CD5 EXPRESSION LEVELS AND HUMAN IMMUNODEFICIENCY VIRUS-SPECIFIC T CELLS

STEPHEN JOHN PENNEY

CD5 Expression Levels And Human Immunodeficiency Virus-Specific T Cells

By

©Stephen John Penney

A thesis submitted to the

School of Graduate Studies

In partial fulfillment of the

requirements for the degree of

Master of Science

School of Graduate Studies, Immunology and Infectious Disease Program,
Division of BioMedical Sciences, Faculty of Medicine,
Memorial University of Newfoundland

September 2010

St. John's Newfoundland

Abstract

Almost all T cells normally express CD5, a transmembrane protein that regulates signaling through the T cell receptor (TCR). CD5 expression on T cells may be tuned to the avidity of TCR interactions with their cognate peptide/ major histocompatibility molecule complex (MHC). In transgenic mouse model systems, T cells expressing receptors with high avidity for self peptides escape negative selection if they have high levels of CD5. Conversely, peripheral T cells with low CD5 levels selectively react with cancer cells expressing low levels of cognate peptide MHC complexes. In human immunodeficiency virus (HIV) infection, CD5 expression is reduced on $CD8^+$ T cells and there is evidence of abnormal $CD8^+$ T cell cross-reactivity. The role of CD5 as a suppressor of TCR signaling suggests that its down-regulation in HIV infection may influence $CD8^+$ T cell cross-reactivity. Our hypothesis is that HIV mutation generates $CD8^+$ T cell epitope variants with lower avidity TCR interactions and that CD5 is down-regulated on memory T cells in adaptation. To test whether the avidity of TCR-peptide/MHC interaction correlates with CD5 expression on $CD8^+$ T lymphocytes, T cell stimulation with non-HIV and HIV-derived peptides spanning 1000 fold range in avidity was carried out. Non-HIV infected controls were tested against Cytomegalovirus (CMV), Influenza (FLU), Epstein Barr virus (EBV) and self peptides. HIV-infected individuals were tested against the same set of peptides and a series of HIV peptides. $CD8^+$ T cells proliferating against different peptides were identified by dilution of carboxyfluorescein diacetate succinimidyl ester

(CFSE) fluorescence intensity and co-stained for CD8 and CD5. In controls and HIV-infected individuals, higher proportions of $CD8^+$ T cells against non-HIV peptides with high TCR-peptide/MHC interaction avidities expressed CD5. In general, lower proportions of $CD8^+$ T cells against HIV-derived peptides expressed CD5, regardless of avidity.

The data suggest that reduced CD5 expression is conducive to promiscuous activation of peptide specific cytotoxic T cells through lower avidity T cell receptor interactions. With reduced CD5 expression there is a lower threshold for activation and T cells can be activated by cross-reactive peptides with lower avidity for the TCR than the index peptide (primary peptide used for initial activation). A greater understanding of this natural immunological occurrence could have potential therapeutic applications. Diseases regulated by cell mediated immunity, including HIV infection, could potentially be treated through control of T cell activation and signal modulation. The elucidation of $CD8^+$ T cells lacking CD5 expression exclusively in HIV infected individuals could help scientists unravel new methodologies for treatment. *Supported by CIHR*

Acknowledgements

I would like to express thanks to my supervisor Dr. Michael Grant for the opportunity to work in his lab. His guidance and expertise through the completion of this project is greatly appreciated. I would like to thank my committee members Dr. George Carayanniotis and Dr. Sheila Drover for their scientific critiquing. I would also like to thank Maureen Gallant for collecting the many tubes of blood required in this study and helping me make most of the reagents. I would like to thank Chungming Dai for teaching me many of the main assays used throughout this project. I would also like to express thanks to all my laboratory mates for their kind help during busy times in the lab. Thanks to the Canadian Institute of Health Research for providing funding. I would like to thank friends and family and lastly the Immunology group in general for being the most social group in BioMedical Sciences.

Contributions

Rosemarie Mason performed the preliminary ELISpot screening of HIV-infected subjects for HIV peptide reactivity. Rosemarie Mason also performed the ELISpot and cytotoxicity assays required for identification of low TCR avidity self peptide IP-30 within the HIV study cohort. Eastern Health clinical staff performed the flow cytometry analysis of patient blood samples for CD5 expression. Maureen Gallant performed the HLA typing on HIV-infected subjects and controls. I performed all other experimentation for the entirety of the thesis project.

Table of contents

Page

List of Tables

List of Figures

List of Abbreviations

Ab	Antibody
Ag	Antigen
APC	Antigen-presenting cells
AIDS	Acquired immunodeficiency syndrome
BCR	B cell receptor
C#	Control subject #
CA	Capsid
CCR5	Chemokine receptor 5
CD	Cluster of differentiation
CD5-APC	CD5-allophycocyanin
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CMV	Cytomegalovirus
CTL	Cytolytic/cytotoxic T lymphocytes
CXCR4	CXC chemokine Receptor, also called fusin
DNA	Deoxyribonucleic acid
ds	Double stranded
EBV	Epstein Barr virus
FCS	Fetal calf serum
FACS	Fluorescence activated cell sorter
FITC	Fluorescein isothiocyanate
Flu	Influenza

GAM	Goat anti-mouse
HIV-1	Human immunodeficiency virus 1
HAART	Highly Active Antiretroviral Therapy
IFN-γ	Interferon γ
Ig	Immunoglobulin
IL-2	Interleukin 2
IL-7	Interleukin 7
IN	Integrase
ITAMs	Immunoreceptor tyrosine-based activation motifs
ITIMs	Immunoreceptor tyrosine-based inhibitory motifs
LEDGF/p75	Lens epithelium-derived growth factor
M	Matrix (Influenza)
MA	Matrix (HIV-1)
MHC	Major histocompatibility complex
NIAID	National Institute of Allergy and Infectious Disease
NA	Neuraminidase
NC	Nucleocapsid
NFκB	Nuclear factor κ B
NK	Natural killer cell
NP	Nucleoprotein
PAMPs	Pathogen-associated molecular patterns
PBL	Peripheral blood lymphocytes

PBS	Phosphate buffered saline
PBMC	Peripheral blood mononuclear cells
PE	Phycoerythrin
PI	Protease Inhibitors
PR	Protease
RNA	Ribonucleic acid
RT	Reverse transcriptase
RTI	Reverse transcriptase inhibitors
SHP-1	Src-Homology 2 domain Phosphatase-1
SP	Single positive
ss	Single stranded
Syk	Spleen tyrosine kinase
TCR	T cell receptor
T_{EM}	Effector memory T cells
UNAIDS	Joint United Nations Program on HIV/AIDS
WHO	World Health Organization

1 Introduction

Human immunodeficiency virus (HIV-1) is a complex lentivirus (a member of the Retroviridae family) that causes acquired immunodeficiency syndrome (AIDS). This condition in humans manifests when the immune system begins to fail, allowing life-threatening opportunistic infections. HIV-1 primarily infects vital cells in the human immune system including helper T cells (specifically $CD4^+$ T cells), macrophages and dendritic cells [1, 2]. HIV infection leads to low levels of $CD4^+$ T cells through at least three mechanisms: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells; and thirdly, killing of infected $CD4^+$ T cells by cytotoxic $CD8^+$ T cells that recognize infected cells [3, 4]. $CD4^+$ T cells have multiple protective roles as depicted in Figure 1.1, and when their numbers decline below a critical protective level, cell-mediated immunity is compromised.

Since HIV was first recognized on December 1, 1981, the World Health Organization estimates that AIDS has killed more than 25 million people. Two million new deaths occurred in 2007 alone. These statistics make it the most destructive pandemic in recorded history [5]. Referred to as the defining public health crisis of our time, the Joint United Nations Program on HIV/AIDS (UNAIDS) as well as the World Health Organization (WHO) identify the AIDS pandemic as a mix of diverse epidemics from all regions of the world. Southern

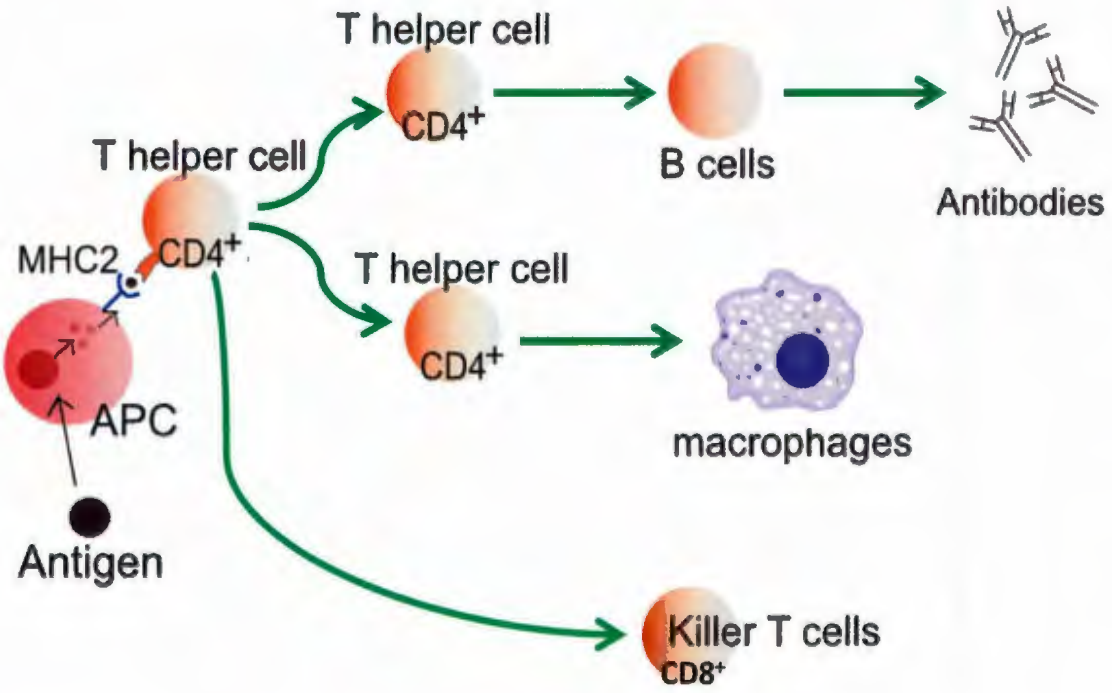


Figure 1.1: Simple schematic for lymphocyte activation. Green arrows represent cytokines and activation signals from T helper cells (T_H cells). These cells stimulate activation of B cell proliferation and induce antibody class switching. T_H cells augment the killing efficacy of macrophages and stimulate the expansion of cytotoxic $CD8^+$ T cells through production of their associated cytokines such as IL-2. Persistent HIV infection leads to the loss of $CD4^+$ helper T cells which in turn, decreases the availability of activation signals. The host becomes progressively more susceptible to opportunistic infections that are potentially lethal [6].

Africa remains the epicenter of the pandemic and continues to have high rates of new HIV-1 infections [7]. The following illustration (Figure 1.2) depicts the latest statistics on the world pandemic of HIV and AIDS published by UNAIDS/WHO in July 2008, and reports to the end of 2007.

With 2.7 million people newly infected in 2007, the total number of people living with HIV in 2009 is over 33 million [5]. Antiretroviral treatment reduces the mortality and morbidity of HIV infection, however, this treatment is not available in all countries. There has been no success in developing a vaccine to prevent infection. HIV has evolved numerous immune evasion mechanisms to allow entry, integration and continuous viral replication inside the host. The diversity of HIV together with these mechanisms and their effects over time hamper design and development of effective vaccines.

1.1 HIV Summary

1.1.1 HIV-1 structural makeup

HIV is enveloped by a lipid bilayer of host origin in which viral glycoproteins are embedded as depicted in Figure 1.3. HIV is ~120 nm in diameter and roughly spherical. HIV has several major genes coding for structural proteins found in all retroviruses, and several nonstructural ("accessory") genes that are unique to HIV. Its genetic material is encoded in two copies of positive single-stranded RNA 9749 bp in size, which harbor the virus' nine genes within a conical capsid. These genes include gag, pol, env, tat,

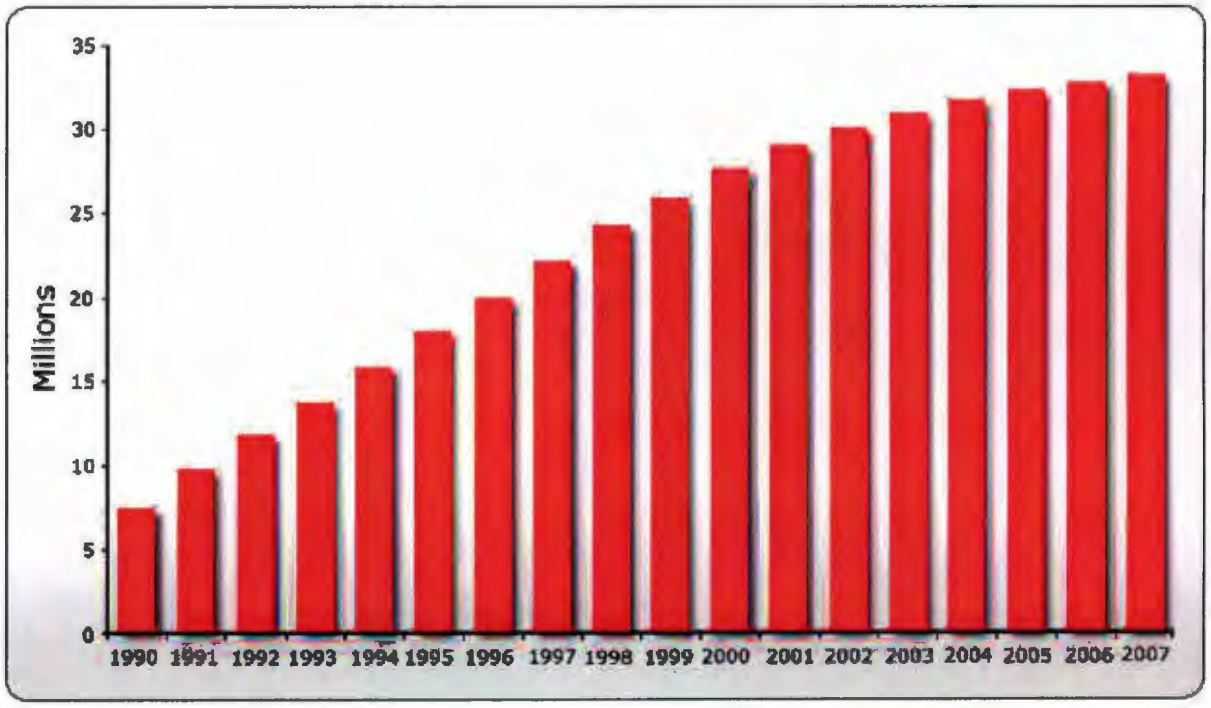


Figure 1.2: People living with HIV infection worldwide. Modified from http://www.avert.org/worldstats.html

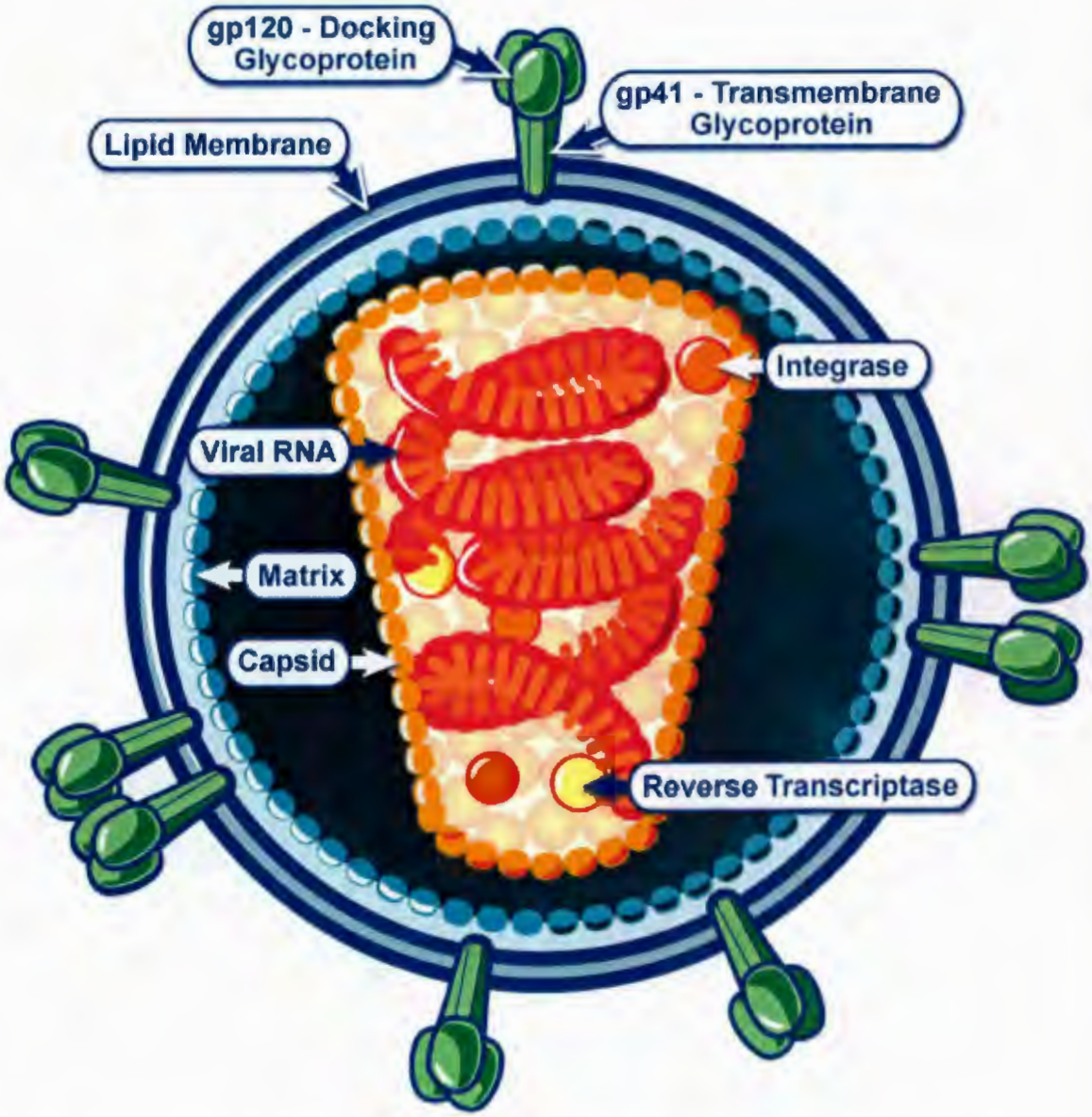


Figure 1.3: Illustration of HIV virion, depicting its two encapsulated copies of positive stranded RNA within its surrounding glycoprotein-embedded lipid membrane. Credit: National Institute of Allergy and Infectious Disease (NIAID) http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/Pages/structure.aspx

rev, nef, vif, vpr, and vpu that collectively encode 19 proteins. Information needed to make the structural proteins for new virus particles is encoded by the gag, and env genes [8]. Gag codes for the nuclear core protein. Its precursor is synthesized as a 55 kDa protein which is cleaved into smaller proteins referred to as matrix 17 kDa (p17), capsid 24 kDa (p24), and nucleocapsid 7kDa (p7 and p6) [9]. The env gene codes for a glycoprotein called gp160, which is broken down to form transmembrane structural component proteins gp120 and gp41 [10]. They are found in the outer membrane of the retrovirus. Pol encodes for the Pol polyprotein that is broken down into protease (PR), reverse transcriptase (RT) and integrase (IN), all of which provide essential enzymatic functions. Similar to other retroviruses, the remaining six proteins encoded by HIV-1 regulatory genes are so-called accessory proteins; tat, rev, nef, vif, vpr, and vpu, that control HIV's ability to infect cells, replicate, and cause disease [11]. The Tat proteins (p16 and p14) are transcriptional transactivators for the long terminal repeat promoter that act by binding the trans-activation response RNA element [12]. The Rev protein (p19) is a regulator of structural gene expression; it is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the Rev responsive RNA element [13]. The Vif protein (p23) promotes infectivity by preventing the action of APOBEC3G. This protein is a cytidine deaminase that induces numerous deoxycytidine to deoxyuridine mutations in the first negative strand of the HIV DNA, primarily expressed as complementary DNA. The hypermutation ultimately destroys the coding and replicative capacity of the virus, resulting in many non

viable virions [13]. The Vpr protein (p14) is a weak transcriptional activator and arrests cell division at G2/M [11]. The Nef protein (p27) down-regulates CD4, the major viral receptor, and MHC class I molecules [14]. Vpu (p16) is a type I integral membrane protein with at least two different biological functions: it promotes degradation of CD4 in the endoplasmic reticulum, and it is required for efficient virion budding and release from the plasma membrane of HIV-1-infected cells [11].

1.1.2 Diagnosis of HIV infection

A diagnosis of acute HIV-1 infection cannot be made with standard serologic tests. The recombinant enzyme-linked immunosorbent assays (ELISAs) commonly used to diagnose established HIV-1 infection are usually negative in persons who present with acute infection. Serologic tests for detection of HIV-specific antibodies first become positive approximately 22 to 27 days after acute infection [15]. The only test licensed for earlier detection of HIV-1 infection is the serum or plasma p24 antigen test, which is used routinely in blood donors to detect viral infection before the development of HIV-1 antibodies. Cases of acute HIV-1 infection have also been accurately diagnosed on the basis of high plasma viral RNA levels [15].

1.1.3 HIV tropism

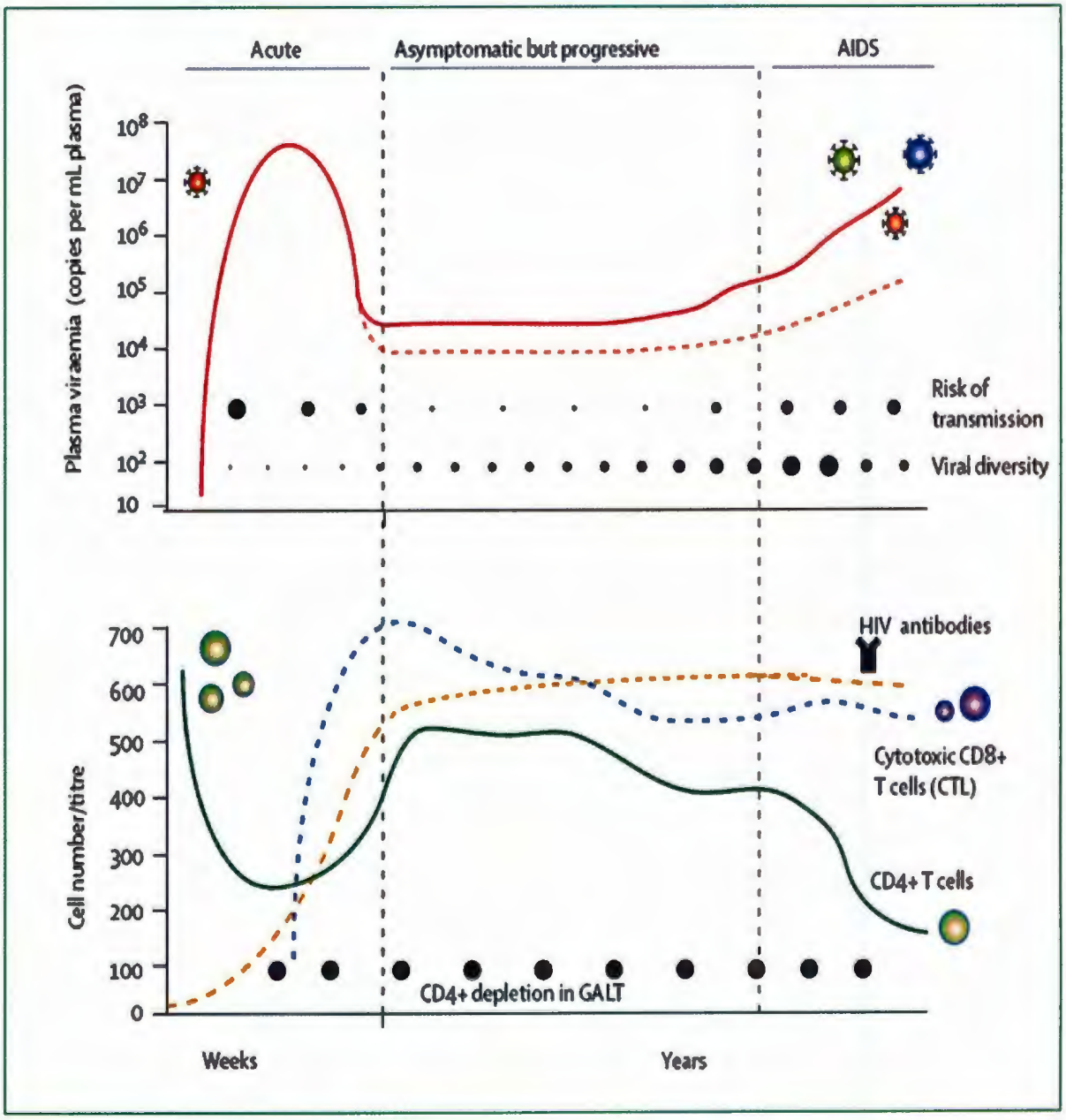
Transmission of HIV occurs through direct contact between the bloodstream and/or mucosa and virions found in infected body fluids. The most common sources are blood, semen, and vaginal secretions [15]. HIV has been detected in saliva, tears, and urine, however, it is found in extremely low levels in these fluids [16].

The cells primarily infected with HIV include T_H cells ($CD4^+$ T cells), dendritic cells and macrophages. The reason for this tropism is that HIV utilizes the CD4 molecule as the primary receptor for entry into target cells. Coreceptors include CCR5, exploited by R5 HIV strains and CXCR4, utilized by the X4 HIV strains for entry [17]. A successful infection of the targeted cell requires that HIV effectively transfer its genetic material into the cytoplasm. The duration and outcome of viral infection at the cellular level is highly dependent on the target cell type and its level of activation [2].

1.1.4 Pathogenesis

The pathogenesis of HIV is complex and not fully understood. Infection with HIV follows three stages: the acute stage of infection followed by the clinical latency stage and lastly by AIDS. The following Figure 1.4 summarizes the course of HIV infection reflecting seroconversion and major changes in viremia as well the involvement of different cell types and changes in their numbers through to the development of AIDS.

Figure 1.4: Illustrated course of HIV-1 infection as defined by the level of viral replication. Plasma viremia (top graph), and dynamic changes of the $CD4^+$ T cell compartments (bottom graph) occur during the course of HIV infection. Primary HIV infection is characterized by high plasma viremia (red line, top), low CD4 cells (green line, bottom), and absence of HIV-1 specific antibodies (orange dotted line, bottom). Seroconversion typically occurs around 4 weeks post exposure. Viremia falls as cytotoxic $CD8^+$ T cells (CTL) develop (blue dotted line, bottom) and the individual's viral-load set point is reached during the chronic infection phase. The viral set points are known to differ greatly among individuals (red dotted line, top) and predict the rate of disease progression. Disease progression can range from a few years to several decades. The viral diversity increases throughout infection (black circles, top). The risk of HIV-1 transmission is highest in the first weeks of infection when viremia peaks (black circles, top). GALT refers to gut-associated lymphoid tissues. Figure adaptation from [2].

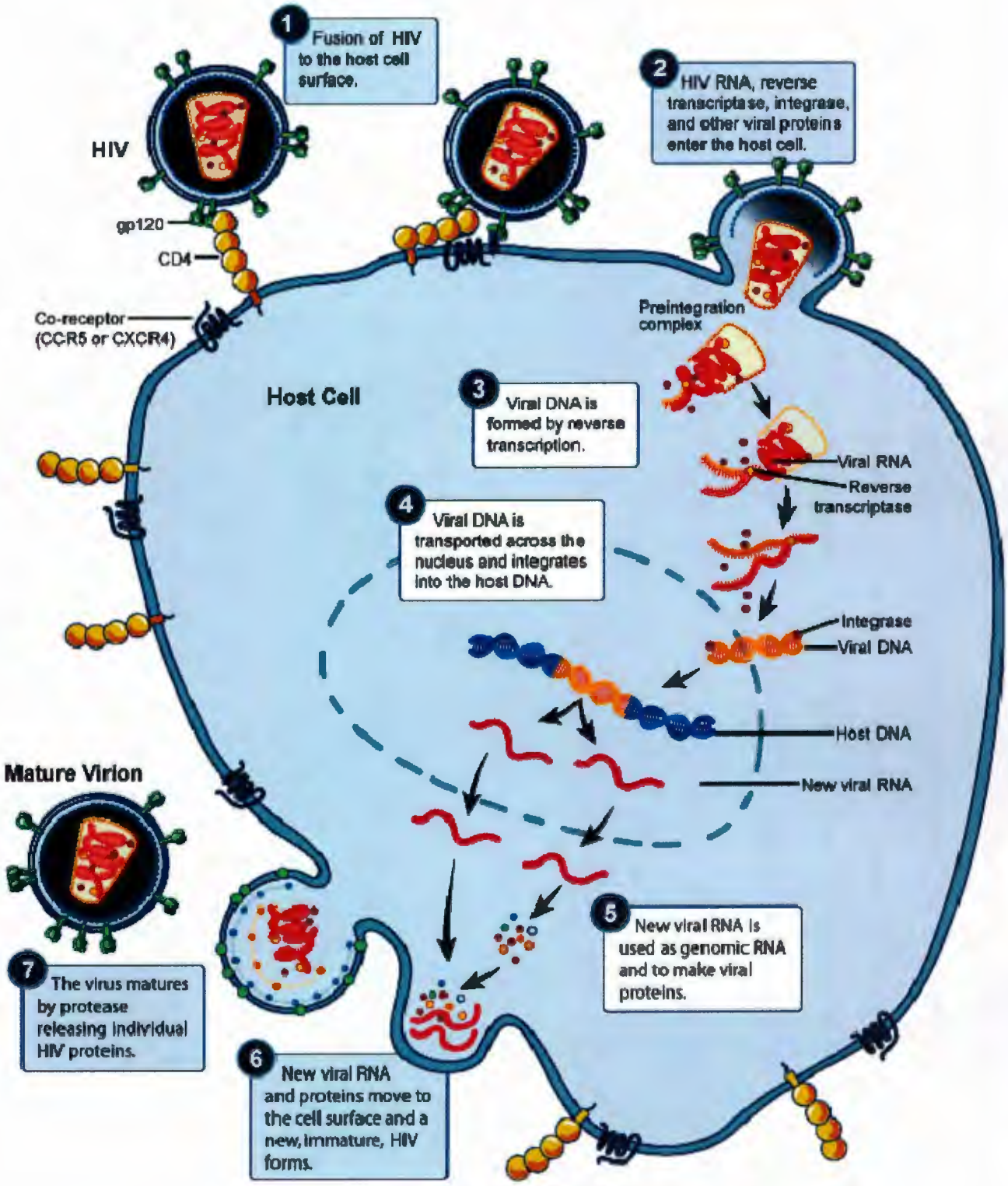

Acute
Asymptomatic but progressive
AIDS
Plasma viraemia (copies per mL plasma)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10
Risk of transmission
Viral diversity
Cell number/titre
700
600
500
400
300
200
100
0
HIV antibodies
Cytotoxic CD8+ T cells (CTL)
CD4+ T cells
CD4+ depletion in GALT
Weeks
Years

Upon exposure to HIV, a rapid series of immunologic, virologic and clinical events take place. As illustrated in Figure 1.5, the primary, or acute infection, is a period of replication, leading to levels of HIV approaching several million viruses per mL in the peripheral blood [15]. The half-life of a single virion is so short that half of the entire plasma virus population is replaced every 30 minutes [18]. Acute stage infection is then followed by a characteristic drop in the number of circulating $CD4^+$ T cells. Acute viremia is uniformly associated with activation of $CD8^+$ T cells, as well as antibody production, or seroconversion. This stage usually occurs during the initial four to eight weeks post-exposure [19].

1.1.5 Hallmark of HIV infection

The hallmark of HIV infection is depletion of $CD4^+$ T cells. Bystander apoptosis induced by viral antigens or cytokines as well as down-regulation of CD4 receptor by Nef protein may be the mechanism for this sudden drop in cells. Activated virus-specific cytotoxic T lymphocytes (CTL) partially keep HIV infection in control, but eventually fail at containing the infection. HIV spread is attributed to its ability to effectively counteract innate, adaptive and intrinsic immunity [20]. HIV's ability to generate escape mutants, and establish latent provirus into the host genome in $CD4^+CD45RO^+$ T cells, facilitates evasion from most immunological attacks, including cellular and antibody-mediated immune responses. During the latent stage of infection, HIV actively replicates in the lymphoid organs.

Figure 1.5: HIV replication cycle depicting all major retroviral replication events giving rise to a mature virion. During the entry process, binding of the viral envelope protein (Env) to CD4 induces conformational changes in the gp120 subunit of Env, enabling it to interact efficiently with the chemokine co-receptors CCR5 or CXCR4. ❶These two binding events then trigger additional irreversible conformational changes within the envelope external glycoprotein and transmembrane proteins that result in fusion of the viral and cellular membranes permitting the viral genetic material to enter the cell [2]. ❷The actual fusion event occurs within minutes of pore formation following release of the viral core into the cell cytoplasm [6]. Once the core disassembles, ❸ the viral genome is reverse transcribed into DNA by the viral encoded reverse transcriptase enzyme. Viral variants can be generated during this process since reverse transcriptase is error prone and has no proofreading activity [3, 21-23]. At the midpoint of infection, ❹ the viral protein integrase in conjunction with host DNA repair enzymes inserts the HIV genome into transcriptionally active, gene rich regions of the host's chromosomal DNA. An integrase binding host factor, LEDGF/p75 (lens epithelium-derived growth factor), facilitates integration, which irreversibly transforms the cell into a potential virus producer [2, 6]. In the late steps, ❺ production of viral particles requires host as well as virus driven transcription. The viral proteins are ❻ transported and assembled in proximity to the cell membrane. Virus egress from the cell is not lytic, taking advantage of the vesicular sorting pathway, ❼ which normally mediates the budding of endosomes into multivesicular bodies [2]. Cleavage of the Gag-Pol polyprotein by the viral protease produces mature infectious virions [9]. Figure is adapted from: NIAID. http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/Pages/hivReplicationCycle.aspx.


1 Fusion of HIV to the host cell surface.
HIV
gp120
CD4
Co-receptor (CCR5 or CXCR4)
2 HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.
Preintegration complex
Host Cell
3 Viral DNA is formed by reverse transcription.
Viral RNA
Reverse transcriptase
4 Viral DNA is transported across the nucleus and integrates into the host DNA.
Integrase
Viral DNA
Host DNA
New viral RNA
Mature Virion
5 New viral RNA is used as genomic RNA and to make viral proteins.
7 The virus matures by protease releasing individual HIV proteins.
6 New viral RNA and proteins move to the cell surface and a new, immature, HIV forms.

1.1.6 Diagnosis: AIDS

AIDS is described as the advanced state of progressive HIV infection. In the absence of effective treatment, AIDS results from $CD4^+$ T cell numbers profoundly decreasing over time. A person is clinically diagnosed with AIDS when their $CD4^+$ T cell count falls below 200 cells per cubic millimeter of blood, the level at which the danger of developing an AIDS-defining illness or infection becomes high. In comparison, healthy individuals have about 1,000 $CD4^+$ T cells per cubic millimeter of blood [2].Once below critical numbers, cell mediated immunity no longer provides protection. The gradual and continuous viral destruction of immune cells severely compromises the host's defenses and allows infections that are otherwise easily dealt with. Thus these infections are said to be "AIDS-defining."

1.1.7 Treatment

There is no safe and effective vaccine against HIV despite years of research. Currently, antiviral drugs with harmful side effects are the only method of controlling HIV infection. Understanding the viral life cycle, structure and function of HIV proteins enabled the development of HIV-specific drugs. There are currently more than 20 drugs available to treat HIV-infected patients [21]. Introduced in 1996, Highly Active Antiretroviral Therapy (HAART) involves the administration of a drug combination including protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (RTI's) and non-nucleoside reverse

transcriptase inhibitors. The implementation of HAART supports suppression of plasma viremia below detectable levels, raises circulating $CD4^+$ T cell counts, and reduces the occurrence of opportunistic infections, thereby prolonging life [24]. RTIs block reverse transcriptase whereas PIs prevent viral replication by inhibiting HIV-1 protease [25].

1.1.8 Persistence

The tremendous diversity of HIV is a result of the fast viral replication cycle, with the generation of 10^9 to 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication [26]. This leads to the generation of many variants of HIV in a single infected patient. When a single cell is simultaneously infected by two or more different strains of HIV, such variability is compounded due to the recombinogenic properties of reverse transcriptase. During simultaneous infection, the genome of progeny virions may be composed of RNA strands from two different strains. These hybrid virions then infect a new cell where they undergo replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes [27]. The host cannot generate an immune response fast enough to keep up with the high genetic variability due to mutation and recombination. As cytotoxic T cell responses target dominant epitopes, the virus mutates and escape occurs [22]. Thus through mutation, HIV infection creates a

highly diverse 'quasispecies' that is a moving target [23]. The disturbing outcome is that the virus cannot be cleared by the host's immune system.

1.1.9 CD8+ T cell response

Virus-specific $CD8^+$ T cell responses are crucial for immune control of viral infections and have been shown to play an important role in HIV infection [6, 28]. $CD8^+$ T cells recognize viral peptides (epitopes) presented by HLA class I molecules on the surface of infected cells through specific binding of their T-cell receptor (TCR) with the HLA class I-peptide complex. Partial control of virus replication is attributed to the cytotoxic T lymphocyte response where infected cells are destroyed by cytolytic activity [6, 29]. T cell abnormalities are a common occurrence in HIV infection [6]. Responding $CD8^+$ T cells in HIV infected individuals exhibit a modified ligand expression. There is evidence that HIV infection down-regulates surface MHC class I expression [30], as well as decreases CD5 expression on $CD8^+$ T cells in HIV infected individuals [31]. Most abnormalities on responding HIV-specific $CD8^+$ T cells have been widely researched, but the effect of CD5 down-regulation has never been functionally examined.

1.2 CD5

1.2.1 Discovery

CD5 was discovered over 30 years ago by Reinherz who produced monoclonal antibodies with selective reactivity against mature human thymocytes and peripheral human T cells [32]. CD5 was later described to be a pan T cell marker belonging to the cysteine-rich scavenger receptor glycoprotein family. It was later determined that CD5 expression increases in parallel with that of the CD3/TCR complex during T-cell development [33] .

1.2.2 Structure

CD5 contains three extracellular cysteine-rich scavenger receptor domains, a hydrophobic transmembrane region and a large cytoplasmic domain as depicted in Figure 1.6. The CD5 cytoplasmic tail is devoid of intrinsic enzymatic activity, but is well adapted for signal transduction. The cytoplasmic domain harbors four tyrosine residues at positions 378, 429, 441, and 463. Tyrosine 378 is contained within an immunoreceptor tyrosine-based inhibitory motif (ITIM). This finding suggests that CD5 interacts with intermediate proteins that are involved in inhibitory signaling [34].

In recent years, CD5 has been recognized as a modulator of TCR signaling [35]. It is recruited to, and tightly co-localizes with CD3 at the immunological synapse so as to inhibit TCR signaling in T cells interacting with APCs, without influencing conjugate formation [36]. CD5-mediated inhibition of

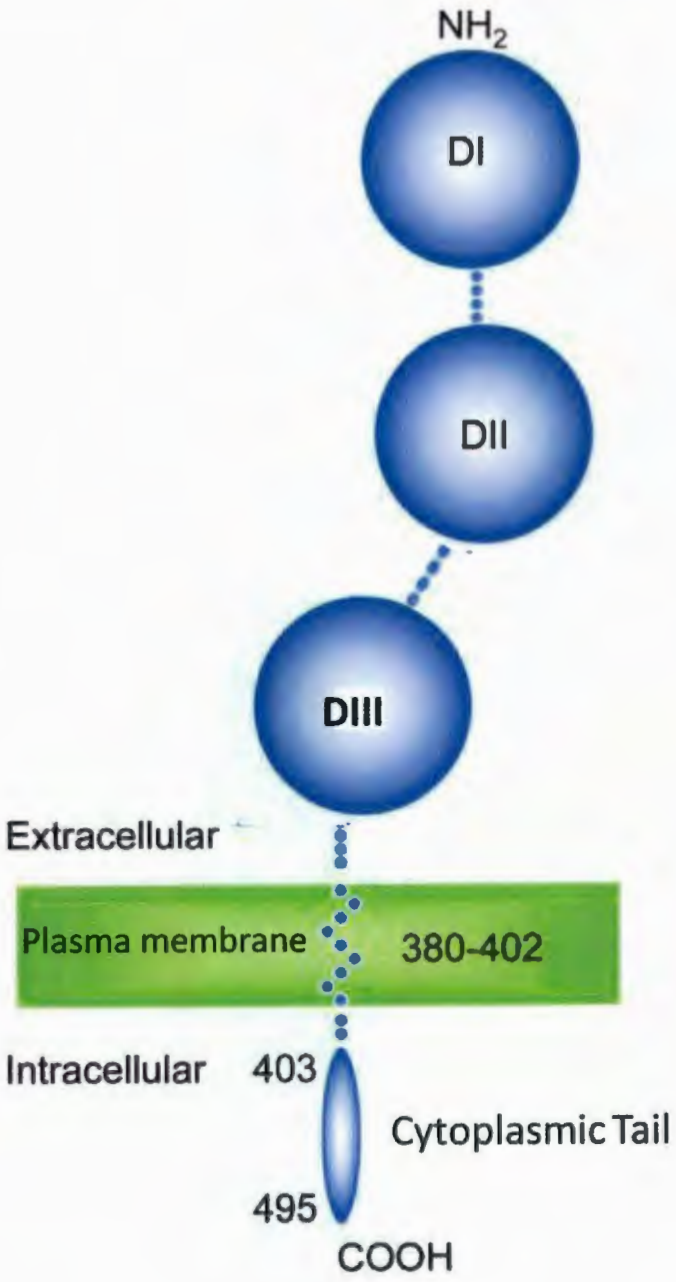


Figure 1.6: Arrangement of CD5 extracellular region with respect to the plasma membrane depicting the three cysteine-rich scavenger receptor domains and cytoplasmic tail [37].

TCR signaling does not require an extracellular domain, but only its cytoplasmic domain, [38] in which an ITIM motif plays an important role [34].

1.2.3 Biochemistry

TCR-CD3-induced CD5 phosphorylation recruits Src-Homology 2 domain Phosphatase-1 (SHP-1), resulting in the activation and tyrosine dephosphorylation of different substrates [34]. The CD5-regulated substrates include CD3ζ, ZAP-70, Syk, and phospholipase C. The cytoplasmic domain of CD5 contains four potential tyrosine phosphorylation sites, including an imperfect immunoreceptor tyrosine-based activation motif (ITAM), and an important functional ITIM [34, 37, 39, 40].

By mutation of all four CD5 intracellular tyrosine residues to phenylalanine, the membrane-proximal tyrosine at position 378, was shown to be crucial for SHP-1 association [34, 37, 39]. Recruited SHP-1 associates with membrane receptors, regulating their function. Substrates dephosphorylated by the cytoplasmic tyrosine phosphatase SHP-1 include; CD3ζ, CD19, CD22, PIR B/p91A, BIT, PLCγ1, and the intracellular protein tyrosine kinases $p56^{lck}$, $p59^{fyn}$, CD3ζ-associated protein 70 (ZAP-70) and Spleen tyrosine kinase (Syk) [41]. The involvement of SHP-1 in such broad dephosphorylation affects the activation threshold for TCR signaling. The level of CD5 expression in combination with its intracellular ITIMs and recruitment of SHP-1 evidently modulates the threshold for activation of T cells, [34] as illustrated in Figure 1.7.

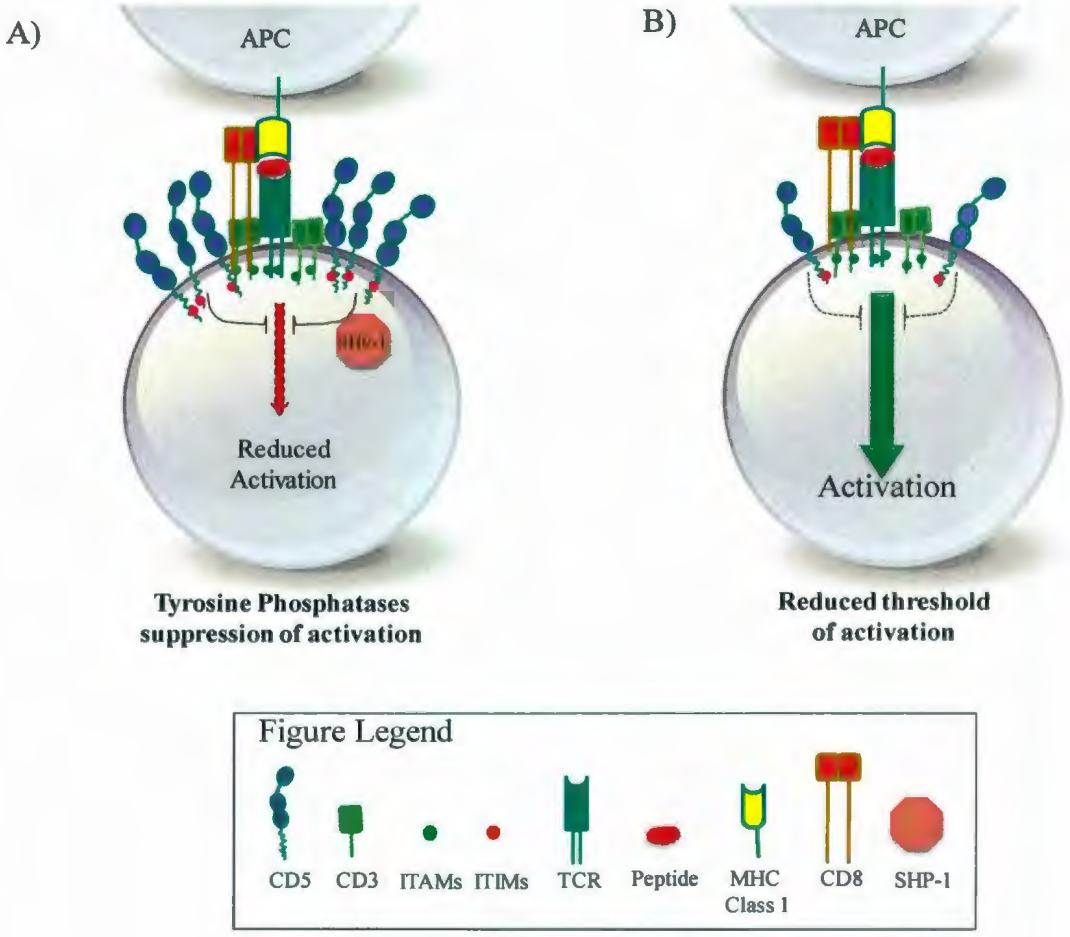


Figure 1.7: The level of CD5 expression affects the activation threshold for TCR signaling. (A) $CD5^{hi}$ expressing T cells have more tyrosine phosphatase-mediated suppression of activation by the molecule SHP-1 (Src-Homology 2 domain Phosphatase-1) [34]. (B) $CD5^{lo}$ T cells have a reduced threshold for activation due to lesser antagonistic activity.

1.2.4 Evidence for CD5 function

No ligand has been clearly identified for CD5 [40]. Researchers agree that the biological role of CD5 is to modulate the intracellular strength of signals induced by antigen receptors in both T cells and B cells. This is achieved by CD5 acting as a negative regulator for TCR signaling whereby CD5 expression level maintains a balance between tolerance and immunity [35, 40, 42]. The molecular mechanisms behind the regulatory functions have only recently been elucidated. The critical role of CD5 ITIM has been made evident through demonstration of binding to SHP-1, mediating the down-regulatory activity of this receptor [34].

Construction of tyrosine mutants via point mutation in CD5 cytoplasmic ITIM removed both SHP-1 binding and the down-regulating activity of CD5 during TCR-CD3 stimulation [34]. These results reinforce a critical role for CD5 ITIM which by binding to SHP-1 mediates the down-regulatory activity of this receptor.

Studies conducted in cell lines have revealed much about CD5 and its function. The regulatory role of CD5 in T cell activation was defined as inhibiting Ca^{2+} mobilization triggered via TCR-CD3 in Jurkat cells [34]. Similarly, $CD8^{+}$ T cells that express high levels of CD5 are anergic until transferred into antigen free hosts where CD5 levels return to normal [43].

Genetic manipulation in transgenic mouse models allows control over CD5 expression and examination of resultant T cell behavior. For instance, single positive thymocytes from CD5-deficient mice stimulated with antibodies to CD3,

proliferate more efficiently than those from wild-type mice, indicating that CD5 in immature T cells attenuates TCR-dependent signals [44]. Further support for CD5 function was discovered when CD5 expression levels on T cells were shown to be tuned to the avidity of TCR interactions with cognate peptide/MHC complexes. It was in transgenic model systems that T cells expressing receptors with high avidity for self peptides were shown to escape negative selection in the thymus if they expressed high levels of CD5 [42]. Conversely, peripheral T cells expressing low CD5 levels selectively react with cancer cells expressing low levels of cognate peptide MHC complexes [45]. It is very apparent that CD5 expression has modulatory effects in T cells.

The absence of a clearly identifiable ligand is a limitation to our understanding of CD5 biology. If a ligand were discovered, perhaps other functions for CD5 may be identified. The knowledge of CD5 function gathered to date suggests why we should consider CD5 a homeostatic regulator of T cells. Therapeutic manipulation of CD5 may be useful in autoimmune disease [40]. High levels of CD5 expression would result in increased thresholds for TCR triggering and could be a means to control autoreactive T cells and avoid overt autoreactivity in the continuous presence of self antigens [43].

1.2.5 $CD3^+CD8^+CD5^-$ subset population

In the peripheral blood there is a normal lymphoid phenotype population of $CD5^-$ T lymphocytes mainly, $CD3^+$ $CD8^+$ [31]. In advanced stages of HIV-1

infection, when viral burden and replication are high, the subset population of $CD3^+CD8^+CD5^-$ has been found to reach a mean 37.6 ± 14.8% of the total $CD3^+CD8^+$ cells [31] . This increase in $CD5^-$ T cells was also evident in subjects with less advanced disease progression (mean 27.4 ± 13.6%). These subset percentage findings are significant when compared to the low mean 5.5 ± 3.2% expression of the total $CD3^+CD8^+$ T cells for HIV seronegative people examined [31].

1.2.6 CD5 expression in HIV infection

In HIV infection, CD5 expression is reduced on $CD8^+$ T cells [31] and there is evidence of viral-specific $CD8^+$ T cell cross-reactivity with self peptides [46]. The role of CD5 as a suppressor of TCR signaling suggests that its down-regulation is involved in the underlying mechanism of $CD8^+$ T cell cross-reactivity in HIV infection. It was found that T cells lacking CD5 were capable of clonal expansion and cytolysis of autologous target cells with proportionately greater efficiency than the $CD8^+$ $CD5^+$ populations [47]. In 1995, Indraccolo et al. described a $CD3^+CD8^+CD5^-$ subset population greatly expanded in the peripheral blood of HIV-1 infected patients. Their investigation was strictly observational and did not include functional experimentation. Our overall aim was to examine CD5 expression in relation to peptide-specific CTL responses of varying avidities and to determine if there is lower CD5 expression in HIV infection.

1.3 Study Design

1.3.1 The importance of MHC

The peptide/MHC complexes act as "signposts" that display fragmented pieces of an antigen on the host cell's surface. In humans, the MHC is divided into three types: Class I, II, and III. The A, B, and C genes belong to MHC class I. MHC class I is expressed on all nucleated cells. MHC class I proteins contain an α heavy chain and β2-microglobulin chain. They present antigen fragments to cytotoxic T cells and bind to CD8 on cytotoxic T cells [8].

In this study, the response of human histocompatibility-linked leukocyte antigen A2 (HLA-A2) positive individuals was chosen to be examined due to the high frequency of this allele in the study population. Over 50% of the HIV-infected study cohort was previously shown to express HLA-A2. HLA-A2 is a good presenter of strong HIV epitopes correlating with disease progression. HIV-uninfected volunteers were recruited, of which some had been previously fully typed for class I expression. As seen in Figure 1.8, flow cytometry was used to screen for HLA- A2 expression in new HIV-uninfected subjects. It was therefore desirable to screen peptides expressed in the context of HLA-A2 restriction due to the high frequency of HLA-A2 in the study population. The immune system of healthy individuals is frequently challenged with viral infections that are normally kept under control. Once primed, the immune system, through the generation of

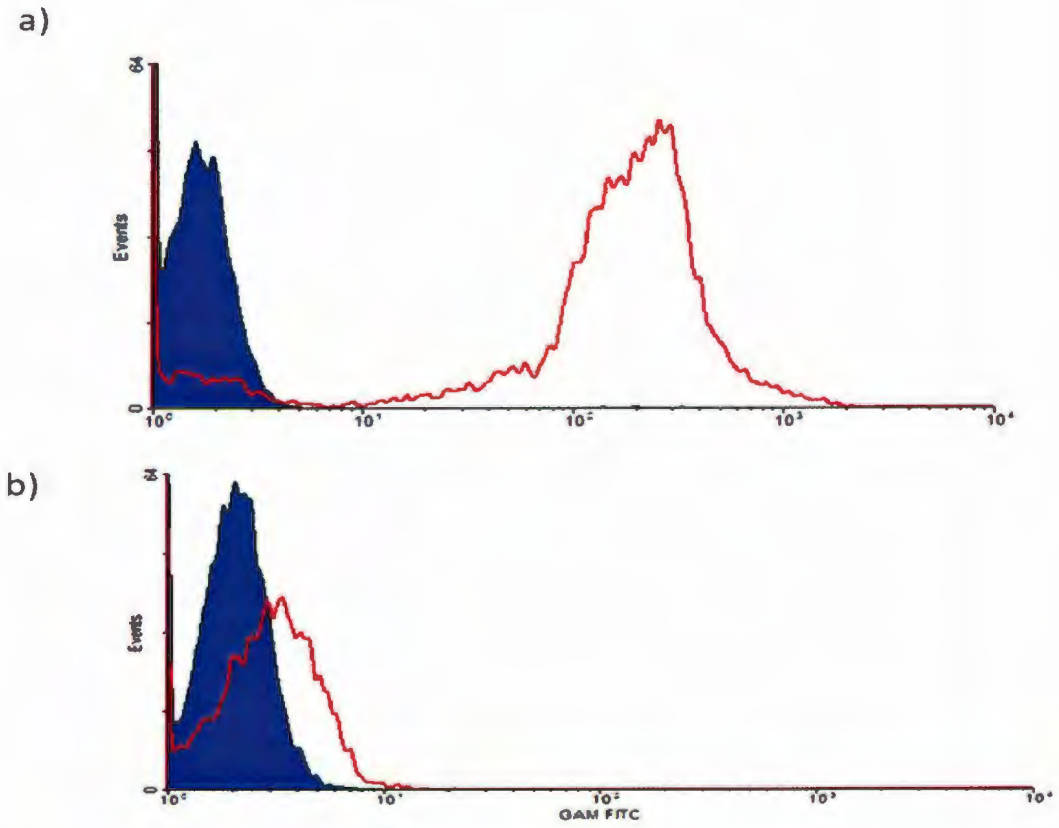


Figure 1.8: Immunofluorescent labeling of fresh peripheral blood mononuclear cells (PBMC) from non-HIV infected control C32, HLA-A2 positive (a) and C34, HLA-A2 negative (b). HLA-A2 specific Mouse anti-Human primary monoclonal antibody is bound and detected by the secondary FITC conjugated goat anti-mouse (GAM) polyclonal antibody. Blue solid peak indicates isotype control and the red peak indicates A2-GAM-FITC positive binding.

memory cells, is able to more rapidly respond against reoccurring attacks from these persistent viral infections. Cytomegalovirus, Influenza virus as well as Epstein Bar virus are among the most common viral infections that challenge the immune system. Through the normal generation and expansion of memory T cells specific to dominant epitopes of viral peptides, cell mediated immunity quickly assists in eliminating infected cells and with it the virus in most cases. Immunodominant viral peptide-specific responses of memory $CD8^+$ T cells were chosen to be examined in individuals due to their high incidence in the screened population. Peptides include those shown in Table 1.1.

1.3.2 Immune response towards common viral infections

Common viral infections that the immune system effectively eliminates or keeps under control include Cytomegalovirus (CMV), Epstein – Barr (EBV), and Influenza viruses (Flu). Conventionally, during acute viral infection, antigen-specific T cells expand as much as 50,000-fold, acquire effector function and mediate clearance of the pathogen [48]. After resolution of the infection, 90%–95% of the antigen-specific T cells die, leaving behind a long-lived population of memory T cells that provide protection upon re-infection [48]. Memory T cells possess several properties crucial for their function, including higher frequencies than naive precursors, the ability to rapidly reactivate upon antigen stimulation, wide tissue distribution, and the ability to survive and self-renew for long periods in the absence of cognate antigen. Memory cells produce effector cytokines including IFN-γ and some memory T cells have been reported to survive for as

Table 1.1: HLA-A*02 restricted HIV and non-HIV peptides examined.

HLA-A*02 – binding peptide	Location name	Sequence
A2-CMV	pp65 495-503	NL[1]VPMV[2]ATV
A2-Flu	Matrix 55-66	GILGFVFTL
A2-EBV	BMLF1 280-288	GLCTLVAML
A2-IP	IP-30 -11 to -3	LLDVPTAAV
A2-4	PR 76-84	LVGPTPVNI
A2-Gag	P17 77-85	SLYNTVATL
A2-1	RT 179-187	VIYQYMDDL
A2-2	**RT 33-41**	ALVEICTEM

Anchors[1]

Auxiliary anchors[2]

long as 75 years [49]. This reservoir of primed $CD8^+$ T cells enables the investigation of peripheral viral-specific memory $CD8^+$ T cells. Examination of these viral specific immune responses is a method to directly study the naturally generated immune response established by the body that is developed specifically towards eliminating viral infection.

CMV, EBV, and FLU viruses generate a variety of different host responses and outcomes. Flu viruses cause a self limiting disease after which the virus is eradicated. CMV and EBV viruses cause pauci-symptomatic or asymptomatic diseases after which the viruses establish lifelong latency in the host cells, but are kept in check by immunity.

CMV of the Herpes virus group is found throughout all geographic locations and socioeconomic groups, and infects between 50% and 80% of adults [50]. CMV is a very potent immunogen, which triggers all arms of the immune system. Humoral immunity is established early in infection. However, the cellular immune response is considered the major mechanism by which viral replication is controlled. The cytotoxic $CD8^+$ T cell response is the most important component of CMV immune response [50, 51]. $CD8^+$ T cell responses focus on two CMV proteins, which are the pp65 tegument protein (protein that lines the space between the envelope and the nucleocapsid) and IE-1 protein (immediate-early regulatory protein) [50]. Immunodominant cellular responses are directed towards pp65 viral peptides, one of which is used in our study.

The Epstein - Barr virus (EBV), also from the Herpes family, is considered one of the most common viruses to infect humans. The majority of people infected with EBV remain asymptomatic but a few develop infectious mononucleosis. During primary infection, there is a massive expansion of activated antigen-specific $CD8^+$ T cells, which can persist in relatively high numbers for at least three years after primary infection [51]. During acute infection the cellular response directed towards lytic cell cycle proteins is about ten times stronger than the response to latent cycle proteins. EBV transactivator gene product BMLF1 (immediate-early promoter) is known to be an immunodominant antigen [52], which is used in our study.

Each year, as many as 60 million people contract Influenza in the United States alone. Commonly known as the flu, this infectious disease is spread by RNA viruses of the family Orthomyxoviridae. The $CD8^+$ T cell response is essential to clear virus. Dendritic cells and macrophages carry the antigen from the lungs to the lymph nodes, where they prime naïve $CD8^+$ T cells. The activated $CD8^+$ T cells then migrate to the lungs to clear the infection. Flu-specific $CD8^+$ T cells primed during primary infection persist and enable a rapid and vigorous response to secondary challenges [53]. Flu Matrix proteins are immunodominant in cellular immune responses and, therefore, are used in this study.

1.3.3 Autoreactive self peptide

Autoreactive $CD8^+$ T cells are selectively expanded in HIV-infected patients [46]. The signal peptide from an IFN-γ-inducible protein, termed IP-30, is a dominant self peptide expressed in the context of HLA-A2. Due to its previously determined low avidity interaction [46] we anticipate low CD5 expression on IP-30 peptide-specific cytotoxic T cells.

The IP-30-specific CTL escape thymic deletion and enter peripheral circulation. Experimentation with IP-30 peptide ascertained failure of the HLA-A2:Ig/IP-30 peptide dimer to stain IP-30 specific CTL. This finding suggests that the TCR interaction with the HLA-A2/IP-30 signal peptide complex is of relatively low avidity in comparison to other peptides examined [46].

1.3.4 The need for a therapeutic vaccine.

Nearly three decades have passed since HIV was first identified and there is still much to learn about its pathogenesis. HIV infection relentlessly continues to spread leaving millions awaiting the discovery of effective treatment. Therapeutic vaccines hold an untapped potential in the treatment of HIV. A therapeutic HIV vaccine would be designed to boost the body's immune response to HIV in order to better control the infection. Currently, there are no therapeutic HIV vaccines approved by the Food and Drug Administration (FDA). However, therapeutic HIV vaccines are being tested in clinical trials to determine efficacy. If therapeutic vaccines are able to strengthen the body's natural anti-HIV immune

response, people with HIV will not have to rely exclusively on the antiretroviral drugs now used to treat HIV infection. At present, antiretroviral drugs must be taken for life, and most cause serious side effects. The need for alternative treatment arises from the multiple failed outcomes at vaccine design. The wait for alternative treatment is compounded by treatment regimes including HAART that pressure HIV to develop drug resistance through rapid turnover of viral mutants. These viral mutants exhibiting high fitness propel the urgency for effective treatment. This demand invites researchers to apply novel concepts and approaches to HIV research that perhaps have been overlooked.

Greater knowledge of how the immune system responds to HIV infection can educate researchers towards developing effective treatment. Our study examines peptide-specific $CD8^+$ T cell memory responses and addresses the critical aspect of T cell activation with regard to varying TCR-peptide/MHC interaction avidity in the context of CD5 expression. We explore a potential role for CD5 in common viral infections and compare these common viral peptide-specific $CD8^+$ T cell responses to HIV-specific $CD8^+$ T cell immune responses. This comparison will be made while keeping in mind that CD5 attenuates TCR signaling [44], and its expression level positively correlates with the strength of TCR-induced signals [42, 54].

1.4 Hypothesis

HIV mutation generates $CD8^+$ T cell epitope variants that have lower avidity TCR interactions and CD5 is down-regulated on memory T cells in adaptation. We therefore expect that HIV-specific T cells as well as T cells specific for self peptides will activate more readily upon peptide presentation when expression of CD5 is reduced.

In an attempt to explore this hypothesis several research aims have been designed to ultimately determine if CD5 expression levels are linked to the avidity of the TCR-peptide/MHC interaction and to ascertain HIV's influence on CD5 expression.

1.5 Specific Aims

1. *Examine CD5 expression on $CD8^+$ T cells from non-HIV-infected individuals against a set of HLA-A2 restricted immunodominant viral peptides.*
2. *Examine CD5 expression on $CD8^+$ T cells from HIV infected individuals against the same set of HLA-A2 restricted non-HIV immunodominant viral peptides.*
3. *Examine CD5 expression on $CD8^+$ T cells from HIV infected individuals against a set of HLA-A2 restricted HIV-derived immunodominant peptides.*

2 Materials and Methods

2.1 Study cohort

Freshly isolated peripheral blood mononuclear cells (PBMC) from healthy uninfected and HIV-infected individuals were used in this study. Healthy uninfected volunteers were recruited from hospital personnel and served as the control group. Dr. Michael Grant's study cohort is comprised of over 240 HIV-infected individuals recruited through the Infectious Disease Clinic of the St John's General Hospital, St. John's, Canada. Ethical approval for this study was obtained from the Memorial University Faculty of Medicine Human Investigation Committee and all participants provided informed consent for blood collection and access to clinical and laboratory records. All HIV-infected subjects have EBV-transformed B lymphoblastoid cell lines (BLCL) generated and had been typed for HLA class I A and B antigens. Nearly all HIV infected subjects had been previously tested by cytotoxicity or ELISPOT assays for HIV-specific $CD8^+$ T cell responses.

2.2 Peptides

We selected a variety of peptides to stimulate memory $CD8^+$ T cell responses within the test subjects. Common viral peptides that are known to elicit $CD8^+$ T cell responses include A2-restricted peptides from CMV, Flu, and EBV. These viral peptides as shown in Table 1.1 were used as controls and HIV

peptides including A2-4, A2-Gag, A2-1 and A2-2 served as the test peptides. Self peptide A2-IP was examined due to its known low TCR-peptide/MHC interaction avidity and ability to activate peptide-specific $CD8^+$ T cells in HIV-infected individuals [46]. Synthetic peptides used were all > 95% pure (EZBiolab, Carmel, IN).

2.3 Lymphocyte isolation and cell culture

Whole blood was collected by venipuncture into Vacutainers containing acid-citrate-dextrose anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque PLUS (Amersham Biosciences AB, Uppsala, Sweden) gradient separation. PBMC were washed once in phosphate-buffered saline (PBS) and again with PBS plus 1% fetal calf serum (FCS). Cells were resuspended in "complete medium" consisting of RPMI 1640 supplemented with 10% FCS, 100 IU/ml of penicillin, 100 µg/ml of streptomycin, 2 mM L-glutamine, 10 mM HEPES buffer solution, and $2x10^{-5}$ M 2-mercaptoethanol (Gibco Invitrogen Corporation, Grand Island, NY). For proliferation studies, AIM V medium was supplemented with 2 mM L-glutamine, 1% non-essential amino acids (Gibco Invitrogen Corporation) and 10% Human AB serum (Atlanta Biological, Lawrenceville, GA),

Autologous BLCL were generated by Epstein-Barr virus transformation of peripheral blood B cells. In brief, 2.5 ml of supernatant from marmoset B95-8 leukocytes (ATCC CRL 1612; Rockland, ME) was passed through a 0.45 µm

pore size sterile filter (Millipore, Bedford, MA) and added to 5 X 10^6 freshly isolated PBMC. These cells were then cultured for 24 h, washed and maintained in complete medium supplemented with 20% FCS and 1 µg/ml of cyclosporine A (Sigma Aldrich, Canada) until sufficient growth occurred for cryopreservation of several aliquots of the cell line.

2.4 HLA-A2 Screening

To identify individuals expressing HLA-A2, screening was carried out using a HLA-A2 specific monoclonal antibody. The primary staining A2-specific-antibody bound to the MHC and was detected with a secondary staining goat anti-mouse IgG fluorescein isothiocyanate (FITC) fluorochrome-conjugated polyclonal antibody. Positive individuals were then identified by using flow cytometry. Most study subjects have been fully typed for HLA class I A and B antigens using commercial kits (One Lambda, Canoga, CA), as previously described [55].

2.5 Identification of peptide-specific IFN-γ production using ELISpot assay

HLA-A2 HIV-uninfected individuals were screened for reactivity with peptides by ELISpot. Microtitre assay plates (Multiscreen; Millipore, Bedford, MA) were coated with 0.75 µg/well of anti-IFN-γ capture MAb, 1-D1K (Mabtech, Stockholm, Sweden) and left overnight at 4°C. The plates were washed six times

with phosphate buffered saline (PBS), and PBMC were diluted in complete medium to $1x10^6$/ml; 200 µL ($2x10^5$ cells) were transferred to duplicate wells containing 0.4 µM peptide of interest. Cells stimulated with 5 µg/mL of purified phytohemagluttinin (PHA) (ICN Biomedicals Inc., Aurora, OH) served as positive controls ($2x10^5$ cells/well). PBMC plated in absence of peptide served as a background control. After 16 hr incubation at 37°C in a 5% CO_2 incubator, plates were washed as above and developed as per test kit instructions. Briefly, 100 µl/well of 1 µg/ml biotinylated anti-IFN-γ MAb 7-B6-1 (Mabtech, Stockholm, Sweden) diluted in PBS with 0.5% FCS was added for 2 h. Wells were washed six more times and 100 µl of streptavidin alkaline phosphatase conjugate (Mabtech, Stockholm, Sweden), diluted 1:1,000 in PBS with 0.5% FCS was added for 1 h. Plates were washed again six times, and 100 µl of chromogenic alkaline phosphatase substrate (Bio-Rad Laboratories, Hercules, CA) diluted 1:100 in colour development solution was added to the wells. The plates were incubated with the substrate until dark spots emerged (usually between 20 min – 1hr), at which point the wells were rinsed with tap water to terminate the color development reaction. The plates were then left to air dry and spots were counted with an automated ELISpot counter (ImmunoScan, Cellular technology Ltd. Cleveland, OH). Wells that produce spots were considered positive if the number of spots were twice the background. PHA was used as a positive control and PBMC alone in complete medium served as background.

2.6 Assessment of CD5 expression on fresh PBMC

The baseline expression of CD5 on $CD8^+$ T cells was assessed on fresh PBMC using triple fluorochrome staining by flow cytometry. Fluorochrome conjugated antibodies included CD5-allophycocyanin (APC), CD8-phycoerythrin (PE) and CD3-FITC (All from Biolegend, San Diego, CA). The results were expressed as percent positive or mean fluorescence intensity of $CD8^+$ T cells that are $CD5^+$.

2.7 Assessment of CD5 levels on peptide-stimulated, proliferating T cells. (CFSE Staining)

$1x10^6$ fresh PBMC/ml were stained with 1 μM carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Molecular Probes, Eugene, OR) diluted in PBS containing 5 mM ethylenediaminetetraacetic acid (EDTA) for 10 minutes at 37°C. After staining, the cells were washed with complete medium chilled on ice. The cells were then pelleted and 100 μl of test peptide at 200 μg/ml was added to the appropriate tubes. Controls were PBMC incubated in the absence of peptide stimulation. The cells were incubated at 37°C, 5% CO_2 for 1 hour, then 2 ml of AIM V culture medium was added to the cultures and the cells were incubated for 7 days. On day 7, the proliferating cells were identified by flow cytometry based on their decreased fluorescence intensity of CFSE. Cells were washed with fluorescence-activated cell sorting (FACS) buffer containing 5 mM EDTA, 0.5% bovine serum albumin, and 0.02% sodium azide (Sigma) in PBS; incubated with

phycoerythrin-labeled anti-human CD8 and allophycocyanin-labelled anti-human CD5 (All from Biolegend, San Diego, CA) at 4°C for 20 min; then washed again with FACS buffer and resuspended in 1% paraformaldehyde. 50,000 cellular events were acquired for analysis. Proliferating $CD8^+$ T cells were gated for analysis and level of CD5 was examined using a FACScalibur flow cytometer.

Peptide-specific proliferation was considered positive if the respective background control was below 2.5% and the proliferation was more than twice the background.

2.8 Generation of Cytotoxic T cells

Cytotoxic T cells were generated to examine functional activity. $5x10^6$ PBMC were exposed to 100 µM peptide in 100 µL of incomplete medium for 1 hour at 37°C. The cells were resuspended at 2.5 x 10^6/mL in complete medium supplemented with 25 ng/mL of recombinant human interleukin 7 (IL-7) (R&D Systems, Minneapolis, MN) and incubated for 3 days, followed by 7-10 days expansion in complete medium supplemented with 10 U/mL IL-2 (Hoffmann-La Roche Ltd., Nutley, NJ).

2.9 Cytotoxicity Assays

Autologous BLCL or partially HLA-matched target cells were labeled with 100 µCi $Na_2{}^{51}CrO_4$ (MP Biomedical, Irvine, CA) for 90 min at 37°C. Labeled cells were washed three times in PBS with 1% FCS, and transferred to U-bottom 96-

well plates (Corning Inc., Corning, NY) at 5 x10^3 cells/well in 50 µl complete medium. To sensitize the target cells, peptides were added to the appropriate wells for 1h at 37°C at a final concentration of 20 µM in a total of 100 µl complete medium. Sufficient effector cells were added to attain desired effector to target cell ratios of either 20, 10, or 5:1 and volume in each well adjusted to 300 µl with medium. The assays incubated at 37°C for 5h at which point 125 µl of supernatant was removed from each well and counted in a Wallac 1280 gamma counter. Spontaneous release was determined from duplicate wells containing ^{51}Cr-labeled target cells in complete medium after 5 hours incubation. Maximum release was determined by examining duplicate wells containing ^{51}Cr-labeled target cells in 1N hydrochloric acid after 5 hours incubation.

Specific lysis was calculated as (experimental ^{51}Cr release – spontaneous ^{51}Cr release) / maximum ^{51}Cr release – spontaneous ^{51}Cr release) X 100. Specific lysis ≥ 10% above background was considered positive.

2.10 Measurement of TCR-peptide/MHC interaction avidities

Effector cells from individuals with effective cytotoxic T cells generated against specific peptides were used to measure TCR-peptide/MHC interaction avidity. More specifically, autologous BLCL were pulsed with 10-fold dilutions of peptide in duplicates starting at 10 µM and going to 0.00001 µM. These BLCL served as targets in cytotoxicity assays. The avidity was derived from the peptide concentration at which specific lysis fell to 50% of maximum.

2.11 Statistical analysis

Data analysis and graphical representations were performed using the GraphPad Prism Ver. 4.0 software package (Graph-Pad Software, San Diego, Ca). Statistical significance (p values) of the results was calculated by using an unpaired, two-tailed parametric Student's *t* test. A two-tailed *p* value of less than 0.05 was considered significant.

3 Results

3.1 Preliminary analyses

In order to establish a solid foundation for the project, it was necessary to assess certain aspects of the background of our control and HIV-infected groups. These included:

1) Baseline percentages of $CD5^+CD8^+$ T cells in the control and the HIV-infected population.

2) Expression of HLA-A2.

3) Responses by HLA-A2 subjects against common viral peptide epitopes from CMV, EBV, and FLU.

3.1.1 Percentage of $CD8^+$ T cells expressing CD5 in control and HIV subjects.

We tested PBMC from 17 HIV-uninfected subjects by flow cytometry to measure the percentage of circulating $CD8^+$ T cells expressing CD5 under normal conditions. Figure 3.1 summarizes the results from all 17 HIV-uninfected subjects. Among total $CD8^+$ T cells in peripheral circulation, an average of 98.2 ± 0.3% expressed CD5. Data collected from PBMC of 32 HIV-infected subjects is also summarized in Figure 3.1, which shows an average of 95.1 ± 0.4% of the

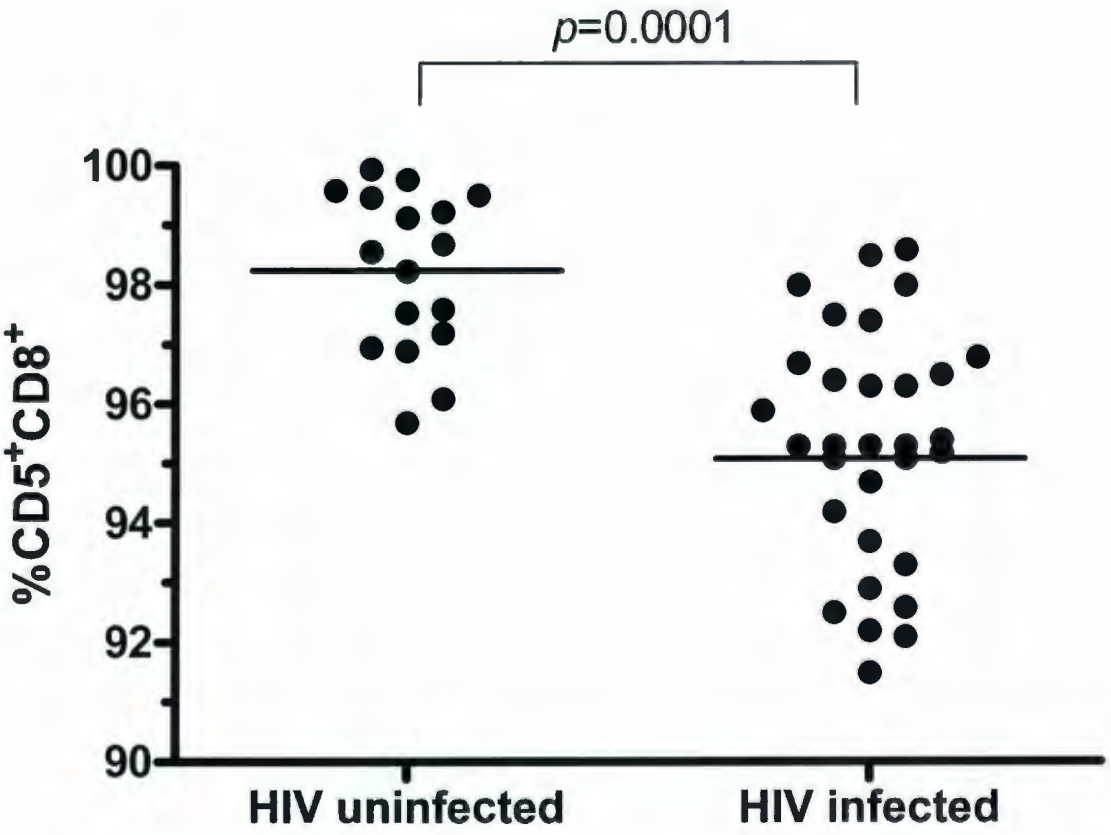


Figure 3.1: Percentage of peripheral blood $CD8^+$ T cells that express CD5 within our study groups.

total $CD8^+$ T cells in peripheral circulation express CD5. Although similar, the fraction of $CD8^+$ T cells expressing CD5 is significantly lower in the HIV infected group ($p = 0.0001$). In both populations, the vast majority of circulating $CD8^+$ T cells express CD5.

3.1.2 HLA-A2 expression in the HIV-uninfected study group

Among the 36 subjects initially screened for HLA-A2 expression, 24 (67%) were found to be $HLA\text{-}A2^+$, as reported in Table 3.1. The PBMC from these subjects were tested for peptide-specific IFN-γ production with common A2-restricted viral peptides using ELISpot assays.

3.1.3 Assessment of IFN-γ production following activation of lymphocytes by immunodominant peptides.

Reactivity against HLA-A2-restricted, immunodominant viral peptides was demonstrated by IFN-γ production. All 24 HLA-A2 controls were tested by ELISpot for reactivity against common viral peptides from CMV, FLU, EBV, and a self peptide A2-IP. PHA was used as a positive control and PBMC were cultured alone to establish background.

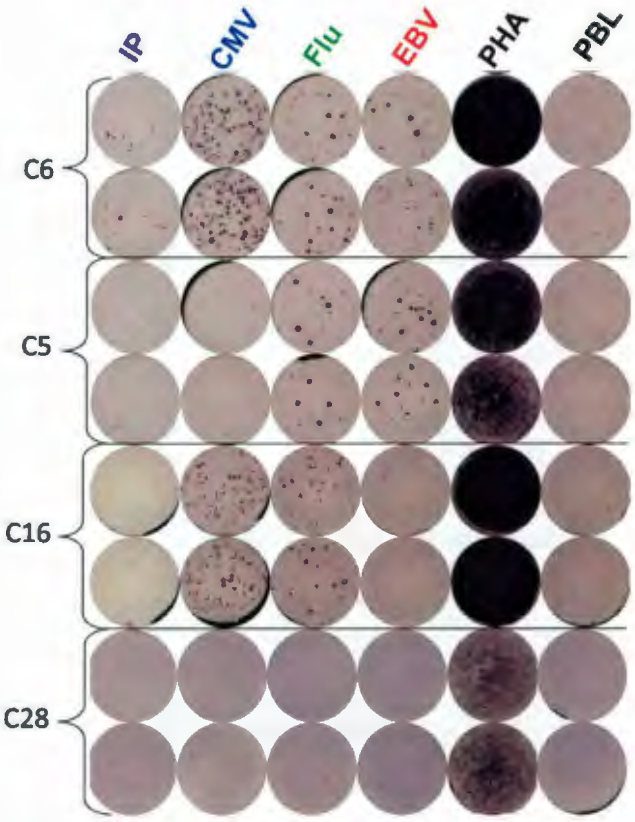
Table 3.1: Selection of HIV-uninfected subjects expressing HLA-A2.

HIV uninfected subject	HLA-A2
C1	Positive
C2	Positive
C3	Positive
C4	Positive
C5	Positive
C6	Positive
C7	Positive
C8	Positive
C9	Positive
C10	Positive
C11	Positive
C12	Positive
C13	Negative
C14	Negative
C15	Negative
C16	Positive
C17	Negative
C18	Positive
C19	Positive
C20	Negative
C21	Positive
C22	Positive
C23	Positive
C24	Positive
C25	Negative
C26	Positive
C27	Negative
C28	Negative
C29	Positive
C30	Negative
C31	Negative
C32	Positive
C33	Positive
C34	Negative
C35	Positive
C36	Negative

A representative ELISpot is shown in Figure 3.2 which illustrates within the screened subjects' PBMC, IFN-γ release stimulated by the viral and self immunodominant peptides examined. Subjects C6 and C16 both demonstrated CMV peptide reactivity. C6 and C5 both showed EBV peptide reactivity. C6, C5 and C16 tested positive for reactivity towards FLU peptide. Although subject C6 appears to have produced a slight detectable response towards IP-30 peptide, the number IFN-γ spots detected was not twice the background therefore, under our set criteria it was not considered a positive peptide-specific response. Subject C28 was shown to express HLA-A3 and not HLA-A2 by serological methods. C28 was examined by ELISpot to confirm peptide restriction presentation by HLA-A2 and not by HLA-A3. This subjects' PBMC served as a true negative control (data not shown) for identifying background.

As summarized in Table 3.2, ELISpot testing revealed 10 (48%) subjects with CMV peptide reactivity, 15 (71%) subjects with FLU peptide reactivity, 14 (67%) subjects with EBV peptide reactivity, 2 (9.5%) subjects with IP-30 peptide reactivity. Three subjects (12.5%) demonstrated weak IFN-γ production (close to background) by PHA stimulation, indicating that those PBMC samples were not viable. The data from those subjects with weakly stimulated PBMC were not included in the analysis.

Figure 3.2: Representative ELISpot assay, identifying PBL stimulated by immunodominant peptides examined in duplicate wells from 4 different HIV uninfected subjects C6, C5, C16, and C28. A positive response is at least twice the negative control (PBL in complete medium).



Peptide	Control subject	IFN-γ Counts		IFN-γ/2x10^5 PBL	IFN-γ/10^6 PBL
		Well 1	Well 2		
CMV	C6	131	137	127	635
	C5	0	0	0	0
	C16	143	151	142	710
	C28	0	0	0	0
EBV	C6	11	13	5	25
	C5	35	16	26	130
	C16	4	3	2	10
	C28	0	0	0	0
Flu	C6	35	29	25	125
	C5	15	11	13	65
	C16	35	32	29	145
	C28	0	0	0	0
IP	C6	7	12	3	15
	C5	0	1	1	5
	C16	1	3	0	0
	C28	0	0	0	0
PHA	C6	TNTC	TNTC	TNTC	TNTC
	C5	TNTC	TNTC	TNTC	TNTC
	C16	TNTC	TNTC	TNTC	TNTC
	C28	TNTC	TNTC	TNTC	TNTC
PBL Background	C6	6	7	0	N/A
	C5	0	0	0	N/A
	C16	5	4	0	N/A
	C28	0	0	0	N/A

TNTC: To numerous to count
PBL: Peripheral blood lymphocytes

Table 3.2: Summary of the ELISpot results for detection of peptide-specific T cell IFN-γ production from PBMC of 24 HIV-uninfected subjects.

HIV uninfected subject	ELISpot, IFN-γ response
C1	FLU
C2	CMV, FLU
C3	CMV, FLU, EBV, IP
C4	CMV, EBV
C5	FLU, EBV
C6	CMV, FLU, EBV
C7	EBV
C8	EBV
C9	CMV, FLU, EBV, IP
C10	CMV
C11	FLU, EBV
C12	Weak PHA
C16	CMV, FLU
C18	Weak PHA
C19	EBV
C21	EBV, FLU
C22	EBV, FLU
C23	Weak PHA
C24	CMV, FLU
C26	FLU
C29	FLU, CMV
C32	FLU, EBV
C33	EBV, FLU
C35	CMV, EBV

3.1.4 TCR-peptide/MHC interaction avidity of immunodominant non-HIV peptides.

^{51}Cr release assays were used to determine the TCR-peptide/MHC interaction avidities as shown in representative Figure 3.3. Table 3.3 lists the non-HIV peptides examined. TCR interaction with the HLA-A2 CMV peptide complex is classified as high avidity. Both EBV and Flu peptides are classified as having intermediate binding avidity. The self peptide IP-30 HLA-A2 complex has the lowest TCR interaction avidity. The overall strength of the interaction determines the activation threshold for the responding cells activation. The anchor residues identified within the amino acid sequence of the peptide are critical for proper binding of the peptide to the HLA-A2 binding cleft. However, to clarify the assay, it is the overall strength (avidity) of the TCR-peptide/MHC interaction that is measured and not the affinity of the peptide binding to HLA-A2.

3.1.5 Summary of preliminary analysis

The HIV-uninfected control population, as previously shown in Figure 3.1, had a significantly higher baseline $CD8^+$ T cell CD5 expression than the HIV-infected group. Sufficient numbers of HLA-A2 expressing subjects were identified within the screened population to allow for an adequate sample distribution in both the HIV-uninfected control and HIV-infected cohort. As previously shown in Table 3.2, ELISpot responses confirmed the high probability of reactivity towards the common viral peptides being examined. This finding allowed selection of peptide-specific responders for further testing.

3.2 The relationship between CD8$^+$ T cell receptor avidity and CD5 expression for non-HIV viral peptides in HIV-uninfected subjects.

AIM 1: Analysis of CD5 expression on CD8$^+$ T cells from HIV-uninfected subjects reactive against HLA-A2 restricted non-HIV viral peptides.

To test whether the avidity of TCR-peptide/MHC interaction correlates to CD5 expression level on CD8$^+$ T lymphocytes, T cells were stimulated with non-HIV peptides spanning a 1000-fold range in TCR interaction avidity. HIV-uninfected subjects were screened for reactivity towards immunodominant HLA-A2-restricted peptides from common viruses including Cytomegalovirus, Influenza A, Epstein Barr and against a prominent self peptide from IFN-γ-inducible protein 30 (IP-30). The avidity of the TCR-peptide/MHC interaction was determined for each of the peptides examined as shown in Figure 3.3 and summarized in Table 3.3. Those subjects exhibiting reactivity were selected for further analysis of CD5 expression on peptide-specific T cells using proliferation assays.

Proliferation assays were performed to allow examination of CD8$^+$ T cells with varying CD5 expression proliferating in response to the relevant peptide. In this way, peptide specific CD8$^+$ T cells can be identified and further characterized for their CD5 expression by flow cytometry. Background proliferation was significantly reduced by using human AB serum instead of conventional FCS, thereby enhancing the sensitivity of this assay.

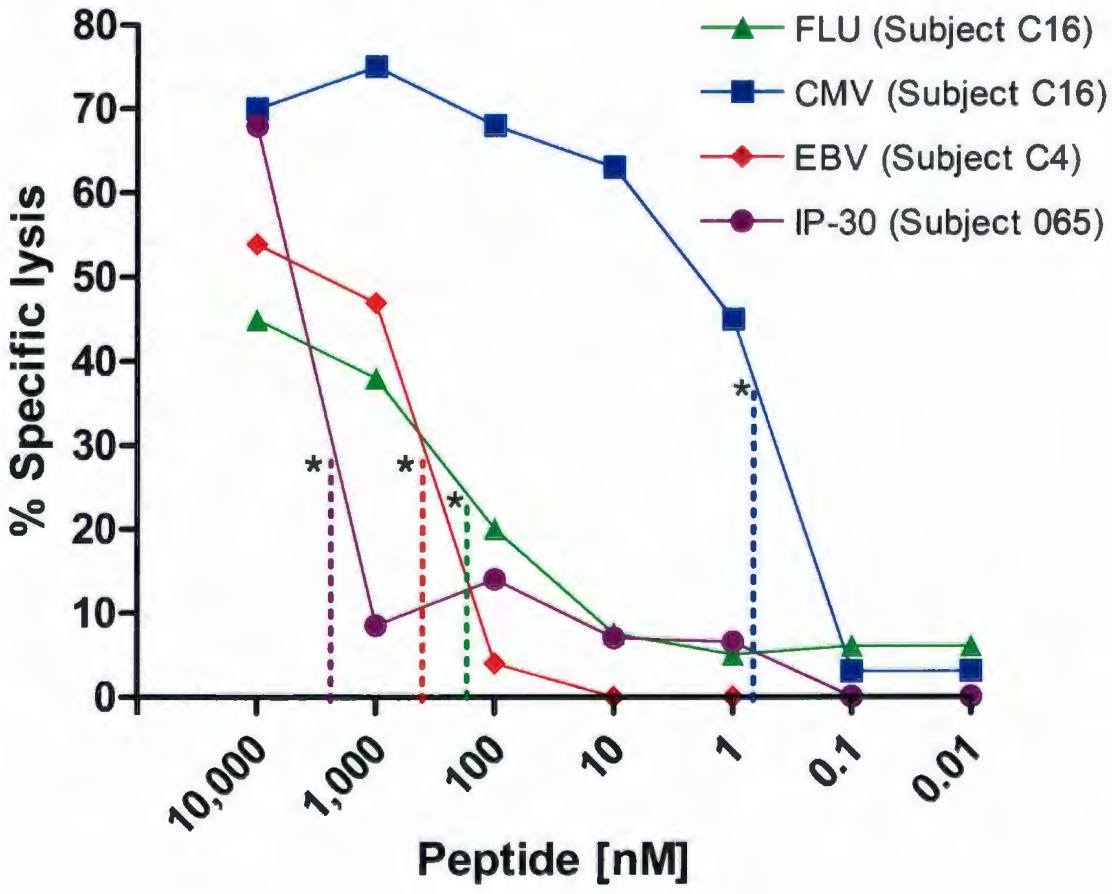


Figure 3.3: Specific lysis of HLA-matched, peptide-pulsed B-lymphoblastoid cells (BLCL). (*) Peptide concentration eliciting 50% of maximal specific lysis was used as a measure of TCR- peptide/MHC interaction avidity. Dashed lines to the X axis indicate estimated avidity.

Table 3.3: TCR-peptide/MHC interaction avidity summary for non-HIV peptides.

Peptide	Amino Acid Sequence	Location	Derived Avidity
A2-CMV	NL[1]VPMV[2]ATV	pp65 495-503	<1 ± 0.38 nM, n=16
A2-EBV	GLCTLVAML	BMLF1 280-288	500 ± 50 nM, n=8
A2-FLU	GILGFVFTL	Matrix 55-66	250 ± 59 nM, n=13
A2-IP-30	LLDVPTAAV	IP-30 -11 to -3	2000 nM* n=1

[1]**Anchors**
[2]Auxiliary anchors
*Verified from literature [46]
n = sample number

Fresh PBMC were stained with CFSE. Confirmation of CFSE staining was carried out using a fluorescent microscope as shown in Figure 3.4. This step was important to assure proper staining intensity before initiation of the 7 day incubation with specific peptides. Responding lymphocytes undergo multiple divisions in culture and can thus be identified by dilution of CFSE fluorescence intensity. Stimulated cells were co-stained for CD8 and CD5, then the $CD8^+$ population was gated for analysis as shown in Figure 3.5 (A). In Figure 3.5 (B) The upper quadrants of the analysis contain the $CD5^+CD8^+$ T cells. The lower quadrants depict the $CD5^-CD8^+$ T cells. The right quadrants represent the combined CFSE high stained parental population and 100% of the non-proliferating cells. All cells found within the left quadrants are presumed to be proliferating peptide-specific $CD8^+$ T cells. The results show that 96% of the parental cells are CD5 positive. The upper left quadrant of the analysis represents the CD5 positive proliferating $CD8^+$ T cells. The lower left quadrant depicts the CD5 negative proliferating $CD8^+$ T cells. Collectively these quadrants depict the CFSE-low daughter populations and 100% of the proliferating cells. In this example, the high avidity TCR-peptide/MHC interactions with HLA-A2 and peptide from CMV produced a $CD8^+$ proliferating T cell population ($CFSE^{low}$) that was 99% positive for CD5 expression.

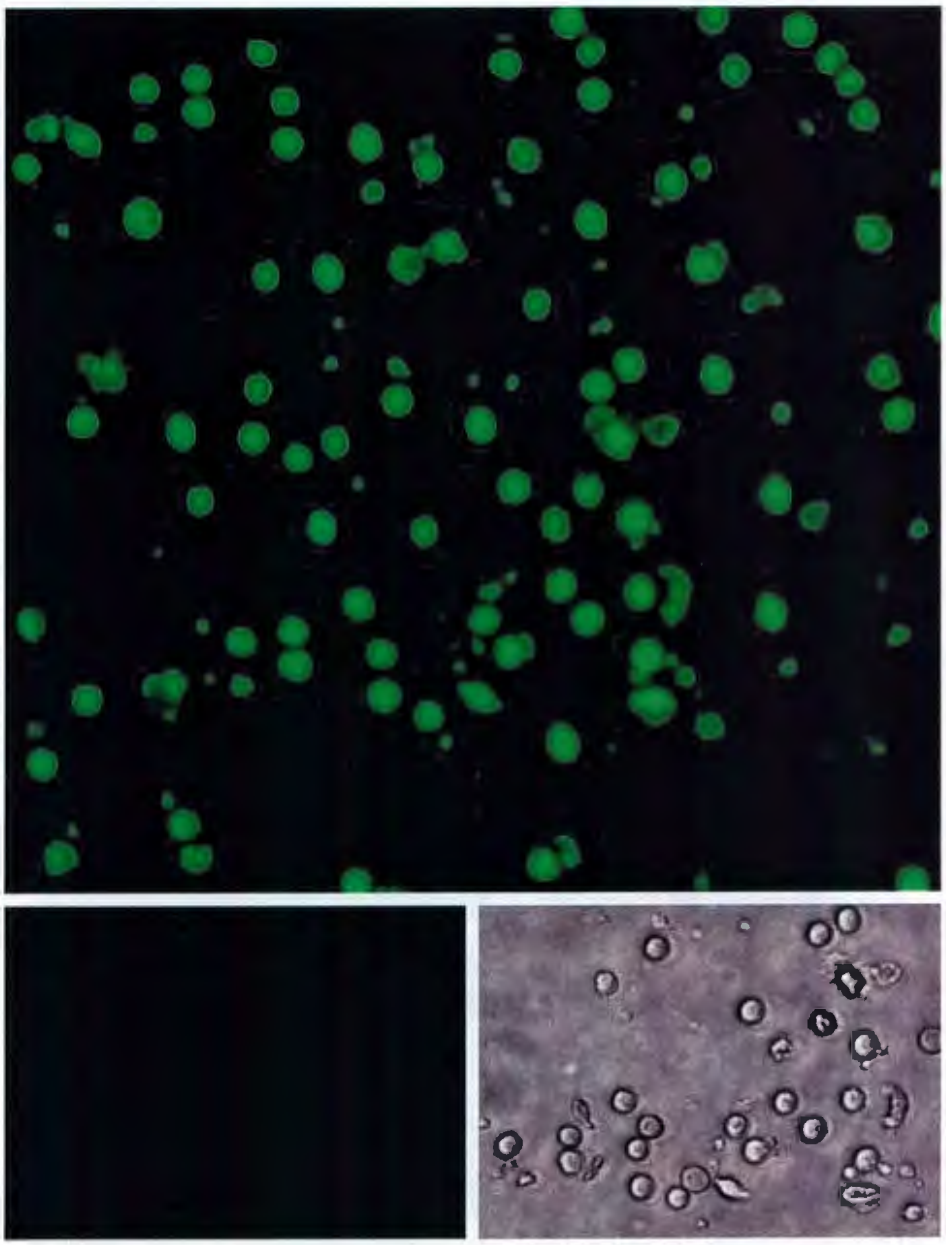


Figure 3.4: Fluorescence of CFSE-stained PBMC. Freshly isolated PBMC stained with 1 μM CFSE proliferation marker and viewed under a fluorescent microscope at 100X magnification (Top). Unstained control PBMC viewed under a fluorescent microscope showing no fluorescence (lower left). Unstained PBMC viewed under a light microscope (lower right).

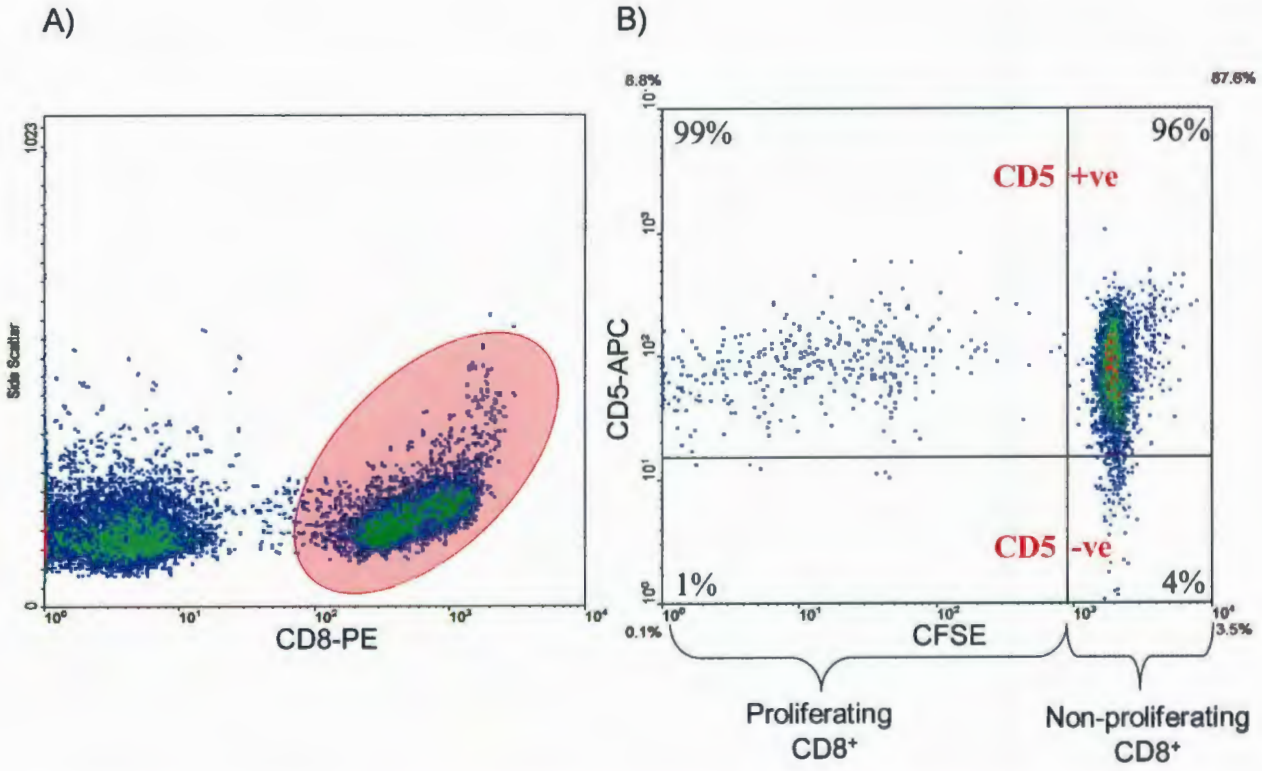


Figure 3.5: Three color flow cytometry analysis of CFSE-stained CMV peptide-specific $CD8^+$ T cells from subject C16. PE-labeled $CD8^+$ T cells are gated for analysis (A). $CD8^+$ T cells responding to A2-CMV peptide are tested for CD5 expression using allophycocyanin-labeled anti-CD5 (CD5-APC) (B). Decreased fluorescence intensity of CFSE shows peptide specific $CD8^+$ T cell proliferation with high CD5 expression in the upper left quadrant.

The results of this part of the study are summarized in Figure 3.6. The CMV peptide-stimulated $CD8^+$ T cells displayed a mean $CD5^+$ expression level of 97.5±1.1%, n=7. CD5 expression was consistently high on the CMV-specific CD8 T cells in all HIV-uninfected subjects tested. The EBV peptide demonstrated a lower TCR avidity compared to the CMV peptide (Table 3.3). This peptide stimulated $CD8^+$ T cell populations with a mean 95±1.5%, $CD5^+$ expression n=7. The FLU peptide examined had a lower TCR avidity than either CMV or EBV. FLU peptide-stimulated $CD8^+$ T cell populations averaged 93.9±1.5% with CD5 expression, n=10. There was no significant difference in percentage CD5 expression between T cells responding to any of the viral peptides. Proliferation was also examined by stimulation with the low TCR avidity self peptide IP-30. IP-30 has a 2000-fold lower TCR avidity compared to CMV peptide, 8-fold lower than FLU peptide and 4-fold lower than EBV peptide. IP-30 peptide stimulated $CD8^+$ T cell populations with an average CD5 expression of 86.5±3.5%, n=4. This is significantly lower than the $CD8^+$ T cell populations responding to CMV peptide ($p=0.009$), EBV peptide ($p=0.03$), or FLU peptide ($p=0.05$). These data suggest that high population levels of $CD8^+$ T cells expressing CD5 are associated with high avidity TCR interactions. Conversely, low avidity TCR interactions are associated with a lower percentage of responding T cells expressing CD5.

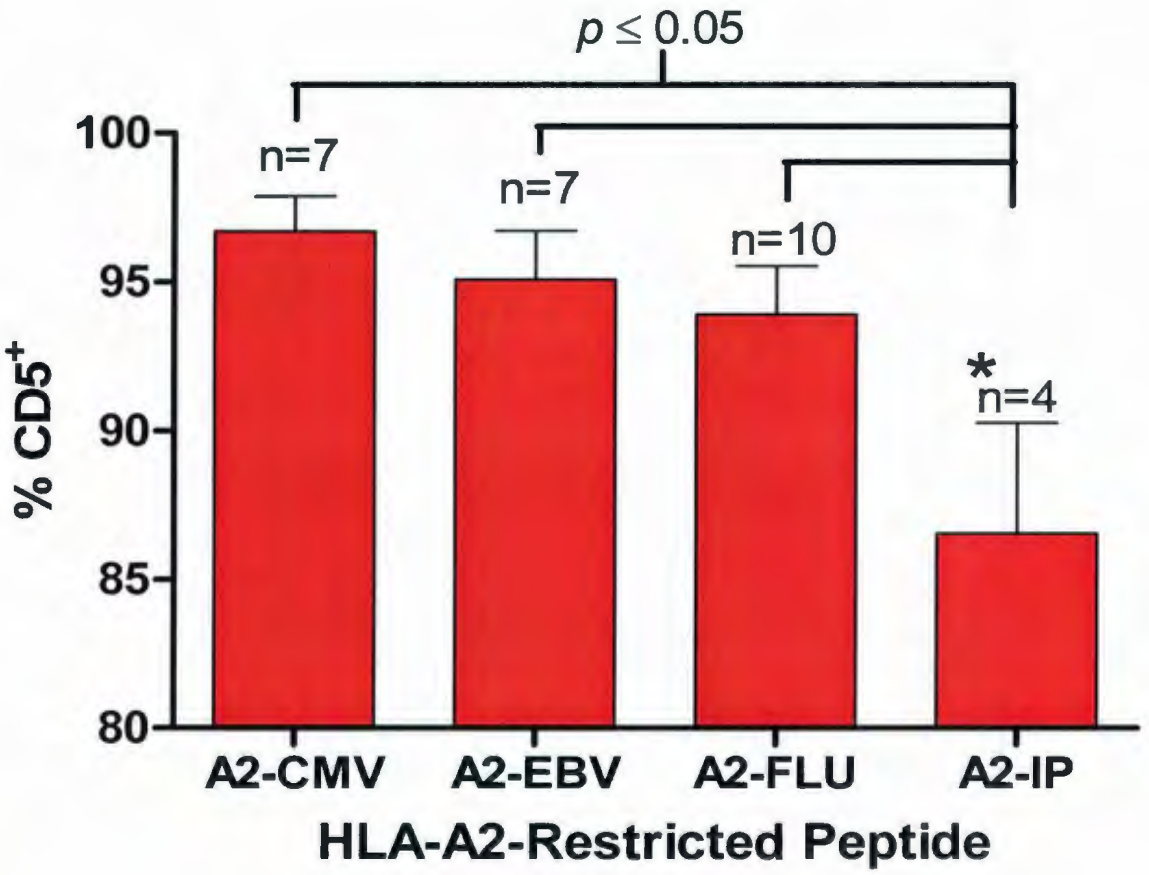


Figure 3.6: Mean percentage of peptide-specific $CD8^+$ T lymphocytes expressing CD5 in HIV-uninfected controls. Error bars indicate standard deviation from the mean. T cell populations responding to non-HIV-derived viral peptides have significantly higher $CD5^+$ expression compared to T cells responding to the low TCR-peptide/MHC interaction avidity self peptide IP-30 *($p \leq 0.05$).

3.3 The relationship between TCR-peptide/MHC interaction avidity and CD5 expression with non-HIV viral peptides in HIV infection.

AIM 2: Analysis of CD5 expression on $CD8^+$ T cells from HIV^+ individuals responding against HLA-A2 restricted non-HIV viral peptides.

In order to investigate CD5 expression on HIV-specific effector cells, we first evaluated whether HIV-infected subjects and HIV-uninfected individuals had similar CD5 expression levels on their $CD8^+$ T cells against non-HIV peptides. If so, any differences observed in the $CD8^+$ T cells responding to HIV peptides would be specific to HIV infection and suggest selective CD5 modulation on HIV-specific $CD8^+$ T cells. CD5 expression may be down regulated on expanding HIV-specific $CD8^+$ T cells or there could be expansion of HIV-specific $CD8^+$ T cells clones that have a pre-existing lack of CD5. Proliferation assays were conducted the same way for both HIV-infected and HIV-uninfected groups. HIV-infected individuals previously screened by ELISPOT for reactivity against non-HIV peptides were tested with the same set of A2-restricted immunodominant peptides as the non-HIV infected group. As illustrated in Figure 3.7 A, the CMV peptide elicited a proliferating population ($CFSE^{low}$) of 99.3% $CD8^+$ T cells expressing CD5. Results with FLU and EBV peptides were similar to CMV responses. Conversely, A2-IP stimulated proliferation of a population of $CD8^+$ T cells with 90% CD5 expression (Figure 3.7, B). This difference in the percent responding $CD8^+$ T cells expressing CD5 between high (A2-CMV, A2-EBV, and A2-FLU) and low (A2-IP) TCR-peptide/MHC interaction avidity peptides was

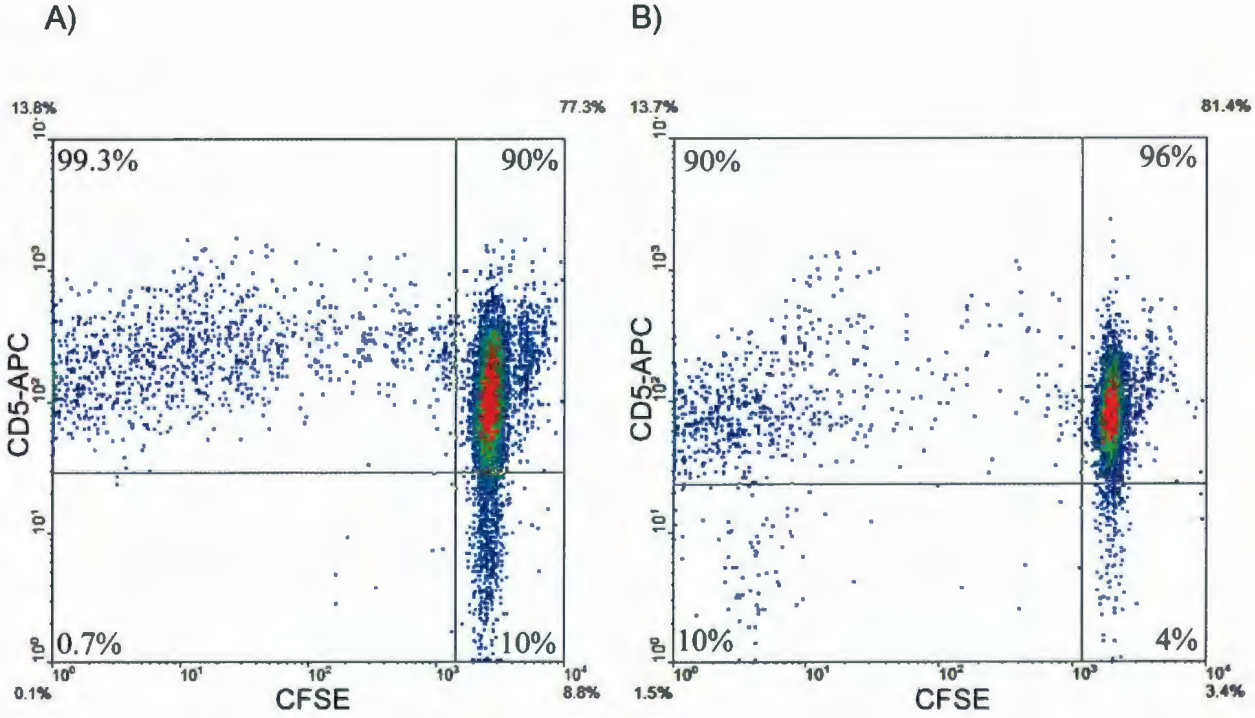


Figure 3.7: Multi color flow cytometry analysis depicting CD5 expression on (A) A2-CMV (subject 117) and (B) A2-IP reactive $CD8^+$ T cells (subject 65). Analysis of proliferating cells with decreased fluorescence intensity of CFSE show a high percentage of CMV-reactive $CD8^+$ T cells expressing CD5 *(left quadrant)* (A) and a comparatively lower percentage of A2-IP-reactive $CD8^+$ T cells expressing CD5 *(left quadrant)* (B).

seen consistently in all 36 subjects responding to peptide stimulation as summarized in Figure 3.8.

$CD8^+$ T cells responding in the context of high TCR-CMV peptide/MHC avidity interaction showed consistently elevated CD5 expression compared to other peptides examined. This observation was evident in all HIV-infected subjects tested. CMV peptide-stimulated $CD8^+$ T cell populations averaged CD5 expression on 96.5±1% of the responding CD8 T cells, n=25. TCR-EBV peptide/MHC interaction avidity is lower than the CMV interaction. EBV peptide stimulated $CD8^+$ T cell populations with 94.3±1.7% $CD5^+$ expression, n=16. The FLU peptide has a lower TCR-peptide/MHC avidity than CMV and EBV peptides, but stimulated proliferating populations with 94.9±1.7% $CD5^+$ expression, n=17. There was no significant difference in CD5 expression observed amongst the responding $CD8^+$ T cells specific for these 3 higher TCR-peptide/MHC avidity viral peptides. Proliferating $CD8^+$ T cells were also examined following stimulation with low TCR-peptide/MHC avidity self peptide IP-30. As seen in Table 3.3, IP-30 peptide has substantially lower TCR-peptide/MHC interaction avidity compared to CMV peptide (2000 fold lower), FLU peptide (8 fold lower) and EBV peptide (4 fold lower). IP-30 peptide stimulated $CD8^+$ T cell populations which averaged a $CD5^+$ expression of 85±3.4%, n=11. Compared to all other tested non-HIV peptides, IP-30 reactivates the lowest percent of $CD8^+$ T cells expressing CD5.

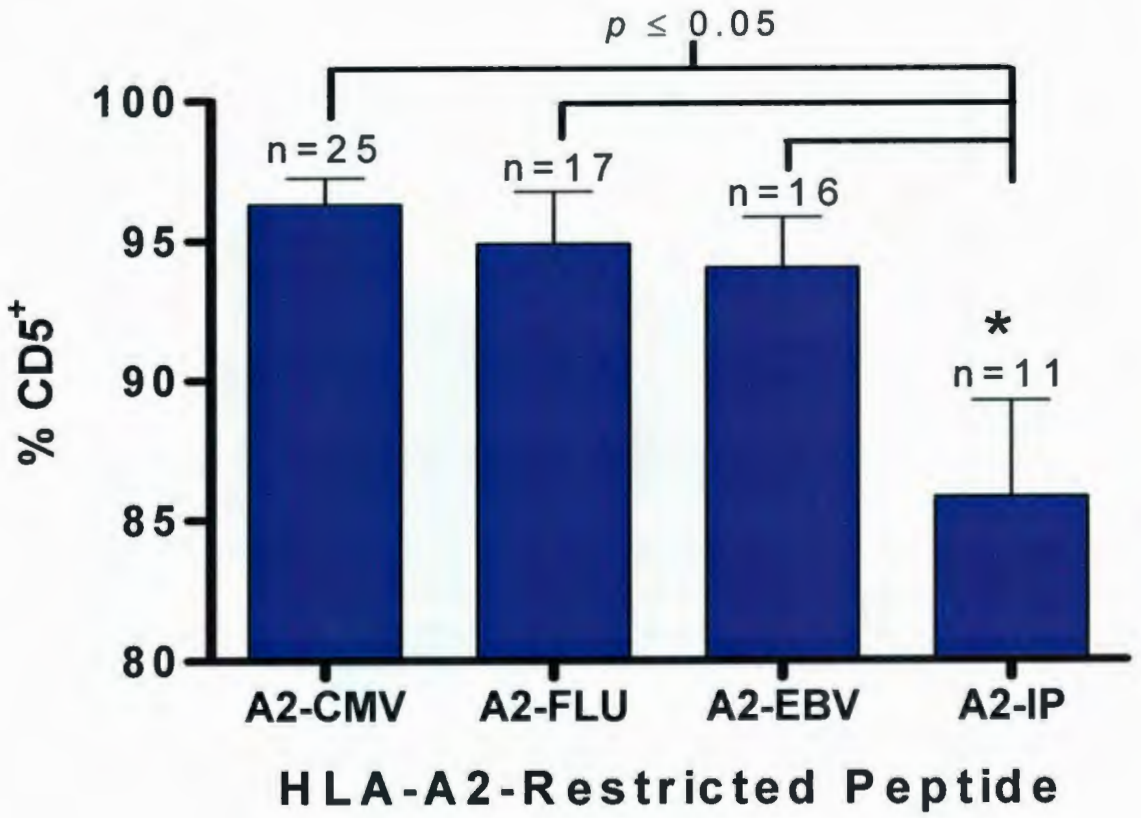


Figure 3.8: Mean percentage of proliferating $CD8^+$ T cells expressing CD5 in a total of 36 HIV^+ subjects responding to 1 or more non-HIV peptides. Error bars indicate standard deviation from the mean. $CD8^+$ T cells, specific for self peptide A2-IP have significantly lower CD5 expression *($p \leq 0.05$) compared to the $CD8^+$ T cells specific for non-HIV derived viral peptides.

The IP-30 stimulated T cells have a significantly lower CD5 expression than $CD8^+$ T cells responding to CMV peptide (p=0.0005), FLU peptide (p=0.001) and EBV peptide (p=0.03). There was no significant difference in CD5 expression on non-HIV viral peptide-specific $CD8^+$ T cells between the HIV-infected and uninfected group. Results in the HIV-infected group were almost identical to the control group. The low TCR-peptide/MHC avidity self peptide A2-IP activated $CD8^+$ T cells with a lower percent CD5 expression when compared to the non-HIV peptides.

3.4 The relationship between TCR-peptide/MHC interaction avidity with HIV viral peptides and CD5 expression.

AIM 3: CD5 expression on $CD8^+$ T cells from HIV-infected individuals reactive against a set of HLA-A2 restricted HIV peptides.

HIV-infected individuals were tested against a set of immunodominant HIV peptides shown in Table 3.4. This set of HIV peptides was previously determined to be optimal HIV-1 epitopes [56], i.e., the minimal amino acid sequence required for T cell stimulation has been established. As previously stated, HIV chronically infects the host and is never cleared by the immune system. During normal viral replication, the HIV genome is mutated, producing variants that consistently challenge the immune system to adapt to them. Aim 3 addresses my hypothesis that HIV mutation generates $CD8^+$ T cell epitope variants with lower avidity TCR interactions and CD5 is down-regulated on memory T cells in response. This

reduction in CD5 expression would enable memory $CD8^+$ T cells to reactivate more readily to peptide stimulation. Reduced CD5 expression removes antagonistic dephosphorylation allowing stronger TCR-peptide/MHC interaction signal transduction to take place. Reduced CD5 expression could occur to maintain the activation potential of HIV-specific memory $CD8^+$ T cells against variant peptides. The fine tuning of the $CD8^+$ T cell activation threshold through altering CD5 expression potentially adapts for the changing viral peptide sequences created by the HIVs mutating genome. Decreased CD5 expression is speculated to compensate for lower TCR avidity due to the peptide amino acid variations generated by HIV mutation. Before examining the level of CD5-expressing cells associated with HIV peptide restimulation, the TCR-peptide/MHC interaction avidity of each HIV peptide was determined.

The TCR-peptide/MHC interaction avidity was measured with several HIV peptides as shown in Figure 3.9. Table 3.4 summarizes the findings. A2-4 had a very high TCR-peptide/MHC avidity interaction (<0.1 nM), followed by peptides A2-1 and A2-2, which both had similar TCR-peptide/MHC avidities of ~10 nM. A2-Gag peptide demonstrated the lowest TCR-peptide/MHC interaction avidity amongst the HIV peptides examined of ~ 50 nM.

Expression of CD5 on the HIV-specific T cells was evaluated in the HIV subjects using the same methodology as in the HIV uninfected subject group. The same screening criteria were applied to the HIV peptide-stimulated $CD8^+$ T

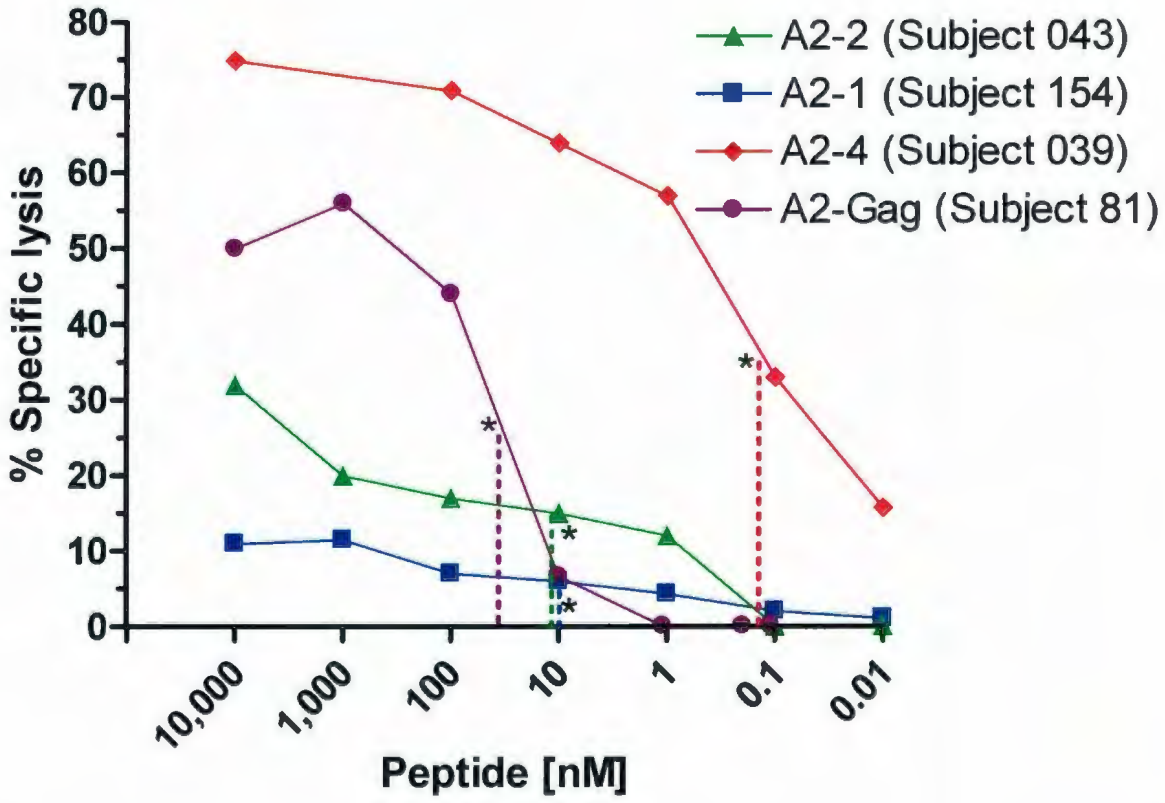


Figure 3.9: TCR-peptide/MHC interaction avidity of HIV peptides. The peptide concentration eliciting 50% of maximal specific lysis is taken to represent avidity (*). Dashed lines to the x axis indicate avidity for each peptide.

Table 3.4: Summary of TCR-peptide/MHC interaction avidities for HIV immunodominant peptides screened.

Peptide	Amino Acid Sequence	Location	Derived Avidity
A2-1	VI[1]YQYM[2]DDL	RT 179-187	10nM** n=1
A2-2	ALVEICTE**M**	RT 33-41	10 ± 2.5 nM, n=4
A2-4	L**V**GPTPVN**I**	PR 76-84	<0.1 ± 0.18 nM, n=8
A2-Gag	SLYNTVAT**L**	P17 77-85	50 ± 16 nM, n=6

[1]**Anchors**
[2]Auxiliary anchors

**Verified from literature [57]
n= Sample number

cell proliferation data. Figure 3.10 shows the staining pattern evident for CD5 expression and CFSE intensity after HIV-specific proliferation.

Evaluation of HIV-specific $CD8^+$ T cell proliferation demonstrates that the responding $CD8^+$ T cells exhibit low CD5 expression similar to $CD8^+$ T cells proliferating in response to self peptide (IP-30). Figure 3.11 summarizes the variability of CD5 expression on HIV-specific $CD8^+$ T cells within the 26 HIV infected subjects, as demonstrated in proliferation assays. Stimulation with HIV peptide A2-4 demonstrated that 89.4±3.6% (n=8) of the proliferating $CD8^+$ T cells expressed CD5. The A2-4 peptide was identified to be a high TCR-peptide/MHC avidity interacting peptide, but, the results do not parallel with the non-HIV peptides responding $CD8^+$ T cells, where we observed a pattern of high CD5 expression in association with high TCR-peptide/MHC avidity. The HIV peptides examined, including A2-1, 86.3 ±5.7% CD5 expression (n=9), may have either down regulated CD5 or selected $CD8^+$ T cells with lower CD5 expression. Similarly, A2-2-stimulated 84.2 ± 4.8% (n=8) $CD5^+CD8^+$ T cells. Both the A2-1 and A2-2 peptides demonstrated approximately the same TCR-peptide/MHC interaction avidity. For HIV peptide A2-Gag, 89.9± 2.3% (n=17) of responding $CD8^+$ T cells expressed CD5. This peptide was confirmed as having the lowest TCR-peptide/MHC avidity amongst the set of HIV peptides examined, as shown in Table 3.4.

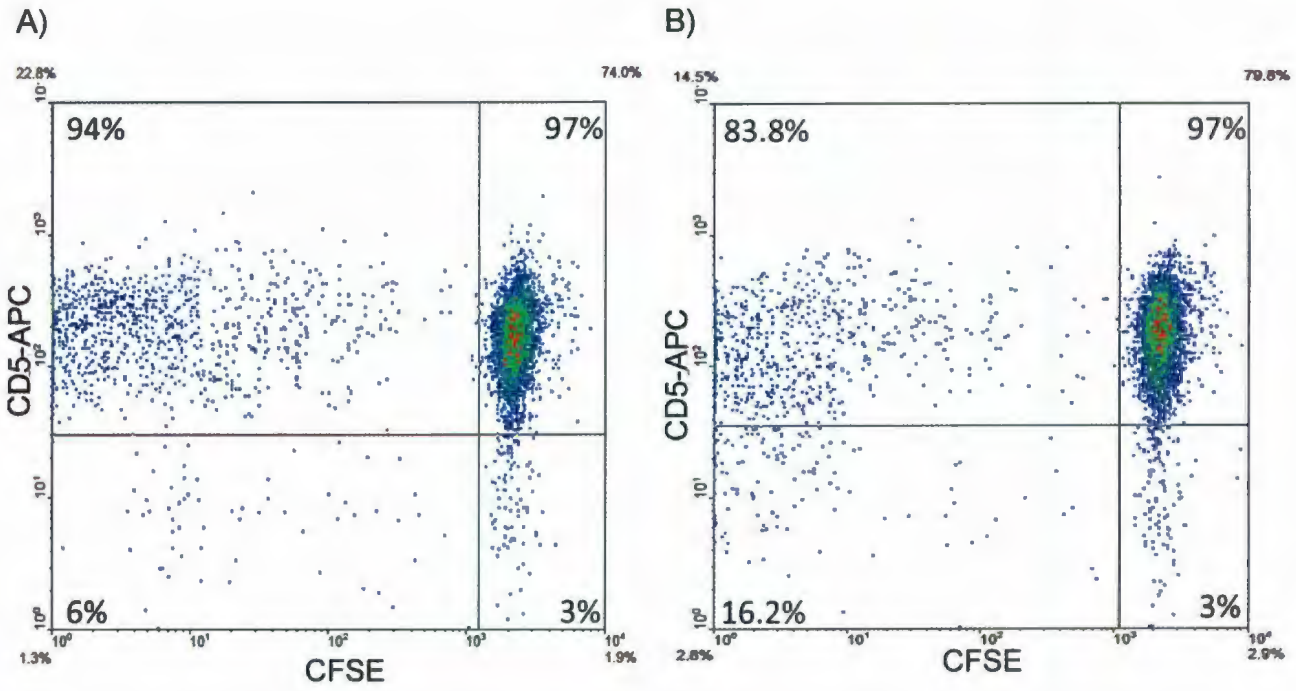


Figure 3.10: Multi color flow cytometry analysis depicting CD5 expression on HIV-specific $CD8^+$ T cells from subject 105. Anti-CD5 labeled with allophycocyanin allowed observation of CD5 expression on A2-4 reactive $CD8^+$ T cells (A) and A2-Gag reactive $CD8^+$ T cells (B).

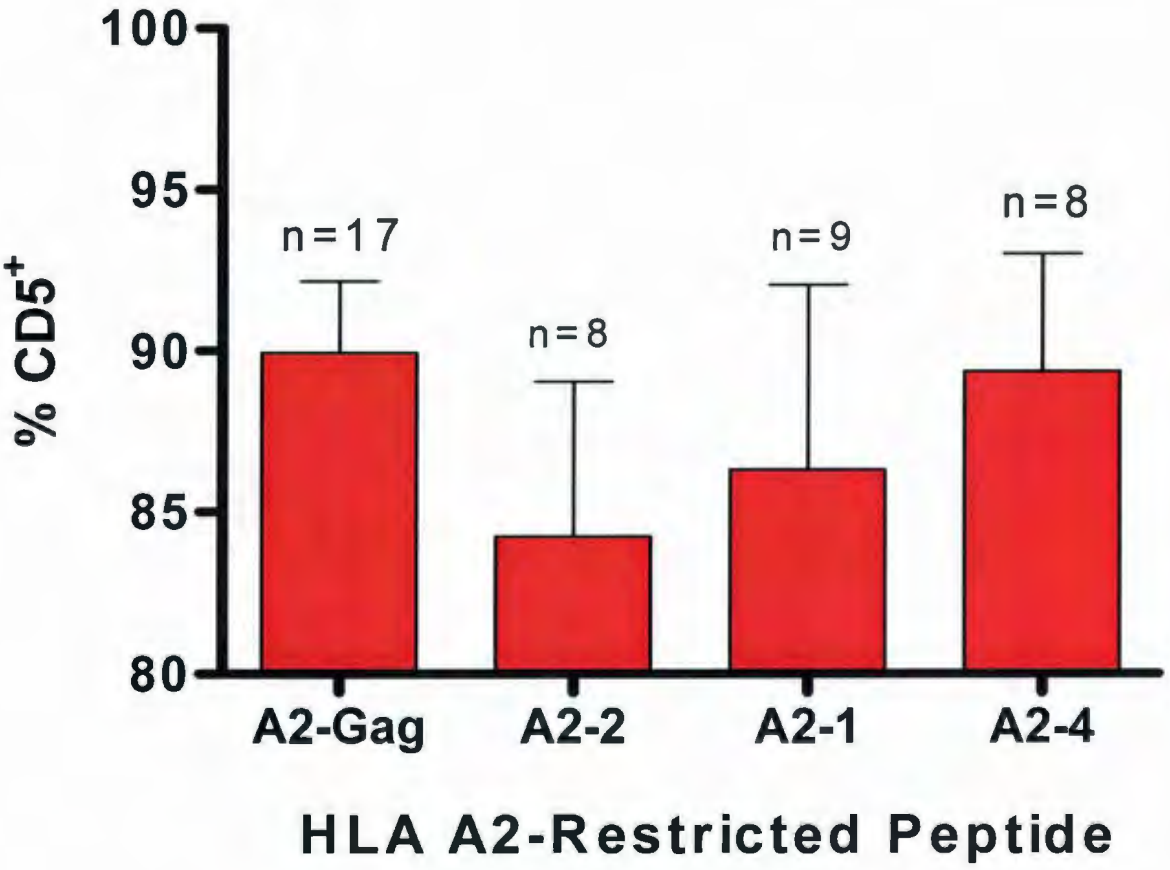


Figure 3.11: Summary of CD5 expression on HIV-specific $CD8^+$ T cells from 26 HIV-infected subjects. Error bars indicate standard deviation from the mean.

All the HIV peptides examined stimulated proliferation of $CD5^+$ $CD8^+$ T cells with a large standard deviation from the mean in terms of CD5 expression. This distinct variance in CD5 expression level observed in responding cells was not evident for the control peptides examined in either the HIV-infected or uninfected groups and therefore, appears unique to HIV peptide-specific stimulation. The reduced expression of CD5 on HIV-specific T cells relative to $CD8^+$ T cells specific for non-HIV viral peptides was apparent in all cases regardless of HIV TCR-peptide/MHC interaction avidity, (Figure 3.8 and Figure 3.11.)

3.5 General Summary of Results

$CD8^+$ T cells proliferating in response to different peptides were identified by dilution of CFSE fluorescence intensity and co-stained for CD8 and CD5. In controls and HIV-infected individuals, a higher proportion of $CD8^+$ T cells reacting against high TCR-peptide/MHC interaction avidity non-HIV peptides expressed CD5. In general, lower proportions of $CD8^+$ T cells reactive against HIV-derived peptides expressed CD5, regardless of the avidity of the TCR-peptide/MHC interaction. Self peptide IP-30-responding $CD8^+$ T cells also demonstrated reduced CD5 expression in both the HIV uninfected and infected groups as shown in Table 3.5. CD5 expression levels in the HIV group with CMV peptide stimulation was significantly higher ($p<0.05$) compared to stimulation by all HIV-derived peptides examined: A2-1 ($p=0.01$), A2-2 ($p=0.0005$), A2-4 ($p=0.01$) and A2-Gag ($p=0.005$). Both A2-Flu and A2-EBV viral peptides also induce

proliferation of $CD8^+$ T cells with significantly higher CD5 expression (p=0.016 and p=0.02) compared to HIV derived A2-2 peptide. It is clear from the collection of proliferation data as summarized in Table 3.5 that there is a significant distinction between the low levels of CD5 expression on HIV-specific $CD8^+$ T cell responses, and the higher CD5 expression levels on $CD8^+$ T cells responses against non-HIV viral peptides. HIV peptide reactivity associated with low CD5 expression may account for the $CD8^+$ T cell cross-reactivity previously observed [46].

Table 3.5: Comparative summary in HIV-infected individuals of proliferating $CD8^+$ T cells expressing CD5 in response to HIV peptides and non-HIV peptides.

	A2-1	A2-2	A2-4	A2-Gag
CMV	p=0.01	p=0.0005	p=0.01	p=0.005
EBV	NS	p=0.02	NS	NS
FLU	NS	p=0.016	NS	NS
IP-30	NS	NS	NS	NS

NS: No significant difference

4 Discussion

4.1 Evidence of reduced CD5 expression in HIV infection.

We studied HLA-A2 positive individuals because this allele occurs at high frequency (>50%) within both our HIV-uninfected and HIV infected study groups. Peptides were chosen based on previous demonstration of their immunodominance. Selected peptides exhibit optimum anchor residues for peptide binding to HLA-A2, preferably comprising leucine and valine at the 2^{nd} and 9^{th} positions of the peptide sequence respectively [58]. We first examined baseline CD5 expression levels on $CD8^+$ T cells in both HIV-infected and HIV-uninfected groups. In HIV-infected subjects, fewer circulating $CD8^+$ T cells expressed CD5 than in uninfected subjects PBMC. This is not the first report of reduced CD5 expression in HIV infection. The reported expression of CD5 in our HIV-infected cohort corresponds to the early stages (WR1-WR3) of the Walter Reed HIV classification [59], as reported in Indraccolo *et al.* 1995 [31]. Indraccolo examined CD5 profiles in HIV infected subjects at all time points of disease progression up to and including the late stages of HIV infection (WR4-WR6) according to the Walter Reed staging classification, as seen in Table 4.1. Our collected data of CD5 expression mirror Indraccolo's published reports of CD5 expression in early HIV infection [31]. Indraccolo indicated that with disease progression there is a significant increase in circulating $CD5^-CD8^+$ T cells, as much as a 65% increase when comparing CD5 expression from HIV-uninfected

Table 4.1: Walter Reed Staging Classification of HIV Infection [59].

	Positive Antibody or viral isolation	Chronic lymph-adenopathy	CD4/mm^3	DHS	Thrush	O.I.
WR0	-	-	>400	NL	-	-
WR1	+	-	>400	NL	-	-
WR2	+	+	>400	NL	-	-
WR3	+	±	<400	NL	-	-
WR4	+	±	<400	P	-	-
WR5	+	±	<400	C and/or +		-
WR6	+	±	<400	PC	±	+

DHS = Delayed hypersensitivity
NL = Normal
P = Partial cutaneous anergy (cutaneous response to only one of four test antigens)
C = Complete cutaneous anergy to four test antigens
O.I = Opportunistic infections

healthy controls with that of CD5 expression found in late stages of HIV infection. Unlike Indraccolo's study, we did not analyze CD5 expression according to the Walter Reed staging classification but, instead compared CD5 expression within the whole HIV-infected group to CD5 expression of the uninfected group. An important distinction would be that our HIV infected cohort are all receiving antiviral treatment to maintain low viral loads and thus, our cohort subjects are at early stages in the Walter Reid HIV staging classification.

4.2 IFN-γ produced by peptide-specific $CD8^+$ T cells

We identified individuals reactive with HLA-A2-restricted immunodominant peptides by ELISpot assays. IFN-γ production is indicative of peptide-specific memory $CD8^+$ T cells. The majority of control individuals tested generated an IFN-γ response to one or more of the non-HIV peptides. These individuals provided PBMC for peptide-specific proliferation assays using CFSE fluorescent staining. HIV-uninfected subjects PBMC that demonstrated peptide-specific IFN-γ production demonstrated $CD8^+$ T cell proliferation when their PBMC were stimulated with the same peptide *in vitro*. To ensure that our examination included a broad spectrum of avidities the self peptide A2-IP with known low TCR-peptide/MHC avidity was also screened for $CD8^+$ T cell reactivity by ELISpot and by CFSE proliferation assays.

4.3 The role of avidity in T cell activation.

Avidity is a critical factor related to the sensitivity of initiation of cellular immune responses. The strength of avidity forms the culmination of T cell activation, influencing the delivery rate of effector cell function. The molecular control of avidity is complex. No single mechanism accounts for the control of functional avidity. The elucidation of the control of avidity would offer a substantial benefit. If these mechanisms were clearly identified, a number of avenues for the optimization of high avidity T cell activation may become apparent. In our study, subject PBMC that generate cytotoxic effector $CD8^+$ T cells in vitro that exhibit high cytolytic activity were used to measure TCR-peptide/MHC avidity. We describe T cell avidity as the measure of the sensitivity of a cell to peptide antigen stimulation. It is the strength of the overall interaction that facilitates transduction of the activation signal within the cell, initiated from recognition by the TCR of peptide bound to MHC.

The avidities derived for all TCR-peptide/MHC interactions examined were reproducible with minimal variation from the mean, as shown in Table 3.3 for non-HIV peptides and Table 3.4 for HIV peptides. Avidities that were derived with low sample numbers, including A2-IP and A2-1, were verified from previous publications [46] and [57].

4.4 CD5 expression on peptide-specific T cells.

Proliferation assays were effective in identifying $CD8^+$ T cells dividing in response to peptide stimulation in both the HIV-uninfected and infected groups.

Background proliferation was significantly reduced by substituting conventional FCS with human AB serum, which increased the sensitivity of this assay for detecting peptide specific proliferation.

Examination of the $CD8^+$ T cell response to non-HIV peptides showed that the TCR-peptide/MHC interaction avidity hierarchy correlated with CD5 expression in non-HIV infected people. While there was no significant difference between CMV, EBV and FLU peptide specific responses, the peptide-specific $CD8^+$ T cells with high TCR avidity contain the highest percentage of CD5 expressing cells. Conversely, low TCR-peptide/MHC interaction avidity with peptides (A2-IP) stimulated a lower percentage of $CD8^+$ T cells expressing CD5. The same pattern was clearly observed for both HIV-infected and uninfected subjects. This finding agrees with the current understanding of the modulatory effects of CD5 and avidity first proposed by Azzam et al in 1998. They suggest that CD5 surface expression is proportional to the signaling capacity of the TCR. Significantly, CD5 surface expression on T cells was found to directly parallel the avidity or signaling intensity of the positively selecting TCR–MHC-ligand interaction [42].

The overall strength of the TCR signal transduction ultimately dictates the activation fate of $CD8^+$ T cells. High avidity CTL are required for fast clearance of common viral infections such as CMV, FLU and EBV [60]. Our investigation identified high TCR-peptide interaction avidity that facilitates functional activity where responding T cells express higher levels of CD5. We observed that as

non-HIV TCR-peptide/MHC interaction avidity drops, so do the responding $CD8^+$ T cells that express high levels of CD5. The self peptide A2-IP had the lowest TCR-peptide/MHC interaction avidity among all peptides examined and reactivated the highest percentage of $CD8^+$ T cells that did not express CD5. We speculate that IP-30 self peptide-specific $CD8^+$ T cells in the periphery that have low CD5 or are devoid of the CD5 receptor are rendered capable of reactivating in response to high amounts of self peptide which break the signaling threshold. If high levels of CD5 were expressed in these T cells, reactivation would most likely not occur.

Our results suggest that while the host is repeatedly encountering novel peptide epitope variants in HIV infection, changes occur at the T cell clonal expansion level. During clonal expansion, selection for the alteration of CD5 expression occurs on HIV-specific T cells compared to non HIV-specific T cells. We observed that the notable correlation of avidity and CD5 expression seen for non-HIV peptides was not evident amongst the HIV peptides examined. It appears that CD5 expression is generally reduced in all HIV-peptide-specific T cells responses. Two scenarios could explain the relationship between HIV infection and CD5 expression. At the level of the T cell population, there is either 1) Selection for and expansion of $CD8^+$ T cells not expressing CD5 or 2) Modulation of CD5 expression taking place within the HIV-specific $CD8^+$ T cell repertoire.

In common viral infections such as CMV and EBV, low TCR-peptide/MHC interaction avidity CTL are thought to require a high peptide/MHC determinant density [61]. It is for this reason that CTL are unable to recognize and lyse virally infected target cells until later in viral development when higher amounts of peptide-MHC complexes are displayed at the cell surface. However, in the case of HIV infection, we speculate that modulation of CD5 in HIV infection fine tunes the signal transduction sensitivity of the responding cells. This resensitization of the activation threshold is believed to enable HIV-specific $CD8^+$ $CD5^-$ T cells to activate with altered peptides, which demonstrate lower TCR-peptide/MHC interaction avidity.

The percentage of $CD8^+$ T cells with CD5 expression in the HIV group is comparable to $CD8^+$ T cells responding to self peptide from both the HIV infected and uninfected groups. Comparatively, the HIV infected group did have a large variability in CD5 expression on responding $CD8^+$ T cells. This may be attributed to different degrees of HIV progression between individuals examined. HIV peptides induce reactivity of a larger population of HIV-specific $CD8^+$ T cells not expressing CD5. These findings contrast with control non-HIV peptides examined in the same individuals that do agree with the avidity and CD5 expression hierarchy. Our results support evidence for the loss of CD5 expression as a T cell adaptation and highlight how it is specific to HIV infection.

Nearly all peripheral blood circulating T cells are inherently CD5 positive under normal conditions [62]. The reduced CD5 expression in our study is clearly noticeable and expanded in HIV infected individuals. The drop in CD5 expression according to our data is specifically attributed to HIV infection, considering that non-HIV viral peptides did not generate the same low CD5 expression trend as the HIV peptides. Cellular immune responses are pressured to maintain active immunity in the face of a mutating HIV genome generating variant epitopes. We speculate that the pressure upon the host's responding T cells towards HIV infection creates cellular immune responses that have reset their activation signaling threshold. More specifically, the HIV-specific $CD8^+$ T cells have reset their activation potentials by reducing their CD5 expression levels. $CD8^+$ T cells that have reduced CD5 levels or none at all have a reduced threshold for activation due to lesser antagonistic activity from tyrosine phosphatase mediated suppression of activation. This resensitization is most likely accomplished through CD5 down regulation or by selection against CD5 expression on T cells at the clonal level, which may play several important roles in the pathogenesis of HIV infection.

4.5 General Significance

It is not clearly known whether CD5 modulation is an intrinsic property of individual cells or if there exists a window during T cell maturation where naïve cells maturing to effector cells modulate CD5 in response to environmental signals, i.e., high peptide concentration, co-stimulatory molecules, or cytokines. If

a T cell can alter its sensitivity to peptide antigen in response to environmental stimuli, this would likely occur during a finite window of development. In support of modulation, it has been observed in previous studies that CD5 levels on peripheral lymphocytes are susceptible to either up- or down- regulation in response to different experimental and/or pathological conditions. For instance, CD5 up-regulation is also observed on peripheral T cells after TCR/CD3 cross-linking [63].

Alexander-Miller et al. (1996), reported that following stimulation with antigen presenting cells (APC) presenting a high (supraoptimal) level of peptide antigen, high avidity cells undergo apoptosis. Similarly, when low avidity lines are stimulated in the presence of low antigen, they undergo death by neglect [64]. A need for T cell activation modulation is a strict requirement for T cell survival and efficient functionality. Absence of CD5 expression is not exclusively restricted to HIV infection. Its disruption or modulation is also found in other clinical settings. Hemophagocytic lymphohistiocytosis (HLH) is a severe condition characterized by uncontrolled activation of T cells. EBV-associated HLH findings suggest that accumulation of $CD5^-$ $CD8^+$ T cells may serve as a useful marker of dysregulated activation and proliferation [65]. Increased subpopulations of $CD5^-$ $CD8^+$ T cells were also described in several clinical settings including allogeneic bone marrow transplantation [66], acute herpes virus infection [67], as well as peripheral T cell neoplasims [68]. It is possible that the expansion of the $CD5^-$ T cell population reflects a general immune dysregulation [66]. The transcription factor controlling

CD5 expression in $CD8^+$ T cells remains unknown [69-71]. Ling et al (2007) report that GATA3 is essential for appropriate TCR up-regulation and CD5 modulation, selectively in developing $CD4^+$ T lineage cells. GATA3 expression is specifically up-regulated during development of CD4 and not CD8 lineage cells [70], implying that the molecular mechanisms for modulation of CD5 expression levels during positive selection are different between the CD4 and the CD8 lineage [71]. Although GATA3 controls the expression of CD5 during $CD4^+$ T cell lineage development [71], GATA3 expression is not induced in the CD8 lineage [69]. It appears that different nuclear factors are responsible for the regulation of the expression levels of CD5 in the CD8 lineage [69].

CD5 is involved in the regulation of Fas/FasL pathway, which is known to play an important role in T cell activation induced cell death (AICD). CD5 protects T cells from TCR activation dependent apoptosis triggered following recognition of the specific target. It provides survival signals to $CD8^+$ T cells through regulation of FasL expression and caspase-8 activation, which supports a role for CD5 in the control of CD8 T cell homeostasis [72]. $CD8^+$ T cells not expressing CD5 may be more susceptible to apoptosis and perhaps do not last as long in the peripheral circulation. The T cells lacking CD5 expression that are specific for self peptides or HIV peptides as presented in this study may be more sensitive to apoptosis. This sensitivity to apoptosis may be a natural mechanism to control overt auto-reactivity of $CD8^+$ T cells with self antigens.

The absence of CD5 is believed to decrease the threshold of activation of HIV-specific $CD8^+$ T cells and may hold functional benefits in HIV infection. Within HIV infection there are escape mutants created that evade the immune system. We suggest that low or absent CD5 expression on $CD8^+$ T cells may facilitate cross-reactive T cell activation and cytolysis of variant peptide-expressing HIV-infected cells. Since CD5 modulates the signaling threshold of T cell activation, when CD5 is reduced on T cells the threshold for activation is also reduced. T cells acquiring activation signals from peptide bound MHC can also recognize variations of peptide amino acid sequences which trigger a signal cascade provided the anchor residues are not disrupted. The activating T cell would then have a broader range of sensitivity, demonstrating promiscuous cytotoxicity. The data on CD5 modulation in T cells, suggests such cross-reactivity is a likely occurrence.

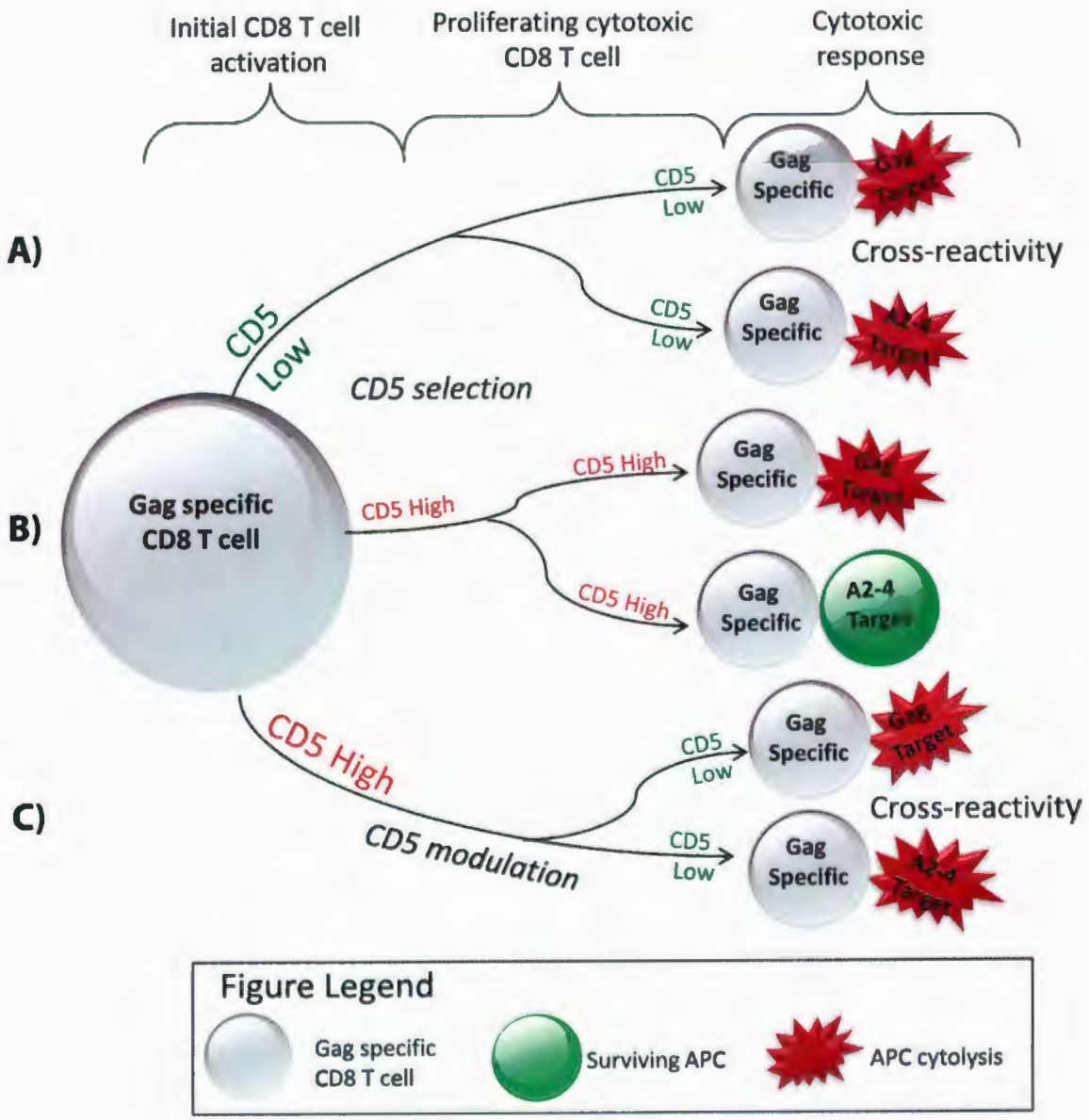
4.6 Potential link between CD5 expression and HIV-specific cross-reactive $CD8^+$ T cells.

We speculate that low CD5 expression on cytotoxic $CD8^+$ T cells allow the cell to interact its TCR productively with multiple peptide/MHC complexes. In the absence of CD5, the reduced avidity interactions occur with uninhibited TCR signaling. The uninhibited signaling transduction may affect cytotoxic $CD8^+$ T cell functions by allowing cytotoxic T cells to kill a broader selection of target cells. The reduced CD5 expression on these T cells is thought to fine tune the TCR

signal transduction and thus perpetuate a reduced threshold for activation of the cytotoxic T cell. As illustrated in Figure 4.1, the relationship between CD5 expression and avidity of HIV specific cytotoxic T lymphocytes may reflect an *in vivo* adaptation of $CD8^+$ T cells to HIV mutant peptides with lower TCR avidities than the original wild type epitope. Such occurrences would explain reported examples of HIV specific cross-reactivity with multiple peptides [46].

The strong signal generated downstream of a high avidity TCR is thought to induce a negative feedback in thymocytes and/or peripheral T cells, dampening their proliferation potential and limiting possible activation by self ligands. In contrast, the weak signal generated by low avidity TCR would still be compatible with normal survival/expansion in the periphery. Adaptation is said to be induced through CD5 modulation and where cells acquire improved functional capacities [73]. Based on the negative regulation exerted by CD5 on TCR signaling, it is expected that an increase in CD5 expression would decrease T cell reactivity and vice versa. Several observations have previously suggested that CD5 levels actually mirror the intensity of TCR signals [35, 54] .

Figure 4.1: Potential CD5 expression level on HIV-Gag-specific $CD8^+$ T cells with cytolytic activity. Gag-specific $CD8^+$ T cells are selected from the T cell repertoire expressing low CD5. These cells interact their TCR with A2-Gag presenting targets and demonstrate cross-reactivity with A2-4 presenting targets (A). Gag-specific $CD8^+$ T cells are selected from the T cell repertoire expressing high CD5. These cells interact their TCR with only index peptide (peptide initiating original activation) presenting targets. Gag-specific effectors are not cross-reactive and lyse only Gag presenting cells (B). Gag-specific $CD8^+$ T cells are selected from the T cell repertoire expressing high CD5. These cells undergo modulation of CD5 and reduce expression. These cells are then able to utilize their TCR with its cross-reactive potential and lyse multiple peptide presenting targets (C).


Initial CD8 T cell activation
Proliferating cytotoxic CD8 T cell
Cytotoxic response
A)
B)
C)
CD5 Low
CD5 selection
Gag specific CD8 T cell
CD5 High
CD5 modulation
Gag Specific
Gag Target
A2-4 Target
Cross-reactivity
Figure Legend
Gag specific CD8 T cell
Surviving APC
APC cytolysis

5 Future Directions

Future experiments should directly address the relationship between CD5 expression on HIV-specific cytotoxic T lymphocytes and their cross-reactivity with multiple peptides. Results from proliferation assays on peptide-specific $CD8^+$ T cells indicate that self peptide and HIV-specific $CD8^+$ T cells have reduced CD5 expression relative to non HIV viral peptide-specific responses. In order to support these findings, broadening the studies to include other HLA-class I molecules such as B44, B35 and A3 would be appropriate. These studies could generalize our evidence for a role of CD5 down regulation in HIV infection. How CD5 expression is regulated in lymphocytes and the outcome of modulation as it relates to cross-reactivity and tolerance requires further investigation.

While much has been elucidated about the structure and function of CD5 [33-40, 42-45, 62, 71-86] there is still an incomplete understanding of the function of CD5 and how it influences the immune response. Much more research is required to clarify the impact of reduced CD5 expression in HIV infection, and how modulation of CD5 manipulates the immune response. Experiments that directly address the relationship between CD5 expression on HIV-specific cytotoxic T lymphocytes and their cross-reactivity with multiple peptides should be conducted. Screening within HIV subjects and testing high and low TCR-peptide/MHC interaction avidities with non HIV and HIV peptides could identify cross-reactive interactions that support our current findings. In cross-reactive instances, examining CD5 level expression on responding $CD8^+$ T cell effectors

for high and low TCR-peptide/MHC interaction could clarify if low avidity interacting peptides generate low CD5 expressing cytotoxic effectors with cross-reactive activity for non index peptides. Data from our lab suggest that there is modulation of CD5 on responding HIV peptide-specific cytotoxic T cells. Addressing the following unanswered questions would help clarify the role of CD5 in the pathogenesis HIV infection.

Do $CD8^+$ T cells with low CD5 expression selectively proliferate in vivo in HIV infection?

Individuals with HIV infection have challenged immune systems that are actively generating cytotoxic T cells against dominant HIV epitopes. We propose there is a relationship between reduced CD5 expression levels and proliferation of HIV-specific $CD8^+$ T cells *in vivo*. Evaluation would involve examining co-stained freshly isolated PBMC from HIV-infected individuals with anti-CD8, anti-CD5 and anti-Ki67 and identifying peptide-specific $CD8^+$ T cells with peptide bound tetramer staining. Ki-67 antigen is the prototypic cell cycle related nuclear protein, expressed by proliferating cells in all phases of the active cell cycle (G1, S, G2 and M phase). It is absent in resting (G0) cells. Identification of peripheral circulating HIV-specific $CD8^+$ T cells by tetramer staining provides an opportunity to assess CD5 expression while using Ki-67 antigen identification *in vivo*. Control peptides can be examined in HIV uninfected individuals. Results may distinguish if CD5 modulation occurs *in vivo* or if peptide-specific $CD8^+$T cells with low CD5 are selectively proliferating.

Do peptides demonstrating high and low TCR-peptide/MHC interaction avidity within subjects expressing HLA-B44, B35, B57, A3 stimulate effector memory $CD8^+$ T cells with high and low CD5 expression?

This examination would broaden our pool of peptide specific responders and potentially confirm an association between low CD5 expression with likelihood of cross-reactivity. This finding will broaden our knowledge of CD5 modulation into other MHC restriction categories besides the currently screened HLA-A2 restriction. Currently in our lab, HIV peptides and self peptides restricted to various HLA groupings have shown peptide specific $CD8^+$ T cell activation from both HIV peptide and self peptide recognition. Unpublished data of CFSE stained PBMC demonstrate very high expansion of CD5 negative peptide specific $CD8^+$ T cells directed towards self peptide, which merit further examination. It is therefore feasible to expand this aspect of the study into other HLA types.

Do CD5 levels affect cross-reactivity spanning known cross-reactive HLA ie A2 and A3 peptides?

TCRs recognize antigenic peptides presented by HLA class I or class II molecules. The set of HLA molecules expressed in a particular person is highly individual and allows each person to only present a specific pattern of foreign peptides to the immune system. Yet, specific HLA class I alleles have a high degree of sequence homology and share similar peptide-binding motifs, resulting in the presentation of identical epitopes [87]. The impact of this promiscuous

antigen presentation within one HLA on the recognition by TCRs, however, is currently not well understood. We propose to examine CD5 levels in cross-reactive instances in known cross-reactive HLA. We predict that there is an increased likelihood that responding $CD8^+$ T cells exhibiting cross-reactive tendencies across HLA may exhibit reduced CD5 expression. In this study, we would analyze cross-recognition of HIV-1 epitopes between major alleles, HLA-A2 and HLA-A3 during natural HIV infection and determine to what degree the promiscuous presentation of a frequently targeted HIV-1 epitope by HLA-A2 and HLA-A3 is associated with promiscuous, cross-reactive recognition by TCRs.

Do CD5 expression levels on HIV-specific $CD8^+$ T cells support a role of CD5 in promoting T cell survival?

It is known that apoptosis is increased in HIV infection [4, 6, 88]. Recently high CD5 levels have been shown to protect T cells from TCR activation-dependent apoptosis [72]. We predict that upon TCR activation following recognition of the specific target, CD5 levels may also influence apoptosis in HIV infected individuals. Recent research reported that CD5 prevents activation induced cell death in T cells through regulation of Fas/FasL pathway as well $CD5^{high}$ T cells express lower FasL mRNA and surface protein than $CD5^{low}$ T cells after specific stimulation [44, 83]. An examination of CD5 in our study design may further provide evidence for a role of CD5 in promoting T cell survival through modulation of FasL expression, as a consequence of TCR-signaling inhibition.

6 Conclusions

Our investigation examined CD5 expression in relation to TCR-peptide/MHC interaction avidity for different viral specific cellular immune responses. It was clear that HIV peptide T cell restimulation generated a significantly higher proportion of $CD5^{-}CD8^{+}$ T cells in contrast to control peptides examined. Proliferating HIV-specific T cell CD5 levels are comparable to self peptide-specific CD5 expression levels. In our study this reduction in CD5 expression was unique to HIV peptide stimulation and may relate to the high occurrence of cross-reactivity in HIV infection. With the confirmation that CD5 expression is reduced in HIV specific immune responses, it would be useful to examine reduced CD5 expression more broadly in HIV as it relates to disease pathogenesis and immune response regulation.

We believe that the examination of the effects of CD5 expression in HIV infection may clarify its function and influence on the immune response. CD5 modulation has never been explored in HIV infection. Examining CD5 in a natural model of infection such as HIV could augment the understanding of HIV pathogenesis as it relates to the immune response, viral control and potential therapies. Inducing CD5 expression on lymphocytes may be protective in autoimmune diseases conversely, inhibiting CD5 expression on HIV-specific lymphocytes may help $CD8^{+}$ T cells target multiple peptide variants of HIV epitopes. Clarifying the specific functional role of CD5 could support therapeutic

vaccine design and contribute to a better understanding of factors determining the nature of the $CD8^+$ T cell immune response against HIV.

7 References

1. Pantaleo, G. and A.S. Fauci, *New concepts in the immunopathogenesis of HIV infection.* Annu Rev Immunol, 1995. **13**: p. 487-512.
2. Simon, V., D.D. Ho, and Q. Abdool Karim, *HIV/AIDS epidemiology, pathogenesis, prevention, and treatment.* Lancet, 2006. **368**(9534): p. 489-504.
3. Paranjape, R.S., *Immunopathogenesis of HIV infection.* Indian J Med Res, 2005. **121**(4): p. 240-55.
4. Gougeon, M.L., *To kill or be killed: how HIV exhausts the immune system.* Cell Death Differ, 2005. **12 Suppl 1**: p. 845-54.
5. Mohammed Ali, M.B., Monika Bloessner,, et al., *World Health Statistics 2009.* 2009: p. 149.
6. Tripathi, P. and S. Agrawal, *Immunobiology of human imunodeficiency virus infection.* Indian J Med Microbiol, 2007. **25**(4): p. 311-22.
7. Hayes, R. and H. Weiss, *Epidemiology. Understanding HIV epidemic trends in Africa.* Science, 2006. **311**(5761): p. 620-1.
8. Murphy, K., et al., *Janeway's immunobiology*. 7th ed. 2008, New York: Garland Science. xxi, 887 p.
9. Göttlinger, H.G., *HIV-1 Gag: a Molecular Machine Driving Viral Particle Assembly and Release.* 2001: p. 2-28
10. Richard Wyatt, P.D.K., Wayne A. Hendrickson, Joseph G. Sodroski, *Structure of the core of the HIV-1 gp120 Exterior Envelope Glycoprotein.* 1998: p. 9.
11. Emerman, M. and M.H. Malim, *HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology.* Science, 1998. **280**(5371): p. 1880-4.
12. Karn, J., *Tat, a novel regulator of HIV transcription and latency.* 2000: p. 2-18.
13. Qin Yu, N.R.L.a.R.K., *Vif and the Role of Antiviral Cytidine Deaminases in HIV-1 Replication.* 2003: p. 2-13.
14. Trono, V.P.a.D., *A Structure-function analysis of the Nef Protein of Primate Lentiviruses.* 1999.
15. Kahn, J.O. and B.D. Walker, *Acute human immunodeficiency virus type 1 infection.* N Engl J Med, 1998. **339**(1): p. 33-9.
16. Lifson, A.R., *Do alternate modes for transmission of human immunodeficiency virus exist? A review.* JAMA, 1988. **259**(9): p. 1353-6.
17. Li, M., et al., *Retroviral DNA integration: reaction pathway and critical intermediates.* EMBO J, 2006. **25**(6): p. 1295-304.
18. Douek, D.C., L.J. Picker, and R.A. Koup, *T cell dynamics in HIV-1 infection.* Annu Rev Immunol, 2003. **21**: p. 265-304.
19. Soogoor, M. and E.S. Daar, *Primary human immunodeficiency virus type 1 infection.* Curr HIV/AIDS Rep, 2005. **2**(2): p. 55-60.
20. Bieniasz, P.D., *Intrinsic immunity: a front-line defense against viral attack.* Nat Immunol, 2004. **5**(11): p. 1109-15.
21. Alcami, J., *[The HIV replication cycle. Established therapeutic targets and potential targets].* Enferm Infecc Microbiol Clin, 2008. **26 Suppl 12**: p. 3-10.

22. Althaus, C.L. and R.J. De Boer, *Dynamics of immune escape during HIV/SIV infection.* PLoS Comput Biol, 2008. **4**(7): p. e1000103.
23. Altman, J.D. and M.B. Feinberg, *HIV escape: there and back again.* Nat Med, 2004. **10**(3): p. 229-30.
24. Autran, B., et al., *Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease.* Science, 1997. **277**(5322): p. 112-6.
25. Paci, P., et al., *Immune control of HIV-1 infection after therapy interruption: immediate versus deferred antiretroviral therapy.* BMC Infect Dis, 2009. **9**(1): p. 172.
26. Robertson, D.L., B.H. Hahn, and P.M. Sharp, *Recombination in AIDS viruses.* J Mol Evol, 1995. **40**(3): p. 249-59.
27. Letvin, N.L., *Progress and obstacles in the development of an AIDS vaccine.* Nat Rev Immunol, 2006. **6**(12): p. 930-9.
28. Bennett, M.S., et al., *Epitope-dependent avidity thresholds for cytotoxic T-lymphocyte clearance of virus-infected cells.* J Virol, 2007. **81**(10): p. 4973-80.
29. Koup, R.A., et al., *Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome.* J Virol, 1994. **68**(7): p. 4650-5.
30. Turnbull, E.L., et al., *HIV-1 epitope-specific CD8+ T cell responses strongly associated with delayed disease progression cross-recognize epitope variants efficiently.* J Immunol, 2006. **176**(10): p. 6130-46.
31. Indraccolo, S., et al., *A CD3+CD8+ T cell population lacking CD5 antigen expression is expanded in peripheral blood of human immunodeficiency virus-infected patients.* Clin Immunol Immunopathol, 1995. **77**(3): p. 253-61.
32. Reinherz, E.L., et al., *A monoclonal antibody with selective reactivity with functionally mature human thymocytes and all peripheral human T cells.* J Immunol, 1979. **123**(3): p. 1312-7.
33. Osman, N., S.C. Ley, and M.J. Crumpton, *Evidence for an association between the T cell receptor/CD3 antigen complex and the CD5 antigen in human T lymphocytes.* Eur J Immunol, 1992. **22**(11): p. 2995-3000.
34. Perez-Villar, J.J., et al., *CD5 negatively regulates the T-cell antigen receptor signal transduction pathway: involvement of SH2-containing phosphotyrosine phosphatase SHP-1.* Mol Cell Biol, 1999. **19**(4): p. 2903-12.
35. Azzam, H.S., et al., *Fine tuning of TCR signaling by CD5.* J Immunol, 2001. **166**(9): p. 5464-72.
36. Brossard, C., et al., *CD5 inhibits signaling at the immunological synapse without impairing its formation.* J Immunol, 2003. **170**(9): p. 4623-9.
37. Rodamilans, B., et al., *Crystal structure of the third extracellular domain of CD5 reveals the fold of a group B scavenger cysteine-rich receptor domain.* J Biol Chem, 2007. **282**(17): p. 12669-77.
38. Bhandoola, A., et al., *CD5-mediated inhibition of TCR signaling during intrathymic selection and development does not require the CD5 extracellular domain.* Eur J Immunol, 2002. **32**(6): p. 1811-7.
39. Lozano, F., et al., *CD5 signal transduction: positive or negative modulation of antigen receptor signaling.* Crit Rev Immunol, 2000. **20**(4): p. 347-58.
40. Raman, C., *CD5, an important regulator of lymphocyte selection and immune tolerance.* Immunol Res, 2002. **26**(1-3): p. 255-63.

41. Neel, B.G., *Role of phosphatases in lymphocyte activation.* Curr Opin Immunol, 1997. **9**(3): p. 405-20.
42. Azzam, H.S., et al., *CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity.* J Exp Med, 1998. **188**(12): p. 2301-11.
43. Stamou, P., et al., *Chronic exposure to low levels of antigen in the periphery causes reversible functional impairment correlating with changes in CD5 levels in monoclonal CD8 T cells.* J Immunol, 2003. **171**(3): p. 1278-84.
44. Tarakhovsky, A., et al., *A role for CD5 in TCR-mediated signal transduction and thymocyte selection.* Science, 1995. **269**(5223): p. 535-7.
45. Dorothee, G., et al., *In situ sensory adaptation of tumor-infiltrating T lymphocytes to peptide-MHC levels elicits strong antitumor reactivity.* J Immunol, 2005. **174**(11): p. 6888-97.
46. Mason, R.D., et al., *Cross-reactive cytotoxic T lymphocytes against human immunodeficiency virus type 1 protease and gamma interferon-inducible protein 30.* J Virol, 2005. **79**(9): p. 5529-36.
47. Bierer, B.E., et al., *Phenotypic and functional characterization of human cytolytic T cells lacking expression of CD5.* J Clin Invest, 1988. **81**(5): p. 1390-7.
48. Williams, M.A. and M.J. Bevan, *Effector and memory CTL differentiation.* Annu Rev Immunol, 2007. **25**: p. 171-92.
49. Hammarlund, E., et al., *Duration of antiviral immunity after smallpox vaccination.* Nat Med, 2003. **9**(9): p. 1131-7.
50. Freeman, R.B., Jr., *The 'indirect' effects of cytomegalovirus infection.* Am J Transplant, 2009. **9**(11): p. 2453-8.
51. Fuse, S., M.J. Molloy, and E.J. Usherwood, *Immune responses against persistent viral infections: possible avenues for immunotherapeutic interventions.* Crit Rev Immunol, 2008. **28**(2): p. 159-83.
52. Kenney, S., et al., *The Epstein-Barr virus BMLF1 promoter contains an enhancer element that is responsive to the BZLF1 and BRLF1 transactivators.* J Virol, 1989. **63**(9): p. 3878-83.
53. Tamura, S. and T. Kurata, *Defense mechanisms against influenza virus infection in the respiratory tract mucosa.* Jpn J Infect Dis, 2004. **57**(6): p. 236-47.
54. Smith, K., et al., *Sensory adaptation in naive peripheral CD4 T cells.* J Exp Med, 2001. **194**(9): p. 1253-61.
55. Terasaki, P.I., et al., *Microdroplet testing for HLA-A, -B, -C, and -D antigens. The Phillip Levine Award Lecture.* Am J Clin Pathol, 1978. **69**(2): p. 103-20.
56. Nicole Frahm, B.B., Christian Brander, *HIV-Derived Cytotoxic T Lymphocyte (CTL) Epitopes for the Study of CTL Escape, Functional Avidity and Viral Evolution.* HIV Molecular Immunology, 2008: p. 24.
57. Okazaki, T., et al., *Epitope-enhanced conserved HIV-1 peptide protects HLA-A2-transgenic mice against virus expressing HIV-1 antigen.* J Immunol, 2003. **171**(5): p. 2548-55.
58. Rammensee, H.G., T. Friede, and S. Stevanoviic, *MHC ligands and peptide motifs: first listing.* Immunogenetics, 1995. **41**(4): p. 178-228.
59. Redfield, R.R., D.C. Wright, and E.C. Tramont, *The Walter Reed staging classification for HTLV-III/LAV infection.* N Engl J Med, 1986. **314**(2): p. 131-2.
60. Snyder, J.T., et al., *Molecular mechanisms and biological significance of CTL avidity.* Curr HIV Res, 2003. **1**(3): p. 287-94.

61. Derby, M., et al., *High-avidity CTL exploit two complementary mechanisms to provide better protection against viral infection than low-avidity CTL.* J Immunol, 2001. **166**(3): p. 1690-7.
62. Dalloul, A., *CD5: a safeguard against autoimmunity and a shield for cancer cells.* Autoimmun Rev, 2009. **8**(4): p. 349-53.
63. Arman, M., et al., *Transcriptional regulation of human CD5: important role of Ets transcription factors in CD5 expression in T cells.* J Immunol, 2004. **172**(12): p. 7519-29.
64. Alexander-Miller, M.A., et al., *Role of antigen, CD8, and cytotoxic T lymphocyte (CTL) avidity in high dose antigen induction of apoptosis of effector CTL.* J Exp Med, 1996. **184**(2): p. 485-92.
65. Wada, T., et al., *Immunophenotypic analysis of Epstein-Barr virus (EBV)-infected CD8(+) T cells in a patient with EBV-associated hemophagocytic lymphohistiocytosis.* Eur J Haematol, 2007. **79**(1): p. 72-5.
66. Bierer, B.E., S.J. Burakoff, and B.R. Smith, *A large proportion of T lymphocytes lack CD5 expression after bone marrow transplantation.* Blood, 1989. **73**(5): p. 1359-66.
67. Borthwick, N.J., et al., *Factors that influence activated CD8+ T-cell apoptosis in patients with acute herpesvirus infections: loss of costimulatory molecules CD28, CD5 and CD6 but relative maintenance of Bax and Bcl-X expression.* Immunology, 1996. **88**(4): p. 508-15.
68. Jamal, S., et al., *Immunophenotypic analysis of peripheral T-cell neoplasms. A multiparameter flow cytometric approach.* Am J Clin Pathol, 2001. **116**(4): p. 512-26.
69. Nawijn, M.C., et al., *Enforced expression of GATA-3 during T cell development inhibits maturation of CD8 single-positive cells and induces thymic lymphoma in transgenic mice.* J Immunol, 2001. **167**(2): p. 715-23.
70. Hernandez-Hoyos, G., et al., *GATA-3 expression is controlled by TCR signals and regulates CD4/CD8 differentiation.* Immunity, 2003. **19**(1): p. 83-94.
71. Ling, K.W., et al., *GATA3 controls the expression of CD5 and the T cell receptor during CD4 T cell lineage development.* Eur J Immunol, 2007. **37**(4): p. 1043-52.
72. Friedlein, G., et al., *Human CD5 protects circulating tumor antigen-specific CTL from tumor-mediated activation-induced cell death.* J Immunol, 2007. **178**(11): p. 6821-7.
73. Marquez, M.E., et al., *CD8 T cell sensory adaptation dependent on TCR avidity for self-antigens.* J Immunol, 2005. **175**(11): p. 7388-97.
74. Lankester, A.C., et al., *CD5 is associated with the human B cell antigen receptor complex.* Eur J Immunol, 1994. **24**(4): p. 812-6.
75. Dasu, T., et al., *CD5 plays an inhibitory role in the suppressive function of murine CD4(+) CD25(+) T(reg) cells.* Immunol Lett, 2008. **119**(1-2): p. 103-13.
76. Huang, Y.C., et al., *CD5-low expression lymphocytes in canine peripheral blood show characteristics of natural killer cells.* J Leukoc Biol, 2008.
77. Simarro, M., et al., *The cytoplasmic domain of CD5 mediates both TCR/CD3-dependent and -independent diacylglycerol production.* J Immunol, 1997. **159**(9): p. 4307-15.
78. Calvo, J., et al., *Human CD5 signaling and constitutive phosphorylation of C-terminal serine residues by casein kinase II.* J Immunol, 1998. **161**(11): p. 6022-9.
79. Calvo, J., et al., *Identification of a natural soluble form of human CD5.* Tissue Antigens, 1999. **54**(2): p. 128-37.
80. Biancone, L., et al., *Identification of a novel inducible cell-surface ligand of CD5 on activated lymphocytes.* J Exp Med, 1996. **184**(3): p. 811-9.

81. Hawiger, D., et al., *Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells in vivo.* Immunity, 2004. **20**(6): p. 695-705.
82. Kassiotis, G., R. Zamoyska, and B. Stockinger, *Involvement of avidity for major histocompatibility complex in homeostasis of naive and memory T cells.* J Exp Med, 2003. **197**(8): p. 1007-16.
83. Pena-Rossi, C., et al., *Negative regulation of CD4 lineage development and responses by CD5.* J Immunol, 1999. **163**(12): p. 6494-501.
84. Bikah, G., et al., *A role for CD5 in cognate interactions between T cells and B cells, and identification of a novel ligand for CD5.* Int Immunol, 1998. **10**(8): p. 1185-96.
85. Simarro, M., et al., *Signaling through CD5 involves acidic sphingomyelinase, protein kinase C-zeta, mitogen-activated protein kinase kinase, and c-Jun NH2-terminal kinase.* J Immunol, 1999. **162**(9): p. 5149-55.
86. McAlister, M.S., et al., *Structural analysis of the CD5 antigen--expression, disulphide bond analysis and physical characterisation of CD5 scavenger receptor superfamily domain 1.* Eur J Biochem, 1998. **257**(1): p. 131-41.
87. Lichterfeld, M., et al., *T cell receptor cross-recognition of an HIV-1 CD8+ T cell epitope presented by closely related alleles from the HLA-A3 superfamily.* Int Immunol, 2006. **18**(7): p. 1179-88.
88. Petrovas, C., Y.M. Mueller, and P.D. Katsikis, *Apoptosis of HIV-specific CD8+ T cells: an HIV evasion strategy.* Cell Death Differ, 2005. **12 Suppl 1**: p. 859-70.