haney

University of Pennsylvania, dhaney2@gmail.com

Follow this and additional works at: <http://repository.upenn.edu/edissertations>

 Part of the [Allergy and Immunology Commons](#), [Immunology and Infectious Disease Commons](#), and the [Medical Immunology Commons](#)

Recommended Citation

Haney, Danielle, "Impact of HAART on T Cell activation in HIV-Infected Adolescents" (2012). *Publicly Accessible Penn Dissertations*. 517.

<http://repository.upenn.edu/edissertations/517>

This paper is posted at ScholarlyCommons. <http://repository.upenn.edu/edissertations/517>

For more information, please contact libraryrepository@pobox.upenn.edu.

Impact of HAART on T Cell activation in HIV-Infected Adolescents

Abstract

Every year, HIV-positive adolescents add to the growing number of HIV-infected individuals. In fact, as of 2005, 50% of newly diagnosed persons with HIV worldwide were youth with an estimated 10.3 million between the ages of 15 and 24. Adolescents will live with HIV 20 years longer than their adult counterparts, but disease progression in them is less understood. It is well known that immune activation is prognostic of chronic disease progression in adults and is often reduced when subjects go on therapy. It is unclear whether immune activation is entirely eliminated in adolescents on therapy. To date, studies of HIV-positive adolescents have been limited by design and methodology, resulting in a lack of research addressing the relationship between outcome of antiretroviral therapy in adolescents and immune activation. To this end, we designed a study to examine the relationship between therapeutic outcomes and immune activation in HIV-positive adolescents. We assembled a cohort of 35 HIV-infected and 12 healthy adolescents and measured immune activation (CD38, CD38/HLA-DR, Ki67) in both baseline and longitudinal data. As expected, cell turnover was greatly increased in memory CD4 T cells over time. We found that levels of immune activation in both CD8 and CD4 T cell subsets was elevated in viremic subjects at baseline and after long-term therapy. One clear distinguishing factor of pathogenic HIV-infection is increased microbial translocation, which can be measured by CD14 release by activated monocytes. We report sustained elevation of sCD14 in viremic adolescents, which directly correlated to immune activation. Our results show that adolescents who are not therapy compliant have elevated immune activation and turnover. Collectively, our results show that in the presence of suboptimal therapy measures associated with disease progression are elevated. Therefore, it is necessary to reconsider current treatment guidelines in adolescents to promote optimal adherence and virologic control in this growing population of HIV-infected individuals.

Degree Type

Dissertation

Degree Name

Doctor of Philosophy (PhD)

Graduate Group

Immunology

First Advisor

Michael R. Betts

Keywords

Activation, adolescents, HIV, T cells

Subject Categories

Allergy and Immunology | Immunology and Infectious Disease | Medical Immunology

**IMPACT OF HAART ON T CELL ACTIVATION IN HIV-INFECTED
ADOLESCENTS**

Danielle Haney

A DISSERTATION

in

Immunology

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2012

Supervisor of Dissertation

Michael R. Betts, Ph.D., Assistant Professor of Microbiology

Graduate Group Chairperson

E. John Wherry, Ph.D., Associate Professor of Microbiology

Dissertation Committee:

Kathleen Sullivan M.D., Ph.D., Professor of Pediatrics

E. John Wherry Ph.D., Associate Professor of Microbiology

Daniel Douek M.D., MRCP, Ph.D., Chief, Human Immunology Section, Vaccine
Research Center, National Institutes of Health

Michael P. Cancro Ph.D., Professor of Pathology and Laboratory Medicine

Yvonne Paterson Ph.D., Professor of Microbiology

DEDICATION

I dedicate this work to my mother, Novelyn, and my grandmother, Louvilla. They are two of the strongest women I have ever known and have been a source of continuous love and support during this entire process.

ACKNOWLEDGMENTS

First I would like to thank my thesis mentor, Mike Betts for helpful discussions, guidance and extreme patience throughout my graduate career. I would like to thank my committee for helpful discussions and suggestions they have provided over the last several years. Lastly, I am grateful to the various members of the Betts Lab who have provided insight and suggestions during my graduate career.

ABSTRACT**IMPACT OF HART ON T CELL ACTIVATION IN HIV-INFECTED
ADOLESCENTS**

Danielle Haney

Michael R. Betts

Every year, HIV-positive adolescents add to the growing number of HIV-infected individuals. In fact, as of 2005, 50% of newly diagnosed persons with HIV worldwide were youth with an estimated 10.3 million between the ages of 15 and 24. Adolescents will live with HIV 20 years longer than their adult counterparts, but disease progression in them is less understood. It is well known that immune activation is prognostic of chronic disease progression in adults and is often reduced when subjects go on therapy. It is unclear whether immune activation is entirely eliminated in adolescents on therapy. To date, studies of HIV-positive adolescents have been limited by design and methodology, resulting in a lack of research addressing the relationship between outcome of antiretroviral therapy in adolescents and immune activation. To this end, we designed a study to examine the relationship between therapeutic outcomes and immune activation in HIV-positive adolescents. We assembled a cohort of 35 HIV-infected and 12 healthy adolescents and measured immune activation (CD38,CD38/HLA-DR, Ki67) in both baseline and longitudinal data. As expected, cell turnover was greatly increased in memory CD4 T cells over time. We found that levels of immune activation in both CD8 and CD4 T cell subsets was elevated in viremic subjects at baseline and after long-term therapy. One clear distinguishing factor of pathogenic HIV-infection is increased microbial translocation, which can be measured by CD14 release by activated monocytes. We report sustained elevation of sCD14 in viremic adolescents, which directly correlated

to immune activation. Our results show that adolescents who are not therapy compliant have elevated immune activation and turnover. Collectively, our results show that in the presence of suboptimal therapy measures associated with disease progression are elevated. Therefore, it is necessary to reconsider current treatment guidelines in adolescents to promote optimal adherence and virologic control in this growing population of HIV-infected individuals.

Table of Contents

CHAPTER 1: INTRODUCTION	1
OVERVIEW	1
HIV HISTORY, BIOLOGY AND PATHOGENESIS	3
IMMUNE RESPONSE TO HIV	5
<i>Humoral Immunity</i>	5
<i>Innate Immunity</i>	6
<i>HIV-specific CD4 T cells</i>	6
<i>HIV-specific CD8 T cells</i>	7
IMMUNE ACTIVATION.....	12
ADOLESCENTS AND IMMUNE ACTIVATION.....	17
THESIS GOALS.....	21
 CHAPTER 2: AN EXAMINATION OF INCREASED CD8+ T CELL ACTIVATION IN HIV+ ADOLESCENTS ON HAART	 23
ABSTRACT.....	23
INTRODUCTION.....	23
MATERIALS AND METHODS	27
RESULTS.....	33
DISCUSSION	41
 CHAPTER 3: ELEVATED IMMUNE ACTIVATION AND TURNOVER IN CD4+ T CELLS OF HIV+ ADOLESCENTS	 48
ABSTRACT.....	48

	vii
TABLE V: ASSESSMENT OF KI67 EXPRESSION IN CD4 T CELL MEMORY SUBSETS	51
MATERIALS AND METHODS	54
RESULTS.....	54
DISCUSSION	62
CHAPTER 4: GAG AND NEF- SPECIFIC CD8 T CELL RESPONSES IN ADOLESCENTS ON HAART	68
ABSTRACT.....	68
INTRODUCTION.....	68
MATERIALS AND METHODS	71
RESULTS.....	73
CHAPTER 5: DISCUSSION AND IMPLICATIONS.....	86
APPENDIX.....	94
BIBILOGRAPHY	97

List of Tables

Table I: Detailed Information on HIV+ Adolescent Subjects.....	104
Table II: Overview of Adolescent Cohort.....	34
Table III: Assessment of CD38 expression in CD8 Effector Cells (CCR7+CD45RO-).....	44
Table IV: Assessment of Ki67 expression on CD8 Central Memory Cells(CCR7+CD45RO+).....	46
Table V: Assessment of Ki67 expression on CD4 T cell memory subsets...	61
Table VI: Assessment of CD38+/HLA-DR- expression on CD4+ T cell subsets	67

List of Figures

<i>Figure 1: Gating Scheme for Activation Markers.....</i>	<i>26</i>
<i>Figure 2: Activation levels in Bulk memory and Naïve populations of CD8+ T cells.....</i>	<i>28</i>
<i>.....</i>	<i>29</i>
<i>Figure 3: Immune Activation is elevated in some T cell subsets</i>	<i>30</i>
<i>Figure 4: Longitudinal assessment of Immune Activation and Turnover.</i>	<i>32</i>
<i>Figure 5: CD4 T cell counts are inversely correlated to viral load.</i>	<i>38</i>
<i>Figure 6: Soluble CD14 expression correlates with biological markers of disease progression.</i>	<i>40</i>
<i>Figure 7: Biomarkers of microbial translocation are associated with viral load and immune activation.....</i>	<i>42</i>
<i>Figure 8: Immune Turnover CD4 T cells memory subsets.</i>	<i>49</i>
<i>Figure 9: Expression of CD38 in Bulk memory and Naïve populations of CD4+ T cells.</i>	<i>53</i>
<i>Figure 10: Expression of CD38 in Memory of CD4 T cell subsets.</i>	<i>55</i>
<i>Figure 11: CD38+HLA-DR+ expression is elevated in some T cell subsets. .</i>	<i>59</i>
<i>Figure 12: Longitudinal assessment of CD38+HLA-DR- expression in CD4 T cells.....</i>	<i>61</i>
<i>Figure 13: Longitudinal assessment of CD38+HLA-DR+ expression in CD4 T cells.....</i>	<i>63</i>

Figure 14: Longitudinal assessment of Ki67+Bcl- expression in CD4 T cells.	
.....	65
Figure 15: HIV-specific CD8 T cell response in adolescents.	70
Figure 16: Gag-specific CD8 T cell response in adolescents.	72
Figure 17: Nef-specific CD8 T cell response in adolescents.	74
Figure 18: Perforin upregulation and polyfunctionality in the total HIV-Specific CD8 T cell response.	76
Figure 19: Perforin upregulation versus polyfunctionality of Gag and Nef-Specific CD8 T cell responses.	78
Figure 20: There is no relationship between age and CD8 T cell parameters.	80
Figure 21: There is no relationship between CD4 T cell percentage and HIV-specific CD8 T cell response.	82
Figure 22: There is no relationship between CD4 T cell count and antigen-specific CD8 T cell response.	85

Chapter 1: Introduction

Overview

Although the immune system is efficient at recognizing and clearing pathogens, certain microorganisms can evade the immune response and ultimately cause death. One such pathogen is Human Immunodeficiency Virus (HIV), which primarily infects and replicates in CD4 T helper cells (Klatzmann et al., 1984b; Douek et al., 2002). HIV replication results in host cell death, latent infection or further infection of surrounding CD4 T cells. To combat infection, CD8 T cells identify and kill target cells by releasing cytotoxic molecules and secreting cytokines (Millard et al., 1984; Podack and Konigsberg, 1984; Podack et al., 1985; Badovinac and Harty, 2006). While CD8 T cells can impact viral replication during acute HIV infection, it is clear from primate studies that HIV/SIV is able to elude the immune system and escape detection from CD8 T cells (Brenchley et al., 2010). As a result of chronic exposure to antigen, CD8 T cells become hyper activated, dysfunctional (Betts et al., 2006; Betts and Harari, 2008), and ultimately exhausted (Day et al., 2006; Yamamoto et al., 2011). As such, the diminished functional capacity of HIV-specific CD8 T cells, coupled with the massive depletion of CD4 T cells renders the HIV-infected host unable to clear HIV infection and combat otherwise benign pathogens.

Over the last thirty years, there have been significant advancements in the design and development of antiretroviral therapies that have allowed HIV-infected patients to gain control of their disease and live relatively normal lives. The most standard course of treatment is highly active retroviral therapy (HAART), a cocktail of drugs that inhibits multiple steps of HIV viral replication. When used effectively, HAART decreases viral

replication and results in partial immune recovery and long term survival. However, in patients on therapy, virus persists in long-lived reservoirs, which are a source of viral replication (Blankson, 2006). Treatment interruption and lapses in adherence allow viral recrudescence that can result in renewed T cell depletion and the appearance of drug resistance mutations that contribute to therapy failure. Thus, HAART ultimately increases the duration of time a patient can survive with the virus, but is incapable of eliminating the viral reservoir within the lifetime of an infected individual (Finzi et al., 1997; Blankson et al., 1999; Finzi et al., 1999). While it is fairly well established how HAART affects viral course in adult HIV-positive subjects, several unanswered questions remain as to how HAART may affect other subject groups, like adolescents and young adults. Some of these questions include: Does HAART effectively reduce levels of immune activation and viral load? If so, are these levels sufficiently reduced to dictate a better disease course? A goal of this thesis is to determine the effectiveness of HAART on viral load and immune activation reduction in adolescents.

Currently, 50% of new incident HIV infections occur in adolescents aged 13-24. Treatment of these subjects is more complex because they are less likely to comply with therapy regimens. Poor adherence to therapy results in increased viral replication, drug mutations and ultimately virologic failure. A large body of work has addressed the effects of HAART in adults, but thus far research in HIV-positive adolescents is limited. Because adolescents will live longer with the disease those infected in adulthood, it is of the utmost importance to understand how their immune systems respond to the virus and how reduced adherence to HAART will impact viral dynamics. The goal of my thesis is to elucidate the effects of HIV on T cell activation and function in HIV+ adolescents.

HIV History, Biology and Pathogenesis

In 1981, an increased incidence of Kaposi Sarcoma (KS), a rare form of benign cancer, appeared in homosexual men in New York (Hymes et al., 1981). Coincident with this increase in KS was the emergence of a rare lung infection, *Pneumocystis carinii*, which appeared in IV drug users and homosexual men (Masur et al., 1981). In addition to pneumonia, infection was associated with substantial depletion of CD4 T cells from the peripheral blood of infected patients. Researchers isolated the infectious agent from the inflamed lymph nodes of individuals and found the agent to possess characteristics common to retroviruses (Barre-Sinoussi et al., 1983; Klatzmann et al., 1984a). Further study revealed that the virus was cytopathic to CD4 T cells (Klatzmann et al., 1984a). This virus is now known as the Human Immunodeficiency Virus (HIV), the virus that causes AIDS (Acquired Immunodeficiency Syndrome). As of 2009, an estimated 33.3 million people are living with HIV/AIDS and 2.6 million people were newly infected. A staggering 1.8 million deaths are attributed to HIV each year (Cohen et al., 2011). Although we know much about the disease and its impact on the body, a successful vaccine has yet to be developed.

In adults, HIV is typically acquired from exposure at mucosal surfaces through unprotected sexual contact or through intravenous or percutaneous inoculations (Cohen et al., 2011). Adolescents acquire HIV through high risk sexual behavior but a large number acquired HIV as infants through mother to child transmission (Catalozzi and Futterman, 2005). Upon exposure, HIV targets and primarily infects CD4 T helper cells, but can also infect macrophages and other antigen presenting cells. In the case of CD4 infection, the HIV envelope protein, gp120 fuses with the CD4 cell surface receptor and either chemokine receptor type 5 (CCR5) (Berson et al., 1996; Deng et al., 1996; Rucker et al., 1996) or CXC chemokine receptor type 4 (CXCR4) (Feng et al., 1996). Once inside

the cell, the virus utilizes the host's cellular machinery to replicate and produce new virions, which bud from the host cell and further propagate in surrounding cells and tissues, including the mucosa and draining lymphoid tissues (Cohen et al., 2011).

The acute phase of infection occurs a few weeks post-transmission and is characterized by high levels of viral replication. Concomitant with massive viral replication is the depletion of mucosal (Brenchley, 2004) and peripheral blood CD4+ T cells (Daar and Ho, 1991). After the initial peak in viremia, HIV viral load decreases to a "set point" of 10^3 to 10^4 copies of viral RNA per milliliter of plasma and typically remains at this level for 5-10 years (Cohen et al., 2011). It was initially thought that set point was directly correlated with progression to AIDS, wherein subjects with high set points progressed to AIDS more quickly than those individuals with lower set points (Mellors et al., 1995). However, it is now known that plasma HIV RNA level is not an independent marker of disease progression (Rodriguez et al., 2006). Although subjects are asymptomatic during chronic infection, there is continuous viral replication and CD4 T cell attrition during this phase. As a result of CD4 T cell depletion, CD4 mediated help to both CD8 T cells and B cells is impaired resulting in decreased killing ability and antibody production, respectively (Rowland-Jones, 2003).

Because the virus is continually present, there is constant pressure on both the innate and adaptive immune systems to produce cytokines, which results in immune hyperactivation. Over time, hyperactivation of CD8 T cells leads to a state of exhaustion, which causes desensitization to antigen (Moir et al., 2011). CD4+ T cell attrition and impaired CD8 T cell function contributes to a dysfunctional immune state. The combination of these factors results in acquisition of opportunistic infections that characterize AIDS and result in death of the host.

Immune Response to HIV

Humoral Immunity

B cells produce antibodies to combat invading pathogens. These antibodies can induce antibody-dependent cell-mediated cytotoxicity (ADCC) or sterically hinder the interaction of the pathogen with an uninfected cell in a process called neutralization. Most vaccines are antibody-based and rely on these antibodies to neutralize the infectious agent. It has recently been suggested that future vaccine design should focus on targeting HIV neutralizing antibodies at mucosal sites in order to block HIV entry (McMichael et al., 2010); however, due to the variability and structure of the HIV envelope, these antibodies would need to be broadly neutralizing to protect against a wide variety of HIV strains. There have been several studies in monkeys where the administration of broadly neutralizing antibodies at mucosal sites has led to protection against subsequent simian immunodeficiency virus-HIV chimera (SHIV) challenge (Hessell et al., 2009); however, similar results have yet to be achieved with humans. In fact, there are several limitations in protections from broadly neutralizing antibodies including rapid escape mutations (Richman et al., 2003; Wei et al., 2003; McElrath and Haynes, 2010) induction of non-neutralizing or neutralizing strain-specific antibodies (Kwong et al., 2002) and antibody binding-induced conformational changes (Kwong et al., 2002). There have, however, been recent studies showing that some HIV infected individuals can develop a range of neutralizing antibodies, a function that was previously thought to be difficult (McElrath et al., 2008; Stamatatos et al., 2009; Mikell et al., 2011). Therefore, future studies should continue to address ways to overcome structural and biological impediments on the effectiveness of broadly neutralizing antibodies for use in an HIV vaccine.

Innate Immunity

Natural Killer (NK) cells, characterized by their large granular structure, are a major arm of the innate immune system. NK cells are multifunctional and make up ~10% of the circulating blood population (Herberman et al., 1975; West et al., 1977). Unlike T and B cells, these cells do not express antigen receptors but do express multiple activating and inhibitory receptors, such as killer cell immunoglobulin like receptors KIRs (Vilches and Parham, 2002) in response to pro-inflammatory stimuli, NK cells migrate to the site of infection where, much like CD8 T cells, they release cytolytic molecules including perforin, granzymes, and granulysin (Timonen et al., 1981). NK cells also produce IFN- γ which activates macrophages, promotes CD4 Th1 differentiation and has several anti-viral effects (Cooper et al., 2001).

Recently, it has been shown that NK cells play a role in the defense against HIV. Expression of KIR3DS1, an activating KIR gene, and its ligand, Bw4 is associated with slower HIV-1 disease progression (Martin et al., 2007). NK cells lyse infected target T cells during HIV infection, although this lysis effect is somewhat weak and dependent on the presence of NK surface inhibitory receptors (De Maria et al., 2003; Mavilio et al., 2003) Furthermore, chemokines secreted by NK cells can block viral entry *in vitro* (Costa et al., 2003). Additionally, elevated antibody dependent cellular toxicity, an important function of NK cells, is correlated with lower risk of death when controlling for outside factors like CD4 T cell count and viral load (Forthal et al., 2001a; Forthal et al., 2001b; Bernstein et al., 2004)

HIV-specific CD4 T cells

CD4 T cells are an integral part of an effective immune response and are often referred to as T helper cells. They promote immune control by providing help to CD8 T cells and B cells. However, HIV-specific CD4 T cells are preferentially infected and killed

by the virus (Douek et al., 2002). Severe depletion of CD4 T cells during HIV infection is tightly linked to opportunistic infections in AIDS patients and increased dysfunction in the CD8 T cell compartment (Kalams et al., 1999a; Altfeld and Rosenberg, 2000). Therefore, several groups have sought to determine the relationship between HIV-specific CD4 T cell responses and viral load control.

HIV subjects can be segmented into two separate groups, those who control viral replication and individuals who do not (progressors)(Pereyra et al., 2010). Several researchers have attempted to understand qualitative and quantitative differences in CD4+ T cell responses between progressors and controllers. The frequency of CD4 T cells is inversely correlated to viral load but the ability for HIV-specific CD4 T cells to proliferate has been identified as one of the key factors in viral control (Rosenberg et al., 1997; Pitcher et al., 1999; Wilson et al., 2000). Indeed, CD4 T cell proliferative capacity is diminished in patients with high viral load (Gea-Banacloche et al., 2000; Iyasere et al., 2003) and is often restored upon initiation of HAART (Autran et al., 1997a; Emu et al., 2005). In addition to proliferative capacity, HIV-specific CD4 cells also secrete cytokines in response to virus; therefore, several studies have focused on identifying cytokine expression patterns that may correlate to protection. Thus far, it has been established that long-term non-progressors (LTNP) possess HIV-specific CD4 T cells that produce both IL-2 and IFN- γ whereas subjects who cannot control the disease do not (Boaz et al., 2002; Harari et al., 2004). It is unclear if diminished functionality observed in HIV-specific CD4 T cells from progressors actually causes AIDS. More than likely low functionality could be a consequence of the immune state during progression.

HIV-specific CD8 T cells

In 1986, HIV-specific CD8 T cells were identified in seropositive individuals but their potential role in combating HIV disease progression was not known (Walker et al.,

1986). Two separate groups reported initial evidence for HIV-specific CD8 T cell mediated control (Borrow et al., 1994; Koup et al., 1994). These studies showed a temporal association between HIV-specific CD8 T cell expansion and the resolution of acute viremia. Individuals with persistent viremia and lower CD4 T cell counts were less likely to have high HIV-specific CD8 T cell counts, suggesting a direct role for HIV-specific CD8 T cells in disease progression. To extend these findings and directly test the hypothesis that CD8 T cells were necessary for viral control, researchers carried out experiments in rhesus macaques using the simian immunodeficiency virus (SIV) model. Using anti-CD8 monoclonal antibodies to deplete CD8 T cells *in vivo*, they showed that CD8-depleted primates were unable to control viremia, but upon restoration of CD8 T cell numbers the ability to control SIV replication was re-established (Jin et al., 1999; Schmitz et al., 1999). This initial work in humans and monkeys demonstrated a potential role for T cell based vaccines and motivated the field to determine potential mechanisms of control mediated by these cells.

Further evidence for HIV-specific CD8 T cell control in HIV infection is the link between MHC Class I allele expression and HIV disease progression. Expression of certain MHC Class I alleles can often predict disease outcome. Subjects who are homozygous at any of the 3 HLA Class I loci (HLA-A, HLA-B, HLA-C) typically progress more rapidly to AIDS than those who are heterozygous (Goulder et al., 1997; Carrington et al., 1999). This heterozygosity is thought to increase the breadth of recognized viral epitopes resulting in better CD8 T cell-mediated control than subjects who are homozygous. However, in certain examples a narrowly directed HIV-specific CD8 T cell response can be effective at controlling viremia over time (Goulder et al., 1997). Subjects who maintain superior control of virus in the absence of therapy express HLA-B27 and B57 alleles (Kaslow et al., 1996; Migueles et al., 2000; Emu et al., 2008). CD8 T cells

recognize peptides presented by MHC Class I molecules therefore the association between certain MHC alleles and viral control further implicates CD8 T cells in the control of viremia.

CD8 T cell-mediated recognition of virus-infected cells induces rapid selection for viral escape mutants. The first evidence for CD8-mediated escape was shown in 1997 by several groups (Borrow et al., 1997; Goulder et al., 1997; Price et al., 1997). In two of these studies, escape was shown to occur during acute infection. Additionally, CD8 T cell escape was also shown to occur late in infection even after effective long-term control of virus and was associated with progression to AIDS. Subsequent studies in the SIV model have supported that CD8 escape occurs in the chronic (Allen et al., 2000; O'Connor et al., 2002) and acute (Evans et al., 1994; Evans et al., 1999) phase of infection. Therefore the evidence that HIV escapes from CD8 T cell mediated pressure is vast. Interestingly, escape mutations often confer negative fitness cost to the virus and may precede effective suppression of viremia.

The expansion of HIV-specific CD8+ T cells and the concomitant decline of viremia has been one of the most convincing arguments for CD8 T cell control of viral replication. Several groups chose to determine if the control induced by HIV-specific CD8 T cells was the result of quantitative or qualitative events. MHC Class I tetramer-based studies demonstrated that the frequency of HIV-specific CD8+ T cells inversely correlated with viral load (Ogg et al., 1998). These results were limited to A2-restricted Gag and Pol-specific CD8 T cell responses and therefore were not reflective of the entire response to HIV. A more comprehensive study went on to establish that there was a positive correlation between viral load and the total HIV-, Env- and Nef-specific CD8 T cell frequency (Betts et al., 2001a). Conversely, other studies reported certain responses to viral proteins inversely correlated to viral load (Edwards et al., 2002). Therefore, it

can be seen that early studies involving a link between frequency and function of CD8 T cells and plasma load were inconclusive. Moreover, the total frequency of HIV-specific CD8 T cells did not explain differential control of viral load in controllers versus progressors.

In 2006, Betts et al. employed the use of polychromatic flow cytometry to characterize the CD8 T cell response in non-progressors. They found that subjects maintaining low viral loads possessed highly functional HIV-specific CD8 T cells. Conversely, the frequency and proportion of HIV-specific CD8 T cell response with the highest functionality inversely correlated with viral load in the progressors (Betts et al., 2006). Furthermore, HIV-specific CD8 T cells in non-progressors were superior in their ability to proliferate and degranulate upon antigen encounter. This study was the first to determine that the quality of the functional response was a better correlate of HIV disease progression (Betts et al., 2006). Although a single correlation factor has yet to be identified, perforin-mediated killing and proliferative capacity is enriched in patients who control viral load (Migueles et al., 2002; Migueles et al., 2008; Hersperger et al., 2010a) suggesting that HIV-specific CD8 T cells from subjects who maintain killing function are most effective at suppressing viral replication.

Finally, when cellular functionality is altered, the importance of CD8 T cells becomes evident. In studies using LCMV, a murine model of acute and chronic viral infection, it was revealed that CD8 T cells become exhausted after prolonged antigen exposure (Blackburn et al., 2009). Exhaustion is characterized by diminished function, proliferation of CD8 T cells and the expression of exhaustion markers like PD-1, a negative regulator of activated T cells. Recently, studies in HIV-positive humans have shown that HIV-specific CD8 T cells expressing higher levels of PD-1 also have impaired effector function and correlate with higher viral loads (Day et al., 2006; Petrovas et al.,

2006; Younes et al., 2007). However, it is becoming increasingly clear that expression of multiple inhibitory receptors indicates an exhausted phenotype (Yamamoto et al., 2011) in chronically infected humans. Future studies are necessary to determine the contribution of immune exhaustion to HIV disease progression.

The role HIV-specific T cell responses in adolescents

HIV-specific CD4 and CD8 T cells play an integral part in the reduction in viral replication and progression to HIV (Rosenberg et al., 1997; Betts et al., 2001a; Boaz et al., 2002; Pantaleo and Koup, 2004; Betts et al., 2006; Duvall et al., 2006). In HIV-positive adults, it is known that the presence of polyfunctional CD8 T cell responses are correlated with reduced viral load (Betts et al., 2006). Adults who have a higher viral load and progress to AIDS are more likely to have a less polyfunctional CD8 T cell response (Betts et al., 2006). While HIV-specific T cell responses have been fairly well characterized in adults, the role of these responses in adolescents remains unclear.

The degree of T cell functionality or the ability of the T cell to respond to antigenic stimulation is related to age (Buseyne et al., 2005a; Prendergast et al., 2011). For HIV-positive newborns, an immature immune system combined with poor antigen presentation impairs the generation of HIV-specific T cells during the first months of life (Buseyne et al., 1998). As a consequence, untreated children have high levels of persistent viremia, which promotes immune activation and decreases the chance for recovery post-therapy initiation. For children on therapy, as well as adults, viral suppression is often associated with a decreased frequency of HIV-specific IFN- γ producing CD8 T cells (Buseyne et al., 2005a). When responses are detected, it is the frequency of gag-specific CD4 and CD8 T cell responses that are associated with virologic control in infants and children (Prendergast et al., 2011). In HIV-positive adolescents antigen-specific CD8 T cell functional responses are even less studied than in children.

When the functionality of CD8 T cells in adolescents has been examined, it is often studied and analyzed in conjunction with T cell functionality in children from 0-9 years of age making it difficult to draw conclusions about the contribution of these responses to viral control

Immune Activation

A hallmark of HIV infection is severe cellular depletion in the CD4 T cell compartment (Hunt et al., 2008). Initially, it was believed that CD4 T cell depletion was due to direct killing of infected CD4 T cells, but the widespread immune damage was far too severe to be solely attributed to HIV-induced CD4 T cell destruction. For this reason, several groups sought to determine the non-antigen specific mechanisms that caused CD4 T cell attrition. In the early 90s, several groups showed that HIV induced immune activation primarily impacted uninfected bystander cells leading to apoptotic cell death (Groux et al., 1992; Finkel et al., 1995). It is now understood that chronic widespread immune activation is the distinguishing characteristic of pathogenic HIV infection (Giorgi et al., 1993; Liu et al., 1997). Immune activation is the key difference between pathogenic and non-pathogenic infection. For example, non-progressive infection is characterized by high viral load, controlled immune activation and a lack of progression to AIDS. In contrast, progressive infection is characterized by uncontrolled immune activation and disease progression (Van Rompay et al., 2004). Immune activation is characterized by accelerated proliferation, exhaustion, and T cell depletion that results in reduced regenerative capacity of the immune system. Administration of antiretroviral therapy reduces immune activation through direct inhibition of HIV viral replication. Importantly, reducing immune activation results in a more favorable disease outcome (Kalams et al., 1999b; Hunt et al., 2003a).

There are several markers that have been used to identify T cell activation *in vitro*, including CD38, HLA-DR, CD25, CD69, CD70, tumor necrosis factor receptor type II and Beta2-microglobulin (Liu et al., 1997). CD38/HLA-DR co-expression was one of the earliest combinations of activation markers identified to directly correlate with increased viral loads (Giorgi et al., 1993). Elevated levels of CD38 have been directly associated with a higher propensity to develop AIDS than those with lower CD38 levels (Liu et al., 1997). Moreover, elevated levels of CD38+CD8+ T cells were found to be better predictors of disease progression than viral load or CD4 T cell count (Wilson et al., 2004). Further, upon therapy initiation, the levels of CD38 in the blood and lymph node decreased coincident with plasma viremia albeit not to levels similar to those at baseline (Benito et al., 2004; Yang et al., 2005).

Increased T cell turnover is now known to be a consequence of increased immune activation during HIV infection. However, it was previously debated whether the rapid rise in CD4 counts in the blood after HAART initiation was due to this process. Some groups postulated that the rise in CD4 T cell counts was largely due to lymphocyte redistribution and not a result of increased turnover (Sprent and Tough, 1995). Supporting this hypothesis, CD4 T cell telomere length in HIV infected persons was not found to be significantly shorter than that of their uninfected counterparts suggesting that these cells were not undergoing rapid turnover (Wolthers et al., 1996). To directly address if T cell turnover occurs during HIV infection, several groups employed the SIV-rhesus macaque model (Mohri et al., 1998; Rosenzweig et al., 1998). Using Bromodeoxyuridine (BRDU), a dye that incorporates into the DNA of proliferating cells, T cell turnover was quantified in living tissues. Animals with high viral loads had increased BRDU incorporation as well as higher rates of decay in CD3+CD4+, CD3+CD4-, CD3-CD8+CD16+ and CD3-CD20 cells, suggesting that increased turnover

occurs in each lymphocyte population. These results were further supported by the observation that CD4 and CD8 T cell turnover rates were 2-3 fold higher in infected primates (Mohri et al., 1998; Rosenzweig et al., 1998). Taken together, these data showed that there was, in fact, increased turnover during HIV-like infection in each lymphocyte subset.

Studies were extended to humans using deuterated glucose (D-glucose) to determine relative rates of turnover *in vivo*. Similar to findings in rhesus macaques, CD4 and CD8 turnover rates in HIV-infected subjects decreased over time with therapy (Hellerstein et al., 1999; Mohri et al., 2001). These results were further confirmed in human *in vitro* studies using Ki67, a marker of proliferation and cell cycle entry. The percentage and absolute number of Ki67+ CD4 and CD8 T cells was elevated in infected subjects compared to uninfected counterparts (Hazenberg et al., 2000c). After HAART therapy, proliferation of CD8 and CD4 T cells decreased, as did viral loads. Theoretically, increased T cell turnover could be a reflection of homeostatic proliferation driven by cell losses induced by the virus. In this study however there was an initial decrease in CD4 T cell count after therapy despite the relative low initial numbers. This suggests that proliferation is a result of immune activation and not homeostasis.

Causes of Immune Activation

To date, the underlying cause of immune activation in HIV-positive humans remains unclear, however, a combination of factors have been implicated as contributing to activation. Firstly, the virus itself is thought to promote immune activation through the binding of gp120 to CD4 or CCR5 (Decrion et al., 2005). This binding event results in increased intracellular signaling thus promoting cellular activation and further infection. Indeed, studies have shown that the addition of CCR5 antagonists result in decreased immune activation (Ahuja et al., 2008) and that elevated CCR5 surface density is linked

to reduced CD4 T cell restoration (Vincent et al., 2006). Additionally, host innate immune responses have been implicated. Macrophages, DCs, and NK cells release pro-inflammatory cytokines like TNF, IL-6, and IL1- β during infection. These cytokines activate adaptive immune responses resulting in HIV-specific and bystander cell activation (Engram et al., 2010). Furthermore, massive CD4 T cell depletion during HIV leads to suboptimal control of persistent viruses like CMV and EBV. Reactivation of these viruses exacerbates the immune response and the inflammatory environment (Bafica et al., 2004). Finally, CD4 T cell depletion during HIV infection occurs primarily in the gut associated lymphoid tissues (GALT) and is associated with the disruption of the mucosal barrier (Brenchley et al., 2004). This causes the translocation of microbial products from the intestinal tract, resulting in detection by the immune system. These by-products, including LPS and CpG, activate immune cells through TLR ligation and contribute to chronic immune activation (Brenchley et al., 2006). Taken together, these studies suggest that one single factor does not contribute to the activated environment during HIV infection but it is most likely a combination of factors that contributes to overall activation.

HAART: Highly Active Antiretroviral Therapy

Presently, the most effective therapy for HIV infection is HAART, a cocktail of different viral inhibitors that each prevent specific steps in viral replication from occurring. These drug classes are: Entry Inhibitors, Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors (PI), and Integrase Inhibitors (II). Entry inhibitors bind to the cell surface receptor, CCR5, and block the fusion of HIV GP120 with the host cell membrane (Lalezari et al., 2003; Root and Hamer, 2003; Liu et al., 2007). The second class of inhibitors (NRTIs) interferes with the reverse transcription step of HIV (St Clair

et al., 1987; Roberts et al., 1988). Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are analogues of deoxynucleotides that are needed to synthesize DNA, but lack the 3'hydroxyl chain, and when incorporated into the growing cDNA genome, result in termination of replication (Jeffries, 1989; Merluzzi et al., 1990; Richman et al., 1991; Hsiou et al., 1998). The third class of viral inhibitors target HIV Protease. Protease inhibitors prevent the cleavage of viral proteins, which are translated as polyproteins, and thus interfere with replication within the host cell (Billich et al., 1995; Reedijk et al., 1995). The final class of inhibitors target HIV integrase. These inhibitors prevent the action of HIV integrase which functions to integrate the viral genome into the host genome allowing production of new viruses (Hazuda et al., 2000; Hazuda et al., 2004; Steigbigel et al., 2008).

While HAART is efficient at inducing low to undetectable viral loads, it is essential that individuals are compliant with their therapy regimens. Without a rigid schedule of medication, HIV viral load is able to rebound, resulting in increased immune activation and decreased CD4 T cell counts and disease progression (Karlsson et al., 2004; Hunt et al., 2006; Agwu et al., 2008). Additionally, while HAART intervention contributes to overall quality and duration of life, it is not a cure and should not be viewed as a perfect solution. When compared to both HIV-negative and long-term non-progressor controls, CD4 T cell counts and T cell activation remain abnormal after therapy intervention (Benito et al., 2004; Gandhi et al., 2010). In fact, while HAART does control viral load, it does not eliminate the virus completely, for the viral reservoir does not appear to decay effectively during therapy (Chun et al., 1997; Finzi et al., 1997; Finzi et al., 1999; Lassen et al., 2006). Residual viremia affects immune recovery by causing elevated immune activation even while patients are on therapy (Karlsson et al., 2004). For these reasons, future studies are needed to determine the cause of prolonged

immune activation and its lasting impact on immune recovery even after therapy intervention.

Adolescents and Immune Activation

Overview

Compared to adults, adolescents will live with HIV on average 20 years longer than infected adults, but disease progression in them is less understood. While adolescents are commonly infected as infants via vertical transmission (mother-child) or through breast milk, new advances in therapy have reduced the rate of transmission from mother to child. In recent years, an increasing number of adolescents are also acquiring infection through behavioral routes, such as risky sexual acts and drug use. In fact, as of 2005, 50% of newly diagnosed persons with HIV worldwide are youth with an estimated 10.3 million between the ages of 15 and 24 infected. While antiretroviral therapies have proven successful at increasing lifespan, effective treatment of adolescents is particularly challenging because of their unique psychosocial issues, including gender power imbalance in sexual relationships, lack of safe sex education, perceived HIV disease stigma, and limited access to healthcare (Futterman, 2005). These factors can have a significant impact on the efficacy of therapeutic strategies to target the virus in adolescents, and thus must be carefully considered, because of the link between adherence to therapy and drug resistance in HIV infection.

T cell turnover and Immune Activation in Adolescents

Maintenance of adequate T cell numbers and diversity of the T cell repertoire are primary requirements for a functional immune system. During HIV infection, T cells are constantly being destroyed causing an imbalance in the T cell population, which leads to immune dysfunction and increased susceptibility to opportunistic infections. The thymus generates new cells which helps to replenish the T cell repertoire. For individuals

infected with HIV, this feature of the immune system can help restore the cellular compartments that were destroyed by the virus. This regenerative capacity is most robust in children and continually declines as the individual ages (Mackall et al., 1995; Douek et al., 1998; Vigano et al., 2000b).

During the typical HIV disease course, the percentage and number of CD4 T cells declines over time whereas CD8 T cell counts either remain stable or rise (Ibegbu et al., 1994; Rabin et al., 1995). When individuals initiate HAART, memory and naïve CD4 T cell counts increase and CD8 T cell numbers normalize. In HIV-infected adults, CD4 T cell levels do not return to pre-infection levels even if viral replication is controlled with therapy (Autran et al., 1997a; Surh and Sprent, 2008). Compared to adults on therapy, HAART-treated adolescents have higher recovery rates in naïve and memory T cell populations, suggesting that increased thymic capabilities in younger subjects promote immune restoration (Cohen Stuart et al., 1998a; Sleasman et al., 1999).

To date there are few definitive studies on the effects of HAART on turnover in HIV-positive adolescents. Using Ki67 as a marker of proliferation to determine relative turnover, studies have shown that HIV-positive adolescents have higher Ki67-expressing naïve and memory T cell subsets than healthy controls, with the highest levels of Ki67 being in the naïve CD8 T compartment (Flynn et al., 2007). Additionally, HIV-infected adolescents have significantly lower numbers of CD4 T cells than controls (Rudy et al., 2002). In one report, increased Ki67 expression correlated with CD38/HLA-DR co-expression on CD8 T cells suggesting that increased rates of proliferation were driven by increased immune activation (Starr et al., 2002b). There was no correlation or relationship found between Ki67 expression and therapy initiation. A possible explanation of this relationship is that therapy in adolescents is ineffective at decreasing turnover. Findings resulting from well defined cohorts, accurately defined activation

parameters, and T cell subsets will prove beneficial in determining therapeutic effects on the adolescent immune system.

Although lymphocytes rebound following therapy in HIV infected adolescents, continued immune activation may negatively impact lymphocyte recovery. A link between immune activation and T cell recovery following HAART in HIV-positive adolescents can be found in a study designed to evaluate predictors of CD4 T cell loss. In this study they measured CD4 T cell counts and markers of immune activation (CD38, Ki67) at baseline and up to week 60 following therapy initiation (Rudy et al., 2006b). A subset of individuals with CD4 counts over 300 cells/ μ l reached viral suppression by week 24. After 24 weeks on therapy, adolescents showed significant increases in CD4 T cell counts and decreases in CD8 T cell counts. However, these individuals were less likely to sustain this suppression if they had elevated levels of activated CD38/HLA-DR expressing CD8 T cells (Rudy et al., 2006a), suggesting that the advantage of higher CD4 counts prior to therapy (Perez et al., 2001) is lost in the presence of immune activation. Indeed, children with lower levels of CD38/HLA-DR CD8 T cells have moderate increases in CD4 T cell counts and lower viral loads (Borkowsky et al., 2000; Starr et al., 2002a). Taken together these studies demonstrate the impact of T cell activation on T cell recovery rates and virologic control in youth. They also suggest that it is necessary to reduce immune activation while on therapy to maintain viral suppression and recover CD4 T cell counts.

Antiretroviral Treatment in Adolescents

Antiretroviral treatment failure and subsequent drug resistance are major obstacles in long-term management of disease in HIV-infected youth. Adolescents who receive HAART have low adherence to therapy with rates ranging from 29-50% (Douglas et al., 2000; Rudy et al., 2001; Agwu et al., 2008a). These rates are substantially lower

than the 90-95% adherence rates necessary for successful long-term viral control and prevention of drug resistance. Consequently, only 58% of youth reach viral suppression on therapy at week 24 of treatment compared to 80-90% of adults (Rudy et al., 2006b). As a result, a growing number of HIV-positive adolescents on HAART will likely undergo viral breakthrough and subsequent drug resistance.

Effective strategies aimed at adolescents must preserve immunity, have a low dosing frequency, a low risk for therapy-associated toxicity, and a minimal risk for drug resistant viruses. As discussed previously, two major drug regimens are NNRTI or PI based. Both of these regimens are equally effective in early therapy, however, these regimens are not perfect. For example, increased drug resistance during NNRTI treatment results in persistent viral variants that can be detrimental in long-term patients (Cohen Stuart et al., 1998; Agwu et al., 2008a). Even after subjects are found to be resistant to therapy, they often continue on the same regimen (Gortmaker et al., 2001). The addition of PI to the NNRTI regimen has been successful at reducing drug mutations in adults, so several groups turned their attention to investigating the effects of different combination therapy in children and adolescents (Hammer et al., 1997; Palella et al., 1998). A three-year prospective study in children (age birth-20) showed a 67% reduction in risk of death when protease inhibitors were used (Gortmaker et al., 2001). Adolescents undergoing NNRTI treatment were more prone to drug resistance mutations than adolescents on protease inhibitors alone (Agwu et al., 2008b). Furthermore, since adolescents usually remain on NNRTI therapy, more mutations are likely to occur. However, while protease inhibitor therapy may be less prone to drug resistance, there are long-term metabolic side effects that should not be ignored. Therefore, careful consideration must be made when choosing therapeutic regimens for adolescents. Future studies should carefully examine the effect of different drug

combinations on immune activation, which is the strongest predictor of disease progression.

Microbial Translocation in HIV+ Adolescents

As discussed previously, microbial translocation is thought to be the driving force of immune activation in adults, but the role of microbial translocation has been understudied in HIV-positive adolescents. In HIV-positive infants other markers of microbial translocation such as sCD14, LBP and Endocab are increased in infants who delay treatment during the first six months of life compared to their HIV-exposed counterparts (Papasavvas et al., 2011). Soluble CD14, a marker of macrophage activation and increased microbial translocation, has been cited as the strongest predictor of death in adult HIV studies (Sandler et al., 2011). Therefore, increased levels of sCD14 even in the absence of immune activation could signify poor disease outcome. A recent study in children aged 0-17 years cited increased LPS and sCD14 levels in infected subjects compared to controls (Wallet et al., 2010) and, in contrast to adult reports, neither of the studies discussed found an association between immune activation and microbial translocation (Brenchley et al., 2006). Due to the long-term nature of the disease in adolescents and children, future studies should work to elucidate the association of sCD14 with HIV disease progression.

Thesis Goals

Adolescents comprise an alarming portion of new HIV cases yearly. Moreover, the duration of their disease will be much longer than that of their adult counterparts. They will also be exposed to the effects of immune activation for the duration of their lifetime. It is unclear whether this immune activation is entirely eliminated even in adolescents that strictly adhere to therapy let alone in the large number of adolescents who do not adhere. To date, studies of HIV-positive adolescents have been limited by

design and methodology resulting in a lack of research addressing the relationship between outcome of antiretroviral therapy in adolescents and immune activation in this patient group.

In order to address this deficit in research of HIV-infected adolescents and to examine the relationship between therapeutic outcomes and immune activation in adolescents, we assembled a cohort of HIV-infected HAART-treated subjects and measured common markers of immune activation on lymphocytes from these subjects. We first chose to investigate the affects of HAART therapy on the activation levels of CD4 T cells and CD8 T cells. To this end, we took a novel approach by examining the simultaneous expression of CD38, HLA-DR and Ki67 in the cohort. To date, most studies have been defined using naïve versus memory analysis and have been limited to 3-color flow cytometry. In my studies, we have taken advantage of polychromatic flow cytometry, which allows the examination of up to 18 colors simultaneously. Using this technology, we investigated the expression of activation markers on multiple memory T cell subsets with the goal of determining if viral load preferentially correlates with activation, as well as, memory T cell definition, and microbial translocation. In addition to studying the effects of HAART on activation in adolescents this body of work also addresses the CD8 T cell response to HIV in adolescents. The goal of this thesis is to increase the understanding of the capacity of the adolescent immune system and its response to therapy, with the ultimate goal of informing future therapy regimens in this long-term patient population.

Chapter 2: An examination of Increased CD8+ T cell Activation in HIV+ adolescents on HAART

Abstract

In 2006, 50% of new HIV infections occurred in adolescents aged 13-24. In adults, immune activation directly correlates to HIV disease progression; however, immune activation in adolescents is not completely understood. We characterized immune activation markers on CD8 T cells from a cohort of adolescent subjects including 15 viremic (>50 copies RNA/ml), 17 aviremic (<50 copies RNA/ml), and 12 HIV uninfected subjects. We used polychromatic flow cytometry to define the immune activation profile of CD8+ T cells by measuring simultaneous expression of CD38, HLA-DR, Ki67 and Bcl-2. We observed increased immune activation on total memory, effector (CCR7-CD45RO-), effector memory (CCR7-CD45RO+) and central memory (CCR7+CD45RO+) CD8 T cell subsets. The frequency of CD38+CD8+ effector T cells directly correlated with viral load ($p=.0366$). However, we did not find a correlation between viral load and CD4 T cell counts. Compared to aviremic subjects, sCD14 levels were higher in viremic subjects and were correlated with CD38 expression on central memory, effector and effector memory populations. Taken together, these results show that there is preferential activation across memory subsets and a direct relationship between viremia, activation and sCD14 levels in HIV+ adolescents.

Introduction

Adolescents and young adults comprise roughly 50% of new HIV infections in the United States (Futterman, 2005) yet current therapy guidelines for this population are based on adult standards. Psychosocial issues in young adults make it difficult for this

Table II: Overview of Adolescent Cohort

	Viremic	Aviremic	Healthy	Healthy Donors for sCD14 measurement
# of Subjects	15	17	12	43
Plasma RNA (median)	6,790 copies RNA per/ml	< 50 copies/RNA/ml	N/A	N/A
CD4 T cell count (mean)	505 cells/ml	732 cells/ml	Not determined	Not determined
CD8 T cell count (mean)	985 cells/ml	886 cell/ml	Not determined	Not determined
Sex	6 males 13 females	10 males 6 females	6 male 6 female	28-male 15-female
Mode of Transmission	4 Behavior 11 Perinatal	2 Behavior 15 Perinatal	N/A	N/A

population to adhere to therapy guidelines (Futterman, 2005) Despite the numerous reports in children and adults, few studies have addressed the successful control of HIV by HAART therapy in adolescents. It has been suggested that adolescents have more thymic capacity than adults (Douek et al., 1998; Douglas et al., 2000). For example, adolescents have higher naïve CD8 and CD4 T cells counts, which may allow for favorable response to therapy (Douglas et al., 2000; Starr et al., 2002a). In fact, it has been shown that individuals with higher pre-treatment CD4 T cell counts are likely to rebound cell numbers close to that of aviremic controls (Perez et al., 2001). Adolescents will be lifelong carriers of the disease; therefore, it is of the utmost importance to understand the effectiveness of current therapies in this expanding population to determine if it is necessary to restructure their treatment regimens.

Immune activation has been identified as a better predictor of disease progression than viral load (Rodriguez et al., 2006) or CD4 T cells counts in HIV-infected adults (Giorgi et al., 1993; Liu et al., 1997; Giorgi et al., 2002; Rodriguez et al., 2006). Immune activation is commonly assessed by measuring expression of Ki67, CD38 or co-expression of CD38/HLA-DR (Giorgi et al., 1993; Sachsenberg et al., 1998a; Hazenberg et al., 2000c). Higher CD38 and HLA-DR memory CD8 T cells in HIV-infected adults is predictive of slower CD4 T cell recovery rates during HAART (Hazenberg et al., 2000c). Long-term therapy reduces the absolute number of CD38+ CD8 T cells; conversely, an increase in CD38 expressing CD8+ cells correlates with progression to AIDS (Giorgi et al., 1993; Liu et al., 1997). Studies in adolescents have revealed analogous results (Starr et al., 2002a; Rudy et al., 2006a). However, these previous adolescent studies have been limited to single activation parameters and examination of total memory (RO+) cells. Qualitative or quantitative differences in

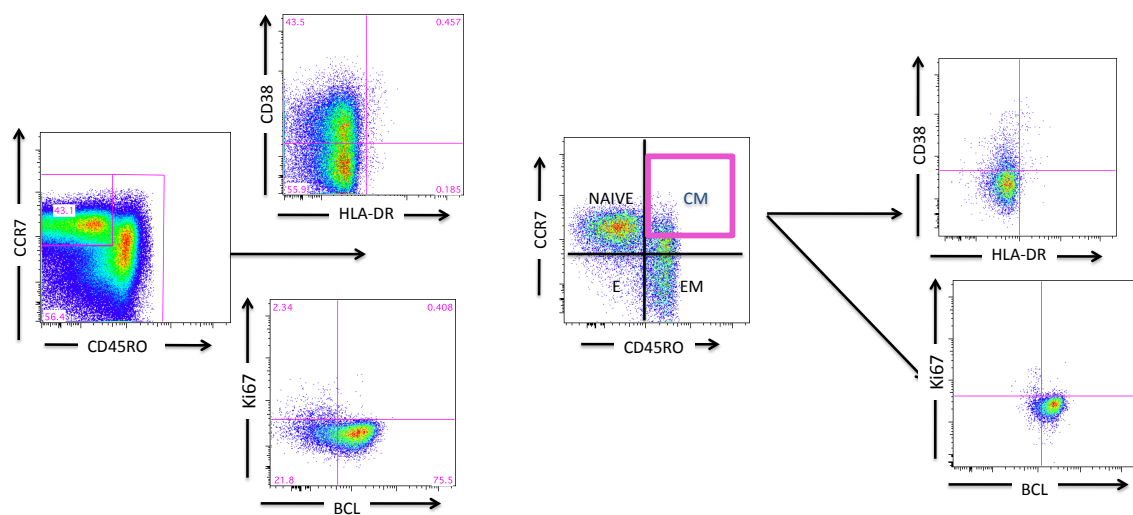


Figure 1: Gating Scheme for Activation Markers A) CD8 or CD4 cells were gated into naïve (CCR7+CD27+CD45RO-) and bulk memory subsets B) memory subsets were then further subdivided to assess expression of the markers Ki67, HLA-DR, CD38, Bcl-2

immune activation between memory subsets could have important implications for disease mediated by HIV in adolescents.

While the definitive causes of chronic immune activation remain unclear, decreased integrity of the intestinal wall during chronic HIV infection in adults has been associated with higher levels of circulating bacterial products such as lipopolysaccharide (LPS) (Brenchley et al., 2006; Papasavvas et al., 2009b; Nixon and Landay, 2010). One means to detect the effect of LPS induced activation is to measure circulating levels of soluble CD14 (sCD14) (Lien et al., 1998). It has been shown that increased LPS directly correlate to increased CD38 expression (Brenchley et al., 2006; Nixon and Landay, 2010). Furthermore, increased sCD14 has been directly correlated to an increased risk of mortality in adult clinical studies (Sandler et al., 2011). To date an examination of the interplay of immune activation, CD4 T cell depletion, CD8 T cell activation and sCD14 has yet to be carried out in adolescent subjects.

Here we examined the link between immune activation and viral control in HIV-infected, HAART treated adolescents by analyzing Ki67, HLA-DR, CD38 and Bcl-2 expression on well-defined CD8+ T memory cell subsets. Our data show that as a consequence of inadequate therapy adherence adolescents sustain increased viral load and immune activation. This work enhances our understanding of the effectiveness of current therapy and informs regimen design in HIV-positive adolescents.

Materials and Methods

2.1 Cells: The cohort consisted of 44 HIV-infected adolescents in the following groups: 15 viremic (>50 copies RNA/ml), 17 aviremic (<50 copies RNA/ml), and 12 HIV uninfected subjects (Table I and Table II: Appendix). 43 additional Healthy subjects were acquired

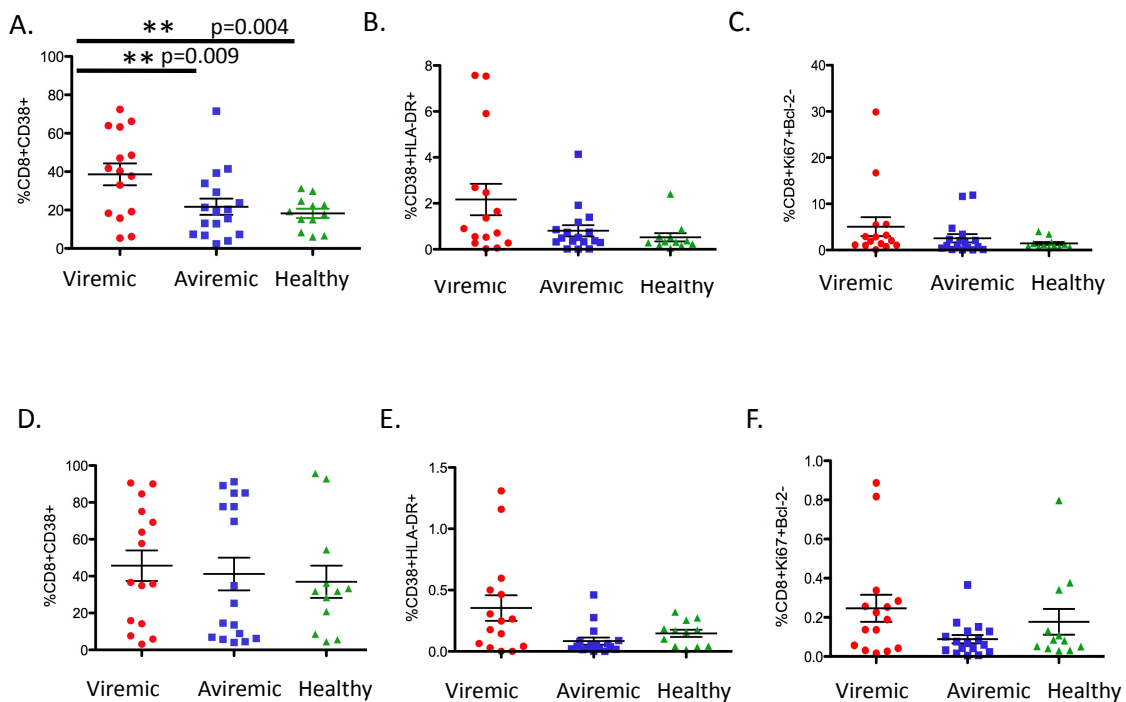


Figure 2: Activation levels in Bulk memory and Naïve populations of CD8+ T cells. Human PBMCs were thawed and stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and activation markers (CD38, HLA-DR, Ki67, Bcl-2) A) CD38 B) Ki67 C) CD38+HLA-DR+ expression was assessed on non-naïve CD8+ T and naïve (D-F) cells using non-parametric t-tests

for soluble CD14 titers in healthy adolescents. Patients acquired HIV from either mother-to-child transmission or high-risk behavior. Healthy, age-matched subjects were collected at two different study sites. The University of South Florida and University of Pennsylvania Institutional Internal Review Boards approved the protocol for collection. PBMC were isolated by standard Hypaque-Ficoll separation and cryopreserved in fetal bovine serum (FBS; ICS Hyclone, Logan, Utah) containing 10% dimethyl sulfoxide (DMSO; Fisher Scientific, Pittsburgh, Pennsylvania).

2.2 Antibodies: Monoclonal antibodies (mAbs) for surface staining included: (i) anti-CD4 PE-Cy5-5, anti-CD27 APC Alexa-750, anti-CD38 APC, and anti-HLA-DR Pacific blue, anti-CD8 605 (Invitrogen, Carlsbad, California); (ii) anti-CD19 Pcy5 and anti-CD16 Pcy5 (Biolegend, San Diego, California); and (iii) anti-CD45RO Qdot 705 (custom). For intracellular staining, mAbs included anti-Ki67 FITC and anti-Bcl-2 PE (BD Pharmingen, San Diego, California) and anti-CD3 TRPE (Beckman Coulter, Fullerton, California). Custom conjugations to quantum dot (Qdot) nanocrystals were performed in our laboratory as described previously (Chattopadhyay et al., 2006) with reagents purchased from Invitrogen.

2.3 Flow cytometry staining assay: Cryopreserved PBMC were thawed and rested overnight at 37°C, 5% CO₂ in cRPMI medium at a concentration of 2x10⁶ cells/ml. Subsequently, the cells were washed with cRPMI and resuspended at a concentration of 1x10⁶ cells/ml. The cells were washed once with PBS and stained with Aqua Blue amine-reactive viability dye (Invitrogen) for 10 minutes in the dark at room temperature; a mAb cocktail was used to stain surface markers for an additional 20 minutes. The cells were washed with PBS containing 1% bovine serum albumin (BSA; Fisher Scientific) and 0.1% sodium azide (Fisher Scientific) and either fixed in paraformaldehyde (Sigma-Aldrich) or fixed/permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) according to the

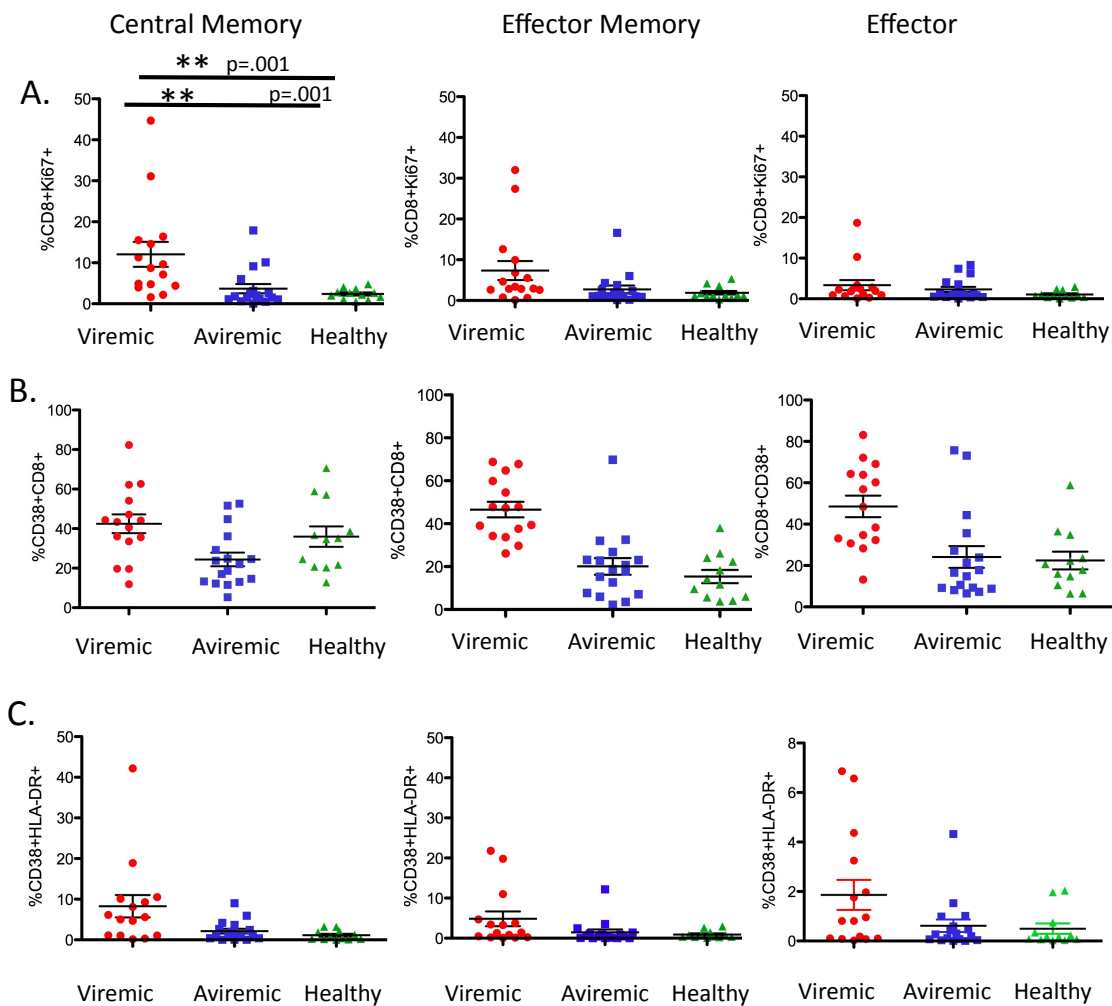


Figure 3: Immune Activation is elevated in some T cell subsets (A) Ki67 (B) CD38 (C) CD38+HLA-DR+ expression were assessed on memory (Effector, Effector Memory, Central Memory) populations across the viremic, aviremic and HIV- subjects.

manufacturer's instructions. A cocktail of mAbs against intracellular markers was added to the fixed/permeabilized cells, which were incubated for 1 hour in the dark at room temperature. These cells were washed once with Perm Wash buffer (BD Biosciences) and fixed in PBS containing 1% paraformaldehyde (Sigma-Aldrich). Fixed cells were stored in the dark at 4°C until the time of collection.

2.4 Flow cytometric analysis: For each specimen, between 0.5 and 1x10⁶ total events were acquired on a modified LSRII flow cytometer (BD Immunocytometry Systems, San Jose, California) equipped for the detection of 18 fluorescent parameters. Anti-mouse Igk antibody capture beads (BD Biosciences) were used to prepare individual compensation tubes for each mAb used in the experiment. Data analysis was performed using FlowJo version 9.1 (TreeStar, Ashland, Oregon).

2.5 sCD14 measurement: Previously frozen (-80°C) plasma and serum samples were thawed at room temperature. Plasma samples were diluted 1:400 and serum samples were diluted 1:100 in calibrator diluent. The samples were analyzed for sCD14 with R & D Systems ELISA kit per kit instructions.

2.6 Statistical Analysis: Initial analyses were restricted to baseline observations. Analysis of variance (ANOVA) or Kruskal-Wallis tests were used to compare the activation levels between the 3 groups, with Tukey adjustment for multiple comparisons. Soluble CD14 levels were compared between two groups using non-parametric tests. Correlation coefficients assessed the association between activation and viral load. Longitudinal analyses were conducted using generalized estimating equations (GEEs) with either a Poisson or normal distribution, as appropriate, and a log-link. Partial correlation coefficients were calculated to assess associations longitudinally. All analyses were conducted using Stata/MP 11.

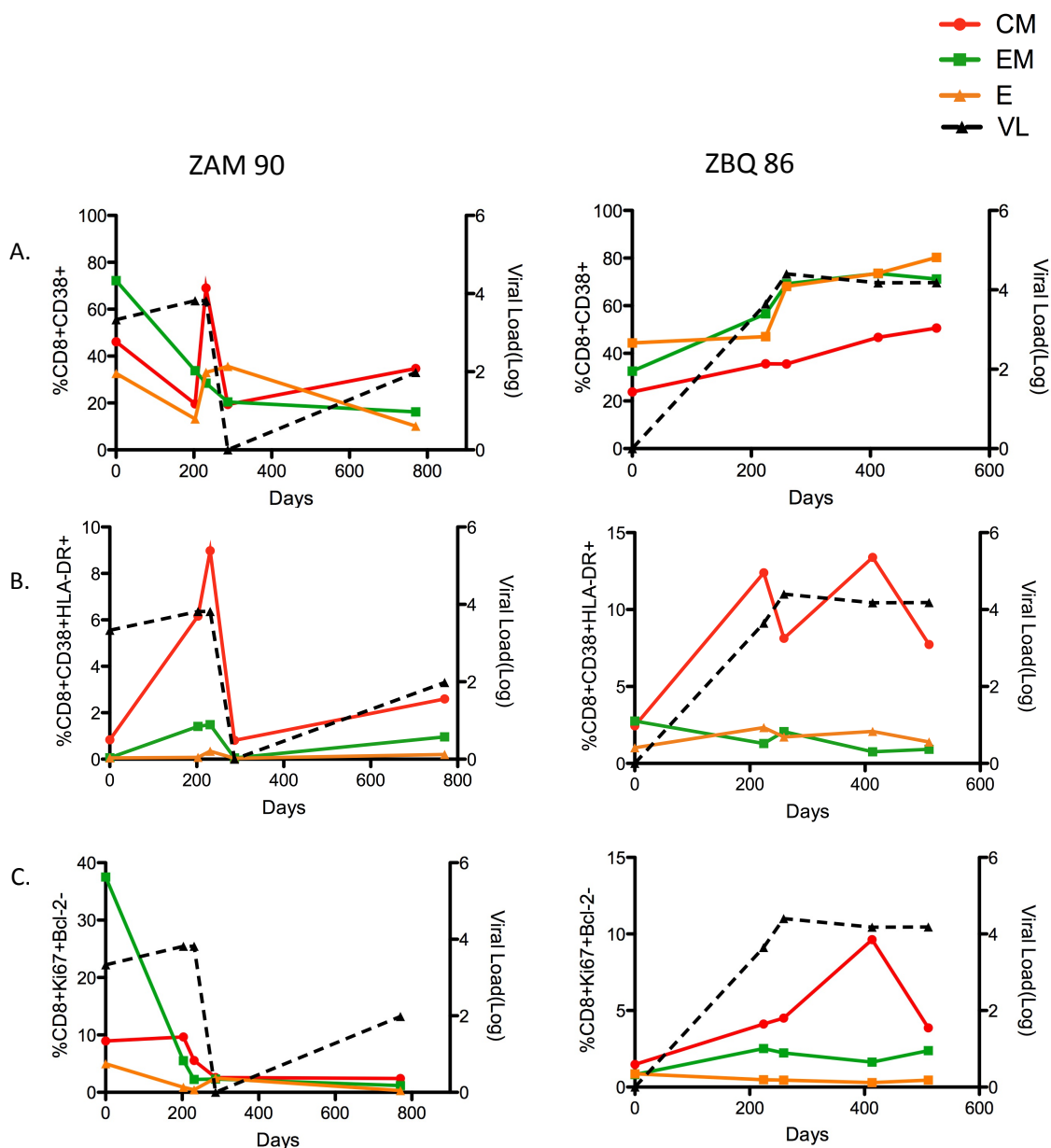


Figure 4: Longitudinal assessment of Immune Activation and Turnover.

(A) CD38+HLA-DR-(B) CD38+HLA-DR+(C)Ki67+BCL- expression in effector (gray squares), effector memory (black circles), and central memory CD8 T cells (gray dash diamond) and viral load (black dash triangle) overtime (visit days). %Activation expressed on the primary y-axis, viral load plotted on secondary y-axis.

Results

Examination of Immune Activation in bulk memory T cell populations

To assess T cell activation and makers of chronic immune activation, we assembled a cohort comprised of 15 viremic (>50 copies RNA/ml), 17 aviremic (<50 copies RNA/ml), and 12 HIV uninfected subjects (Table I, Table II:appendix). The gating scheme used for activation marker discrimination Figure 1. All HIV positive subjects were on HAART. 9 subjects had developed drug resistance and at least two subjects were non-compliant with therapy after the initial time point. Subjects were assessed for expression of common prognostic markers of HIV disease progression and activation: CD38, HLA-DR, Ki67, Bcl-2.

Previous studies in immune activation assessed marker expression on bulk memory and naïve cell subsets in cross-sectional samples. Therefore, we first determined activation levels in the naïve and memory CD8 T cell populations. CD38 expression in memory cells was elevated in viremic compared to both aviremic subjects ($p= 0.009$) and healthy controls ($p= 0.004$) (Figure 2A). Co-expression of CD38 and HLA-DR was elevated in memory cells but did not reach significance (Figure 2B). We found no significant difference in Ki67 expression on memory cells (Figure 2C) though the viremic group displayed almost 3-fold higher levels than the aviremic group ($p= 0.075$) (Figure 2C). We analyzed CD38, CD38/HLA-DR and Ki67 expression in the memory CD8 T cell population and found no correlation between levels of activation and viral load. Although there were no significant differences in activation in the naïve cell population (Figure 2D-F) we did observe a bimodal distribution of CD38 levels.

Increased Turnover in HIV+ individuals on HAART

Next we subdivided the memory population into central memory (CCR7+CD45RO+), effector memory (CCR7-CD45RO+) and effector (CCR7-CD45RO-)

**Table III: Assessment of CD38 expression in CD8 Effector Cells (CCR7-
CD45RO-)**

Subject Group	N	Mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	28	24.99	18.54	1.9
Viremic	32	48.81	20.96	1
Healthy	12	22.42	14.94	2.1

phenotypes in order to determine whether there was differential activation between these subsets. We assessed Bcl-2 expression to delineate cells undergoing turnover because studies have demonstrated activated T cells are pro-apoptotic. We found increased expression of Ki67 in the central memory subset (CCR7+CD45RO+), of viremic adolescents compared to aviremic ($p= 0.001$) and healthy subject groups ($p= 0.001$) (Figure 3A). We did not find turnover in either the effector memory (CCR7-CD45RO+), effector (CCR7-CD45RO-) (Figure 3A). Thus in agreement with previous findings in adults, the central memory CD8+ T cells are more likely to be actively dividing in viremic adolescents compared to other memory phenotypes.

Immune Activation in Memory Subsets

We next examined CD38 expression in the different memory subsets. CD38 expression on central memory CD8 T cells of aviremic adolescents was significantly lower than viremic ($p= 0.001$) and healthy controls ($p= 0.046$). CD38 expression was also increased on effector and effector memory CD8+ T cells of viremic adolescents when compared to aviremic ($p= 0.001$) and healthy controls ($p= 0.001$) (Figure 3B). Elevated CD38/HLA-DR co-expression was not observed in memory subsets (Figure 3C). Of note, we observed a much lower frequency of CD38/HLA-DR CD8 T cells than has been previously reported in adults (Giorgi et al., 1993; Hunt et al., 2003b). While we do find a correlation between CD8+ T cell expression of CD38 and viral load in effector CD8 T cells, we do not see a correlation in total memory or other cell subsets.

Longitudinal Changes in Activation

We assessed longitudinal changes in activation levels on CD8+ T cells in relation to changes in viral load due to poor adherence or escape. Figure 4 shows representative data from subjects with the largest changes in viral load and drug resistance mutations. CD38, CD38/HLA-DR and Ki67 expression per CD8 memory subset tracked closely with

Table IV: Assessment of Ki67 expression in CD8 Central Memory Cells (CCR7+CD45Ro+)

Subject Group	N	mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	28	3.32	4.06	3.8
Viremic	32	10.20	9.14	1.0
Healthy	12	2.40	1.29	4.5

changes in viral load. In subject ZBQ 86, as the viral load increases, the percent of CD38, CD38/HLA-DR, Ki67 expression on total memory CD8+ T cells increases as well (Figure 4A, right graphs). A notable exception to this trend was seen in donor ZAM 90. The level of activation increases in subject ZAM 90 as does viral load but when the viral load becomes undetectable at time point C, the percentage of CD38 expressing T cells continues to increase (Figure 4, left graph). Additionally, within each individual, specific memory subsets appear to be differentially affected by changes in viral load. For example, in subject ZBQ 86, immune activation on effector CD8 T cells does not increase in the presence of high viral load. The central memory subset of this donor demonstrates a marked increase in Ki67 levels following the viral load increase.

We next analyzed the data longitudinally for the entire cohort using generalized estimating equations (GEE) for the entire cohort. CD38 expression is 1.9 and 2.1 fold greater on effector cells in viremic compared to aviremic subjects ($p < 0.001$) and uninfected subjects ($p = 0.004$), respectively (Table III). Similarly, Ki67 expression is 3.8 fold higher on central memory cells from viremic subjects compared to aviremic ($p = 0.001$) and 4.5 fold higher than on cells in this subset found in healthy controls ($p = 0.059$) (Table IV). There significant differences between groups in the effector cell subset with the viremic group having 1.6 fold higher expression than the aviremic group ($p = 0.002$). However, once the data are adjusted for differences in age, the changes do not reach statistical significance. Therefore, we find that despite therapy intervention activation levels remain elevated in viremic adolescent subjects.

CD4 T cell counts and Immune Activation

Increased immune activation is highly predictive of CD4 T cell decline; therefore, we sought to determine if there was a correlation between cell count and activation. We

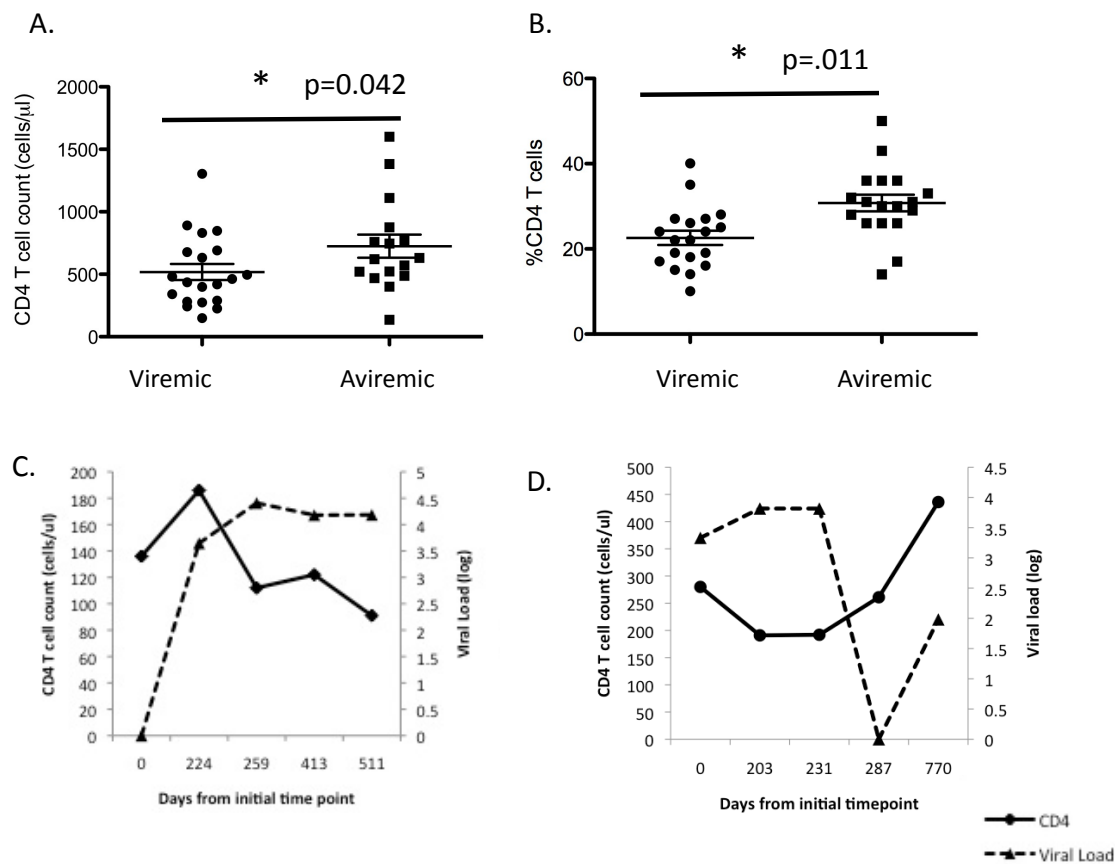


Figure 5: CD4 T cell counts are inversely correlated to viral load. (A) CD4 T cell counts/ml of blood (B) CD4 T cell percentage in viremic (n=20) and aviremic (n=16) subject (C) ZBQ86 (D) ZAM 90 longitudinal assessment of CD4 T cell count and viral load in subjects over time

found negative correlations between CD4 T cell count and Ki67 ($r=-0.40$ $p= 0.049$) and CD38 expression ($r=-0.5425$, $p= 0.005$) on CD8 memory T cells. CD38 expressing central memory cells ($r=-0.4776$, $p= 0.016$) and CD4 T cell count were also negatively correlated. Similar to other studies we also so higher levels of CD4 T cell counts and percentages in avireemics. Therefore, immune activation and CD4 counts correlate negatively.

Because adolescents have increased thymic output we sought to determine if this advantage would allow them to maintain high counts even in the presence of increased viral loads. We observed that at baseline CD4 T cell counts (Figure 5A) and percentages (Figure 5B) are higher in aviremic subjects. We next examined changes in CD4 T cell numbers in combination with viral load in two patients over time. In Figure 5C, the CD4 count in subject ZBQ 86 rapidly declines from time point the first time point to the second in direct correlation to an increase in viral load. The steady increase in viral load observed in subject ZBQ 86 this subject coincides with the decline of CD4 T cell counts. In ZAM 90, a subject with a steady decline in viral load, the CD4 T cell count rebounds (Figure 5D). Overall, CD4 T cell counts track with viral load both in the subjects shown here and in the majority of our cohort. Indeed, when looking at the entire cohort we found aviremic subjects to have higher CD4 T cell counts than viremic subjects. Therefore, increased thymic output may not compensate for increased viral load in the presence of increased activation in adolescents.

A characteristic of HIV disease progression is increased microbial translocation resulting in increased levels of serum sCD14. We therefore measured sCD14 levels to determine if there is an association with high viral load, immune activation and CD4 T cell count in the adolescent cohort. Higher levels of sCD14 would indicate a similar

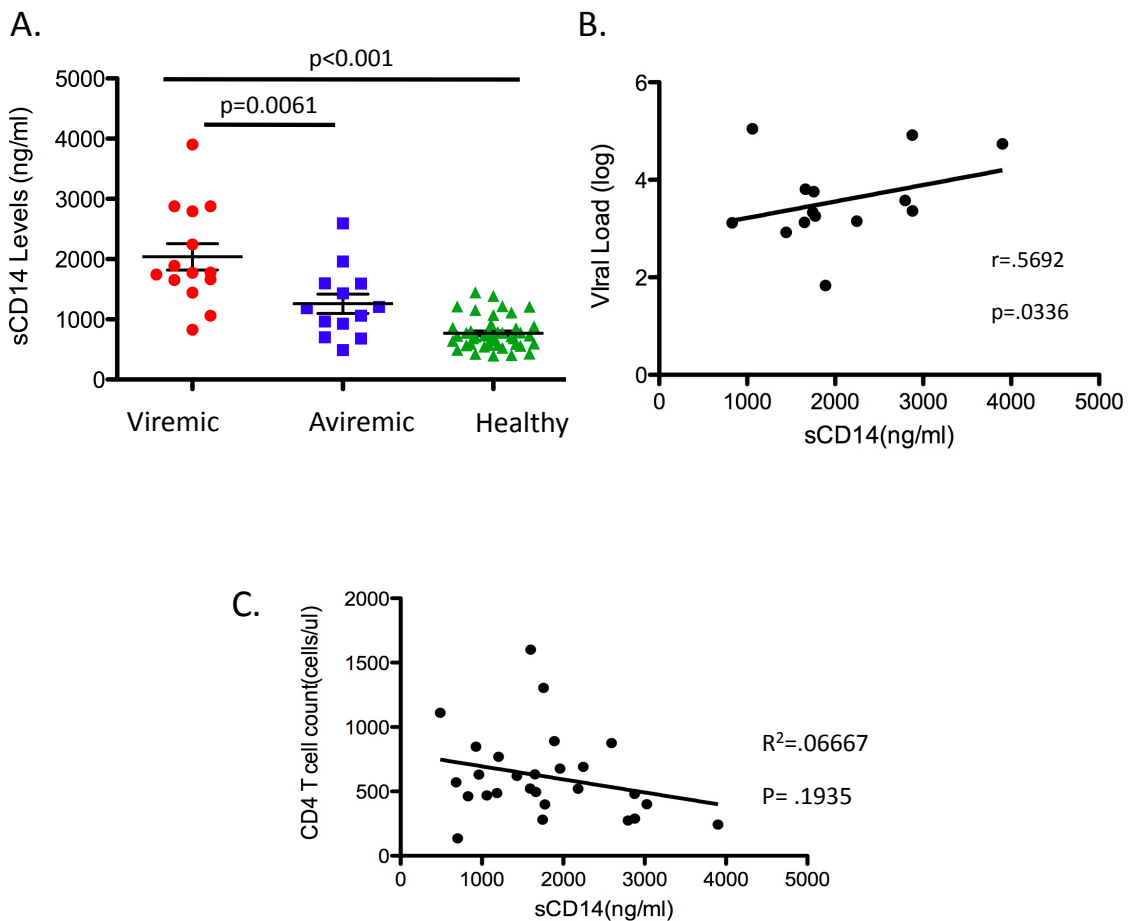


Figure 6: Soluble CD14 expression correlates with biological markers of disease progression. Previously frozen plasma and serum samples were thawed and analyzed for soluble CD14 (sCD14) with the R and D systems ELISA kit (A) sCD14 levels in aviremic, viremic and healthy subjects (B) correlation between viral load and sCD14 levels in viremic subjects (C) correlation between CD4 T cell count and sCD14 levels in aviremic and viremic patients

mechanism for immune activation exists in both HIV-infected adolescents and adults. As shown in Figure 6A, similar to previous findings in HIV-infected adults, sCD14 levels in viremic adolescents are significantly higher than in aviremic subjects ($p= 0.0061$) and healthy controls ($p<.0001$). There is a positive correlation between viral load and sCD14 levels ($r=.569$, $p= 0.034$) (Figure 6B), and a trend towards lower CD4 T cell counts as sCD14 increases (Figure 6C). When examining representative subjects, we see a direct relationship between sCD14 levels and viral load (Figure 7 A-B); as viral load increases, so does sCD14. Similarly, as activation levels change, sCD14 levels remain directly associated. We also see a direct relationship when levels of activation are plotted against sCD14 levels (Figure 7C-D). These data are confirmed when analyzing the entire cohort. In fact, sCD14 is directly correlated to immune activation, as defined by CD38 expression, in each memory subset. Therefore, these data indicate that, similar to HIV-infected adults, chronic immune activation and viral load are directly associated with markers of microbial translocation within HIV-infected adolescents.

Discussion

In the present study, we examined activation levels (CD38+, HLA-DR+ and Ki67+) on CD8 T cells in infected adolescents. This is the first report in adolescents to simultaneously examine each respective immune activation marker and to do so on defined memory subsets. We also take the first step to identify factors related to microbial translocation (sCD14) in adolescents. We found that in the presence of suboptimal therapy adolescents do not control immune activation, viral load or microbial translocation. Several studies suggest that CD38 is a less optimal activation marker in younger population but it is the most consistent in our study. Our data

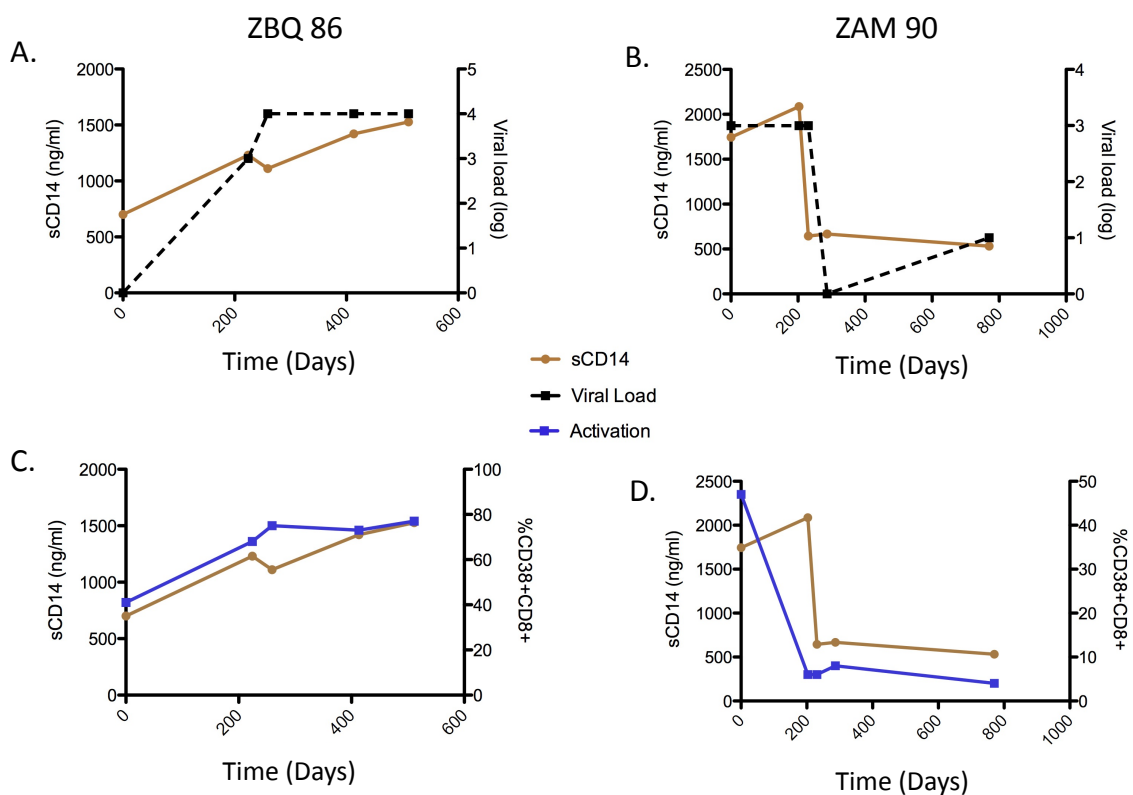


Figure 7: Biomarkers of microbial translocation are associated with viral load and immune activation. Previously frozen plasma and serum samples were thawed and analyzed for soluble CD14 (sCD14) with the R and D systems ELISA kit. Soluble CD14 (sCD14) expression coincides with (A-B) viral load (dashed diamond line) sCD14 (solid triangle line) (C-D) and CD38 expression (black squares) sCD14 (gray diamond) in donors ZBQ 86 and ZAM 90.

demonstrates, CD38 appears to be a pan-activation marker whereas Ki67 activation is limited to the central memory subset in CD8 cells. Unlike other studies in adults and adolescents we do not see elevated CD38/HLA-DR expression in viremic adolescents nor do the levels of this activation pattern correlate with viral load.

In HIV infection, CD38 remains upregulated on T cells and associates with HIV disease progression. This association holds true for HIV+ adults, adolescents and children. We confirm these findings and extend the analysis to distinctly defined memory subsets and show that CD38 is elevated across the effector, central memory and effector memory subsets. Adolescents and children are expected to have higher numbers of CD38 due to its role as a marker of immature T cells. Indeed we observe levels of almost 90% in the naïve cell population, a stark contrast between the 10% expression reported in adults (Giorgi et al., 1993; Emu et al., 2005). We believe that these cells are likely to be recent thymic emigrants and therefore express more CD38. Because there is increased CD38 expression in the memory compartment in infected subjects compared to the healthy subjects it is most likely a consequence of viral load and not age differences.

CD38/HLA-DR co-expression marks immune activation and higher levels of expression on CD8 T cells of HIV-infected adults and children predict disease progression (Giorgi et al., 1993; Giorgi et al., 2002; Rudy et al., 2006a). Contrary to what has previously been shown in adults and adolescents we do not observe significantly higher levels of CD38/HLA-DR expressing cells in our cohort. Furthermore, the percentages we report are quite low compared to those observed in previous studies in adults (Giorgi et al., 1999). Globally, we see lower levels of HLA-DR in our experiments and attribute this to our highly stringent flow cytometric gating strategy. We exclude

monocytes, B cells and NK cells from the final analysis to prevent false positives and reduce background. We have noted that a large portion of HLA-DR is found on macrophages and once these cells are excluded from the analysis the levels of apparent HLA-DR on CD8 T cells can be reduced in some instances. Together, these results may suggest that CD38/HLA-DR expression may not be the best marker of activation in HIV+ adolescents.

Several previous studies have conclusively indicated that T cell turnover is increased in HIV-infected individuals. In HIV-positive adults, Ki67 expression is elevated indicating increased turnover. In adolescents, Ki67 expression has been reported to be 5.1 fold and 2.8 fold higher in memory and naïve CD8 T cells respectively (Starr et al., 2002a). Conversely, we observed no significant differences in Ki67 expression levels or fold change in total memory and naïve CD8 T cells.

To date, studies have not addressed immune activation in distinct memory subsets in adolescents, although a significant body of work exists for adult cohorts. In 1999, Hazenberg et al, delineated CD8 memory subsets on the basis of CD27 expression in adults to determine if turnover rates were different in memory population (Hazenberg et al., 2000c). The percentage of Ki67 expressing cells was increased in the CD27- and CD27+ memory subsets as well as within the naïve compartment of HIV infected individuals. Ki67 expression was elevated up to 20-fold in the naive CD8 T cell population and 7-fold in the CD27- and CD27+ memory populations. We observed significantly higher percentages of Ki67 in the central memory compartment but report no differences in other subsets. Our smaller sample size may account for the lack of difference. We found a lower percentage of Ki67+ cells than reported in some studies but our data is consistent with the numbers in a recent report (Lederman et al., 2011). Another differentiating factor in our study was the use of Bcl-2 , an apoptotic marker. By

using Bcl-2 we are able to identify not just cells undergoing increased turnover but also those that are most likely activated and therefore more susceptible to apoptosis.

We also examined other predictors of disease progression like viral load and CD8 T cell percentages. Similar to previous studies, we reported a positive correlation between viral load and CD8/CD4 T cell percentage. This direct relationship may easily reflect increased turnover in the presence of viremia. Immune activation in the total memory population correlates with viral load in adults, but we saw no correlation with CD38/CD38+HLA-DR+ and viral load in the adolescent cohort. We do observe a correlation between viral load and the effector T cell subset. A lack of correlation between CD38 and viral load may reflect the small sample size and viral load variation in our cohort. Nonetheless, performing correlations studies in defined memory subsets gives a picture of immune activation and T cell depletion patterns.

Recent studies have suggested that initial CD4 T cell counts above 300 cells/ μ l before therapy equate to faster cell count rebound (Perez et al., 2001). Additionally, adolescents have increased thymic output and higher absolute T cell counts than their adult counterparts, even while infected. With this in mind several clinical trials are incorporating early therapy initiation to increase favorable therapy outcomes. While this strategy may prove useful, conflicting results (El-Sadr et al., 2006) suggest early therapy initiation will not be the only key to the successful therapy intervention. Nonetheless, increased numbers of naïve CD4 and CD8 T cells in adolescents may allow for better cell rebound than their adult counterparts. Importantly, activation is not elevated in naïve CD8 T cell subsets in our study. This may prove beneficial for adolescents who are long-term carriers of the disease.

Microbial translocation defined by increased LPS circulation in the plasma has been directly correlated to increased immune activation (Brenchley et al., 2006). LPS

levels directly correlate with soluble CD14 detectable in the plasma (Papasavvas et al., 2009b). Thus, we used sCD14 as an indirect marker of microbial translocation. Similar to what has been observed in adults, we found elevated levels of sCD14 in viremic subjects. These levels of sCD14 are lower in our cohort than those in adults but are relatively similar to those reported in children (Wallet et al., 2010). Furthermore, sCD14 levels were directly correlated to immune activation in each memory subset. It is important to note that even in HAART treated subjects levels of sCD14 do not return to baseline. Indeed, increased levels of microbial translocation persist even in the presence of decreased viral load and increased CD4 T cell counts in treated individuals (Brenchley et al., 2006; Jiang et al., 2009; Papasavvas et al., 2009b; Wallet et al., 2010; Sandler et al., 2011). Recently, sCD14 has been associated with T cell activation and mortality in HIV infected adults (Sandler et al., 2011). This data suggests that increased baseline levels of sCD14 in adolescents, even in the presence of therapy can increase the risk of death. Given what we know about immune activation and maintenance of microbial translocation, it is necessary to rethink how successful therapy outcomes are evaluated. Presently, successful therapy is defined by CD4 T cell count rebound, lower CD8 T cell numbers after treatment and reduced immune activation. These are all proven factors involved in HIV disease progression. Yet, as we expand our understanding of immune activation it is necessary to include factors that may better predict disease mortality. It is important to reduce immune activation and viral load in order to promote immune restoration but simultaneous reduction of microbial translocation may prove to be a key factor in maintaining this immune state.

Adolescents are life-long carriers HIV and are subject to life-long therapy. Without compliance, success levels dramatically decrease and drug resistance susceptibility increases. Adolescents, who are less likely to adhere to therapy, were

recently reported to have increased percentages of circulating CD38 T cells and to have had virologic failure regardless of CD4 T cell count number (Flynn et al., 2004; Rudy et al., 2006a). In support of this data, the majority of the subject pool in our adolescent cohort has been on therapy for a number of years and has yet to control viral load or activation while on therapy. Adolescents must remain adherent or else activation levels will remain high and drug resistance will occur.

These data indicate that increased immune activation in adolescents is not solely due to viral load or CD4 T cell counts but rather results from a combination of complex interactions in the gut lumen and peripheral blood. Most of our cohort was on long-term therapy but the majority of these subjects still maintain high levels of immune activation. This is likely caused by incomplete therapy compliance, a key hindrance in successful treatment in adolescents. Our current study was not designed to address improvement strategies in therapy adherence for adolescents but this is a key point. Therapy guidelines must be streamlined to promote maximal adherence without jeopardizing protection. We believe this study will inform current therapy strategies and approaches in adolescent HIV research.

Chapter 3: Elevated Immune Activation and Turnover in CD4+ T cells of HIV+ Adolescents

Abstract

During HIV infection massive CD4 T cell depletion occurs as a result of direct viral killing, bystander killing, and activation-induced cell death. Depletion and prolonged immune activation leads to severe immune dysfunction. In adults, immune activation directly correlates to HIV disease progression; however, immune activation in adolescents is not completely understood. Here, we characterized markers of immune activation on CD4 T cells in a cohort of adolescent subjects including 15 viremic (>50 copies HIV RNA/ml), 17 aviremic (<50 copies HIV RNA/ml), and 12 HIV uninfected subjects. We used polychromatic flow cytometry to define the immune activation profile of CD4+ T cells by measuring simultaneous expression of CD38, HLA-DR, Ki67 and Bcl-2. We observed increased immune activation on total memory, effector (CCR7-CD45RO-), effector memory (CCR7-CD45RO+) and central memory (CCR7+CD45RO+) CD4 T cell subsets. Increased expression of CD38+ in each memory compartment negatively correlated with CD4 T cell counts. CD38 expression in non-naïve ($r=0.5797$ $p=0.002$), central memory ($r=0.4246$ $p=0.034$) and effector memory ($r=0.5339$ $p=0.0060$) correlated directly to levels of soluble CD14, a marker of microbial translocation. Taken together, these results show that there is preferential activation across memory subsets and a direct relationship between viremia, activation and soluble CD14 levels in HIV+ adolescents.

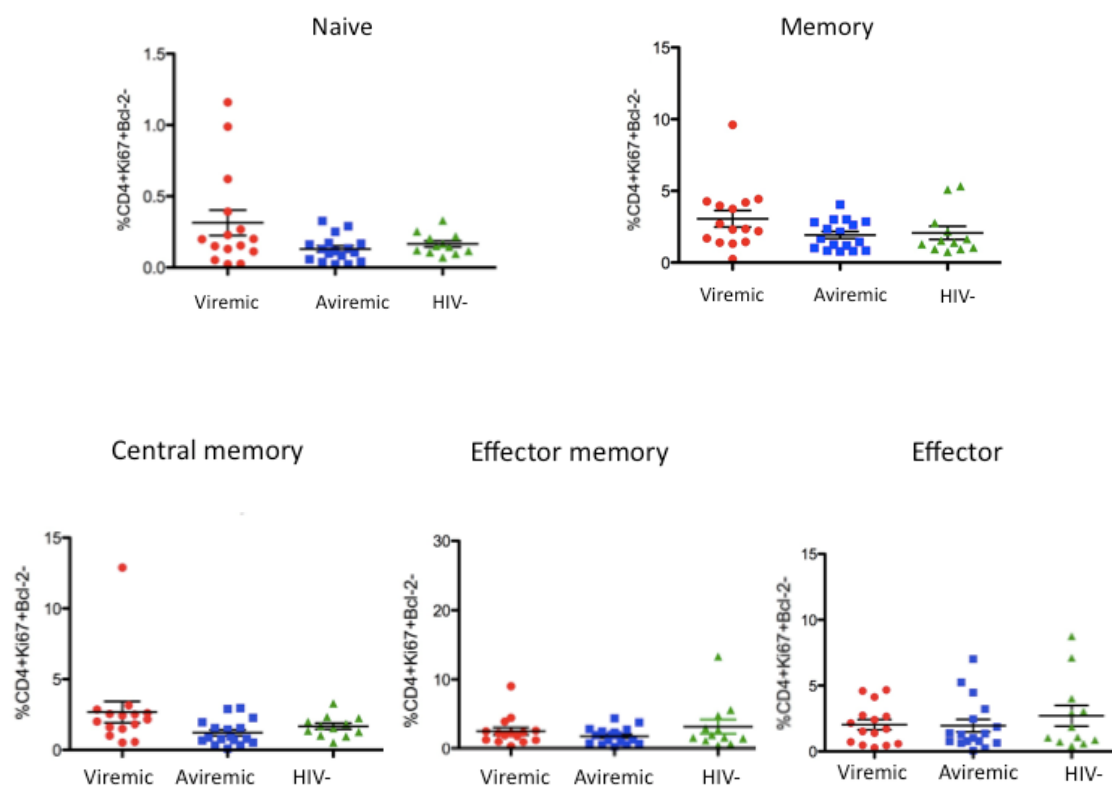


Figure 8: Immune Turnover CD4 T cells memory subsets. Human PBMCs were thawed and stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27,CD45RO,CCR7) and activation markers (CD38,HLA-DR,Ki67,Bcl-2) Ki67 expression was assessed on CD4+ T memory subsets using non-parametric t-tests

Introduction

Immune activation is now known to be a major underlying cause of CD4 T cell depletion during HIV infection and the strongest known predictor of HIV disease progression. Immune activation is commonly measured by elevated expression of Ki67, CD38 and HLA-DR. Specifically, increased Ki67 indicates entry into cell cycle, a characteristic of HIV infection (Sachsenberg et al., 1998a; Douek et al., 2001), and higher levels of circulating CD38 and CD38/HLADR expressing CD8 and CD4 T cells are directly associated with high viral loads and decreased CD4 T cell counts (Giorgi et al., 1993; Liu et al., 1997; Lien et al., 1998; Hazenberg et al., 2000b; Vigano et al., 2000a). Persistent immune activation can severely deplete CD4 T cells and result in impaired immune responses.

During acute HIV infection, and persisting into chronic infection, severe CD4 T cell depletion occurs in gut associated lymphoid tissues (GALT) compromising the integrity of the mucosal membrane (Brenchley 2004). This results in microbial translocation, a process that has been implicated as a key driver of immune activation (Brenchley 2006). In clinical studies, soluble CD14 (sCD14), a measure of LPS-induced activation (Lien et al., 1998) has been directly correlated to increased risk of mortality in adults (Sandler et al., 2011). Although levels of microbial translocation may be reduced after therapy administration, injury to the gut is irreversible. As a result, repopulation of gut memory T cells is impaired (Papasavvas et al., 2009a). These factors contribute to disease progression even in the presence of HAART-suppressed viral control and ultimately lead to immune failure (Lederman et al., 2011).

Table V: Assessment of Ki67 Expression in CD4 T cell memory Subsets

Naïve	Mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	.13	.11	2.7
Viremic	.31	.30	1
Bulk-Memory			
Aviremic	1.83	.99	1.9
Viremic	3.30	20.96	1
Central Memory			
Aviremic	1.19	.89	3.7
Viremic	2.79	2.99	1
Effector Memory			
Aviremic	1.71	1.16	1.7
Viremic	2.71	1.78	1

* N=28 aviremics and 32 viremics

Although HIV research has progressed greatly over the past 20 years, there are still understudied areas. For example, HIV disease progression is understudied in adolescents, a group that makes up a large number of new infections. Unique biological and psychological parameters in adolescents have the potential to greatly influence subsequent HIV disease progression. For example, adolescents are less likely to adhere to strict therapy guidelines and may therefore be more susceptible to drug resistance and viral rebound (Futterman, 2005). In contrast, the heightened regenerative capacity of the immune system is more robust in young children and adolescents (Vigano et al., 2000b) due in part to increased thymic output in adolescents compared to their adult counterparts. Young adults have been shown to rebound CD4 T cell counts more efficiently than adults after therapy initiation (Cohen Stuart et al., 1998a; Slesman et al., 1999). Unfortunately, this advantage may be lost due to increased immune activation caused by incomplete therapy compliance. Furthermore, increased microbial translocation as a result of reduced gut integrity in adolescents may mark this subject group for immune failure even in the presence of optimal therapy.

The response to therapy is understudied in adolescents; here we designed a study to address several resulting concerns. Firstly, we examined the activation levels on memory and naïve CD4 T cells in adolescents on HAART. We assessed activation in central memory, effector memory and effector T cell populations. Determining if certain CD4 T cell memory subsets are selectively activated could lead to a better understanding of HIV disease progression in adolescents. Unlike other studies, which are mostly cross-sectional in nature, we assessed activation from baseline on longitudinal samples. Lastly, we assessed the relationship between sCD14 and CD4 T cell activation to determine if microbial translocation contributes to disease progression in HIV positive adolescents.

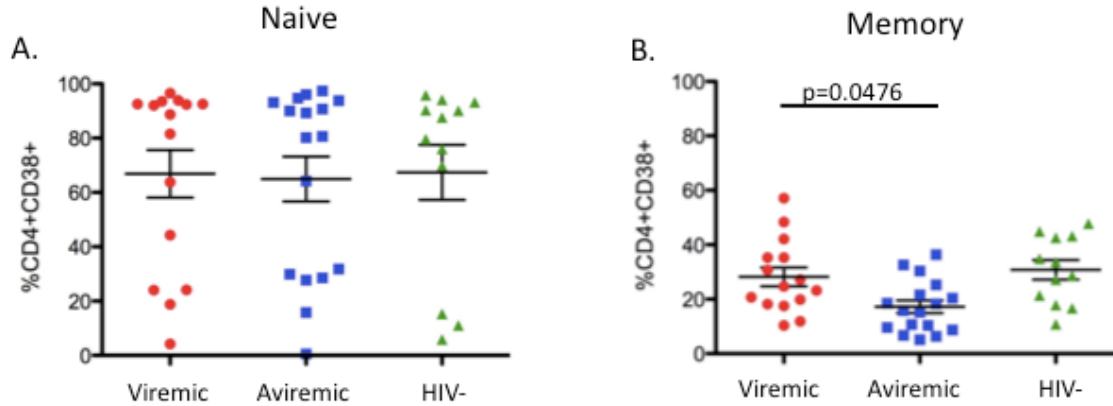


Figure 9: Expression of CD38 in Bulk memory and Naïve populations of CD4+ T cells. Human PBMCs were thawed and stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and activation markers (CD38, HLA-DR, Ki67, Bcl-2) CD38 expression is shown on A) Naïve (B) Bulk memory cells p values calculated using non-parametric t-tests.

Materials and Methods

3.1 Cells: The cohort consisted of PBMCs from 44 HIV-infected adolescents 15 viremic, 17 aviremic and 12 HIV uninfected subjects (appendix). Subjects and cells were acquired as detailed in Chapter 2.

3.2 Antibodies: Antibody-staining panel was employed as described in Chapter 2

3.3 Flow cytometry staining assay: See chapter 2

3.4 Flow cytometric analysis: For each specimen, between 0.5 and 1×10^6 total events were acquired on a modified LSRII flow cytometer (BD Immunocytometry Systems, San Jose, California) equipped for the detection of 18 fluorescent parameters. Data analysis was performed using FlowJo version 9.1 (TreeStar, Ashland, Oregon).

3.5 sCD14 measurement: Previously frozen (-80) plasma and serum samples were thawed at room temperature and analyzed for sCD14 with R&D Systems Elisa kit per instructions.

2.6 Statistical Analysis: See Chapter 2

Results

Turnover and activation in naïve and memory CD4 T cells

To assess T cell activation and markers of chronic immune activation, we assembled a cohort comprised of 15 viremic (>50 copies RNA/ml), 17 aviremic (<50 copies RNA/ml), and 12 HIV uninfected subjects (Appendix: Table I). The gating scheme of this study is shown in Figure 1. Subjects were assessed for expression of the common prognostic markers of HIV disease progression and activation: CD38, HLA-DR and Ki67.

Increased Ki67 expression is a characteristic of heightened immune activation in both adults and adolescents. Because activated T cells are more prone to apoptosis we further delineated cells by lower BCL-2 expression. We observed no differences in Ki67

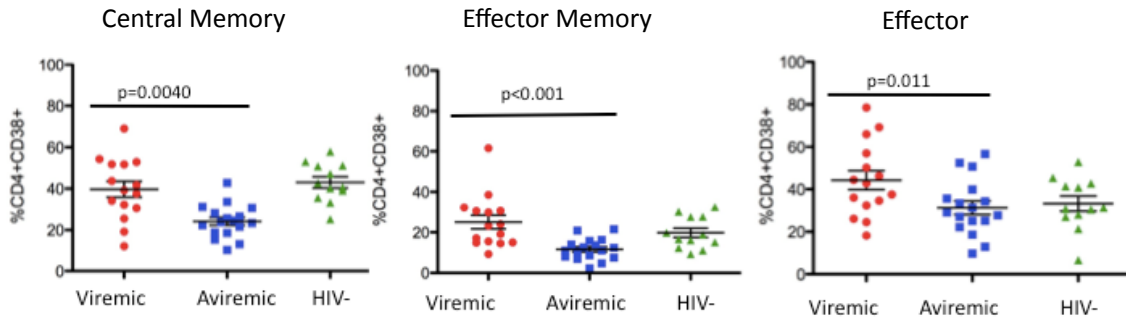


Figure 10: Expression of CD38 in Memory of CD4 T cell subsets. Human PBMCs were thawed and stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and activation markers (CD38, HLA-DR, Ki67, Bcl-2). Cells were then gated on memory phenotype and then further gated on CD38 expression, p-values calculated using non-parametric t-tests.

expression in the bulk memory, naïve or other cell subsets in cross-sectional data (Figure 8); however, we found significant differences in Ki67 expression in all CD4 T cell subsets over time. We see 2.7-fold higher expression of Ki67 in CD4 naïve cells compared to avireemics ($p=0.005$). In bulk memory cells, we observe a 1.9 fold difference between vireemics and avireemics ($P<0.001$). CD4 subsets were divided into central memory (CCR7+CD45RO+), effector memory (CCR7-CD45RO+) and effector phenotypes. We saw a 3.7-fold increase in the central memory ($p=0.001$) and a 1.7-fold increase in the effector memory ($p=0.038$) subset compared to avireemics (Table V). Thus, unlike previous studies in adolescents and adults, we did not observe a difference in CD4 T cell turnover at baseline. However, when data was analyzed longitudinally, we identified increased T cell turnover in each subset with the exception of effector CD4 T cells. Ultimately, increased turnover in the CD4 compartment could lead to increased apoptosis and immune senescence.

Increased expression of CD38 or CD38/HLA-DR on T cells is a hallmark of immune activation and is often associated with high viral loads (Giorgi et al., 1993; Giorgi et al., 1999). We first looked at baseline levels of CD38 in each CD4 T cell subset. We did not observe a significant increase in CD38 expression on naïve CD4 T cells but we did find a bimodal distribution of CD38 expression in this cell type (Figure 9A). There higher CD38 expression in viremic subjects compared to aviremic in the bulk memory group ($p=.0476$) (Figure 9B). We next examined expression of CD38 on memory subsets at baseline. Expression of CD38 was increased on effector memory ($p<0.001$) and effector ($p=0.011$) cell subsets in viremic subjects compared to the avireemics (Figure 10). We did not find a difference in the percentage of CD38/HLA-DR expressed in naïve (Figure 11A), central memory (Figure 11C), effector memory (Figure 11D) or effector cells (Figure 11E), but observed a 2-fold increase in the percentage of

Table VI: Assessment of CD38+/HLA-DR- expression on CD4+ T cell subsets

Central Memory	Mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	28.47	15.12	7.4
Viremic	41.76	14.15	1
Effector	Mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	32.50	14.67	6.9
Viremic	44.33	16.56	1
Effector Memory	Mean	Standard Deviation	Fold Change of Viremic compared to subject group
Aviremic	13.17	8.61	1.8
Viremic	25.59	10.36	1

CD38/HLA-DR expressing bulk memory cells in viremic compared to aviremic at baseline ($p=0.027$) (Figure 11B). Thus, it can be seen that activation levels remain elevated in viremic adolescents on therapy.

We next assessed longitudinal changes in activation levels on CD4+ T cells in relation to changes in viral load due to poor adherence or escape. Figure 5 shows representative data from subjects with large changes in viral load and drug resistance mutations. CD38 expression (Figure 12), CD38/HLA-DR (Figure 13) and Ki67+Bcl-2 (Figure 14) expression per CD8 memory subset tracked closely with changes in viral load in subjects ZAN89 and ZBQ86. The pattern of activation was not as uniform in subject ZAM 90, who had intermittent therapy compliance and consequent drug resistance. The level of CD38 expressing cells increased in ZAM 90 with viral load but expression of the other activation markers was inconsistent. When we examined the entire cohort using GEE, CD38 expression was 6.9-fold higher in effector cells of viremics compared to aviremics ($p=0.025$). The effector memory compartment in the viremic group has 1.8 fold higher levels of CD38 than the aviremic group ($p<0.001$). In the central memory compartment, aviremics had 7.4-fold lower CD38 levels than the viremic group ($p=0.062$). We found no significant differences in CD38/HLA-DR expression in CD4 T cells.

Increased CD4 T cell activation and turnover correlates with lower CD4 T cell counts and higher CD8 T cell counts

Several studies have shown a correlation between activated CD4 T cells, viral load and CD4 T cell count, but none have determined this difference in memory subsets. We chose to examine the expression of all activation markers and their relationship to cell count. CD38 expression was negatively correlated to lower CD4 T cell counts in each memory subset: central memory ($p=0.0216$, $r=0.440$), effector memory ($p=0.0014$ $r=-$

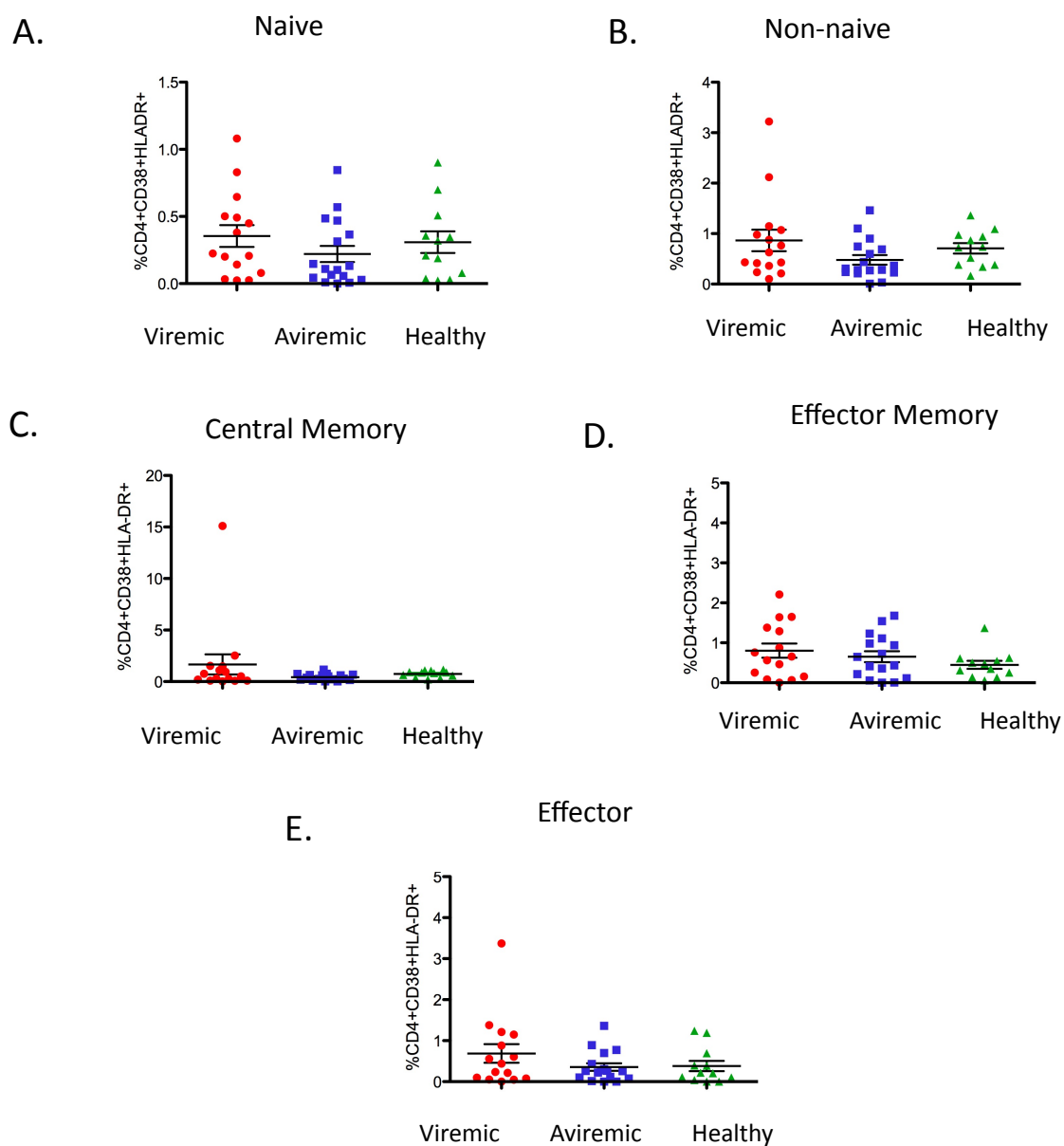


Figure 11: CD38+HLA-DR+ expression is elevated in some T cell subsets.

CD38+HLA-DR+ expression were assessed on memory (A) Naive (B) Bulk memory (C) Central Memory (D) Effector Memory (E) Effector populations across the viremic, aviremic and healthy subjects.

0.5826) and effector ($p=0.0064$, $r=-0.5111$). Lower absolute CD4 T cell counts also correlated with increased turnover in the central memory and effector cell compartments. In contrast to other studies, we did not observe correlations between increased activation and viral load.

During HIV infection, CD8 T cell counts and percentages typically increase dramatically as a result of increasing HIV viral load (Koup et al., 1994; Rudy et al., 2006a). After successful HART therapy however, the CD8 T cell count declines coincident with both decreased viral load and immune activation. However, these initial studies were primarily performed on total memory and naïve T cell population (Hazenberg et al., 2000c; Flynn et al., 2004; Rudy et al., 2006a). We therefore examined the association between the CD8 T cell count and immune activation in CD4 T cells. We found a direct correlation between Ki67+CD4 T cells and the percentage of CD8 T cells ($r=0.467$ $p=0.0163$). We next assessed the association between CD8 T cell count and activation. The CD8 T cell count positively correlated with CD38 expression in the total memory ($p=0.0378$ $r=0.4094$) and effector memory ($r=0.446$ $p=0.022$). CD4 T cell compartments. Thus CD8 T cell counts are directly associated with increased Ki67 expression and immune activation in the CD4 T cell compartment.

A characteristic of HIV-induced immune activation and disease progression is decreased integrity of the intestinal wall, which can be measured by increased levels of sCD14 in the plasma. In Chapter 2, we have shown that the percentage of total CD38+CD8 and viral load directly correlate to sCD14 in adolescents and that CD4 T cell percentage inversely correlates with sCD14. Here, we examined how sCD14 levels correlated to CD4 T cell activation in the adolescent subjects. We found that CD38+ levels in non-naïve ($r=0.5797$ $p=0.002$), central memory($r=0.4246$ $p=0.034$) and effector memory ($r=0.5339$ $p=0.0060$). CD4 T cells positively correlated with sCD14

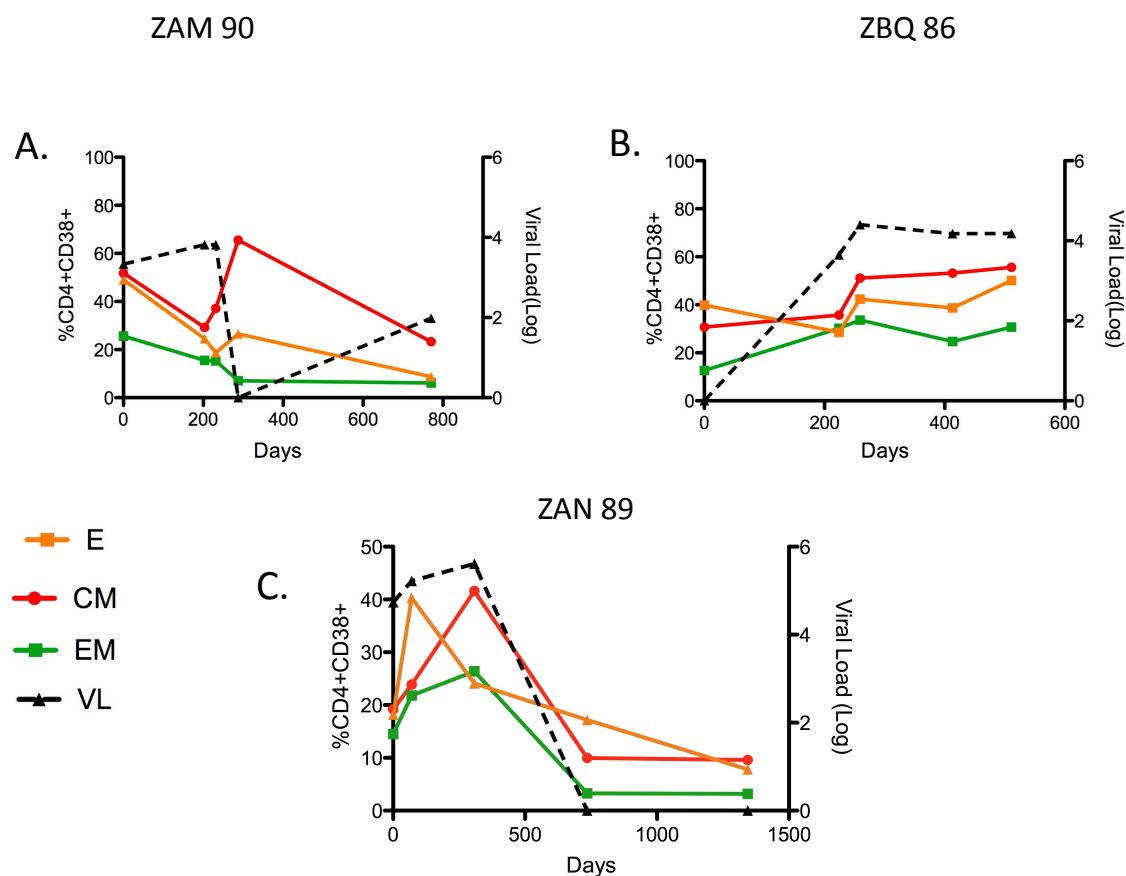


Figure 12: Longitudinal assessment of $CD38+HLA-DR-$ expression in $CD4$ T cells. (A-C) Changes in immune activation in effector (gray squares), effector memory (black circles), and central memory cells (gray dash diamond) and viral load (black dash triangle) overtime (visit days). %Activation expressed on the primary y-axis, viral load plotted on secondary y-axis

levels in the plasma. Thus, microbial translocation may be a factor driving immune activation in adolescents.

Discussion

Immune activation is the strongest predictor of disease progression in adults, however; the global impact of HIV-induced immune activation is understudied in adolescents. In the present study we assessed the activation status of CD4⁺ T cells in HIV-infected adolescents in order to define the CD4 T cell activation signature in this group. In an effort to understand the dynamics of T cell turnover and immune activation in CD4 T cells, we used both cross-sectional and longitudinal data. Similar to our findings in CD8 T cells (CHAPTER 2) we find that in the presence therapy adolescents do not control viral replication or immune activation. We believe this result reflects the consequence of suboptimal therapy compliance common in adolescent subjects. Unlike our CD8 data, we find increased T cell activation is more prevalent in the CD4 T cell naïve, total memory, effector memory and central memory compartments, which is consistent with several studies (Orendi et al., 1998; Hazenberg et al., 2000c; Flynn et al., 2004).

Increased T cell turnover is a hallmark of immune activation in HIV infection. In adolescents, it has been shown that CD4 turnover (as measured by Ki67 expression) is increased in the memory and naïve T cell compartments (Cohen Stuart et al., 1998a; Sleasman et al., 1999; Rudy et al., 2006b). Moreover, untreated HIV-infected adults with increased turnover have lower CD4 T cell counts, suggesting that neither homeostatic nor viral induced proliferation are sufficient to sustain CD4 T cell counts (Hazenberg et al., 2000b; Hunt et al., 2003b). In our adolescent cohort, we did not find an increase in CD4 T cell turnover at baseline but observed increased expression of Ki67 in all memory

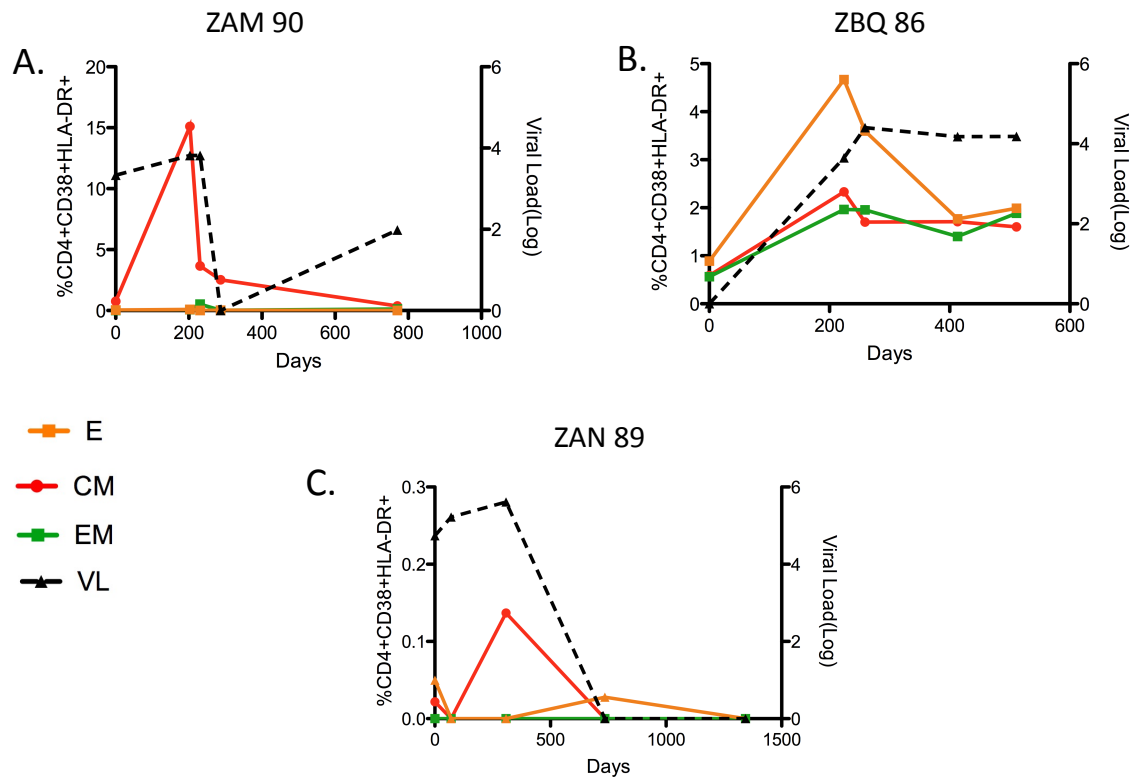


Figure 13: Longitudinal assessment of CD38+HLA-DR+ expression in CD4 T cells. (A-C) Changes in immune activation in effector (gray squares), effector memory (black circles), and central memory cells (gray dash diamond) and viral load (black dash triangle) overtime (visit days). %Activation expressed on the primary y-axis, viral load plotted on secondary y-axis.

compartments longitudinally. Of note, increased turnover in the central and effector memory compartment was associated with lower CD4 T cell counts over time. These findings are similar to a recent study in HIV-infected adults (Lederman et al., 2011) where cell cycling was increased in these compartments. These reports imply that increased turnover of central memory and effector memory cells contribute to decreased CD4 T cell counts. Further evidence suggests that failure to replenish memory T cell numbers in the central or effector memory compartment will result in immune failure (Teixeira et al., 2001; Robbins et al., 2009; Lederman et al., 2011). Our data shows increased turnover in the CD4 memory and naïve T cell compartment of adolescents. This suggests that CD4 T cells will either be severely depleted or exhausted before therapy-noncompliant adolescents reach mid-adulthood making them more prone to rapid disease progression.

In HIV infection, CD38 remains upregulated on T cells and associates with HIV disease progression (Vigano et al., 2000a; Wilson et al., 2004). This association holds true for HIV positive adults, adolescents and children. Our work has confirmed these findings and extended them to well defined memory subsets. We observe increased CD38 expression in effector, central memory, and effector memory subsets. Similar to our findings in naïve CD8 T cells, there appears to be a bimodal distribution of CD38 in naïve CD4 T cells. This expression pattern is most closely associated with age in that younger subjects express more CD38 and is seen regardless of HIV infection status. The addition of CD31, a marker of recent thymic emigrants, may help determine if these CD38+ naïve CD4 cells are immature T cells or truly activated.

Many studies use HLA-DR in addition to CD38 to determine the activated state of T cells. We see a significant difference in CD38-HLA-DR expression on bulk memory CD4 T cells at baseline. This is similar to published studies in adults, which report

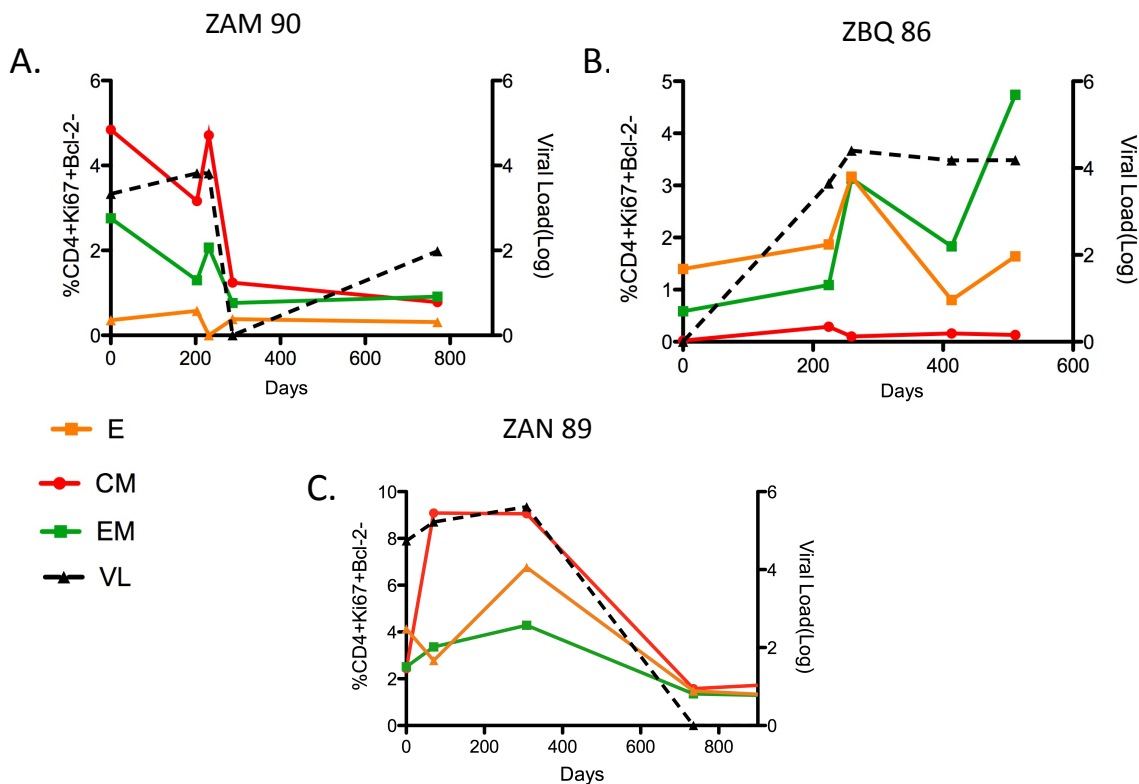


Figure 14: Longitudinal assessment of Ki67+Bcl- expression in CD4 T cells. (A-C) Changes in immune activation in effector (gray squares), effector memory (black circles), and central memory cells (gray dash diamond) and viral load (black dash triangle) overtime (visit days). %Activation expressed on the primary y-axis, viral load plotted on secondary y-axis

elevated expression in treatment failures or HIV-infected (Orendi et al., 1998; Hunt et al., 2006) subjects compared to controls. The levels we report are much lower than that of 10-30% CD38/HLA-DR co-expression shown in other studies. Furthermore we do not observe differences in CD38/HLA-DR expression levels in any CD4 memory subset. We believe both lower expression and lack of statistical significance with CD38/HLA-DR expression may be attributed to the rigorous manner of our flow cytometric gating technique.

Microbial translocation defined by increased LPS circulation in the plasma has been directly correlated to increased immune activation (Brenchley 2006). LPS levels directly correlate with soluble CD14 detectable in the plasma. Thus, we used sCD14 as a surrogate marker of microbial translocation. We showed previously (CHAPTER 2) that sCD14 was heightened in the viremic subjects compared to aviremic. Therefore similar to published studies in adults and our study in adolescents sCD14 is directly correlated with immune activation. Being that sCD14 is correlated to increased mortality, future studies in adolescents should address the effects of sCD14 in the rate of disease progression.

In this study we identified that a subset of HIV+ adolescents on therapy do not control immune activation. When therapy adherence is between 90 and 95% viral replication is controlled and immune activation is reduced. Unfortunately, adolescents are less likely to adhere to therapy guidelines than their adult counterparts. As a result they often possess higher numbers of circulating CD38+ T cells and undergo virologic failure even in the presence of high CD4 T cell counts. Our data demonstrate that viremic adolescents on HARRT have increased activation and turnover in the majority of CD4 T cell subsets compared to aviremic and HIV-negative counterparts. A few questions remain like, what other factors account for differences in activation and viral control in HIV-positive adolescents? Are aviremic subjects more adherent to therapy or do they

possess certain biological determinants that allow them to effectively control viral replication in the absence of adherence? If both the HIV epidemic and potential burden of disease in adolescents is to be reduced, future studies must be designed to properly address these questions.

Chapter 4: Gag and Nef- specific CD8 T cell responses in adolescents on HAART

Abstract

Defining correlates of immune protection is a key factor in informing effective vaccine design. Robust HIV-specific CD8 T cell responses have been observed in adult subjects who control viral load; responses in HIV-positive adolescents, however, are less understood. Here, we examined HIV-specific CD8 T cell responses to Gag and Nef in 26 HIV-positive adolescents who were intermittently compliant to HAART. While we could identify Gag-and Nef specific responses in many of these subjects, we found no significant differences in the magnitude or proportion of cytokine producing nef or gag specific CD8 T cells between aviremic or viremic subjects. There was also no relationship observed between age and the magnitude of the total HIV-specific CD8 T cell response in our cohort. Our data show that adolescents on intermittent therapy produce functional responses.

Introduction

Since the identification of a temporal correlation between HIV-specific CD8 T cell expansion and acute viral resolution (Borrow et al., 1994; Koup et al., 1994), several groups have sought to identify the role of HIV-specific CD8 T cells in disease progression. Multiple independent reports have demonstrated an inverse correlation between the magnitude of the Gag-specific CD8+ T cell response and viremia (Riviere et al., 1995; Ogg et al., 1998; Kiepiela et al., 2007; Rolland et al., 2008). Moreover, subjects who control viral load in the absence of therapy have HIV-specific CD8 T cell responses directed against multiple epitopes and enriched for perforin-mediated killing ability

(Betts et al., 2001a; Migueles et al., 2002; Betts et al., 2006; Emu et al., 2008; Hersperger et al., 2010a). Together these findings suggest that HIV-specific CD8⁺ T cells play an important role in controlling HIV infection. Evidence for the importance of CD8 mediated control has motivated the field to develop T cell based vaccines against HIV, although this strategy has not yet yielded an efficacious vaccine (Watanabe, 2003; McElrath et al., 2008).

The majority of work on HIV-specific CD8 T cell responses has been performed in adults, and little is known about the protective role of these responses in adolescents, a population that makes up 50% of new HIV cases yearly (Futerman, 2005). Because these subjects will be lifelong carriers of HIV, it is important to understand the protective capacity of HIV-specific CD8⁺ T cells in adolescents. Whether the quality of the HIV-specific CD8⁺ T cell response is associated with disease control in adolescents understudied. Most reports have focused primarily on identifying antigen specificity through cytolytic function or IFN- γ responses (Luzuriaga et al., 1995; Buseyne et al., 1998; Spiegel et al., 2000; Scott-Algara et al., 2001; Buseyne et al., 2005b), but a more detailed assessment of total HIV-specific CD8 T cell response has not been performed.

Here, we used polychromatic flow cytometry to examine Gag and Nef-specific CD8 T cell responses in viremic and aviremic adolescents on therapy, focusing on defining the polyfunctional and cytolytic marker expression capacity of HIV-specific CD8 T cells from these individuals. Because higher CD4 T cells counts are indicative of stronger CD8 responses in adults and children (Sandberg et al., 2003), we additionally performed analysis to determine if this trend was also apparent in adolescents. Our preliminary findings are not significant but show a dichotomy in the functional response: viremic subjects appear to have a higher percentage of Nef-responding CD8 T cell than their aviremic counterparts; conversely, aviremic subjects mount a more robust

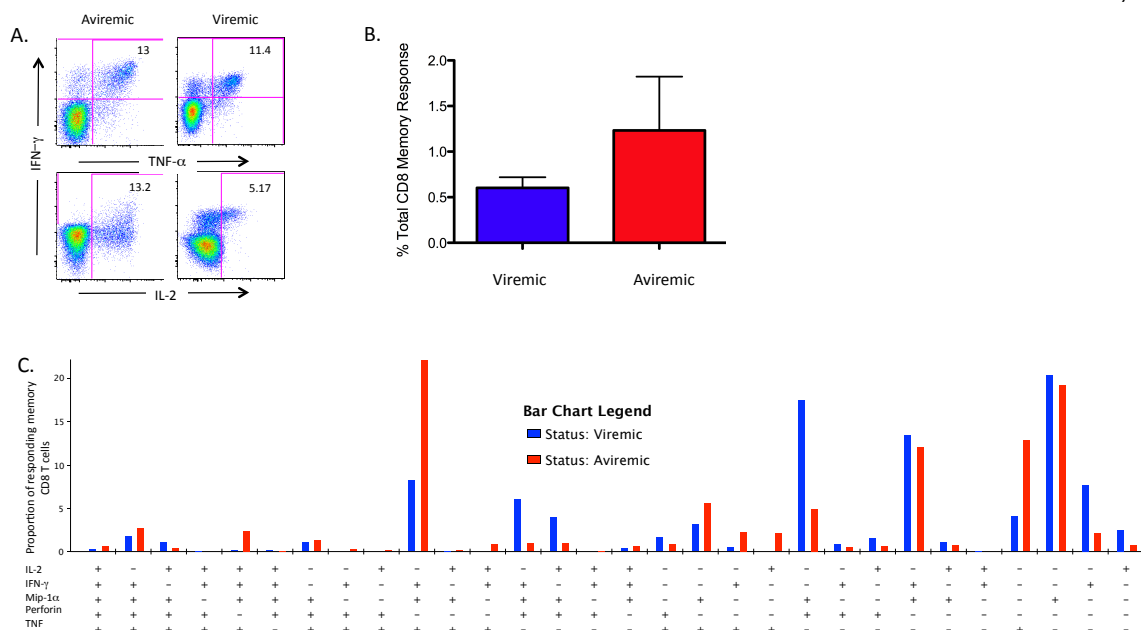


Figure 15: HIV-specific CD8 T cell response in adolescents. Cryopreserved PBMCs were stimulated with Gag or Nef peptide for 6 hours in the presence of BFA. Cells were then stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and cytokines (IL-2, IFN-g, TNF, Perforin, MIP-1a). CD27+CD45RO- (naïve) cells were excluded from the analysis for the memory population. (A) SEB- stimulated memory CD8 T cell responses from an aviremic and viremic subject. (B) The % total cytokine producing (Gag+Nef responding) populations were graphed for viremic (blue) and aviremic (red) subjects. (C) The average expression profile for the memory CD8 T cell response is graphed for IL-2, IFN-g, TNF, Perforin, MIP-1a

polyfunctional response to Gag. These results suggest that CD8 T cells in HIV-positive adolescents are functional and may respond differently based on specificity.

Materials and Methods

4.1 Subjects: Donor peripheral blood mononuclear cells were obtained from the laboratory of Dr. John Sleasman at the University of South Florida in compliance with the guidelines set by the internal review boards of both institutions.

4.2 Staining and Stimulation Assay: Cryopreserved human PBMCs were thawed and subsequently rested overnight @ 37°, 5% CO₂ in complete medium supplemented with 10% FBS, 1% L-glutamine. Cells were then washed with complete medium and resuspended at a concentration of 1 x10⁶ cells/mL if sufficient numbers were available. Brefeldin A and co-stimulatory molecules CD28 and CD49d were added at a final concentration of 1µg/ml. PBMCs were incubated at 37°C, 5% CO₂ with overlapping 15-mer peptide pools encompassing HIV-1 (Clade B) Gag and Nef proteins. At the conclusion of the incubation period conventional surface staining and intracellular cytokine staining was done as described in CHAPTER 2 and published previously (Betts et al., 2001b; Betts et al., 2006; Chattopadhyay et al., 2006).

4.3 Antibodies: To determine cell viability, we used Aqua Blue viability dye (Invitrogen, Carlsbad, California). Monoclonal antibodies (mAbs) for surface staining included: (i) anti-CD8 Qdot 605, anti-CD19 APC-Alexa-750 and anti-CD16 APC-Alexa-750 (Invitrogen, Carlsbad, California); (ii) anti-CD45RO TRPE and anti-CD27 PeCy5 (Beckman Coulter, Fullerton, California); and (iii) anti-CD4 PeCy5-5 (eBioscience) For intracellular staining, mAbs included anti-CD3 Q-dot 655 (Invitrogen, Carlsbad, California) anti-TNF PeCy7, anti-IL-2 APC, anti-Mip-1a FITC (eBioscience) and anti-IFN-γ Alexa 700 (Invitrogen, Carlsbad, California); Perforin-Pacific Blue (Custom).

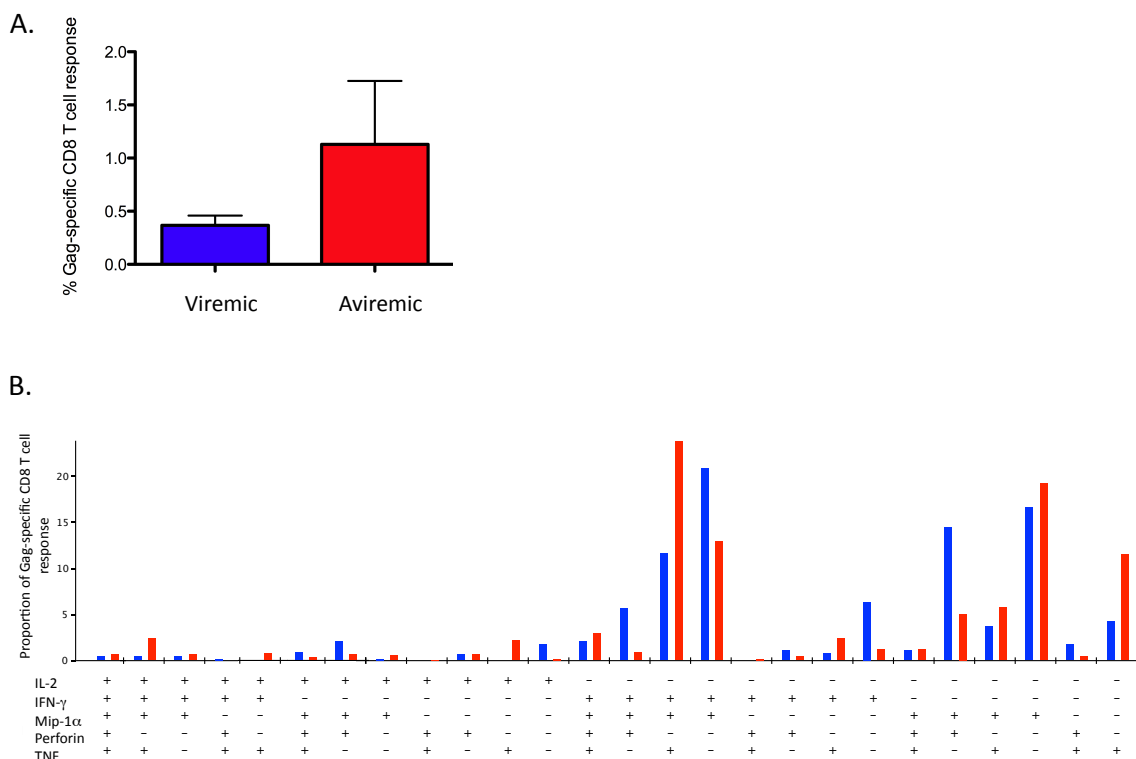


Figure 16: Gag-specific CD8 T cell response in adolescents. Cryopreserved PBMCs were stimulated with Gag for 6 hours in the presence of BFA. Cells were then stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and cytokines (IL-2, IFN-g, TNF, Perforin, MIP-1a). CD27+CD45RO- (naïve) cells were excluded from the analysis for the memory population. (A) The % Gag-specific responding populations were graphed for viremic (blue) and aviremic (red) subjects. (B) The average expression profile for the HIV-Gag-specific CD8 T cell response is graphed for IL-2, IFN-g, TNF, Perforin, MIP-1a

4.4 Flow cytometric analysis: Analysis was performed as discussed in Chapter 2.

Briefly, for each specimen, between 300,000 and one million total events were acquired on a modified LSRII flow cytometer (BD Immunocytometry Systems, San Jose, California) equipped for the detection of 18 fluorescent parameters. Anti-mouse Igk antibody capture beads (BD Biosciences) were used to prepare individual compensation tubes for each mAb used in the experiment. Data analysis was performed using FlowJo version 9.1 (TreeStar, Ashland, Oregon) and SPICE version 5.21. Boolean gating analysis was performed for each functional parameter. This analysis resulted in 32 possible permutations of the 5 measured functions. Events measured negative for all functional parameters and perforin single positive cells were ignored for analysis. By excluding perforin single positive cells it can be assured that perforin examined within the analysis is HIV-specific and present in activated memory CD8 T cells expressing at least one other function. Naïve CD8 T cells (CD27+CD45R0+) were excluded from the analysis

4.5 Statistics: Graphing and Statistical analysis was performed using GraphPad Prism software (version 5.0a). Total magnitude and functionality were compared between the two subject groups using non-parametric t-tests (Mann-Whitney). Correlations were based on Spearman correlation coefficients.

Results

Studies in HIV elite controllers have shown that two types of CD8⁺ T cell responses correlate with control of viremia: highly polyfunctional cells characterized by simultaneous production of IL2, IFN- γ , TNF, MIP1- β , and CD107a, and also CD8⁺ T cells producing perforin in combination with other measured functions (Zimmerli et al., 2005; Betts et al., 2006; Saez-Cirion et al., 2007; Migueles et al., 2009; Hersperger et al., 2010b). As shown in Figure 15A, our panel was able to detect polyfunctional CD8⁺ T cell responses after stimulation with SEB, indicating that both viremic and aviremic

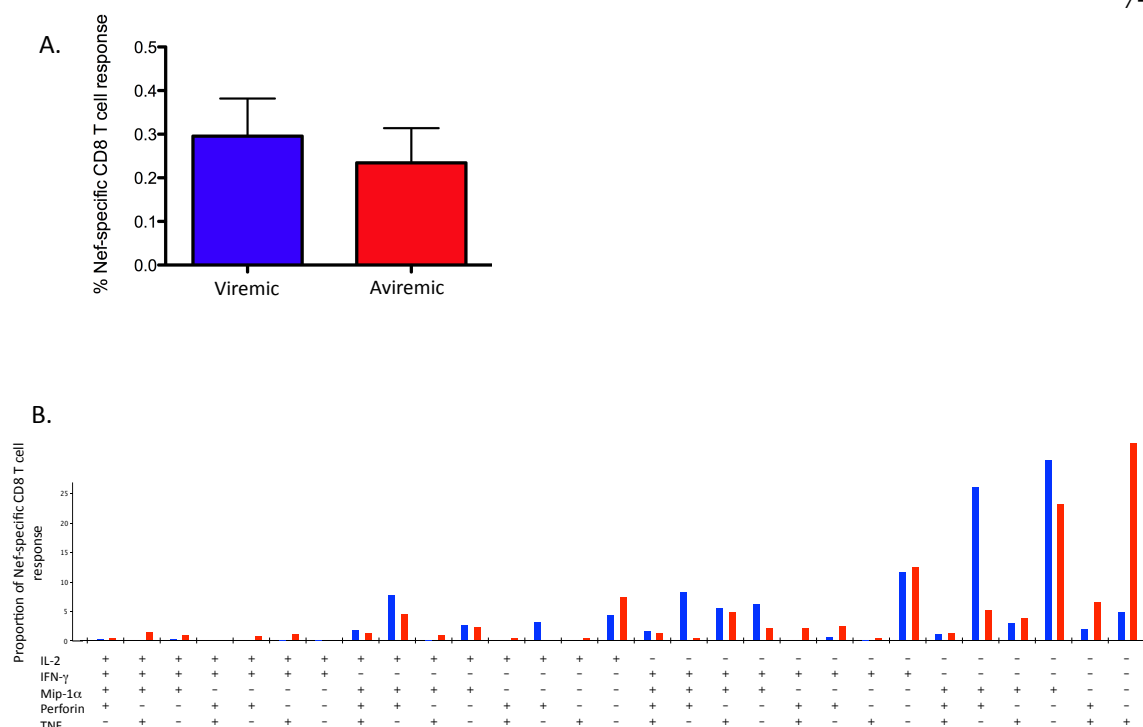
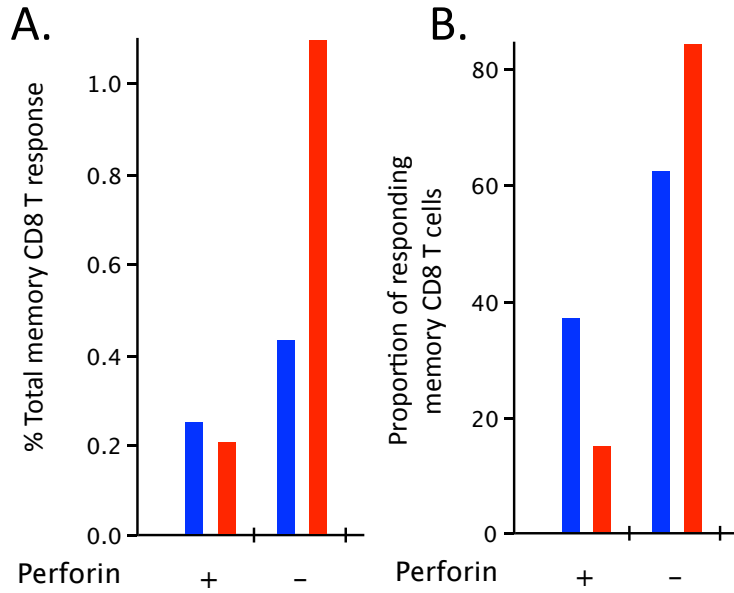


Figure 17: Nef-specific CD8 T cell response in adolescents. Cryoperserved PBMCs were stimulated with Nef for 6 hours in the presence of BFA. Cells were then stained for lineage markers (CD3, CD4, CD8, CD19, CD16, CD14) memory markers (CD27, CD45RO, CCR7) and cytokines (IL-2, IFN- γ , TNF, Perforin, MIP-1 α). CD27+CD45RO- (naïve) cells were excluded from the analysis for the memory population. (A) The Gag-specific responding populations were graphed for viremic (blue) and aviremic (red) subjects. (B) The average expression profile for the HIV-Nef-specific CD8 T cell response is graphed for IL-2, IFN- γ , TNF, Perforin, MIP-1 α

adolescents were capable of eliciting polyfunctional T cell responses. Although the aviremic subjects appeared to have higher responses, we found no statistical difference in the total CD8⁺ T cell response frequency in the two subject groups (Figure 15B). We next examined the polyfunctional profile of the HIV-specific CD8 T cells in aviremic and viremic subjects. The aviremic group appeared to have a higher proportion of 3-function producers (IFN- γ +MIP+TNF+) but this difference was not significant (Figure 15C). We next assessed the total response (Gag+Nef) in viremic and aviremic subjects. Therefore, we do not see a difference in total magnitude or polyfunctionality between viremic and aviremic subjects on therapy.

We next examined the relative contribution of Gag and Nef peptide pools to the HIV-specific CD8 T cell response. Aviremics appear to have a higher Gag-specific CD8 T cell response than their viremic counterparts (Figure 16A) but this difference was not significant. Furthermore, the response was dominated by 3- (IFN- γ +MIP-1 α +TNF+), 2- (IFN- γ +MIP-1 α +), and 1-function producers (MIP-1 α) (Figure 16B). These same functional combinations were also dominant within viremic subjects. When we examined the Nef-specific response, it appears as if a higher proportion of CD8 T cells in viremics responded compared to the aviremics, but this difference was not significant (Figure 17A). Unlike the aviremic Gag-specific response, single function cells dominated the viremic Nef-specific CD8 T cell response.

We therefore specifically examined the relative contribution of these two different populations to the CD8 response in the viremic and aviremic subjects. First we assessed the presence of the most highly polyfunctional cells, measured here as those cells producing IFN- γ , TNF, IL-2, and MIP1- α with or without perforin (CD107a was not measured in our panel). As shown in Figure 18A, very few highly polyfunctional HIV-specific CD8⁺ T cell responses were detected in the viremics and aviremics. Interestingly,



Bar Chart Legend

- Status: Viremic
- Status: Aviremic

Figure 18: Perforin upregulation and polyfunctionality in the total HIV-Specific CD8 T cell response. (A) The magnitude and (B) proportion of the HIV-specific CD8 T cell response to Gag and Nef peptide pools was calculated for viremic (blue) and aviremic (red) subjects and plotted.

viremic subjects had a larger proportion of perforin-producing CD8 T cells than their aviremic counterparts (Figure 18B). Therefore, this suggests that even though viremic subjects have cytolytic function they may be less likely to control viral load in the absence of robust polyfunctional responses.

We next looked at the proportion of cells producing perforin in response to Gag or Nef peptide. A higher proportion of Nef- and Gag-specific CD8 T cells from viremic subjects produce perforin compared to their aviremic counterparts (Figure 19A-B). It is of note that a smaller proportion of Nef and Gag responding CD8 T cells in viremic subjects are polyfunctional compared to those in aviremic subjects (19A-B). As shown in Figure 19C, the magnitude of the Nef response is low in both subject groups but the viremic subjects display a more robust and polyfunctional response. Conversely, Gag-specific CD8 T cells respond with higher magnitude than the aviremic subjects (Figure 19D). Similar to studies in adults (Betts et al., 2006; Hersperger et al., 2010b; Hersperger et al., 2011), these data suggest that a more polyfunctional response to HIV proteins is a characteristic of subjects with low viral loads. Unlike these previous studies we show subjects with higher viral load trend towards a higher proportion of perforin-producing cells.

In children and young adults, age is often a strong correlate with functional responses and disease parameters (Buseyne et al., 1998). However, these studies were limited to IFN- γ ELISpot assays that do not accurately depict the complexity of an immune response. Therefore, we examined whether age was related to total T cell functionality. We found no significant correlation between age and HIV-specific CD8 T cell responses when including multiple functional outputs (20A-C). We next assessed the relationship between CD4 T cell percentage and the HIV-specific CD8 T cell response. We found a trend towards an inverse relationship between the CD4 T cell percentage and CD8⁺ T cell responses to the HIV peptides (Gag, Gag+Nef) (Figure 21A-B). We observed

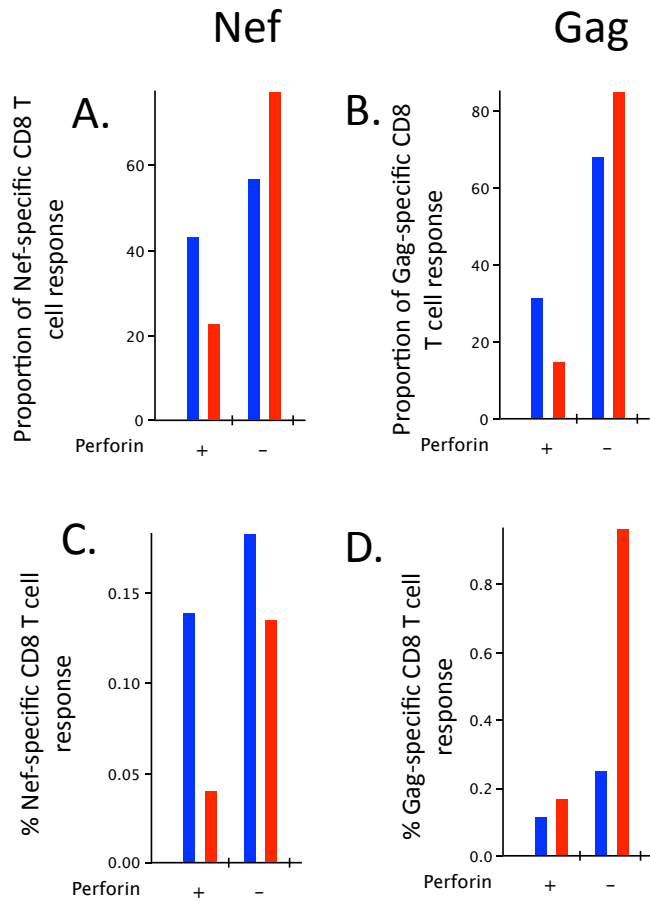


Figure 19: Perforin upregulation versus polyfunctionality of Gag and Nef-Specific CD8 T cell responses. (A-B) The proportion and (C-D) magnitude of the HIV-specific CD8 T cell response to Gag and Nef peptide in viremic (blue) and aviremic (red) subjects. Where (+) indicates the proportion of the response belonging to perforin production and (-) is calculated by summing across all functional combinations with the exclusion of perforin

a trend towards a direct relationship between CD4 T cell percentage and the Nef response (Figure 21C). We did not find a relationship between CD4 T cell count and antigen response (Figure 22A-C). Together these results indicate that HIV-specific CD8 T cell functionality is not directly influenced by subject age or CD4 T cell count.

Discussion

HIV-specific CD8 T cell responses are extensively studied and are a likely correlate of immune protection in HIV-positive adults. While studies have advanced over the past few years in our understanding of the CD8 T cell response, there is a paucity of similar studies in adolescents. Being that adolescents will be long term carriers of the disease, it is therefore important to establish what key factors in the CD8 T cell response correspond to viral control. Determining these factors will help tailor future vaccine design by exposing what specific responses are necessary for efficient viral control. In this study, we measured Gag and Nef-specific responses in HIV-positive adolescents on therapy. We find that adolescents on therapy are capable of making functional responses to both Gag and Nef peptides.

Gag-specific CD8 T cell responses have been shown to be a correlate of viral control in HIV-positive adults and infants. These responses are enriched in slow progressors who maintain low viral loads (Klein et al., 1995; Riviere et al., 1995; Edwards et al., 2002). We did not observe a difference between Gag-specific responses across subject groups. The reasons for this lack of significance were likely low subject number or the high variability in response patterns. We did however observe that the aviremic subjects appeared to exhibit a more robust and polyfunctional response to Gag. This is supported by previous reports in elite controllers where subjects with lower viral loads demonstrate responses with higher magnitude and polyfunctionality (Betts et al., 2006; Hersperger et al., 2010a).

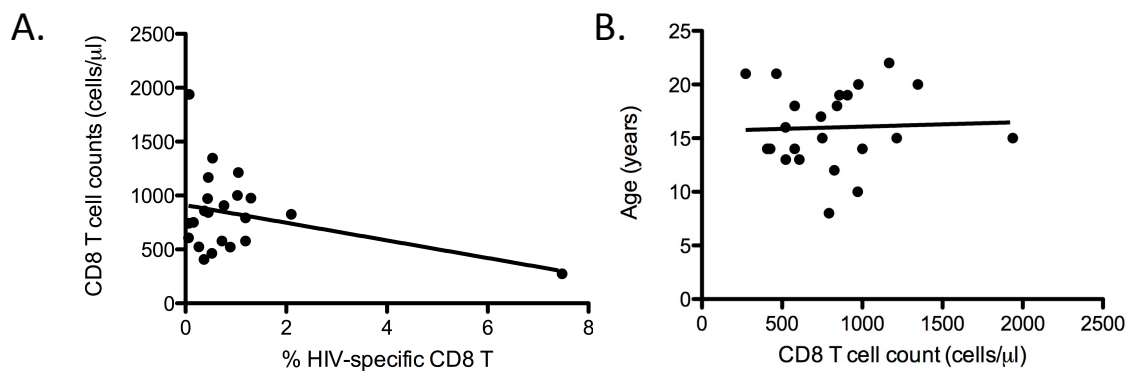


Figure 20: There is no relationship between age and CD8 T cell parameters.

(A) The average proportion of cytokine producing HIV-specific CD8 T cells (B) CD8 T cell count within each subject was plotted against each subjects age.

Nef-specific CD8⁺ T cell responses as detected by IFN- γ ELISpot have been shown to be robust in HIV-positive children on therapy (Sandberg et al., 2003). We therefore chose to examine the Nef-specific response in HIV-positive adolescents. We did not find any significant differences in Nef-specific responses between the viremic and aviremic groups. Unlike the Sandberg study, which used ELISpot analysis, we determined Nef-specific CD8 responses using multiparameter flow cytometry. As such, accounting for non-IFN- γ producing HIV-specific CD8⁺ T cells in HIV infected adolescents may change our understanding of the association between HIV-specific CD8⁺ T cells and viral control in HIV infected adolescents.

Adolescents have higher thymic output of naïve T cells, which allows for better naïve CD4 T cell recovery while adherent to therapy (Cohen Stuart et al., 1998b; Douek et al., 1998; Sleasman et al., 1999; Vigano et al., 2000b). This advantage can often correlate to decreased viral load and improved CD8 T cell responses. Indeed subjects with CD4 T cell counts ranging 400-1500 cells/ μ l have increased breadth and magnitude of HIV-CD8 T specific T cell responses as determined by ELISpot (Sandberg et al., 2003). The results of this report are not surprising in that higher circulating CD4 T cells often correlate to a more polyfunctional CD8 T cell response in adults (Klein et al., 1995; Pantaleo et al., 1995; Betts et al., 2006). Although the subjects in our cohort had CD4 T cell counts ranging from 136-1383 cells/ μ l and a mean CD4 T cell count of 577 cells/ μ l, we did not observe robust responses as seen in previous reports. A key difference between our study and studies in adults is that the subjects in our cohort were on therapy- and notably were often only intermittently compliant. It has been shown that functional responses decay after therapy initiation, providing the subjects are compliant (Casazza et al., 2001). Therefore, the presence of healthy CD4 count levels may only predict polyfunctionality in the absence of therapy. This area should be investigated

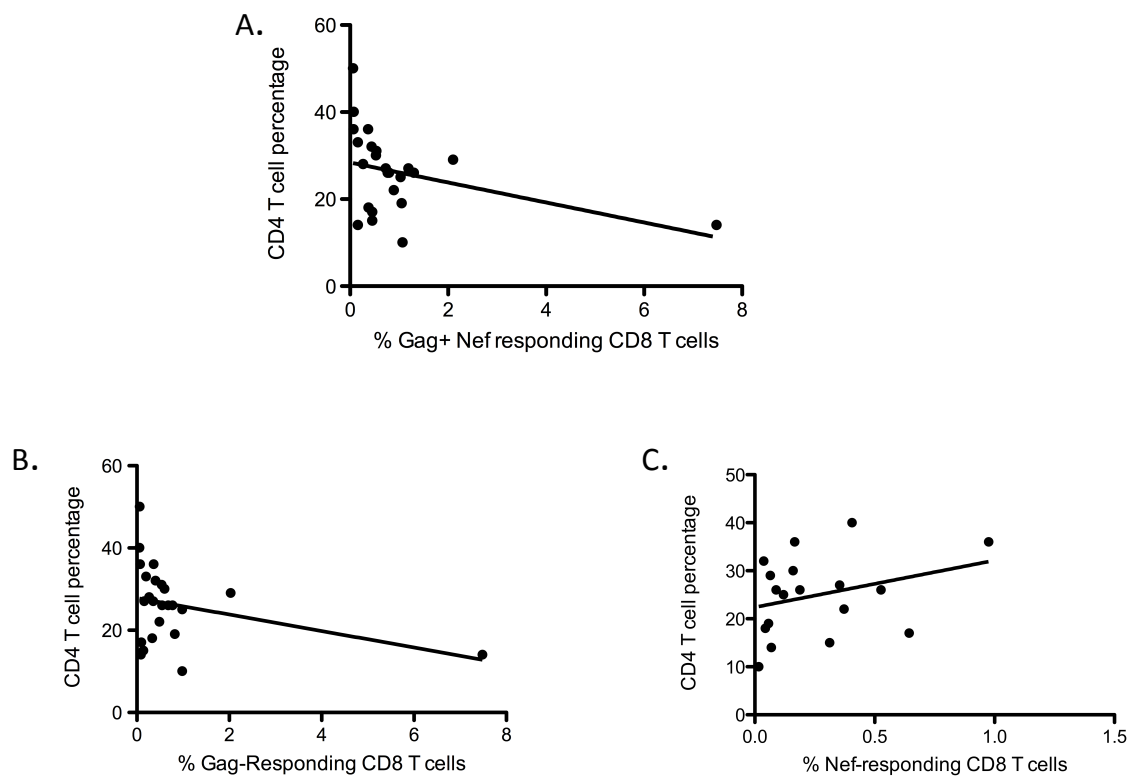


Figure 21: There is no relationship between CD4 T cell percentage and HIV-specific CD8 T cell response. (A) The average proportion of cytokine producing HIV-specific CD8 T cells (B) Gag-specific (C) Nef-specific are plotted versus the CD4 T cell percentage from each respective subject

further to determine if higher CD4 T cell counts correlate to a more functional HIV-specific CD8 T cell population in HIV-positive adolescents.

As stated previously, CD8 T cells in subjects on HAART retain functionality but have reduced response magnitude to HIV peptides. Aviremic subjects within our study have robust CD8 T cell responses. Additionally, a large proportion of these responses are polyfunctional. It is possible that the functional responses we see in aviremics are due to intermittent rounds of replication due to non-compliance. However, we do not have a pre-therapy time point available to compare pre-treatment functionality, which would allow us to determine if aviremics initially had higher responses that are now steadily decreasing as expected.

A growing body of literature supports the hypothesis that cytolytic killing ability is a correlate of immune control in HIV-positive subjects (Migueles et al., 2002; Hersperger et al., 2010a; Hersperger et al., 2011). A subset of individuals termed elite controllers sustain viral control and have superior ability to upregulate cytolytic molecules like perforin in response to HIV stimulation. Here, we identified a higher proportion of perforin production in the viremic adolescents compared to aviremic subjects in response to both Gag and Nef. In fact, the proportion of perforin production in the viremics for both peptides is similar to that of the perforin proportion in elite controllers (Hersperger et al., 2010a). Moreover, the proportion of the response that is perforin positive in aviremic subjects is similar to that of chronic progressors. Further study of additional adolescents, particularly ones compliant to therapy, will be necessary to determine the relationship between perforin and viral load, and whether the associations observed in adults are different than those found in adolescents.

CD8 T cell mediated control is thought to be a potential factor in controlling viral replication and dictating disease outcome. Although we do not see any significant

associations of polyfunctionality in HIV-positive adolescents, this area should continue to be researched. A larger study population should be used to address the potential impact of HIV-specific CD8 T cells in HIV-positive adolescents. Future studies should be longitudinal in design and include assessment of several factors that correspond to CD8 T cell mediated control like HLA types, escape mutations and virus specific responses to determine if certain adolescents are more prone to disease progression than others.

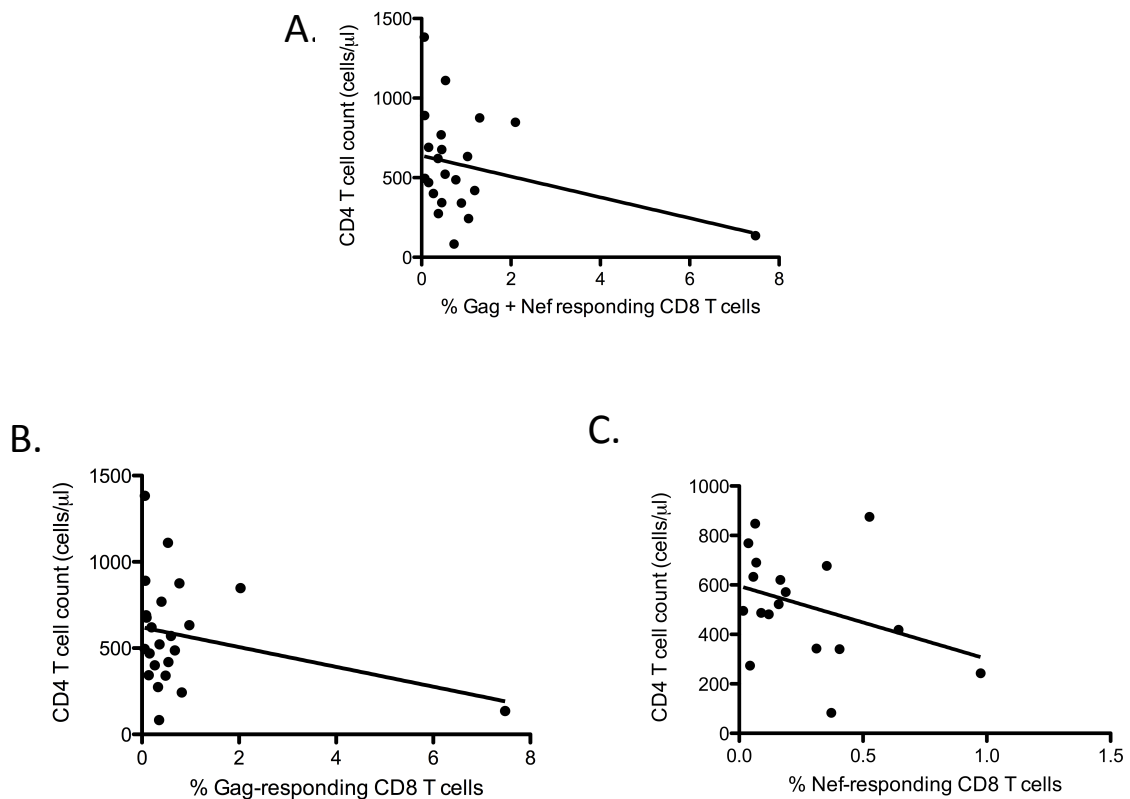


Figure 22: There is no relationship between CD4 T cell count

and antigen-specific CD8 T cell response. (A) The average proportion of cytokine producing HIV-specific CD8 T cells (B) Gag-specific (C) Nef-specific are plotted against the CD4 T cell count from each respective subject.

Chapter 5: Discussion and Implications

Immune Activation and Turnover in adolescents

In HIV infection, increased immune activation as indicated by elevated expression of CD38 and CD38/HLA-DR co-expression in memory CD4 and CD8 T cells is a key driver of disease progression in adults (Giorgi et al., 1993; Liu et al., 1997; Orendi et al., 1998; Giorgi et al., 2002; Emu et al., 2005; Hunt et al., 2008). Here, we assessed immune activation in memory T cell subsets of HIV positive adolescents in order to assess how differential therapy adherence coupled with enhanced thymic output may influence HIV-induced immune activation. Our results show increased expression of CD38 in all memory subsets of viremic adolescents that does not decrease over time in the presence or absence of effective antiretroviral therapy. Unlike other reports, activation defined by CD38/HLA-DR is less pronounced in our study and confined to the bulk memory CD4 T cell subset.

In addition to chronic immune activation in adolescents, we observed increased cell cycling (Ki67) in central memory CD8 T cells and all CD4 T cell subsets. Increased T cell turnover or cycling as indicated by Ki67 expression is a hallmark of HIV infection and subsequent disease progression (Mohri, 1998; Sachsenberg et al., 1998b; Cohen Stuart et al., 2000). T cell cycling typically normalizes after therapy initiation (Hazenberg et al., 2000c); however, in our study, the subjects were poorly adherent to therapy and therefore maintain elevated levels of Ki67 expressing CD8 T cells. This suggests that suboptimal therapy compliance impairs T cell turnover normalization. These results are similar to previous works by Rudy, et al, where continued activation and turnover were characteristics of adolescents who were not adherent to HAART (Rudy et al., 2006a; ATN 061 B.Rudy, J. Sleasman, C. Wilson). It is important to note

that despite high CD4 T cell counts and viral suppression, subjects with high Ki67 expression are characteristically more prone to immune failure.

As a result of increased activation and turnover in HIV infected adults, CD4 and CD8⁺ T cells are driven to immune senescence which impairs the immune response (Sieg et al., 2008). It is unclear, however, whether immune senescence occurs similarly in HIV-infected adolescents. In future studies, we could assess immune senescence through the assessment of CD57 and CD28 expression, as well as telomere length and telomerase activity in HIV infected adolescents (Effros et al., 1996; Brenchley et al., 2003; Appay et al., 2007).

In the setting of HIV infection, a large number of CD4⁺ T cells are depleted due to a number of reasons including direct killing by virus, bystander cell death, activation induced cell death, and CD8 T cell mediated killing (Hazenberg et al., 2000a; Douek et al., 2002; Silvestri and Feinberg, 2003). However, because adolescents have increased thymic output, heightened production of naïve CD4⁺ and CD8⁺ T cells (Smith et al., 2000; Vigano et al., 2000b; Rudy et al., 2001) they may be able to better compensate for HIV-induced depletion. Indeed, while on therapy HIV-infected adolescents recover their CD4⁺ T cell counts more effectively and to slightly higher levels than their adult counterparts. Moreover, in the presence of controlled viral replication and decreased immune activation, adolescent subjects experience decreased instances of viral recrudescence (Douglas et al., 2000; Starr et al., 2002a; Rudy et al., 2006a). However, when activation is not controlled, the advantage of increased thymic output is lost and virus rebounds. These findings highlight the immunological importance of maintaining effective antiretroviral therapy in HIV infected adolescents.

De-intensification: A means to promote adherence

HIV-infected adolescents are less likely to adhere to therapy guidelines compared to HIV-infected adults (Futterman, 2005). Suboptimal therapy compliance can lead to drug resistance, viral breakthrough and increased immune activation. Our data in CHAPTERS 2 and 3 confirm increased immune activation in adolescents on suboptimal therapy. Importantly, our results further highlight the critical need to ensure that HIV-infected adolescents maintain effective therapy. These results suggest that poor adherence in adolescents subjects them to continuous rounds of virologic failure, making them more prone to rapid disease progression than therapy adherent adults. Therefore, one of the key short-term factors to successful treatment of adolescents is increasing adherence.

De-intensification strategies are being explored in order to promote adherence in adolescents (ATN 061, B. Rudy, J. Sleasman, C. Wilson study PIs). The goal of de-intensification is to identify the minimum optimal drug combination and dosage strategy needed to establish and sustain viral control. Once virologic control is established, drugs are removed from the regimen to decrease side effects and promote adherence. A goal of this thesis was to determine the levels of immune activation in adolescent subjects currently on therapy in order to serve as a baseline comparison for de-intensification studies. However, one must consider whether the results from carefully controlled de-intensification studies will translate to success in the clinic. As such, to ensure success, de-intensification strategies should be introduced coincident with both adolescent health counseling to ensure adherence and careful monitoring of the immunologic and virologic status of individuals receiving de-intensified therapy.

Is adherence the ultimate answer?

Adherence to medication has been shown to be the only factor that predicts viral control in adolescents (Sleasman et al., 1999; Starr et al., 2002a; Rudy et al., 2006a). Collectively, our data support the need to improve therapy adherence in HIV-positive adolescents. However, if adherence is achieved, it may not produce the same benefits as seen in chronically infected adult counterparts long-term.

The factors contributing to long-term success in HAART-treated adults are unknown and fairly understudied. A recent report in HAART-suppressed subjects showed that increased CD4 T cell depletion, microbial translocation and immune activation were linked to immune failure in therapy adherent adult subjects. Not only were these studies conducted 7 years post therapy initiation, but all subjects durably suppressed viral replication (Lederman et al., 2011). This suggests that therapy adherence may not be the only key to long-term survival. Considering that virologic control may need to be maintained in adolescents for over 20 years (and throughout adulthood), it is premature to assume that adherence alone will equate to long-term protection.

Functional Responses

Although polyfunctional HIV-specific CD8 T cell responses have been shown to correlate with viral control in infected adults (Betts et al., 1999; Emu et al., 2005; Betts et al., 2006), we did not find this relationship in our adolescent cohort. There was no significant difference between Gag or Nef-specific CD8 T cell responses in adolescents who controlled virus versus those who did not. This could be for several reasons; (i) adolescents have less robust functional responses than adults (Buseyne et al., 2005a); (ii) HIV exposure may cause some basic T cell functions like proliferation and cytokine production to be impaired (Autran et al., 1997b; Connick et al., 2000); (iii) our study

power was too low to properly assess differences in function. To determine if there is a correlation between function and viral control future studies should be of the appropriate power. Functional responses to other HIV viral proteins, CMV and EBV should be determined to understand the breadth of the CD8 response in HIV-positive adolescents. Viral control is not solely dictated by functional responses. Indeed, HLA types have been shown to confer protection against disease progression (Carrington et al., 1999; Altfeld et al., 2006; Emu et al., 2008). Therefore, future studies should also involve HLA typing to determine if the presence of certain MHC loci equate to superior viral control.

Microbial Translocation

This body of work is the first to define the relationship between immune activation, viral load and microbial translocation in adolescents. Similar to results in adults, my findings indicate elevated sCD14 levels in viremic adolescent subjects and direct correlations between sCD14 and viral load. Contrary to what has been seen in other reports concerning adolescents (Papasavvas et al., 2009b; Wallet et al., 2010), we see a correlation between sCD14 and CD38 expression in T cell memory subsets. This thesis has thus laid the groundwork for future studies looking to determine a relationship between microbial translocation and disease progression in adolescents.

Soluble CD14 has been associated with increased risk of mortality in adults, but it is not the only marker of microbial translocation (Nixon and Landay, 2010). LPS, a potent immunostimulatory toxin, is the most direct indicator of microbial translocation. Several studies have reported elevated levels of LPS in chronically infected subjects and those with AIDS. In the few studies concerning microbial translocation in HIV-positive children and infants, levels of LPS and sCD14 are elevated (Wallet et al., 2010; Papasavvas et al., 2011). These levels often persist in the presence of suppressed viremia,

increased CD4 T cell counts and decreased immune activation. This suggests that gut injury during HIV-infection promotes ongoing translocation of microbial products even under conditions of relative viral control (Nixon and Landay, 2010). Inflammatory factors like IL-6 and hs-CRP are associated with disease progression (El-Sadr et al., 2006) and increased risk of opportunistic infections in HIV infected-adults (Rodger et al., 2009). Long-term studies in adolescents are needed to determine the overall effects these factors may have on HIV disease prognosis.

The secondary lymphatic tissue plays a pivotal role in immune system homeostasis (Kaldjian et al., 2001). It is estimated that ~90 % of CD4 T cell reside in secondary lymphoid tissue, poised to respond to immunological threats. During HIV infection massive CD4 T cell depletion occurs in the intestinal mucosal tissue and lymphoid tissue resulting in impaired adaptive immune responses and the disruption of lymph node architecture (Brenchley et al., 2004; Zeng et al., 2011). Widespread damage to the gut is thought to be the driving force behind microbial translocation, a hallmark of pathogenic infection (Brenchley et al., 2006). The mucosal CD4 T cell compartment is not restored effectively by HAART and will greatly impact subjects longitudinally even if they are adherent to therapy. The impact of prolonged gut damage is an issue in understanding adolescent HIV disease progression.

Our cohort consisted of individuals who acquired HIV either at birth or through risky sexual behaviors. Key differences in the routes of infection in these subjects may warrant independent studies in each population. It has been shown that early cycles of viremia during infancy may destroy collagen networks needed for normal development of the human gut (Schacker et al., 2002; Neu, 2007; Estes et al., 2008). This suggests that early on the immune response in perinatally infected youth may be impaired due to insufficient development of the intestinal microenvironment. Therefore, studies should

be designed to specifically address the competence of the immune system in behaviorally infected adolescents compared to perinatally infected adolescents to determine the long-term effects resulting from gut damage. It is possible that the immunodepletion that takes place in the gut will have differential effects on immune responses and disease progression in these two subject groups.

Prolonged sCD14 production induced by microbial translocation has been linked to deficient CD4 T cell reconstitution (Marchetti et al., 2008) and HIV associated inflammatory disorders (Ancuta et al., 2008; Nixon and Landay, 2010). As stated previously, injury to the intestinal epithelium is not reversed by therapy. Being that microbial translocation is one of the underlying causes of immune activation, the strongest predictor of disease progression, future studies should address ways to repair intestinal damage incurred by HIV infection. Probiotics may be the first step to repairing injured gut and have been used in individuals with gastrointestinal disorders to restore an effective gut barrier. Researchers are presently examining the use of probiotics as a means to reconstitute immune cells in the gut. A recent study showed that probiotic consumption boosted CD4 T cell recovery (Irvine et al., 2010). Extension of these probiotic studies could help with immune reconstitution and minimize extended damage to the gut.

Conclusion

This body of work has shown that increased immune activation is present in adolescents undergoing suboptimal therapy. Follow up studies should involve larger populations of subjects to allow for increased statistical power and inter-cohort comparisons between immune capabilities of behavioral and perinatally infected subjects. This study highlights the importance of finding alternate strategies for the control of HIV replication in HIV-positive adolescents. Improving adherence,

identifying alternative treatments and understanding gut dynamics in HIV-positive adolescents is pivotal if we hope to reduce disease progression and improve the health quality of the growing HIV-positive population.

Appendix

Table I: Detailed information of HIV+ Adolescent Subjects

Subject	Date	Age	Gender	Ethnicity	Transmission Mode	CD8%	Absolute CD8	CD4%	Absolute CD4	Virus RNA	Date of Initial ART
ZAB90	06/01/10	16	F	B	perinatal	55	1001	24	481	82,600	2003
ZAF92	06/22/11	15	F	B	perinatal	65	1938	10	11/04	838	2003
	11/29/11	15				ND	ND	ND	ND	15,100	
ZAG89	08/24/10	17	M	B	perinatal	52	805	26	399	1,820	2003
	03/30/11	18				46	274	32	189	55,889	
	04/18/12	19				46	511	38	426	39618	
ZAJ90	07/13/10	16	F	W	perinatal	51	895	36	631	<50	2004
ZAL88	06/28/11	19	F	B	perinatal	49	908	26	487	<50	2005
ZAM90	09/27/09	15	F	B	behavioral	50	751	19	280	2,150	2003
	04/18/10	16				57	679	16	191	6,556	
	05/16/10	16				52	587	17	192	6,550	
	07/11/10	16				49	610	21	261	<50	
	11/06/11	17				53	1082	22	436	96	
ZAN89	03/07/10	17	F	B	perinatal	58	742	19	243	54,300	2005?
	05/16/10	17				62	768	16	198	167,000	
	01/09/11	18				59	797	14	194	407,000	
	03/11/12	19				49	1165	18	416	<50	
	03/10/13	20				38	1522	26	1,044	<48	
	11/10/13	20				ND	ND	33	837	<48	
ZAO93	05/23/10	13	F	B	perinatal	63	2762	17	745	<50	ND
	12/05/10	13				62	2012	19	621	1,690	
	03/03/13	16				57	1920	21	708	<48	
ZAQ91	02/06/11	16	F	B	perinatal	42	521	40	495	6,450	1992
	08/19/12	17				35	470	31	419	<48	
ZAR93	09/19/10	13	M	B	perinatal	36	523	28	401	<50	2002
	08/19/12	15				37	552	28	415	<48	

ZBQ86	06/26/11	21	F	B	perinatal	29	273	14	136	<50	2002
	02/05/12	22				44	763	11	186	4,422	
	03/11/12	22				45	383	13	112	25,716	
	08/12/12	22				50	618	10	122	15,134	
	11/18/12	22				41	392	9	91	15,300	
ZBS93	12/14/11	14	M	B	perinatal	45	579	26	571	<50	1994
ZBV93	10/11/11	14	M	W	perinatal	30	425	33	469	<50	1997
ZBW88	11/01/11	19	M	H	perinatal	35	857	36	891	69	1998
ZBX87	12/11/11	20	M	W	perinatal	38	1346	31	1,110	<50	ND
ZBY86	01/17/12	22	F	B	perinatal	38	1167	27	831	169	1996
	09/12/12	22				ND	ND	29	1,207	<50	
ZCA92	12/14/11	15	F	W	perinatal	64	1882	15	435	26,100	1998
ZCB91	12/20/11	16	F	B	perinatal	ND	ND	17	677	13,800	2007
ZCE95	03/08/12	13	M	B	perinatal	55	842	18	274	3,789	1995
	08/12/12	13				49	716	26	371	<48	
	09/09/12	13				48	698	21	307	<400	
ZCG96	02/26/12	12	M	W	perinatal	28	825	29	848	<400	1996
	04/15/12	12				27	766	32	901	<50	
	02/10/13	13				28	665	31	731	<48	
ZCL86	08/15/12	22	M	B	behavioral	ND	ND	22	240	3,984	N/A
ZCM88	02/13/13	21	M	B	perinatal	26	464	30	522	<48	1995
ZCN95	09/01/13	14	F	H	perinatal	24	408	36	620	<48	ND
ZCR91	03/13/13	18	F	B	behavioral	48	1112	21	497	29,800	N/A
ZDA92	09/29/13	17	F	W	behavioral	44	893	20	407	31,800	N/A
ZDE90	09/29/13	19	M	B	behavioral	45	901	35	713	>750,000	N/A
	01/12/14	20				34	460	43	587	1,130	

ZAS93	03/14/10	13	F	B	perinatal	22	608	50	1,383	<50	1994
	09/18/11	14				20	484	54	1,284	<50	
ZAU93	05/09/10	13	M	B	perinatal	41 (4/3/06)	689 (4/3/06)	31 (4/3/06)	521 (4/3/06)	<50	1998
ZAV92	02/23/10	14	F	B	perinatal	54	976	16	289	2,310	1994
	08/02/11	15				52	894	18	313	2,460	
	10/19/11					45	1061	16	388	<50	
ZAW95	11/08/09	10	M	B	perinatal	35	1312	35	1,304	5,730	1996
	01/23/11	12				30	700	46	1,079	10,300	
	02/05/12	13				43	1172	33	907	574	
ZAX87	11/22/09	18	M	B	behavioral	37	579	22	340	55,400	2006
	08/15/10	19				16	264	30	499	<50	
ZAZ86	01/31/10	20	M	B	perinatal	29	976	26	875	<50	2002
ZBA86	08/24/10	20	F	B	behavioral	61	530	17	149	7,130	2006
ZBB85	02/23/10	21	F	B	perinatal	ND	ND	24	226	111,033	2006
	07/13/10	21				43	663	26	401	1,410	
ZBC90	06/08/10	16	F	B	behavioral	29	1079	43	1,600	<50	2006
ZBE85	05/07/10	21	M	W	behavioral	53	1340	30	759	<50	2003
	08/07/10	21				55	1485	27	749	<50	
	11/21/10	21				55	1445	27	701	<50	
ZBF91	06/15/10	15	M	B	perinatal	48	1214	25	633	1,340	1997
	10/11/11	16				48	898	23	428	8,720	
ZBG97	07/31/11	10	M	B	perinatal	41	971	32	769	<50	2007
ZBI89	09/07/10	17	F	W	behavioral	ND	ND	14	690	48,130	2006
ZBJ99	08/09/11	8	F	H	perinatal	52	792	27	419	1,420	2007
	08/23/11	8				49	689	ND	ND	<50	
ZBL91	02/01/11	16	F	B	perinatal	53	851	28	462	1,310	1996

Bibliography

- Agwu, A., Lindsey, J.C., Ferguson, K., Zhang, H., Spector, S., Rudy, B.J., Ray, S.C., Douglas, S.D., Flynn, P.M. and Persaud, D. (2008) Analyses of HIV-1 drug-resistance profiles among infected adolescents experiencing delayed antiretroviral treatment switch after initial nonsuppressive highly active antiretroviral therapy. *AIDS Patient Care STDS* 22, 545-52.
- Ahuja, S.K., Kulkarni, H., Catano, G., Agan, B.K., Camargo, J.F., He, W., O'Connell, R.J., Marconi, V.C., Delmar, J., Eron, J., Clark, R.A., Frost, S., Martin, J., Ahuja, S.S., Deeks, S.G., Little, S., Richman, D., Hecht, F.M. and Dolan, M.J. (2008) CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1-infected individuals. *Nat Med* 14, 413-20.
- Allen, T.M., O'Connor, D.H., Jing, P., Dzuris, J.L., Mothe, B.R., Vogel, T.U., Dunphy, E., Liebl, M.E., Emerson, C., Wilson, N., Kunstman, K.J., Wang, X., Allison, D.B., Hughes, A.L., Desrosiers, R.C., Altman, J.D., Wolinsky, S.M., Sette, A. and Watkins, D.I. (2000) Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature* 407, 386-90.
- Altfeld, M., Kalife, E.T., Qi, Y., Streeck, H., Lichterfeld, M., Johnston, M.N., Burgett, N., Swartz, M.E., Yang, A., Alter, G., Yu, X.G., Meier, A., Rockstroh, J.K., Allen, T.M., Jessen, H., Rosenberg, E.S., Carrington, M. and Walker, B.D. (2006) HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1. *PLoS Med* 3, e403.
- Altfeld, M. and Rosenberg, E.S. (2000) The role of CD4(+) T helper cells in the cytotoxic T lymphocyte response to HIV-1. *Curr Opin Immunol* 12, 375-80.
- Ancuta, P., Kamat, A., Kunstman, K.J., Kim, E.Y., Autissier, P., Wurcel, A., Zaman, T., Stone, D., Mefford, M., Morgello, S., Singer, E.J., Wolinsky, S.M. and Gabuzda, D. (2008) Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One* 3, e2516.
- Appay, V., Almeida, J.R., Sauce, D., Autran, B. and Papagno, L. (2007) Accelerated immune senescence and HIV-1 infection. *Exp Gerontol* 42, 432-7.
- Autran, B., Carcelain, G., Li, T.S., Blanc, C., Mathez, D., Tubiana, R., Katlama, C., Debre, P. and Leibowitch, J. (1997a) Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 277, 112-6.
- Autran, B., Malphettes, M., Dhedin, N., Gorochoy, G., Leblond, V. and Debre, P. (1997b) Studies of T cell reconstitution after hematopoietic stem cell transplant. *Hematol Cell Ther* 39, 252-6.
- Badovinac, V.P. and Harty, J.T. (2006) Programming, demarcating, and manipulating CD8+ T-cell memory. *Immunol Rev* 211, 67-80.
- Bafica, A., Scanga, C.A., Schito, M., Chaussabel, D. and Sher, A. (2004) Influence of coinfecting pathogens on HIV expression: evidence for a role of Toll-like receptors. *J Immunol* 172, 7229-34.
- Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dautuet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. and Montagnier, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220, 868-71.
- Benito, J.M., Lopez, M., Lozano, S., Martinez, P., Gonzalez-Lahoz, J. and Soriano, V. (2004) CD38 expression on CD8 T lymphocytes as a marker of residual virus

- replication in chronically HIV-infected patients receiving antiretroviral therapy. *AIDS Res Hum Retroviruses* 20, 227-33.
- Bernstein, H.B., Kinter, A.L., Jackson, R. and Fauci, A.S. (2004) Neonatal natural killer cells produce chemokines and suppress HIV replication in vitro. *AIDS Res Hum Retroviruses* 20, 1189-95.
- Berson, J.F., Long, D., Doranz, B.J., Rucker, J., Jirik, F.R. and Doms, R.W. (1996) A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. *J Virol* 70, 6288-95.
- Betts, M.R., Ambrozak, D.R., Douek, D.C., Bonhoeffer, S., Brenchley, J.M., Casazza, J.P., Koup, R.A. and Picker, L.J. (2001a) Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. *J Virol* 75, 11983-91.
- Betts, M.R., Casazza, J.P. and Koup, R.A. (2001b) Monitoring HIV-specific CD8+ T cell responses by intracellular cytokine production. *Immunol Lett* 79, 117-25.
- Betts, M.R. and Harari, A. (2008) Phenotype and function of protective T cell immune responses in HIV. *Curr Opin HIV AIDS* 3, 349-55.
- Betts, M.R., Krowka, J.F., Kepler, T.B., Davidian, M., Christopherson, C., Kwok, S., Louie, L., Eron, J., Sheppard, H. and Frelinger, J.A. (1999) Human immunodeficiency virus type 1-specific cytotoxic T lymphocyte activity is inversely correlated with HIV type 1 viral load in HIV type 1-infected long-term survivors. *AIDS Res Hum Retroviruses* 15, 1219-28.
- Betts, M.R., Nason, M.C., West, S.M., De Rosa, S.C., Migueles, S.A., Abraham, J., Lederman, M.M., Benito, J.M., Goepfert, P.A., Connors, M., Roederer, M. and Koup, R.A. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 107, 4781-9.
- Billich, A., Fricker, G., Muller, I., Donatsch, P., Ettmayer, P., Gstach, H., Lehr, P., Peichl, P., Scholz, D. and Rosenwirth, B. (1995) SDZ PRI 053, an orally bioavailable human immunodeficiency virus type 1 proteinase inhibitor containing the 2-aminobenzylstatine moiety. *Antimicrob Agents Chemother* 39, 1406-13.
- Blackburn, S.D., Shin, H., Haining, W.N., Zou, T., Workman, C.J., Polley, A., Betts, M.R., Freeman, G.J., Vignali, D.A. and Wherry, E.J. (2009) Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 10, 29-37.
- Blankson, J., Persaud, D. and Siliciano, R.F. (1999) Latent reservoirs for HIV-1. *Curr Opin Infect Dis* 12, 5-11.
- Blankson, J.N. (2006) Viral reservoirs and HIV-specific immunity. *Curr Opin HIV AIDS* 1, 147-51.
- Boaz, M.J., Waters, A., Murad, S., Easterbrook, P.J. and Vyakarnam, A. (2002) Presence of HIV-1 Gag-specific IFN-gamma+IL-2+ and CD28+IL-2+ CD4 T cell responses is associated with nonprogression in HIV-1 infection. *J Immunol* 169, 6376-85.
- Borkowsky, W., Stanley, K., Douglas, S.D., Lee, S., Wiznia, A., Pelton, S., Yogev, R., McIntosh, K. and Nachman, S. (2000) Immunologic response to combination nucleoside analogue plus protease inhibitor therapy in stable antiretroviral therapy-experienced human immunodeficiency virus-infected children. *J Infect Dis* 182, 96-103.
- Borrow, P., Lewicki, H., Hahn, B.H., Shaw, G.M. and Oldstone, M.B. (1994) Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 68, 6103-10.
- Borrow, P., Lewicki, H., Wei, X., Horwitz, M.S., Peffer, N., Meyers, H., Nelson, J.A., Gairin, J.E., Hahn, B.H., Oldstone, M.B. and Shaw, G.M. (1997) Antiviral

- pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* 3, 205-11.
- Brenchley, J.M. (2004) CD4+ T Cell Depletion during all Stages of HIV Disease Occurs Predominantly in the Gastrointestinal Tract. *Journal of Experimental Medicine* 200, 749-759.
- Brenchley, J.M., Karandikar, N.J., Betts, M.R., Ambrozak, D.R., Hill, B.J., Crotty, L.E., Casazza, J.P., Kuruppu, J., Migueles, S.A., Connors, M., Roederer, M., Douek, D.C. and Koup, R.A. (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* 101, 2711-20.
- Brenchley, J.M., Price, D.A., Schacker, T.W., Asher, T.E., Silvestri, G., Rao, S., Kazzaz, Z., Bornstein, E., Lambotte, O., Altmann, D., Blazar, B.R., Rodriguez, B., Teixeira-Johnson, L., Landay, A., Martin, J.N., Hecht, F.M., Picker, L.J., Lederman, M.M., Deeks, S.G. and Douek, D.C. (2006) Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 12, 1365-71.
- Brenchley, J.M., Schacker, T.W., Ruff, L.E., Price, D.A., Taylor, J.H., Beilman, G.J., Nguyen, P.L., Khoruts, A., Larson, M., Haase, A.T. and Douek, D.C. (2004) CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 200, 749-59.
- Brenchley, J.M., Silvestri, G. and Douek, D.C. (2010) Nonprogressive and progressive primate immunodeficiency lentivirus infections. *Immunity* 32, 737-42.
- Buseyne, F., Burgard, M., Teglas, J.P., Bui, E., Rouzioux, C., Mayaux, M.J., Blanche, S. and Riviere, Y. (1998) Early HIV-specific cytotoxic T lymphocytes and disease progression in children born to HIV-infected mothers. *AIDS Res Hum Retroviruses* 14, 1435-44.
- Buseyne, F., Scott-Algara, D., Bellal, N., Burgard, M., Rouzioux, C., Blanche, S. and Riviere, Y. (2005a) The frequency of HIV-specific interferon- gamma -producing CD8 T cells is associated with both age and level of antigenic stimulation in HIV-1-infected children. *J Infect Dis* 192, 1781-6.
- Buseyne, F., Scott-Algara, D., Bellal, N., Burgard, M., Rouzioux, C., Blanche, S. and Riviere, Y. (2005b) The frequency of HIV-specific interferon- gamma -producing CD8 T cells is associated with both age and level of antigenic stimulation in HIV-1-infected children. *The Journal of Infectious Diseases* 192, 1781-1786.
- Carrington, M., Nelson, G.W., Martin, M.P., Kissner, T., Vlahov, D., Goedert, J.J., Kaslow, R., Buchbinder, S., Hoots, K. and O'Brien, S.J. (1999) HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* 283, 1748-52.
- Casazza, J.P., Betts, M.R., Picker, L.J. and Koup, R.A. (2001) Decay kinetics of human immunodeficiency virus-specific CD8+ T cells in peripheral blood after initiation of highly active antiretroviral therapy. *J Virol* 75, 6508-16.
- Catalozzi, M. and Futterman, D.C. (2005) HIV in Adolescents. *Curr Infect Dis Rep* 7, 401-5.
- Chattopadhyay, P.K., Price, D.A., Harper, T.F., Betts, M.R., Yu, J., Gostick, E., Perfetto, S.P., Goepfert, P., Koup, R.A., De Rosa, S.C., Bruchez, M.P. and Roederer, M. (2006) Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. *Nat Med* 12, 972-7.
- Chun, T.W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., Kuo, Y.H., Brookmeyer, R., Zeiger, M.A., Barditch-Crovo, P. and Siliciano, R.F. (1997) Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387, 183-8.

- Cohen, M.S., Shaw, G.M., McMichael, A.J. and Haynes, B.F. (2011) Acute HIV-1 Infection. *N Engl J Med* 364, 1943-54.
- Cohen Stuart, J.W., Hazebergh, M.D., Hamann, D., Otto, S.A., Borleffs, J.C., Miedema, F., Boucher, C.A. and de Boer, R.J. (2000) The dominant source of CD4+ and CD8+ T-cell activation in HIV infection is antigenic stimulation. *J Acquir Immune Defic Syndr* 25, 203-11.
- Cohen Stuart, J.W., Slieker, W.A., Rijkers, G.T., Noest, A., Boucher, C.A., Suur, M.H., de Boer, R., Geelen, S.P., Scherpbier, H.J., Hartwig, N.G., Hooijkaas, H., Roos, M.T., de Graeff-Meeder, B. and de Groot, R. (1998a) Early recovery of CD4+ T lymphocytes in children on highly active antiretroviral therapy. Dutch study group for children with HIV infections. *AIDS* 12, 2155-9.
- Cohen Stuart, J.W., Slieker, W.A., Rijkers, G.T., Noest, A., Boucher, C.A., Suur, M.H., de Boer, R., Geelen, S.P., Scherpbier, H.J., Hartwig, N.G., Hooijkaas, H., Roos, M.T., de Graeff-Meeder, B. and de Groot, R. (1998b) Early recovery of CD4+ T lymphocytes in children on highly active antiretroviral therapy. Dutch study group for children with HIV infections. *AIDS* 12, 2155-2159.
- Connick, E., Lederman, M.M., Kotzin, B.L., Spritzler, J., Kuritzkes, D.R., St Clair, M., Sevin, A.D., Fox, L., Chiozzi, M.H., Leonard, J.M., Rousseau, F., D'Arcy, J., Martinez, A., Kessler, H. and Landay, A. (2000) Immune reconstitution in the first year of potent antiretroviral therapy and its relationship to virologic response. *J Infect Dis* 181, 358-63.
- Cooper, M.A., Fehniger, T.A. and Caligiuri, M.A. (2001) The biology of human natural killer-cell subsets. *Trends Immunol* 22, 633-40.
- Costa, P., Rusconi, S., Fogli, M., Mavilio, D., Murdaca, G., Puppo, F., Mingari, M.C., Galli, M., Moretta, L. and De Maria, A. (2003) Low expression of inhibitory natural killer receptors in CD8 cytotoxic T lymphocytes in long-term non-progressor HIV-1-infected patients. *AIDS* 17, 257-60.
- Daar, E.S. and Ho, D.D. (1991) Relative resistance of primary HIV-1 isolates to neutralization by soluble CD4. *Am J Med* 90, 22S-26S.
- Day, C.L., Kaufmann, D.E., Kiepiela, P., Brown, J.A., Moodley, E.S., Reddy, S., Mackey, E.W., Miller, J.D., Leslie, A.J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E.J., Coovadia, H.M., Goulder, P.J., Klenerman, P., Ahmed, R., Freeman, G.J. and Walker, B.D. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443, 350-4.
- De Maria, A., Fogli, M., Costa, P., Murdaca, G., Puppo, F., Mavilio, D., Moretta, A. and Moretta, L. (2003) The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (Nkp46, Nkp30 and Nkp44). *Eur J Immunol* 33, 2410-8.
- Decrion, A.Z., Dichamp, I., Varin, A. and Herbein, G. (2005) HIV and inflammation. *Curr HIV Res* 3, 243-59.
- Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., Davis, C.B., Peiper, S.C., Schall, T.J., Littman, D.R. and Landau, N.R. (1996) Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381, 661-6.
- Douek, D.C., Betts, M.R., Hill, B.J., Little, S.J., Lempicki, R., Metcalf, J.A., Casazza, J., Yoder, C., Adelsberger, J.W., Stevens, R.A., Baseler, M.W., Keiser, P., Richman, D.D., Davey, R.T. and Koup, R.A. (2001) Evidence for increased T cell turnover and decreased thymic output in HIV infection. *J Immunol* 167, 6663-8.

- Douek, D.C., Brenchley, J.M., Betts, M.R., Ambrozak, D.R., Hill, B.J., Okamoto, Y., Casazza, J.P., Kuruppu, J., Kunstman, K., Wolinsky, S., Grossman, Z., Dybul, M., Oxenius, A., Price, D.A., Connors, M. and Koup, R.A. (2002) HIV preferentially infects HIV-specific CD4⁺ T cells. *Nature* 417, 95-8.
- Douek, D.C., McFarland, R.D., Keiser, P.H., Gage, E.A., Massey, J.M., Haynes, B.F., Polis, M.A., Haase, A.T., Feinberg, M.B., Sullivan, J.L., Jamieson, B.D., Zack, J.A., Picker, L.J. and Koup, R.A. (1998) Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396, 690-5.
- Douglas, S.D., Rudy, B., Muenz, L., Starr, S.E., Campbell, D.E., Wilson, C., Holland, C., Crowley-Nowick, P. and Vermund, S.H. (2000) T-lymphocyte subsets in HIV-infected and high-risk HIV-uninfected adolescents: retention of naive T lymphocytes in HIV-infected adolescents. The Adolescent Medicine HIV/AIDS Research Network. *Arch Pediatr Adolesc Med* 154, 375-80.
- Duvall, M.G., Jaye, A., Dong, T., Brenchley, J.M., Alabi, A.S., Jeffries, D.J., van der Sande, M., Togun, T.O., McConkey, S.J., Douek, D.C., McMichael, A.J., Whittle, H.C., Koup, R.A. and Rowland-Jones, S.L. (2006) Maintenance of HIV-specific CD4⁺ T cell help distinguishes HIV-2 from HIV-1 infection. *J Immunol* 176, 6973-81.
- Edwards, B.H., Bansal, A., Sabbaj, S., Bakari, J., Mulligan, M.J. and Goepfert, P.A. (2002) Magnitude of functional CD8⁺ T-cell responses to the gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. *J Virol* 76, 2298-305.
- Effros, R.B., Allsopp, R., Chiu, C.P., Hausner, M.A., Hirji, K., Wang, L., Harley, C.B., Villeponteau, B., West, M.D. and Giorgi, J.V. (1996) Shortened telomeres in the expanded CD28-CD8⁺ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *AIDS* 10, F17-22.
- El-Sadr, W.M., Lundgren, J.D., Neaton, J.D., Gordin, F., Abrams, D., Arduino, R.C., Babiker, A., Burman, W., Clumeck, N., Cohen, C.J., Cohn, D., Cooper, D., Darbyshire, J., Emery, S., Fatkenheuer, G., Gazzard, B., Grund, B., Hoy, J., Klingman, K., Losso, M., Markowitz, N., Neuhaus, J., Phillips, A. and Rappoport, C. (2006) CD4⁺ count-guided interruption of antiretroviral treatment. *N Engl J Med* 355, 2283-96.
- Emu, B., Sinclair, E., Favre, D., Moretto, W.J., Hsue, P., Hoh, R., Martin, J.N., Nixon, D.F., McCune, J.M. and Deeks, S.G. (2005) Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. *J Virol* 79, 14169-78.
- Emu, B., Sinclair, E., Hatano, H., Ferre, A., Shacklett, B., Martin, J.N., McCune, J.M. and Deeks, S.G. (2008) HLA class I-restricted T-cell responses may contribute to the control of human immunodeficiency virus infection, but such responses are not always necessary for long-term virus control. *J Virol* 82, 5398-407.
- Engram, J.C., Cervasi, B., Borghans, J.A., Klatt, N.R., Gordon, S.N., Chahroudi, A., Else, J.G., Mittler, R.S., Sodora, D.L., de Boer, R.J., Brenchley, J.M., Silvestri, G. and Paiardini, M. (2010) Lineage-specific T-cell reconstitution following in vivo CD4⁺ and CD8⁺ lymphocyte depletion in nonhuman primates. *Blood* 116, 748-58.
- Estes, J., Baker, J.V., Brenchley, J.M., Khoruts, A., Barthold, J.L., Bantle, A., Reilly, C.S., Beilman, G.J., George, M.E., Douek, D.C., Haase, A.T. and Schacker, T.W. (2008) Collagen deposition limits immune reconstitution in the gut. *J Infect Dis* 198, 456-64.

- Evans, C.F., Borrow, P., de la Torre, J.C. and Oldstone, M.B. (1994) Virus-induced immunosuppression: kinetic analysis of the selection of a mutation associated with viral persistence. *J Virol* 68, 7367-73.
- Evans, D.T., O'Connor, D.H., Jing, P., Dzuris, J.L., Sidney, J., da Silva, J., Allen, T.M., Horton, H., Venham, J.E., Rudersdorf, R.A., Vogel, T., Pauza, C.D., Bontrop, R.E., DeMars, R., Sette, A., Hughes, A.L. and Watkins, D.I. (1999) Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nat Med* 5, 1270-6.
- Feng, Y., Broder, C.C., Kennedy, P.E. and Berger, E.A. (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272, 872-7.
- Finkel, T.H., Tudor-Williams, G., Banda, N.K., Cotton, M.F., Curiel, T., Monks, C., Baba, T.W., Ruprecht, R.M. and Kupfer, A. (1995) Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat Med* 1, 129-34.
- Finzi, D., Blankson, J., Siliciano, J.D., Margolick, J.B., Chadwick, K., Pierson, T., Smith, K., Lisziewicz, J., Lori, F., Flexner, C., Quinn, T.C., Chaisson, R.E., Rosenberg, E., Walker, B., Gange, S., Gallant, J. and Siliciano, R.F. (1999) Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 5, 512-7.
- Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D. and Siliciano, R.F. (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295-300.
- Flynn, P.M., Rudy, B.J., Douglas, S.D., Lathey, J., Spector, S.A., Martinez, J., Silio, M., Belzer, M., Friedman, L., D'Angelo, L., McNamara, J., Hodge, J., Hughes, M.D., Lindsey, J.C. and Team, P.A.C.T.G.S. (2004) Virologic and immunologic outcomes after 24 weeks in HIV type 1-infected adolescents receiving highly active antiretroviral therapy. *The Journal of Infectious Diseases* 190, 271-279.
- Flynn, P.M., Rudy, B.J., Lindsey, J.C., Douglas, S.D., Lathey, J., Spector, S.A., Martinez, J., Silio, M., Belzer, M., Friedman, L., D'Angelo, L., Smith, E., Hodge, J., Hughes, M.D. and Team, P.S. (2007) Long-term observation of adolescents initiating HAART therapy: three-year follow-up. *AIDS Research and Human Retroviruses* 23, 1208-1214.
- Forthal, D.N., Landucci, G. and Daar, E.S. (2001a) Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. *J Virol* 75, 6953-61.
- Forthal, D.N., Landucci, G. and Keenan, B. (2001b) Relationship between antibody-dependent cellular cytotoxicity, plasma HIV type 1 RNA, and CD4+ lymphocyte count. *AIDS Res Hum Retroviruses* 17, 553-61.
- Futterman, D.C. (2005) HIV in adolescents and young adults: half of all new infections in the United States. *Top HIV Med* 13, 101-5.
- Gandhi, R.T., Zheng, L., Bosch, R.J., Chan, E.S., Margolis, D.M., Read, S., Kallungal, B., Palmer, S., Medvik, K., Lederman, M.M., Alatrakchi, N., Jacobson, J.M., Wiegand, A., Kearney, M., Coffin, J.M., Mellors, J.W., Eron, J.J. and team, A.C.T.G.A. (2010) The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. *PLoS Medicine* 7.
- Gea-Banacloche, J.C., Migueles, S.A., Martino, L., Shupert, W.L., McNeil, A.C., Sabbaghian, M.S., Ehler, L., Prussin, C., Stevens, R., Lambert, L., Altman, J.,

- Hallahan, C.W., de Quiros, J.C. and Connors, M. (2000) Maintenance of large numbers of virus-specific CD8+ T cells in HIV-infected progressors and long-term nonprogressors. *J Immunol* 165, 1082-92.
- Giorgi, J.V., Hultin, L.E., McKeating, J.A., Johnson, T.D., Owens, B., Jacobson, L.P., Shih, R., Lewis, J., Wiley, D.J., Phair, J.P., Wolinsky, S.M. and Detels, R. (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.
- Giorgi, J.V., Liu, Z., Hultin, L.E., Cumberland, W.G., Hennessey, K. and Detels, R. (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.
- Giorgi, J.V., Lyles, R.H., Matud, J.L., Yamashita, T.E., Mellors, J.W., Hultin, L.E., Jamieson, B.D., Margolick, J.B., Rinaldo, C.R., Jr., Phair, J.P. and Detels, R. (2002) Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J Acquir Immune Defic Syndr* 29, 346-55.
- Gortmaker, S.L., Hughes, M., Cervia, J., Brady, M., Johnson, G.M., Seage, G.R., 3rd, Song, L.Y., Dankner, W.M. and Oleske, J.M. (2001) Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. *N Engl J Med* 345, 1522-8.
- Goulder, P.J., Phillips, R.E., Colbert, R.A., McAdam, S., Ogg, G., Nowak, M.A., Giangrande, P., Luzzi, G., Morgan, B., Edwards, A., McMichael, A.J. and Rowland-Jones, S. (1997) Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* 3, 212-7.
- Groux, H., Torpier, G., Monte, D., Mouton, Y., Capron, A. and Ameisen, J.C. (1992) Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. *J Exp Med* 175, 331-40.
- Hammer, S.M., Squires, K.E., Hughes, M.D., Grimes, J.M., Demeter, L.M., Currier, J.S., Eron, J.J., Jr., Feinberg, J.E., Balfour, H.H., Jr., Deyton, L.R., Chodakewitz, J.A. and Fischl, M.A. (1997) A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. *N Engl J Med* 337, 725-33.
- Harari, A., Petitpierre, S., Vallelian, F. and Pantaleo, G. (2004) Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. *Blood* 103, 966-72.
- Hazenberg, M.D., Hamann, D., Schuitemaker, H. and Miedema, F. (2000a) T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol* 1, 285-9.
- Hazenberg, M.D., Otto, S.A., Cohen Stuart, J.W., Verschuren, M.C., Borleffs, J.C., Boucher, C.A., Coutinho, R.A., Lange, J.M., Rinke de Wit, T.F., Tsegaye, A., van Dongen, J.J., Hamann, D., de Boer, R.J. and Miedema, F. (2000b) Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nat Med* 6, 1036-42.
- Hazenberg, M.D., Stuart, J.W., Otto, S.A., Borleffs, J.C., Boucher, C.A., de Boer, R.J., Miedema, F. and Hamann, D. (2000c) T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a

- longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood* 95, 249-55.
- Hazuda, D.J., Felock, P., Witmer, M., Wolfe, A., Stillmock, K., Grobler, J.A., Espeseth, A., Gabryelski, L., Schleif, W., Blau, C. and Miller, M.D. (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 287, 646-50.
- Hazuda, D.J., Young, S.D., Guare, J.P., Anthony, N.J., Gomez, R.P., Wai, J.S., Vacca, J.P., Handt, L., Motzel, S.L., Klein, H.J., Dornadula, G., Danovich, R.M., Witmer, M.V., Wilson, K.A., Tussey, L., Schleif, W.A., Gabryelski, L.S., Jin, L., Miller, M.D., Casimiro, D.R., Emini, E.A. and Shiver, J.W. (2004) Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science* 305, 528-32.
- Hellerstein, M., Hanley, M.B., Cesar, D., Siler, S., Papageorgopoulos, C., Wieder, E., Schmidt, D., Hoh, R., Neese, R., Macallan, D., Deeks, S. and McCune, J.M. (1999) Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med* 5, 83-9.
- Herberman, R.B., Nunn, M.E., Holden, H.T. and Lavrin, D.H. (1975) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer* 16, 230-9.
- Hersperger, A.R., Martin, J.N., Shin, L.Y., Sheth, P.M., Kovacs, C.M., Cosma, G.L., Makedonas, G., Pereyra, F., Walker, B.D., Kaul, R., Deeks, S.G. and Betts, M.R. (2011) Increased HIV-specific CD8+ T-cell cytotoxic potential in HIV elite controllers is associated with T-bet expression. *Blood* 117, 3799-808.
- Hersperger, A.R., Pereyra, F., Nason, M., Demers, K., Sheth, P., Shin, L.Y., Kovacs, C.M., Rodriguez, B., Sieg, S.F., Teixeira-Johnson, L., Gudonis, D., Goepfert, P.A., Lederman, M.M., Frank, I., Makedonas, G., Kaul, R., Walker, B.D. and Betts, M.R. (2010a) Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. *PLoS Pathog* 6, e1000917.
- Hersperger, A.R., Pereyra, F., Nason, M., Demers, K., Sheth, P., Shin, L.Y., Kovacs, C.M., Rodriguez, B., Sieg, S.F., Teixeira-Johnson, L., Gudonis, D., Goepfert, P.A., Lederman, M.M., Frank, I., Makedonas, G., Kaul, R., Walker, B.D. and Betts, M.R. (2010b) Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. *PLoS Pathogens* 6, e1000917.
- Hessell, A.J., Poignard, P., Hunter, M., Hangartner, L., Tehrani, D.M., Bleeker, W.K., Parren, P.W., Marx, P.A. and Burton, D.R. (2009) Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat Med* 15, 951-4.
- Hsiou, Y., Das, K., Ding, J., Clark, A.D., Jr., Kleim, J.P., Rosner, M., Winkler, I., Riess, G., Hughes, S.H. and Arnold, E. (1998) Structures of Tyr188Leu mutant and wild-type HIV-1 reverse transcriptase complexed with the non-nucleoside inhibitor HBY 097: inhibitor flexibility is a useful design feature for reducing drug resistance. *J Mol Biol* 284, 313-23.
- Hunt, P.W., Brenchley, J., Sinclair, E., McCune, J.M., Roland, M., Page-Shafer, K., Hsue, P., Emu, B., Krone, M., Lampiris, H., Douek, D., Martin, J.N. and Deeks, S.G. (2008) Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis* 197, 126-33.
- Hunt, P.W., Deeks, S.G., Bangsberg, D.R., Moss, A., Sinclair, E., Liegler, T., Bates, M., Tsao, G., Lampiris, H., Hoh, R. and Martin, J.N. (2006) The independent effect of drug resistance on T cell activation in HIV infection. *AIDS* 20, 691-699.

- Hunt, P.W., Deeks, S.G., Rodriguez, B., Valdez, H., Shade, S.B., Abrams, D.I., Kitahata, M.M., Krone, M., Neilands, T.B., Brand, R.J., Lederman, M.M. and Martin, J.N. (2003a) Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. *AIDS* 17, 1907-15.
- Hunt, P.W., Martin, J.N., Sinclair, E., Brecht, B., Hagos, E., Lampiris, H. and Deeks, S.G. (2003b) T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *The Journal of Infectious Diseases* 187, 1534-1543.
- Hymes, K.B., Cheung, T., Greene, J.B., Prose, N.S., Marcus, A., Ballard, H., William, D.C. and Laubenstein, L.J. (1981) Kaposi's sarcoma in homosexual men-a report of eight cases. *Lancet* 2, 598-600.
- Ibegbu, C., Spira, T.J., Nesheim, S., Mendez, H., Lee, F., Polliotti, B., Caba, J. and Nahmias, A. (1994) Subpopulations of T and B cells in perinatally HIV-infected and noninfected age-matched children compared with those in adults. *Clin Immunol Immunopathol* 71, 27-32.
- Irvine, S.L., Hummelen, R., Hekmat, S., Looman, C.W., Habbema, J.D. and Reid, G. (2010) Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. *J Clin Gastroenterol* 44, e201-5.
- Iyasere, C., Tilton, J.C., Johnson, A.J., Younes, S., Yassine-Diab, B., Sekaly, R.P., Kwok, W.W., Migueles, S.A., Laborico, A.C., Shupert, W.L., Hallahan, C.W., Davey, R.T., Jr., Dybul, M., Vogel, S., Metcalf, J. and Connors, M. (2003) Diminished proliferation of human immunodeficiency virus-specific CD4+ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. *J Virol* 77, 10900-9.
- Jeffries, D.J. (1989) Targets for antiviral therapy of human immunodeficiency virus infection. *J Infect* 18 Suppl 1, 5-13.
- Jiang, W., Lederman, M.M., Hunt, P., Sieg, S.F., Haley, K., Rodriguez, B., Landay, A., Martin, J., Sinclair, E., Asher, A.I., Deeks, S.G., Douek, D.C. and Brenchley, J.M. (2009) Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis* 199, 1177-85.
- Jin, X., Bauer, D.E., Tuttleton, S.E., Lewin, S., Gettie, A., Blanchard, J., Irwin, C.E., Safrit, J.T., Mittler, J., Weinberger, L., Kostrikis, L.G., Zhang, L., Perelson, A.S. and Ho, D.D. (1999) Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189, 991-8.
- Kalams, S.A., Buchbinder, S.P., Rosenberg, E.S., Billingsley, J.M., Colbert, D.S., Jones, N.G., Shea, A.K., Trocha, A.K. and Walker, B.D. (1999a) Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. *J Virol* 73, 6715-20.
- Kalams, S.A., Goulder, P.J., Shea, A.K., Jones, N.G., Trocha, A.K., Ogg, G.S. and Walker, B.D. (1999b) Levels of human immunodeficiency virus type 1-specific cytotoxic T-lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. *J Virol* 73, 6721-8.
- Kaldjian, E.P., Gretz, J.E., Anderson, A.O., Shi, Y. and Shaw, S. (2001) Spatial and molecular organization of lymph node T cell cortex: a labyrinthine cavity bounded by an epithelium-like monolayer of fibroblastic reticular cells anchored to basement membrane-like extracellular matrix. *Int Immunol* 13, 1243-53.

- Karlsson, A.C., Younger, S.R., Martin, J.N., Grossman, Z., Sinclair, E., Hunt, P.W., Hagos, E., Nixon, D.F. and Deeks, S.G. (2004) Immunologic and virologic evolution during periods of intermittent and persistent low-level viremia. *AIDS* 18, 981-989.
- Kaslow, R.A., Carrington, M., Apple, R., Park, L., Munoz, A., Saah, A.J., Goedert, J.J., Winkler, C., O'Brien, S.J., Rinaldo, C., Detels, R., Blattner, W., Phair, J., Erlich, H. and Mann, D.L. (1996) Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med* 2, 405-11.
- Kiepiela, P., Ngumbela, K., Thobakgale, C., Ramduth, D., Honeyborne, I., Moodley, E., Reddy, S., de Pierres, C., Mncube, Z., Mkhwanazi, N., Bishop, K., van der Stok, M., Nair, K., Khan, N., Crawford, H., Payne, R., Leslie, A., Prado, J., Prendergast, A., Frater, J., McCarthy, N., Brander, C., Learn, G.H., Nickle, D., Rousseau, C., Coovadia, H., Mullins, J.I., Heckerman, D., Walker, B.D. and Goulder, P. (2007) CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. *Nat Med* 13, 46-53.
- Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M.T., Danquet, C., Vilmer, E., Griscelli, C., Brun-Veziret, F., Rouzioux, C., Gluckman, J.C., Chermann, J.C. and et al. (1984a) Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* 225, 59-63.
- Klatzmann, D., Champagne, E., Chamaret, S., Gruet, J., Guetard, D., Hercend, T., Gluckman, J.C. and Montagnier, L. (1984b) T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* 312, 767-8.
- Klein, M.R., van Baalen, C.A., Holwerda, A.M., Kerkhof Garde, S.R., Bende, R.J., Keet, I.P., Eeftink-Schattenkerk, J.K., Osterhaus, A.D., Schuitemaker, H. and Miedema, F. (1995) Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J Exp Med* 181, 1365-72.
- Koup, R.A., Safrit, J.T., Cao, Y., Andrews, C.A., McLeod, G., Borkowsky, W., Farthing, C. and Ho, D.D. (1994) Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol* 68, 4650-5.
- Kwong, P.D., Doyle, M.L., Casper, D.J., Cicala, C., Leavitt, S.A., Majeed, S., Steenbeke, T.D., Venturi, M., Chaiken, I., Fung, M., Katinger, H., Parren, P.W., Robinson, J., Van Ryk, D., Wang, L., Burton, D.R., Freire, E., Wyatt, R., Sodroski, J., Hendrickson, W.A. and Arthos, J. (2002) HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. *Nature* 420, 678-82.
- Lalezari, J.P., Henry, K., O'Hearn, M., Montaner, J.S., Piliero, P.J., Trottier, B., Walmsley, S., Cohen, C., Kuritzkes, D.R., Eron, J.J., Jr., Chung, J., DeMasi, R., Donatucci, L., Drobnes, C., Delehanty, J. and Salgo, M. (2003) Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *N Engl J Med* 348, 2175-85.
- Lassen, K.G., Ramyar, K.X., Bailey, J.R., Zhou, Y. and Siliciano, R.F. (2006) Nuclear retention of multiply spliced HIV-1 RNA in resting CD4+ T cells. *PLoS Pathog* 2, e68.
- Lederman, M.M., Calabrese, L., Funderburg, N.T., Clagett, B., Medvik, K., Bonilla, H., Gripshover, B., Salata, R.A., Taeye, A., Lisgaris, M., McComsey, G.A., Kirchner, E., Baum, J., Shive, C., Asaad, R., Kalayjian, R.C., Sieg, S.F. and Rodriguez, B.

- (2011) Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis* 204, 1217-26.
- Lien, E., Aukrust, P., Sundan, A., Muller, F., Froland, S.S. and Espevik, T. (1998) Elevated levels of serum-soluble CD14 in human immunodeficiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events. *Blood* 92, 2084-92.
- Liu, S., Wu, S. and Jiang, S. (2007) HIV entry inhibitors targeting gp41: from polypeptides to small-molecule compounds. *Curr Pharm Des* 13, 143-62.
- Liu, Z., Cumberland, W.G., Hultin, L.E., Prince, H.E., Detels, R. and Giorgi, J.V. (1997) Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J Acquir Immune Defic Syndr Hum Retrovirol* 16, 83-92.
- Luzuriaga, K., Holmes, D., Hereema, A., Wong, J., Panicali, D.L. and Sullivan, J.L. (1995) HIV-1-specific cytotoxic T lymphocyte responses in the first year of life. *J Immunol* 154, 433-43.
- Mackall, C.L., Fleisher, T.A., Brown, M.R., Andrich, M.P., Chen, C.C., Feuerstein, I.M., Horowitz, M.E., Magrath, I.T., Shad, A.T., Steinberg, S.M. and et al. (1995) Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med* 332, 143-9.
- Marchetti, G., Bellistri, G.M., Borghi, E., Tincati, C., Ferramosca, S., La Francesca, M., Morace, G., Gori, A. and Monforte, A.D. (2008) Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy. *AIDS* 22, 2035-8.
- Martin, M.P., Qi, Y., Gao, X., Yamada, E., Martin, J.N., Pereyra, F., Colombo, S., Brown, E.E., Shupert, W.L., Phair, J., Goedert, J.J., Buchbinder, S., Kirk, G.D., Telenti, A., Connors, M., O'Brien, S.J., Walker, B.D., Parham, P., Deeks, S.G., McVicar, D.W. and Carrington, M. (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 39, 733-40.
- Masur, H., Michelis, M.A., Greene, J.B., Onorato, I., Stouwe, R.A., Holzman, R.S., Wormser, G., Brettman, L., Lange, M., Murray, H.W. and Cunningham-Rundles, S. (1981) An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 305, 1431-8.
- Mavilio, D., Benjamin, J., Daucher, M., Lombardo, G., Kottlil, S., Planta, M.A., Marcenaro, E., Bottino, C., Moretta, L., Moretta, A. and Fauci, A.S. (2003) Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. *Proc Natl Acad Sci U S A* 100, 15011-6.
- McElrath, M.J., De Rosa, S.C., Moodie, Z., Dubey, S., Kierstead, L., Janes, H., Defawe, O.D., Carter, D.K., Hural, J., Akondy, R., Buchbinder, S.P., Robertson, M.N., Mehrotra, D.V., Self, S.G., Corey, L., Shiver, J.W. and Casimiro, D.R. (2008) HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* 372, 1894-905.
- McElrath, M.J. and Haynes, B.F. (2010) Induction of immunity to human immunodeficiency virus type-1 by vaccination. *Immunity* 33, 542-54.
- McMichael, A.J., Borrow, P., Tomaras, G.D., Goonetilleke, N. and Haynes, B.F. (2010) The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol* 10, 11-23.

- Mellors, J.W., Kingsley, L.A., Rinaldo, C.R., Jr., Todd, J.A., Hoo, B.S., Kokka, R.P. and Gupta, P. (1995) Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* 122, 573-9.
- Merluzzi, V.J., Hargrave, K.D., Labadia, M., Grozinger, K., Skoog, M., Wu, J.C., Shih, C.K., Eckner, K., Hattox, S., Adams, J. and et al. (1990) Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. *Science* 250, 1411-3.
- Migueles, S.A., Laborico, A.C., Shupert, W.L., Sabbaghian, M.S., Rabin, R., Hallahan, C.W., Van Baarle, D., Kostense, S., Miedema, F., McLaughlin, M., Ehler, L., Metcalf, J., Liu, S. and Connors, M. (2002) HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol* 3, 1061-8.
- Migueles, S.A., Osborne, C.M., Royce, C., Compton, A.A., Joshi, R.P., Weeks, K.A., Rood, J.E., Berkley, A.M., Sacha, J.B., Cogliano-Shutta, N.A., Lloyd, M., Roby, G., Kwan, R., McLaughlin, M., Stallings, S., Rehm, C., O'Shea, M.A., Mican, J., Packard, B.Z., Komoriya, A., Palmer, S., Wiegand, A.P., Maldarelli, F., Coffin, J.M., Mellors, J.W., Hallahan, C.W., Follman, D.A. and Connors, M. (2008) Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. *Immunity* 29, 1009-21.
- Migueles, S.A., Sabbaghian, M.S., Shupert, W.L., Bettinotti, M.P., Marincola, F.M., Martino, L., Hallahan, C.W., Selig, S.M., Schwartz, D., Sullivan, J. and Connors, M. (2000) HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci U S A* 97, 2709-14.
- Migueles, S.A., Weeks, K.A., Nou, E., Berkley, A.M., Rood, J.E., Osborne, C.M., Hallahan, C.W., Cogliano-Shutta, N.A., Metcalf, J.A., McLaughlin, M., Kwan, R., Mican, J.M., Davey, R.T., Jr. and Connors, M. (2009) Defective human immunodeficiency virus-specific CD8+ T-cell polyfunctionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy. *J Virol* 83, 11876-89.
- Mikell, I., Sather, D.N., Kalams, S.A., Altfeld, M., Alter, G. and Stamatatos, L. (2011) Characteristics of the earliest cross-neutralizing antibody response to HIV-1. *PLoS Pathog* 7, e1001251.
- Millard, P.J., Henkart, M.P., Reynolds, C.W. and Henkart, P.A. (1984) Purification and properties of cytoplasmic granules from cytotoxic rat LGL tumors. *J Immunol* 132, 3197-204.
- Mohri, H. (1998) Rapid Turnover of T Lymphocytes in SIV-Infected Rhesus Macaques. *Science* 279, 1223-1227.
- Mohri, H., Bonhoeffer, S., Monard, S., Perelson, A.S. and Ho, D.D. (1998) Rapid turnover of T lymphocytes in SIV-infected rhesus macaques. *Science* 279, 1223-7.
- Mohri, H., Perelson, A.S., Tung, K., Ribeiro, R.M., Ramratnam, B., Markowitz, M., Kost, R., Hurley, A., Weinberger, L., Cesar, D., Hellerstein, M.K. and Ho, D.D. (2001) Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J Exp Med* 194, 1277-87.
- Moir, S., Chun, T.W. and Fauci, A.S. (2011) Pathogenic mechanisms of HIV disease. *Annu Rev Pathol* 6, 223-48.
- Neu, J. (2007) Gastrointestinal maturation and implications for infant feeding. *Early Hum Dev* 83, 767-75.
- Nixon, D.E. and Landay, A.L. (2010) Biomarkers of immune dysfunction in HIV. *Current Opinion in HIV and AIDS* 5, 498-503.

- O'Connor, D.H., Allen, T.M., Vogel, T.U., Jing, P., DeSouza, I.P., Dodds, E., Dunphy, E.J., Melsaether, C., Mothe, B., Yamamoto, H., Horton, H., Wilson, N., Hughes, A.L. and Watkins, D.I. (2002) Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. *Nat Med* 8, 493-9.
- Ogg, G.S., Jin, X., Bonhoeffer, S., Dunbar, P.R., Nowak, M.A., Monard, S., Segal, J.P., Cao, Y., Rowland-Jones, S.L., Cerundolo, V., Hurley, A., Markowitz, M., Ho, D.D., Nixon, D.F. and McMichael, A.J. (1998) Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 279, 2103-6.
- Orendi, J.M., Bloem, A.C., Borleffs, J.C., Wijnholds, F.J., de Vos, N.M., Nottet, H.S., Visser, M.R., Snippe, H., Verhoef, J. and Boucher, C.A. (1998) Activation and cell cycle antigens in CD4+ and CD8+ T cells correlate with plasma human immunodeficiency virus (HIV-1) RNA level in HIV-1 infection. *J Infect Dis* 178, 1279-87.
- Palella, F.J., Jr., Delaney, K.M., Moorman, A.C., Loveless, M.O., Fuhrer, J., Satten, G.A., Aschman, D.J. and Holmberg, S.D. (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 338, 853-60.
- Pantaleo, G. and Koup, R.A. (2004) Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nature Medicine* 10, 806-810.
- Pantaleo, G., Menzo, S., Vaccarezza, M., Graziosi, C., Cohen, O.J., Demarest, J.F., Montefiori, D., Orenstein, J.M., Fox, C., Schrager, L.K. and et al. (1995) Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med* 332, 209-16.
- Papasavvas, E., Azzoni, L., Foulkes, A., Violari, A., Cotton, M.F., Pistilli, M., Reynolds, G., Yin, X., Glencross, D.K., Stevens, W.S., McIntyre, J.A. and Montaner, L.J. (2011) Increased Microbial Translocation in ≤ 180 Days Old Perinatally Human Immunodeficiency Virus-Positive Infants as Compared With Human Immunodeficiency Virus-exposed Uninfected Infants of Similar Age. *The Pediatric Infectious Disease Journal*, 1.
- Papasavvas, E., Pistilli, M., Reynolds, G., Bucki, R., Azzoni, L., Chehimi, J., Janmey, P.A., DiNubile, M.J., Ondercin, J., Kostman, J.R., Mounzer, K.C. and Montaner, L.J. (2009a) Delayed loss of control of plasma lipopolysaccharide levels after therapy interruption in chronically HIV-1-infected patients. *AIDS* 23, 369-375.
- Papasavvas, E., Pistilli, M., Reynolds, G., Bucki, R., Azzoni, L., Chehimi, J., Janmey, P.A., DiNubile, M.J., Ondercin, J., Kostman, J.R., Mounzer, K.C. and Montaner, L.J. (2009b) Delayed loss of control of plasma lipopolysaccharide levels after therapy interruption in chronically HIV-1-infected patients. *AIDS* 23, 369-75.
- Pereyra, F., Jia, X., McLaren, P.J., Telenti, A., de Bakker, P.I., Walker, B.D., Ripke, S., Brumme, C.J., Pulit, S.L., Carrington, M., Kadie, C.M., Carlson, J.M., Heckerman, D., Graham, R.R., Plenge, R.M., Deeks, S.G., Gianniny, L., Crawford, G., Sullivan, J., Gonzalez, E., Davies, L., Camargo, A., Moore, J.M., Beattie, N., Gupta, S., Crenshaw, A., Burt, N.P., Guiducci, C., Gupta, N., Gao, X., Qi, Y., Yuki, Y., Piechocka-Trocha, A., Cutrell, E., Rosenberg, R., Moss, K.L., Lemay, P., O'Leary, J., Schaefer, T., Verma, P., Toth, I., Block, B., Baker, B., Rothchild, A., Lian, J., Proudfoot, J., Alvino, D.M., Vine, S., Addo, M.M., Allen, T.M., Altfield, M., Henn, M.R., Le Gall, S., Streeck, H., Haas, D.W., Kuritzkes, D.R., Robbins, G.K., Shafer, R.W., Gulick, R.M., Shikuma, C.M., Haubrich, R., Riddler, S., Sax, P.E., Daar, E.S., Ribaud, H.J., Agan, B., Agarwal, S., Ahern, R.L., Allen, B.L., Altidor, S., Altschuler, E.L., Ambardar, S., Anastos, K., Anderson, B., Anderson,

- V., Andraday, U., Antoniskis, D., Bangsberg, D., Barbaro, D., Barrie, W., Bartzak, J., Barton, S., Basden, P., Basgoz, N., Bazner, S., Bellos, N.C., Benson, A.M., Berger, J., Bernard, N.F., Bernard, A.M., Birch, C., Bodner, S.J., Bolan, R.K., Boudreaux, E.T., Bradley, M., Braun, J.F., Brndjar, J.E., Brown, S.J., Brown, K., Brown, S.T., et al. (2010) The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 330, 1551-7.
- Perez, E.E., Rose, S.L., Peyser, B., Lamers, S.L., Burkhardt, B., Dunn, B.M., Hutson, A.D., Sleasman, J.W. and Goodenow, M.M. (2001) Human immunodeficiency virus type 1 protease genotype predicts immune and viral responses to combination therapy with protease inhibitors (PIs) in PI-naive patients. *J Infect Dis* 183, 579-88.
- Petrovas, C., Casazza, J.P., Brenchley, J.M., Price, D.A., Gostick, E., Adams, W.C., Precopio, M.L., Schacker, T., Roederer, M., Douek, D.C. and Koup, R.A. (2006) PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med* 203, 2281-92.
- Pitcher, C.J., Quittner, C., Peterson, D.M., Connors, M., Koup, R.A., Maino, V.C. and Picker, L.J. (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.
- Podack, E.R. and Konigsberg, P.J. (1984) Cytolytic T cell granules. Isolation, structural, biochemical, and functional characterization. *J Exp Med* 160, 695-710.
- Podack, E.R., Young, J.D. and Cohn, Z.A. (1985) Isolation and biochemical and functional characterization of perforin 1 from cytolytic T-cell granules. *Proc Natl Acad Sci U S A* 82, 8629-33.
- Prendergast, A., Goodliffe, H., Clapson, M., Cross, R., Tudor-Williams, G., Riddell, A., Daniels, J., Williams, A. and Goulder, P. (2011) Gag-specific CD4+ T-cell responses are associated with virological control of paediatric HIV-1 infection. *AIDS* 25, 1329-31.
- Price, D.A., Goulder, P.J., Klenerman, P., Sewell, A.K., Easterbrook, P.J., Troop, M., Bangham, C.R. and Phillips, R.E. (1997) Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc Natl Acad Sci U S A* 94, 1890-5.
- Rabin, R.L., Roederer, M., Maldonado, Y., Petru, A. and Herzenberg, L.A. (1995) Altered representation of naive and memory CD8 T cell subsets in HIV-infected children. *J Clin Invest* 95, 2054-60.
- Reedijk, M., Boucher, C.A., van Bommel, T., Ho, D.D., Tzeng, T.B., Sereni, D., Veyssier, P., Jurriaans, S., Granneman, R., Hsu, A. and et al. (1995) Safety, pharmacokinetics, and antiviral activity of A77003, a C2 symmetry-based human immunodeficiency virus protease inhibitor. *Antimicrob Agents Chemother* 39, 1559-64.
- Richman, D., Rosenthal, A.S., Skoog, M., Eckner, R.J., Chou, T.C., Sabo, J.P. and Merluzzi, V.J. (1991) BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. *Antimicrob Agents Chemother* 35, 305-8.
- Richman, D.D., Wrin, T., Little, S.J. and Petropoulos, C.J. (2003) Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci U S A* 100, 4144-9.
- Riviere, Y., McChesney, M.B., Porrot, F., Tanneau-Salvadori, F., Sansonetti, P., Lopez, O., Pialoux, G., Feuillie, V., Mollereau, M., Chamaret, S. and et al. (1995) Gag-specific cytotoxic responses to HIV type 1 are associated with a decreased risk of

- progression to AIDS-related complex or AIDS. *AIDS Res Hum Retroviruses* 11, 903-7.
- Robbins, G.K., Spritzler, J.G., Chan, E.S., Asmuth, D.M., Gandhi, R.T., Rodriguez, B.A., Skowron, G., Skolnik, P.R., Shafer, R.W. and Pollard, R.B. (2009) Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. *Clin Infect Dis* 48, 350-61.
- Roberts, J.D., Bebenek, K. and Kunkel, T.A. (1988) The accuracy of reverse transcriptase from HIV-1. *Science* 242, 1171-3.
- Rodger, A.J., Fox, Z., Lundgren, J.D., Kuller, L.H., Boesecke, C., Gey, D., Skoutelis, A., Goetz, M.B. and Phillips, A.N. (2009) Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. *J Infect Dis* 200, 973-83.
- Rodriguez, B., Sethi, A.K., Cheruvu, V.K., Mackay, W., Bosch, R.J., Kitahata, M., Boswell, S.L., Mathews, W.C., Bangsberg, D.R., Martin, J., Whalen, C.C., Sieg, S., Yadavalli, S., Deeks, S.G. and Lederman, M.M. (2006) Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. *JAMA* 296, 1498-506.
- Rolland, M., Heckerman, D., Deng, W., Rousseau, C.M., Coovadia, H., Bishop, K., Goulder, P.J., Walker, B.D., Brander, C. and Mullins, J.I. (2008) Broad and Gag-biased HIV-1 epitope repertoires are associated with lower viral loads. *PLoS One* 3, e1424.
- Root, M.J. and Hamer, D.H. (2003) Targeting therapeutics to an exposed and conserved binding element of the HIV-1 fusion protein. *Proc Natl Acad Sci U S A* 100, 5016-21.
- Rosenberg, E.S., Billingsley, J.M., Caliendo, A.M., Boswell, S.L., Sax, P.E., Kalams, S.A. and Walker, B.D. (1997) Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 278, 1447-50.
- Rosenzweig, M., DeMaria, M.A., Harper, D.M., Friedrich, S., Jain, R.K. and Johnson, R.P. (1998) Increased rates of CD4(+) and CD8(+) T lymphocyte turnover in simian immunodeficiency virus-infected macaques. *Proc Natl Acad Sci U S A* 95, 6388-93.
- Rowland-Jones, S.L. (2003) Timeline: AIDS pathogenesis: what have two decades of HIV research taught us? *Nat Rev Immunol* 3, 343-8.
- Rucker, J., Samson, M., Doranz, B.J., Libert, F., Berson, J.F., Yi, Y., Smyth, R.J., Collman, R.G., Broder, C.C., Vassart, G., Doms, R.W. and Parmentier, M. (1996) Regions in beta-chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. *Cell* 87, 437-46.
- Rudy, B.J., Crowley-Nowick, P.A. and Douglas, S.D. (2001) Immunology and the REACH study: HIV immunology and preliminary findings. *Reaching for Excellence in Adolescent Care and Health. The Journal of adolescent health : official publication of the Society for Adolescent Medicine* 29, 39-48.
- Rudy, B.J., Lindsey, J.C., Flynn, P.M., Bosch, R.J., Wilson, C.M., Hughes, M.E. and Douglas, S.D. (2006a) Immune reconstitution and predictors of virologic failure in adolescents infected through risk behaviors and initiating HAART: week 60 results from the PACTG 381 cohort. *AIDS Res Hum Retroviruses* 22, 213-21.
- Rudy, B.J., Lindsey, J.C., Flynn, P.M., Bosch, R.J., Wilson, C.M., Hughes, M.E., Douglas, S.D. and Team, P.A.C.T.G.S. (2006b) Immune reconstitution and predictors of virologic failure in adolescents infected through risk behaviors and initiating HAART: week 60 results from the PACTG 381 cohort. *AIDS Research and Human Retroviruses* 22, 213-221.

- Rudy, B.J., Wilson, C.M., Durako, S., Moscicki, A.B., Muenz, L. and Douglas, S.D. (2002) Peripheral blood lymphocyte subsets in adolescents: a longitudinal analysis from the REACH project. *Clin Diagn Lab Immunol* 9, 959-65.
- Sachsenberg, N., Perelson, A.S., Yerly, S., Schockmel, G.A., Leduc, D., Hirschel, B. and Perrin, L. (1998a) Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. *J Exp Med* 187, 1295-303.
- Sachsenberg, N., Perelson, A.S., Yerly, S., Schockmel, G.A., Leduc, D., Hirschel, B. and Perrin, L. (1998b) Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. *The Journal of experimental medicine* 187, 1295-1303.
- Saez-Cirion, A., Lacabaratz, C., Lambotte, O., Versmisse, P., Urrutia, A., Boufassa, F., Barre-Sinoussi, F., Delfraissy, J.F., Sinet, M., Pancino, G. and Venet, A. (2007) HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S A* 104, 6776-81.
- Sandberg, J.K., Fast, N.M., Jordan, K.A., Furlan, S.N., Barbour, J.D., Fennelly, G., Dobroszycki, J., Spiegel, H.M., Wiznia, A., Rosenberg, M.G. and Nixon, D.F. (2003) HIV-specific CD8+ T cell function in children with vertically acquired HIV-1 infection is critically influenced by age and the state of the CD4+ T cell compartment. *J Immunol* 170, 4403-10.
- Sandler, N.G., Wand, H., Roque, A., Law, M., Nason, M.C., Nixon, D.E., Pedersen, C., Ruxrungtham, K., Lewin, S.R., Emery, S., Neaton, J.D., Brenchley, J.M., Deeks, S.G., Sereti, I. and Douek, D.C. (2011) Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis* 203, 780-90.
- Schacker, T.W., Nguyen, P.L., Beilman, G.J., Wolinsky, S., Larson, M., Reilly, C. and Haase, A.T. (2002) Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. *J Clin Invest* 110, 1133-9.
- Schmitz, J.E., Kuroda, M.J., Santra, S., Sasseville, V.G., Simon, M.A., Lifton, M.A., Racz, P., Tenner-Racz, K., Dalesandro, M., Scallon, B.J., Ghayeb, J., Forman, M.A., Montefiori, D.C., Rieber, E.P., Letvin, N.L. and Reimann, K.A. (1999) Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 283, 857-60.
- Scott-Algara, D., Buseyne, F., Blanche, S., Rouzioux, C., Jouanne, C., Romagne, F. and Riviere, Y. (2001) Frequency and phenotyping of human immunodeficiency virus (HIV)-specific CD8+ T cells in HIV-infected children, using major histocompatibility complex class I peptide tetramers. *J Infect Dis* 183, 1565-73.
- Sieg, S.F., Bazdar, D.A. and Lederman, M.M. (2008) S-phase entry leads to cell death in circulating T cells from HIV-infected persons. *J Leukoc Biol* 83, 1382-7.
- Silvestri, G. and Feinberg, M.B. (2003) Turnover of lymphocytes and conceptual paradigms in HIV infection. *J Clin Invest* 112, 821-4.
- Sleasman, J.W., Nelson, R.P., Goodenow, M.M., Wilfret, D., Hutson, A., Baseler, M., Zuckerman, J., Pizzo, P.A. and Mueller, B.U. (1999) Immunoreconstitution after ritonavir therapy in children with human immunodeficiency virus infection involves multiple lymphocyte lineages. *J Pediatr* 134, 597-606.
- Smith, K.Y., Valdez, H., Landay, A., Spritzler, J., Kessler, H.A., Connick, E., Kuritzkes, D., Gross, B., Francis, I., McCune, J.M. and Lederman, M.M. (2000) Thymic size and lymphocyte restoration in patients with human immunodeficiency virus infection after 48 weeks of zidovudine, lamivudine, and ritonavir therapy. *J Infect Dis* 181, 141-7.

- Spiegel, H.M., Chandwani, R., Sheehy, M.E., Dobroszycki, J., Fennelly, G., Wiznia, A., Radding, J., Rigaud, M., Pollack, H., Borkowsky, W., Rosenberg, M. and Nixon, D.F. (2000) The impact of early initiation of highly active antiretroviral therapy on the human immunodeficiency virus type 1-specific CD8 T cell response in children. *J Infect Dis* 182, 88-95.
- Sprent, J. and Tough, D. (1995) HIV results in the frame. CD4+ cell turnover. *Nature* 375, 194; author reply 198.
- St Clair, M.H., Richards, C.A., Spector, T., Weinhold, K.J., Miller, W.H., Langlois, A.J. and Furman, P.A. (1987) 3'-Azido-3'-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. *Antimicrob Agents Chemother* 31, 1972-7.
- Stamatatos, L., Morris, L., Burton, D.R. and Mascola, J.R. (2009) Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? *Nat Med* 15, 866-70.
- Starr, S.E., Sarr, M., Campbell, D.E., Wilson, C.M. and Douglas, S.D. (2002a) Increased proliferation within T lymphocyte subsets of HIV-infected adolescents. *AIDS Res Hum Retroviruses* 18, 1301-10.
- Starr, S.E., Sarr, M., Campbell, D.E., Wilson, C.M. and Douglas, S.D. (2002b) Increased Proliferation within T Lymphocyte Subsets of HIV-Infected Adolescents. *AIDS Research and Human Retroviruses* 18, 1301-1310.
- Steigbigel, R.T., Cooper, D.A., Kumar, P.N., Eron, J.E., Schechter, M., Markowitz, M., Loutfy, M.R., Lennox, J.L., Gatell, J.M., Rockstroh, J.K., Katlama, C., Yeni, P., Lazzarin, A., Clotet, B., Zhao, J., Chen, J., Ryan, D.M., Rhodes, R.R., Killar, J.A., Gilde, L.R., Strohmaier, K.M., Meibohm, A.R., Miller, M.D., Hazuda, D.J., Nessler, M.L., DiNubile, M.J., Isaacs, R.D., Nguyen, B.Y. and Tepller, H. (2008) Raltegravir with optimized background therapy for resistant HIV-1 infection. *N Engl J Med* 359, 339-54.
- Surh, C.D. and Sprent, J. (2008) Homeostasis of naive and memory T cells. *Immunity* 29, 848-62.
- Teixeira, L., Valdez, H., McCune, J.M., Koup, R.A., Badley, A.D., Hellerstein, M.K., Napolitano, L.A., Douek, D.C., Mbisa, G., Deeks, S., Harris, J.M., Barbour, J.D., Gross, B.H., Francis, I.R., Halvorsen, R., Asaad, R. and Lederman, M.M. (2001) Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS* 15, 1749-56.
- Timonen, T., Ortaldo, J.R. and Herberman, R.B. (1981) Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J Exp Med* 153, 569-82.
- Van Rompay, K.K., Singh, R.P., Pahar, B., Sodora, D.L., Wingfield, C., Lawson, J.R., Marthas, M.L. and Bischofberger, N. (2004) CD8+-cell-mediated suppression of virulent simian immunodeficiency virus during tenofovir treatment. *J Virol* 78, 5324-37.
- Vigano, A., Saresella, M., Villa, M.L., Ferrante, P. and Clerici, M. (2000a) CD38+CD8+ T cells as a marker of poor response to therapy in HIV-infected individuals. *Chem Immunol* 75, 207-17.
- Vigano, A., Vella, S., Saresella, M., Vanzulli, A., Bricalli, D., Di Fabio, S., Ferrante, P., Andreotti, M., Pirillo, M., Dally, L.G., Clerici, M. and Principi, N. (2000b) Early immune reconstitution after potent antiretroviral therapy in HIV-infected children correlates with the increase in thymus volume. *AIDS* 14, 251-61.
- Vilches, C. and Parham, P. (2002) KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 20, 217-51.

- Vincent, T., Portales, P., Baillat, V., Eden, A., Clot, J., Reynes, J. and Corbeau, P. (2006) The immunological response to highly active antiretroviral therapy is linked to CD4+ T-cell surface CCR5 density. *J Acquir Immune Defic Syndr* 43, 377-8.
- Walker, C.M., Moody, D.J., Stites, D.P. and Levy, J.A. (1986) CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* 234, 1563-6.
- Wallet, M.A., Rodriguez, C.A., Yin, L., Saporta, S., Chinratanapisit, S., Hou, W., Sleasman, J.W. and Goodenow, M.M. (2010) Microbial translocation induces persistent macrophage activation unrelated to HIV-1 levels or T-cell activation following therapy. *AIDS* 24, 1281-90.
- Watanabe, M.E. (2003) Skeptical scientists skewer VaxGen statistics. *Nat Med* 9, 376.
- Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., Komarova, N.L., Nowak, M.A., Hahn, B.H., Kwong, P.D. and Shaw, G.M. (2003) Antibody neutralization and escape by HIV-1. *Nature* 422, 307-12.
- West, W.H., Cannon, G.B., Kay, H.D., Bonnard, G.D. and Herberman, R.B. (1977) Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. *J Immunol* 118, 355-61.
- Wilson, C.M., Ellenberg, J.H., Douglas, S.D., Moscicki, A.B. and Holland, C.A. (2004) CD8+CD38+ T cells but not HIV type 1 RNA viral load predict CD4+ T cell loss in a predominantly minority female HIV+ adolescent population. *AIDS Res Hum Retroviruses* 20, 263-9.
- Wilson, J.D., Imami, N., Watkins, A., Gill, J., Hay, P., Gazzard, B., Westby, M. and Gotch, F.M. (2000) Loss of CD4+ T cell proliferative ability but not loss of human immunodeficiency virus type 1 specificity equates with progression to disease. *J Infect Dis* 182, 792-8.
- Wolthers, K.C., Bea, G., Wisman, A., Otto, S.A., de Roda Husman, A.M., Schaft, N., de Wolf, F., Goudsmit, J., Coutinho, R.A., van der Zee, A.G., Meyaard, L. and Miedema, F. (1996) T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover. *Science* 274, 1543-7.
- Yamamoto, T., Price, D.A., Casazza, J.P., Ferrari, G., Nason, M., Chattopadhyay, P.K., Roederer, M., Gostick, E., Katsikis, P.D., Douek, D.C., Haubrich, R., Petrovas, C. and Koup, R.A. (2011) Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. *Blood* 117, 4805-15.
- Yang, O.O., Ferbas, J.J., Hausner, M.A., Hultin, L.E., Hultin, P.M., McFadden, D., Sawicki, M., Detels, R., Majchrowicz, M., Matud, J.L., Giorgi, J.V. and Jamieson, B.D. (2005) Effects of HIV-1 infection on lymphocyte phenotypes in blood versus lymph nodes. *J Acquir Immune Defic Syndr* 39, 507-18.
- Younes, S.A., Trautmann, L., Yassine-Diab, B., Kalfayan, L.H., Kernaleguen, A.E., Cameron, T.O., Boulassel, R., Stern, L.J., Routy, J.P., Grossman, Z., Dumont, A.R. and Sekaly, R.P. (2007) The duration of exposure to HIV modulates the breadth and the magnitude of HIV-specific memory CD4+ T cells. *J Immunol* 178, 788-97.
- Zeng, M., Smith, A.J., Wietgreffe, S.W., Southern, P.J., Schacker, T.W., Reilly, C.S., Estes, J.D., Burton, G.F., Silvestri, G., Lifson, J.D., Carlis, J.V. and Haase, A.T. (2011) Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. *J Clin Invest* 121, 998-1008.

Zimmerli, S.C., Harari, A., Cellerai, C., Vallelian, F., Bart, P.A. and Pantaleo, G.
(2005) HIV-1-specific IFN-gamma/IL-2-secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells. Proc Natl Acad Sci U S A 102, 7239-44.