# HIV-1 R5 TROPISM: DETERMINANTS, MACROPHAGES, AND DENDRITIC CELLS

A Dissertation Presented

By

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May 14, 2012

Immunology and Virology Program

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# Acknowledgements

This thesis is dedicated in loving memory to Josephine M. Horn,

February 7, 1922 – April 15, 2012.

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## Abstract

Around thirty years ago HIV-1 was identified, and from that point the known epidemic has grown to over 30 million infected individuals. Early on in the course of HIV-1 research, viruses were classified as either syncytia inducing, CXCR4-using, T-cell tropic or non-syncytia inducing, CCR5-using, macrophage tropic. Since that time, several groups have shown that this is an oversimplification. There is a great deal of diversity amongst CCR5-using HIV-1 variants. There remains a great deal to be discovered regarding HIV-1 CCR5-tropism and how this affects other aspects of HIV-1 infection.

The CD4 binding site (CD4bs) on the HIV-1 envelope plays a major role in determining the capacity of R5 viruses to infect primary macrophages. Thus, envelope determinants within or proximal to the CD4bs have been shown to control the use of low CD4 levels on macrophages for infection. These residues affect the affinity for CD4 either directly or indirectly by altering the exposure of CD4 contact residues. In this thesis, a single amino acid determinant is described in the V1 loop that also modulates macrophage tropism. I identified an E153G substitution that conferred high levels of macrophage infectivity for several heterologous R5 envelopes, while the reciprocal G153E substitution abrogated infection. Shifts in macrophage tropism were associated with dramatic shifts in sensitivity to the V3 loop monoclonal antibody (MAb), 447-52D and soluble CD4, as well as more modest changes in sensitivity to the CD4bs MAb,

b12. These observations are consistent with an altered conformation or exposure of the V3 loop that enables the envelope to use low CD4 levels for infection. The modest shifts in b12 sensitivity suggest that residue 153 impacts on the exposure of the CD4bs. However, the more intense shifts in sCD4 sensitivity suggest additional mechanisms that likely include an increased ability of the envelope to undergo conformational changes following binding to suboptimal levels of cell surface CD4. In summary, a conserved determinant in the V1 loop modulates the V3 loop to prime low CD4 use and macrophage infection.

In addition to determinants, this thesis seeks to evaluate the roles of macrophage tropic and non-macrophage tropic envelopes during the course of infection. Non-macrophage tropic virus predominates in immune tissue throughout infection, even in individuals suffering from HIV-associated dementia (HAD) who are known to carry many macrophage tropic viruses. There must be some advantage for these non-macrophage tropic viruses allowing them to persist in immune tissue throughout the disease. This thesis demonstrates that there is no advantage for these viruses to directly infect CD4+ T-cells, nor is there an advantage for them to be preferentially transmitted by dendritic cells to CD4+ T-cells. Given that transmitted/founder (T/F) viruses may preferentially interact with $\alpha4\beta7$, and T/F viruses are non-macrophage tropic, I tested whether non-mac viruses could utilize $\alpha4\beta7$ to their advantage. These experiments show

that macrophage tropism does not play a role in gp120 interactions with $\alpha 4\beta 7$. I evaluated whether there was a distinct disadvantage to macrophage tropic Envs, given their ability to infect dendritic cells and possibly stimulate the innate immune response. Using infected monocyte-derived dendritic cells (MDDCs), it was shown that mac-tropic Envs do not generate a significant immune response. These experiments demonstrate that there does not appear to be any advantage to non-macrophage tropic Envs, and that macrophage tropic Envs are able to infect CD4+ T-cells more efficiently, as well as DCs.

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# ABBREVIATIONS

| | |
|---|---|
| ADCC | Antibody-dependent cell-mediated cellular cytotoxicity |
| ADCVI | Antibody-dependent cell-mediated viral inhibition |
| AIDS | Acquired Immune Deficiency Syndrome |
| CA | HIV-1 capsid |
| CRF | circulating recombinant form |
| CTL | cytotoxic T lymphocyte |
| ELISA | enzyme linked immuno-sorbant assay |
| Env | HIV-1 envelope |
| ER | endoplasmic reticulum |
| ESCRT | endosomal sorting complex required for transport |
| FFU | Focus forming units |
| Gag | HIV-1 Pr55gag polyprotein |
| GFP | green fluorescent protein |
| HAART | highly active antiretroviral therapy |
| HAD | HIV-associated dementia |
| HIV | human immunodeficiency virus |
| IFN-$\alpha$ | interferon alpha |
| INT | integrase |
| Kb | kilobases |
| LPS | lipopolysaccharide |

| | |
|---|---|
| LTR | long terminal repeat |
| MA | HIV-1 matrix |
| MAb | monoclonal antibody |
| mDC | myeloid dendritic cell |
| MDDC | monocyte derived dendritic cell |
| MHC | major histocompatibility complex |
| MVB | multivesicular bodies |
| NC | HIV-1 nucleocapsid |
| NIH | National Institute of Health |
| Nef | negative factor |
| ORF | open reading frame |
| PBMC | peripheral blood mononuclear cells |
| PCR | polymerase chain reaction |
| PIC | pre-integration complex |
| PR | HIV-1 protease |
| R5 | CCR5 |
| RLU | relative luciferase units |
| RRE | Rev-responsive element |
| RT | reverse transcriptase |
| sCD4 | soluble CD4 |
| SIV | simian immunodeficiency virus |
| SU | surface unit |

| | |
|---|---|
| TAR | transactivation response |
| T/F | transmitted/founder |
| TM | transmembrane |
| Vif | viral infectivity factor |
| Vpr | viral protein r |
| Vpu | viral protein u |
| VSV-G | Vesicular Stomatitis Virus glycoprotein |
| X4 | CXCR4 |

# CHAPTER I: INTRODUCTION

## 1-1 Acquired Immunodeficiency Syndrome:

Beginning in the late 1970s and early 1980s, individuals began presenting to physicians with severe immunological dysfunction. These individuals generally had significantly depleted CD4+ T-cells, opportunistic infections, and uncommon cancers. The Center for Disease Control brought several California men with severe immunodeficiency to the attention of the medical community in the summer of 1981 [1]. Several other groups of people began presenting with similar immunodeficiency in addition to male homosexuals and intravenous drug users, including transfusion recipients, and sexual partners and children of individuals in the aforementioned groups. The disease was termed acquired immunodeficiency syndrome (AIDS), and eventually, in 1983, Barré-Sinoussi et al. were able to isolate what they termed lymphadenopathy-associated virus from patients with acquired immunodeficiency [2]. Soon after, Gallo and others at the National Institutes of Health isolated a retrovirus from a patient with AIDS and named it human T-cell leukemia virus type III (HTLV-III), and confirmed exposure to the retrovirus resulted in the observed immunodeficiency [3]. Eventually, the virus was named Human Immunodeficiency Virus (HIV), and classified as a lentivirus [4].

## 1-2 HIV Variability:

Soon after HIV-1 was discovered, it was shown to have extensive genetic variability throughout its genome, and particularly in the envelope glycoprotein, *env*, in comparison to other retroviruses. There are several factors contributing to the extensive variability seen in HIV-1. Viral DNA synthesis during reverse transcription is highly error prone ($3x10^{-4}$ mutations per nucleotide each replication cycle) [5]. The frequency of recombination of the HIV genome associated with reverse transcription is also high [6]. This variability is compounded further by a high rate of viral replication in the infected host ($10^9$ particles produced/day) [7]. As a result of this diversity, HIV-1 has extensive genetic phylogenetic heterogeneity. HIV-1 has been classified into four groups, main (M), outlier (O), non-main and non-outlier (N), and recently group (P) derived from gorillas [8]. Globally, the majority of HIV-1 infections can be attributed to group M viruses, which can further be divided into 9 distinct clades, A-D, F-H, J and K [9]. In geographic areas where multiple clades are present in the population, circulating recombinant forms (CRFs) can be found as a result of the aforementioned propensity for recombination (Fig. 1-1). The variability of the HIV-1 genome poses a significant challenge to the development of effective treatment and vaccines worldwide.

1-3 HIV-1 Replication:

The HIV-1 replication cycle is generally similar to the replication of other retroviruses. The process begins when the envelope glycoprotein on the virion

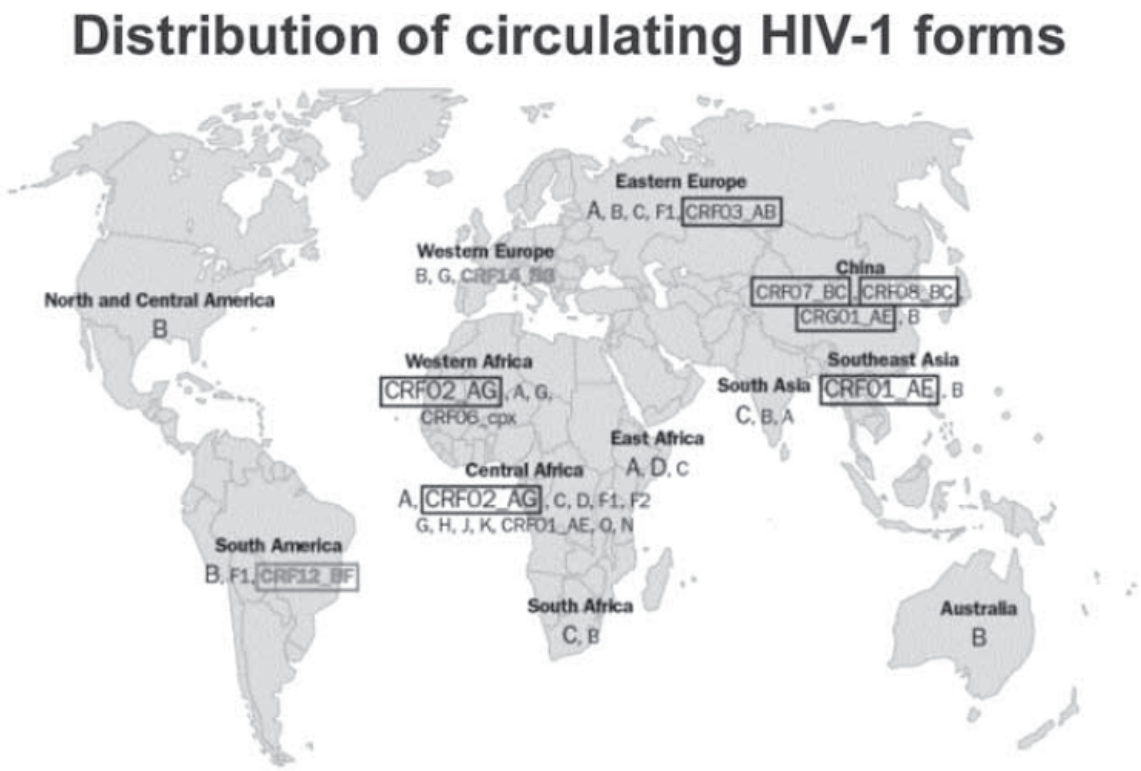



FIG. 1-1. Geographic distribution of HIV-1. Most of the global HIV-1 epidemic can be attributed to group M virus. The majority of infections present in North and South America, Australia, and Europe can be attributed to clade B viruses. Clade C is highly prevalent in Southern Africa and Asia. Every clade can be found in Africa. The propensity towards recombination has led to several circulating recombinant forms (CRF) of HIV-1. This worldwide diversity of HIV-1 contributes to the difficulty of combating the epidemic.

Figure reproduced from Stebbing J and Moyle G: The Clades of HIV: Their Origins and Clinical Significance. AIDS Rev. 2003 Oct-Dec;5(4):205-13, with permission from Permanyer Publications [9].

binds to CD4 on the surface of the target cell. This triggers a conformational change to occur in the gp120 region of Env, exposing the co-receptor binding site. Binding to a co-receptor, either CCR5 or CXCR4, both seven-transmembrane-spanning chemokine receptors, is required to facilitate further conformational changes to gp41, the membrane proximal external region of Env, in order to allow membrane fusion to progress [10]. HIV-1 may also enter target cells via endocytosis [11]. Fusion of the virus, whether at the plasma membrane or via an endosome, releases the viral core into the cytoplasm. The viral core is made up of the capsid, which encloses the viral genome, and several other HIV-1 proteins. This viral core associates with components of the cytoskeleton of the cell to move towards the nucleus [12, 13].

The uncoating of the capsid from the viral core reveals the reverse transcription complex (RTC) consisting of matrix (MA), nucleocapsid (NC), the viral genomic RNA, integrase (INT), reverse transcriptase (RT), and viral protein R (vpr) [14]. The viral genome is reverse transcribed in the RTC by RT into double stranded DNA, and this mature RTC is then referred to as the pre-integration complex, which is translocated into the nucleus [15]. Integrase then catalyzes the integration of the HIV-1 DNA into the chromosomal DNA of the infected cell. The integrated HIV DNA, now called the provirus, is then used as a template for RNA polymerase II directed viral RNA synthesis. Transcription begins at the 5'-end of the integrated provirus and at first produces fully spliced mRNAs that encode Tat, Rev, and Nef [16, 17]. Tat, NF-κB, and Sp1 act along

with RNA pol II to produce large amounts of viral transcripts. Rev then facilitates the export of unspliced and partially spliced transcripts into the cytoplasm [16].

Synthesis of Gag and Gag-Pol polyproteins are synthesized on cytoplasmic ribosomes. Gag is made from the unspliced viral RNA, as are the products of the Pol gene, which are fused to the gag polyprotein due to translational readthrough by way of a frameshift mechanism [18]. The MA domain of Gag serves to direct the Gag and Gag-Pol precursor proteins to the plasma membrane with the help of the host's trafficking machinery [19]. Gag polyproteins converge at the plasma membrane and are associated with dimers of genomic RNA, and begin to bud. Translation of the gp160 Env glycoprotein occurs in the rough endoplasmic reticulum. It is glycosylated and cleaved into mature envelope in the Golgi apparatus, and transported by vesicular trafficking to the plasma membrane [20]. A small fraction of the mature trimeric envelope glycoprotein spikes are incorporated into the budding virion. Scission of the budding virion is accomplished by a sequence in the p6 domain of Gag that interacts with the host cell membrane budding ESCRT machinery [21]. The protease of the Gag-Pol precursor then becomes active during or after viral particle release and proteolytically processes the gag and pol proteins, resulting in the cone-like structure of the viral core and maturing the virus into an infectious virus particle (Fig. 1-2) [22].

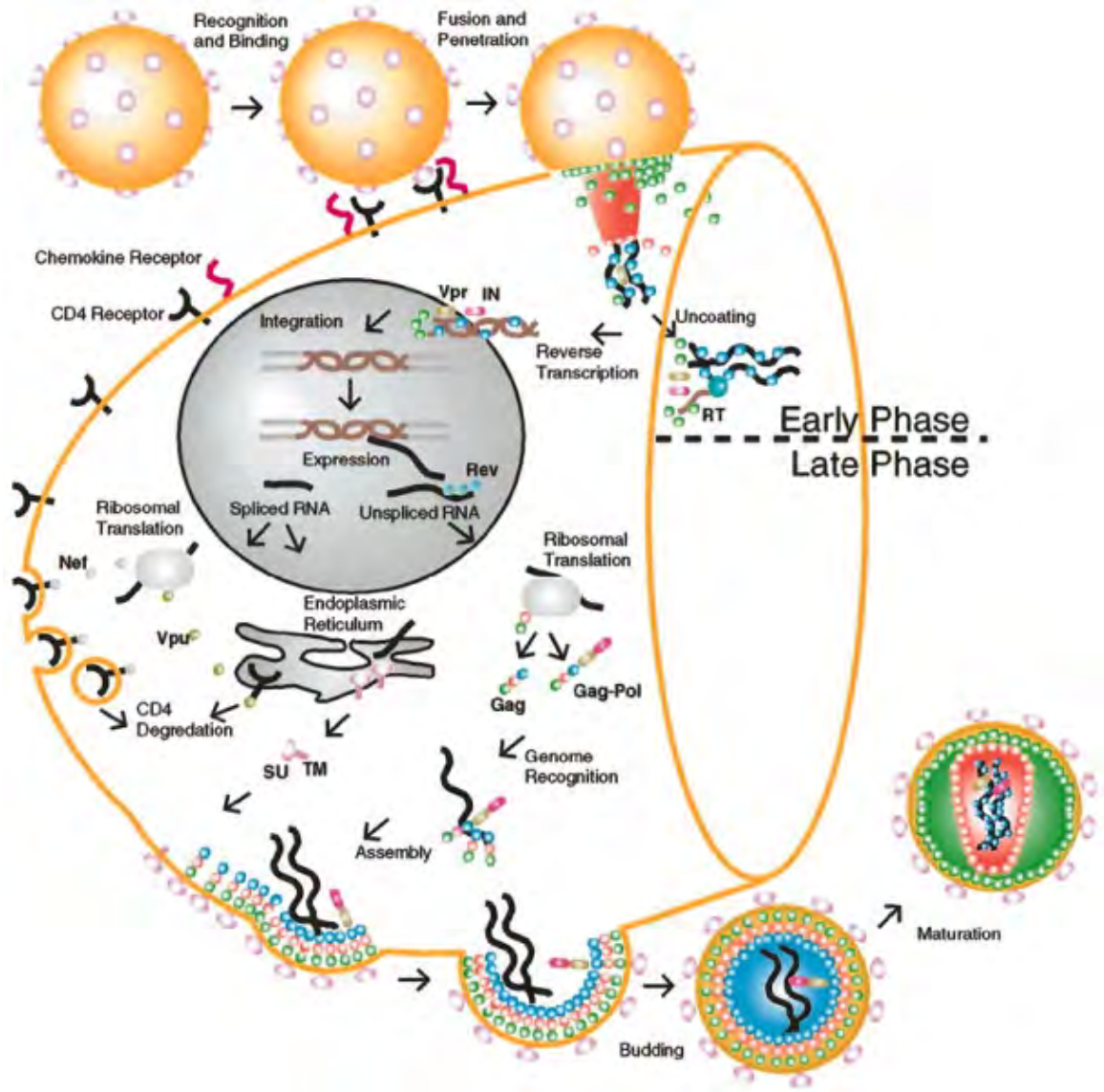



FIG. 1-2. HIV-1 replication cycle. The early phase of HIV-1 replication begins with the recognition and binding of CD4 by gp120. Following fusion, the viral capsid enters the cell and uncoats. Reverse transcription then occurs, followed by nuclear import of the reverse transcribed viral genome. The early phase of replication then culminates with integration. The late phase begins with transcription of the proviral DNA to produce mRNAs, which are then translocated from the nucleus to the cytoplasm. Translation of gag and pol occurs, and then the translation products move to the plasma membrane to participate in virion assembly. Env is translated in the rough ER, and further process in the golgi before it is shuttled to the the plasma membrane for assembly. The late phase ends with the budding of the virus and the maturation of the budded virion.

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1-4 HIV-1 Genes:

The HIV-1 genome is around 9.7 kB in size, and is present in the virion as a dimer of single stranded RNA. It encodes structural, enzymatic, regulatory, and accessory proteins (Fig. 1-3).

1-4-1 Enzymatic proteins:

The pol gene is downstream of Gag, and encodes three enzymes, protease (PR), reverse transcriptase (RT), and integrase (IN). It is part of the Gag-Pol protein initially translated as $Pr160^{gag\text{-}pol}$. Inefficient ribosomal frameshifting during the translation of $Pr55^{gag}$ results in lower levels of expression, about five percent compared to Gag [22].

Protease functions as a dimer to proteolytically cleave the polyprotein precursors of HIV-1 [24]. PR cleaves the gag and gag-pol polyproteins during the budding process, facilitating virus maturation. This cleavage and the resultant morphological change are essential for the virus particle to become infectious. Due to its importance for viral maturation, protease inhibitors were developed to inhibit the active site of the enzyme, and are a major class of drugs utilized clinically (saquinivir, darunivir, etc.) to treat HIV-1 patients, despite the emergence of escape mutations in PR to evade certain drugs. Used in conjunction with other drugs, they contribute greatly to overall control of an HIV-1 infection [25].

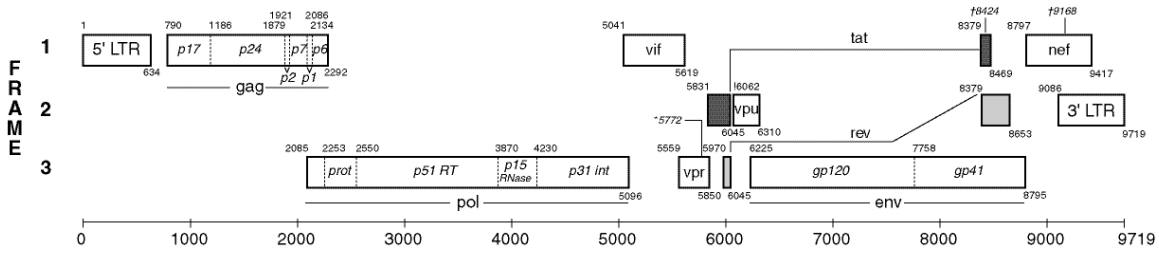



FIG. 1-3. HIV-1 genomic organization. The HIV-1 genome is made up of 9 genes that encode 15 proteins. The schematic above is the specifically the HXB2 genome. The genome is flanked by long terminal repeat regions present in the provirus. The structural genes are gag and env. The enzymatic proteins are encoded by pol. The regulatory proteins are tat and rev. The accessory proteins are vif, vpr, vpu, and nef. Detailed descriptions of these genes are found in the text.

Figure adapted from the HIV Los Alamos Database http://www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark.html

Reverse transcriptase is the enzyme that gives retroviruses their name, and the ability to convert ssRNA into dsDNA. RT performs three different functions, minus-strand DNA synthesis directly from RNA, RNaseH activity, and plus-strand DNA synthesis from DNA. The DNA polymerase activity is catalyzed by $tRNA^{Lys3}$. RT is a heterodimer of p51 and p66. p66 contains the RNaseH domain [26]. As was previously mentioned, reverse transcriptase has a high rate of error, roughly $3x10^{-4}$ per replication cycle, exacerbated by the low affinity between RT and its template, that contributes to template switching during reverse transcription. As a unique enzyme specific to retroviruses, RT makes a viable drug target. Nucleoside analogs have been developed as chain terminators, such as AZT, and non-nucleoside inhibitors are directed towards a pocket near the active site of the p66 protein, such as nevirapine [27]

Integrase is the enzyme responsible for inserting the proviral DNA into the host cell's DNA. It has three domains, an N-terminal zinc-finger domain, a core domain, and a C-terminal domain. Initially, IN creates 3'-recessed ends of the linear viral DNA as a pre-integration substrate. Strand transfer is then accomplished when IN joins the 3'-recessed ends to the 5'-overhangs it creates in the host DNA. DNA repair mechanisms then complete the integration process [28]. Similar to the other proteins derived from pol, IN has also become a target for antiviral drugs. Some drugs directed towards IN function to intercalate into DNA, while others inhibit strand transfer, such as raltegravir [29].

1-4-2 Regulatory Proteins:

Tat, the trans-activator protein, is essential for the processivity of the RNA Pol II transcription complex. It is a nuclear protein encoded by two exons. The activation domain of Tat interacts with cyclin T1, which is part of the P-TEFb complex (cyclin T1/CDK9). This binding induces a conformational change in Tat enabling efficient binding to the transactivation response region, TAR, of the HIV-1 RNA. The P-TEFb associated with Tat then hyperphosphorylates the RNA Pol II C-terminal domain, facilitating the processivity required for efficient transcription [30].

Rev, regulator of expression of virion proteins, allows the export of HIV-1 mRNAs that possess introns or that are incompletely spliced. Similar to Tat, it is encoded by two exons, and consists of two domains; an arginine rich domain that serves as both the RNA binding domain and the nuclear localization sequence, the leucine rich C-terminal domain serving as the nuclear export signal. Rev binds to the rev response element, RRE, which is present in all viral RNAs, with the exception of tat, rev and nef fully spliced mRNAs. Interaction with RanGTPase and CRM1 facilitate HIV-1 transcripts export through the nuclear pore [31].

1-4-3 Accessory Proteins:

The HIV-1 accessory proteins consist of Vif, virus infectivity factor, Vpu, viral protein u, Vpr, viral protein r, and Nef, negative factor. These proteins were originally considered to be accessory proteins because they were thought to not

be necessary for viral replication, although recent research challenges that contention for some of these proteins.

Vif is known to interact with the host factor APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G) and induce its degradation. APOBEC3G is a cytidine deaminase that is incorporated into the nascent virion in the absence of vif. It causes C-to-U (ctosine to uracil) mutations during DNA minus strand synthesis, and causes G-to-A (guanine to adenine) hypermutation during reverse transcription of the HIV genome, resulting in an abortive infection. Vif acts through an E3 ubiqutin ligase complex to target APOBEC3G for 26S proteasomal degradation, thereby preventing its incorporation into virions [32].

Vpu is a type-1 membrane associated protein that contributes to CD4 downregulation and antagonizes Tetherin (BST-2 or bone marrow stromal cell antigen 2). The expression of CD4 at the infected cell's surface is detrimental to viral replication and spread by promoting superinfection and hampering viral release. Vpu first retains CD4 in the ER, and subsequently causes the dislocation of CD4 back across the ER membrane where it is susceptible to proteasomal degradation [33]. Vpu also counteracts tetherin, which sequesters viral particles at the plasma membrane. It may do this by forming complexes with vpu upon encountering it in the trans-Golgi network, by binding tetherin at the plasma membrane, by inducing ubiquitination through β–TrCP-2, and ultimately through proteasomal or lysosomal degradation. The exact method of vpu's antagonism of tetherin is yet to be elucidated [33].

Vpr is thought to contribute significantly to the infection of CD4+ T-cells and macrophages and performs several functions. It is incorporated into viral particles through an interaction with the C-terminus of the p6 domain of gag [34]. Vpr then facilitates the binding of the pre-integration complex (PIC) to importins and nucleoporins, allowing its transport into the nucleus in non-dividing cells. Its interaction with the p300 transcription factor complex and the glucocorticoid receptor may increase HIV-1 production. Vpr causes the phosphorylation of Chk1, which results in $G_2$ cell cycle arrest [35]. These functions compound to contribute to better virus production.

Nef is another HIV-1 accessory protein with a number of functions that help the viral life cycle and enhance infectivity. It also plays a role in CD4 downregulation via a different mechanism. Nef is myristoylated and thereby located at the plasma membrane, where it promotes the detachment of Lck from the cytoplasmic tail of CD4, and binds in its place. AP-2 then promotes clathrin-dependent internalization [36]. Nef also downregulates MHC class I from the surface of infected cells by again binding to its cytoplasmic tail and AP-1 to prevent normal trafficking to the plasma membrane. In addition to downregulation, nef causes the activation of T-cells by phosphorylating Pak2 and possibly myeloid cells via Hck [37].

1-4-4 Structural Proteins:

The Gag and envelope proteins are both initially expressed as polyproteins in the infected cell. They are cleaved by different mechanisms

respectively. The gag precursor, $Pr55^{gag}$, is cleaved by PR to yield p17 matrix (MA), p24 capsid (CA), p7 nucleocapsid (NC), and p6 [38].

MA is the N-terminal portion of gag and is involved in targeting gag to the plasma membrane, early entry events, and the incorporation of the Env glycoprotein. The major purpose of MA is directing binding and assembly of the viral particle at the plasma membrane. This assembly may take place in different cellular compartments depending on the cell type, as is in the case of myeloid cells [39, 40]. The incorporation of full length Env into nascent virions is mediated by MA, specifically through interaction with gp41, the transmembrane portion of Env [38].

CA forms the outer layer of the viral core, and is immediately downstream of MA in uncleaved gag. It has two domains, an N-terminal core, and a C-terminal dimerization domain. CA is important for structural gag-gag and gag-pol interaction during virus formation and post-entry during the uncoating and initiating of reverse transcription. The N-terminal domain also binds cyclophillin A (CypA). TRIM5α is a known restriction factor against HIV-1 known to target CA and interfere with proper uncoating [41].

NC is important for incorporating full length genomic RNA into virions and for gag assembly. NC contains two zinc-finger domains, which along with the basic residues flanking these domains, interact with the ψ-site for encapsidation of the genomic RNA. In addition to these functions, NC plays a role in chaperoning nucleic acids, as well as influencing proper reverse transcription and

integration. NC has also been shown to potentially play a role in plus and minus strand DNA transfers [42].

p6 is a proline rich protein on the C-terminus of gag. It is important for virus release, which specifically maps to a highly conserved Pro-Thr-Ala-Pro (PTAP) motif near the N-terminus of p6 [43]. This protein plays a role in interacting with the budding machinery of the cell to facilitate virion release via the ESCRT-I component Tsg101. Other late domain motifs also on p6 may interact with Alix, a protein important for endosomal sorting [44]. It also binds vpr and allows for its incorporation into virions.

Env is responsible for initiating infection by binding to CD4 and a co-receptor to facilitate fusion. It is synthesized as a precursor, gp160, which is eventually cleaved into gp41, the transmembrane region, and gp120, the globular surface protein. As stated previously, gp160 is translated in the rough ER, where it is concurrently glycosylated [45]. Once in the Golgi, gp160 is cleaved by furin-like proteases at at a highly conserved K/R-X-K/R-R motif into mature gp120 and gp41 proteins [46]. The Env proteins then form heterotrimers that are trafficked to the plasma membrane, where they will be incorporated into virions or endocytosed.

About half of the total mass of gp120 is made up of glycans, which function to shield Env from the host immune machinery, aid virion binding the surface of target cells, and contribute to the overall structure of Env [47]. gp120 has five variable domains and five constant regions. There are a number of

conserved cysteines that impart tertiary structure and delineate the different variable domains. Variable domain 1, (V1), and V2 are variable loops within the context of a larger loop via disulfide bonds, and are the most variable regions of the envelope [48].

Gp120 is made up of a highly glycosylated outer domain composed of a double β-barrel, and an inner domain consisting of a 3-helix, 4-strand bundle along with a 7-stranded β-sandwich. There is also a 4-strand bridging sheet that is composed of two strands from the inner domain (β2-β3) and two strands from the outer domain (β20-β21) [49]. The CD4 binding site is formed by the folding of different conserved regions of gp120 into a conformation receptive to CD4, specifically in C1, C3, and C4 [50]. CD4 binds in a depression between the inner and outer domains and the bridging sheet. It is the binding of CD4 that catalyzes the conformational change that forms the bridging sheet. This conformational change entails a change in orientation of the α1 helix from the inner domain and the significant movement of the β2-β3 strands from the inner domain around 40 Å to a closely adjacent postion to β20-β21 (Fig. 1-4, A) [51]. CD4 binding also induces changes in V1/V2 and V3, exposing epitopes not accessible in the unliganded form. Co-receptor binding via CCR5 or CXCR4 is facilitated by the CD4-bound gp120. The V3 loop is known to be of particular importance in contributing to membrane fusion and determining co-receptor specificity [52-54]. In addition to the V3 loop, the bridging sheet is also known to play a role in co-receptor usage [55].

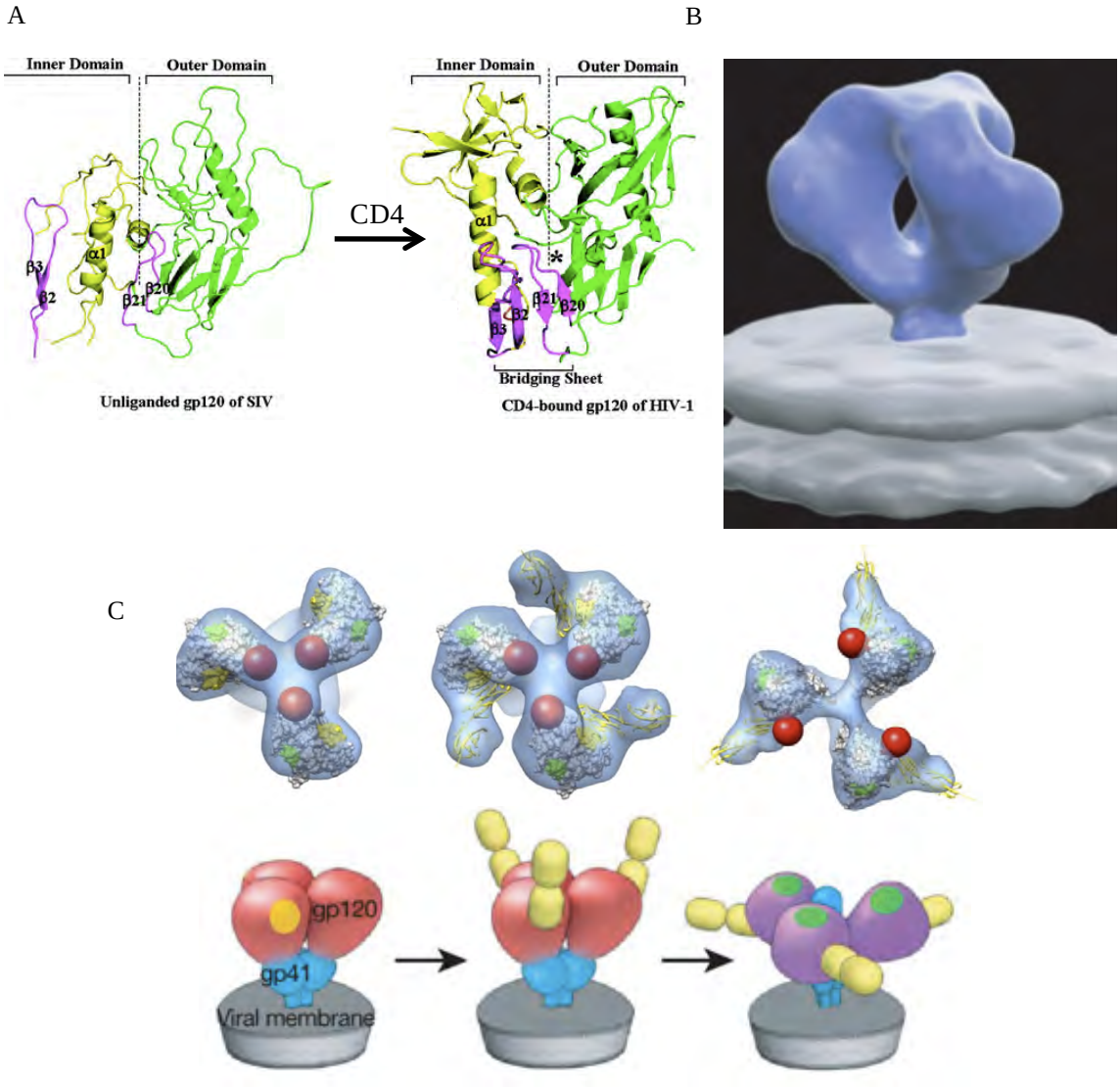



FIG. 1-4. HIV-1 Envelope Structure. (A) The structure of the unliganded gp120 of SIV has been resolved, and here it shows the inner domain in yellow and the outer domain in green. The portions of the bridging sheet are in purple. The drastic conformational change between the unbound SIV and the CD4-bound structure of gp120 of the bridging sheet is caused by the binding of CD4. (B) An electron tomographic structure of the native unbound HIV-1 envelope trimer. (C) The conformational change of the overall trimeric glycoprotein spike upon binding CD4 is shown. The trimer opens, moving V1/V2 loops away from the center of the trimer. On top are electron density maps top view fitted with gp120 structures, with V1/V2 in red. In the model on the bottom the yellow circle represents the CD4 binding site in the unbound trimer, and the green circle is the V3 loop after conformational rearrangement subsequent to CD4 binding. CD4 is in yellow, gp120 is in red/purple, and gp41 is in blue. Figure adapted with permission from Da LT et al. J Phys Chem B. 2009 Oct 29;113(43):14536-43 [51]. Copyright year 2009 American Chemical Society; and Copyright license # 2881940918046,[56] and White TA et al. J Virol. 2011 Dec;85(23):12114-23 [57]

gp41 is responsible for fusing the viral membrane with the host cell's plasma membrane. It is comprised of an extracellular domain, a transmembrane domain, and a cytoplasmic tail. The extracellular domain is composed of the fusion peptide, which is the N-terminal hydrophobic region, a polar region, HR1 and HR2, which are coiled-coil heptad repeat regions, and the membrane proximal external region (MPER), which is Trp-rich [58]. When CD4 binds gp120, it causes a conformational change in gp41 that exends the fusion peptide, which then inserts into the target cell membrane. The HR motifs from the trimer fold over one another in an antiparallel manner to form the six-helix bundle responsible for bringing the membranes close enough to initiate fusion [59]. The cytoplasmic tail of gp41 is involved in Env incorporation into virions, gp120 shedding, and the expression of Env at the cell surface. A Y-X-X-L motif in the CT directs clathrin-mediated endocytosis of Env via adaptor protein complex 2 (AP-2) [60]. gp41 plays a significant role in overall Env structure and function.

Crystal structures of HIV-1 monomeric gp120 have been resolved. However they represent the conformation in the CD4 bound form and not the unliganded structure present in the native trimer. There is currently no crystal structure of the native trimer, although a high resolution electron tomographic structure of the unliganded, uncleaved trimer was presented this year (J. Sodroski, CROI 2012). There are also several lower resolution electron tomographic structures of the trimer, including an unliganded form and forms

bound to sCD4 (Fig. 1-4, B & C) [56]. The unbound trimer shows distinct gp120 subunits with the variable loops V1/V2 and V3 near the apex. Upon binding of CD4, the gp120 subunits move further apart, and the V1/V2 loops swing laterally, with the V1/V2 loops and the CD4 binding site moving away from the center of the trimer (Fig. 1-4 C). There also appears to be gp41 conformational changes. In the unbound state, taking the many glycans into consideration, the CD4 binding site seems to be the only portion of the trimer significantly accessible [61].

1-5 HIV-1 Pathogenesis:

HIV-1 is known to infect CD4+ cells resulting in their death and the virus' propagation, but this is an oversimplification of what occurs in the host throughout the course of the infection. The course of HIV-1 infection can be divided into three distinct stages of infection, acute, chronic, and late-stage or AIDS. The time period shortly after the host is infected and prior to the adaptive immune response is referred to as the acute phase of infection. CD4+ effector T-cells in the gut and associated lymphatic tissue are severely depleted [62]. A significant portion of these cells express CCR5, the co-receptor predominantly used by transmitted virus. This accompanies flu-like symptoms and a large spike in plasma HIV-1 RNA titers in plasma (up to $10^7$ copies/mL). This phase of high viral replication quickly declines in several weeks to a steady state level, termed

the viral set point [63]. During the acute phase, HIV-1's ability to evade the innate immune response allows it to burgeon in the host and establish the infection.

Following the resolution of acute infection, which coincides with the detection of HIV-1 specific cytotoxic T lymphocytes (CTLs) [64, 65], a chronic phase begins where most infected individuals are relatively asymptomatic. It is during this phase that the number of CD4+ T-cells detected in blood gradually declines, although throughout this latent phase of infection, individuals are generally able to mount an immune response to other infections. The virus is most likely infecting many CD4+ T-cells, and large amounts of these cells are likely turning over, and this many eventually leads to immune exhaustion due to chronic activation and inflammation [66, 67]. The adaptive immune response is able to robustly generate antibodies to the virus. However, this adaptive response is usually unable to cope with the diversity of the virus or penetrate all of the tissue reservoirs *in vivo* [68].

During late-stage disease, or AIDS, plasma viremia increases and CD4+ T-cells decline even further. Without treatment, individuals are unable to mount an effective cellular immune response when their CD4+ T-cell count drops below 200 cells/$mm^3$ [69]. Eventually, without treatment, the individual will succumb to opportunistic infections. A co-receptor switch can occur during this late disease stage from CCR5 using to CXCR4 using virus. The availability of CCR5+ target cells may be significantly depleted. The co-receptor switch allows CCR5-CXCR4+ CD4+ T-cells, naïve cells, to be colonized and is often associated with

faster disease progression [70, 71]. Once an individual reaches this late stage disease, they will most likely succumb to the disease in a short period of time without treatment. The main form of treatment is currently highly active antiretroviral therapy (HAART), which is a generally accepted combinatorial approach to utilize different classes of antiretroviral drugs to address the pathogenic effects of HIV infection [72].

Numerous factors influence immunopathogenesis. Innate immunity plays an important role in combating HIV infection, and the HIV-1 virus has a number of methods of subverting the innate immune response as a result. The activation of immune cells by HIV-1 leads to IFN-$\alpha$ production, which in turn leads to the up-regulation of a number of cellular restriction factors, such as APOBEC3G and SAMHD1 [73-75]. Activation of the host factor Trex1 helps to prevent innate signaling in response to HIV nucleic acids [76]. Acute infection by HIV-1 also promotes NK-cell expansion and activation, which in turn leads to better control over disease progression by the host [77-79].

Cellular responses to HIV infection contribute significantly to pathogenesis. Certain individuals, referred to as elite controllers or elite supressors, are able to successfully control viral replication for extended periods of time, as well as not showing other signs of disease progression [80]. The CD4+ T-cells of these individuals proliferate in response to infection and secrete multiple cytokines, similar to non-pathogenic infections seen in primates. Chronic progressors, however, lack this CD4+ T-cell proliferation, and only

secrete IFN-γ [81]. The CD8+ T-cells of these elite controllers also appear to contribute greatly to their ability to control disease progression by secreting multiple cytokines and exhibiting more efficient proliferation and killing of infected cells, whereas these characteristics are not observed in chronic progressors [81]. Cellular immunity to HIV infection is therefore an important factor contributing to overall pathogenesis.

Antibodies also play a role in HIV-1 pathogenesis, both neutralizing and non-neutralizing. Neutralizing antibodies arise several weeks after infection upon activation of the adaptive immune response to HIV [82]. Broadly neutralizing antibodies generally arise after a much longer period of infection, potentially needing chronic antigen exposure, higher plasma viral loads, and increased affinity maturation [81]. Non-neutralizing antibodies can facilitate immune recognition by binding HIV-1 antigen, and recruiting immune cells via their Fc fragment, leading to antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cell-mediated virus inhibition (ADCVI) [83].

1-6 HIV-1 Tropism:

The term “macrophage tropism” was first used early on in the course of HIV research. Two distinct groups of HIV-1 isolates were described. One group was capable of efficiently infecting and inducing syncytia in T-cell lines and T-cell cultures, and was termed T-tropic. A second group was capable of infecting primary macrophage cultures, did not induce syncytium formation in T-cell cultures, and was termed mac-tropic or M-tropic [84, 85]. CXCR4 was eventually

identified as the co-receptor for the T-tropic, syncytium-inducing viruses, and CCR5 was identified as the co-receptor for M-tropic, non-syncytium inducing viruses [86]. It has since been shown that in infected patients, HIV-1 can utilize CCR5, CXCR4, or both, (now described as R5, X4, or R5X4 viruses) and that ascribing M and T tropism to the viruses exclusively on their usage of a specific co-receptor is an over-simplification [87, 88].

There is significant variation amongst R5 using viruses. As early as 1987, R5 isolates were found within the same individual that differed significantly in their ability to infect macrophages. JR-FL and JR-CSF, both used in these studies, were isolated from brain and cerebrospinal fluid respectively [89]. There have since been a number of primary isolates identified that differ drastically in their ability to infect macrophages, but still use R5 as their primary co-receptor [90-92].

CCR5 is the main coreceptor for transmitted HIV-1 [93], [10], while CXCR4-using variants become detectable in up to around 50% of AIDS patients and are associated with a rapid decline in CD4+ T cells, leading to death [94]. It has also been shown that R5 using isolates can vary in their ability to infect target cells expressing low levels of CCR5 [95, 96]. R5 isolates obtained from brain tissue display a greater incidence of macrophage tropism, or an ability to infect macrophages, than do isolates from the blood and immune tissue [91, 96-98]. There is also evidence that macrophage tropic isolates are more prevalent in late stage disease or AIDS [99-101]. The ability of R5 viruses to infect

macrophages correlates with an ability to infect cells expressing low levels of CD4 [102] and to sensitivity to inhibition by reagents specifically targeting the CD4-gp120 interaction [97, 103]. This indicates that Env is a major determinant of macrophage tropism.

There are several Env determinants that have been identified that affect the macrophage tropism of *env*s, and accordingly some of these are found in or around the CD4 binding site. An asparagine at position 283, within the CD4 binding site within C2, was identified as conferring macrophage infectivity in certain Envs [104]. Further determinants were mapped to regions flanking the CD4 binding site, reinforcing the fact that residues affecting the CD4 binding of gp120 are responsible for macrophage tropism [105]. Residues outside and apart from the CD4 binding loop have also been found to influence macrophage tropism, such as residues within the V3 loop, and an N-linked glycosylation at position 386 of the V4 loop [105, 106]. While these residues and glycans are distinct from the CD4 binding site, they may still play a role in the gp120-CD4 interaction by altering accessibility or conformation, seen by their effects on b12 sensitivity, a broadly neutralizing monoclonal antibody directed against an epitope overlapping the CD4 binding site [107]. In addition to specific determinants, recent work has shown that late-stage disease mac-tropic Env possess lower gp120 charges, while non-mac tropic Envs in immune tissue have a more positive gp120 charge [108].

The effect of macrophage tropism on HIV-1 in the context of the infection can be significant. Viruses that interact more efficiently with CD4 and are able to use low levels of CD4 may confer more efficient infection of all CD4+ cells, including T-cells. The emergence of macrophage tropic variants could contribute to the late stage loss of CD4+ T-cells, acting to exacerbate the overall T-cell decline [99, 101]. During the course of disease progression, the gradual loss of central memory T-cells is observed. These cells express significantly lower levels of CCR5, and are generally less susceptible to infection by R5-tropic viruses [109]. It may be possible that viruses emerge during the chronic stage that are able to exploit these lower levels of CCR5. Consistent with this, one group reported a greater tolerance for variability in the N-terminus of CCR5 by macrophage tropic envelopes [110], while another study finds that mac-tropic envelopes more efficiently use low levels of CCR5 [111].

Macrophage tropic Env can frequently be detected in patients suffering from neurological effects of HIV-1 infection. Virus can be detected in brain tissue early after infection, but significant macrophage infiltration into neurological tissue occurs in the later stages of disease [112]. In late stage disease, CD16+ monocytes expand, become activated, and have the propensity to traffick across the blood-brain barrier [113, 114]. Some of the macrophages that traffic into the brain may be infected, which has been seen in the case of SIV [115]. The infection of macrophages in the brain, and their contribution to inflammation most likely contributes to dementia in HIV patients.

Mac-tropism does not seem to play an important role in the initial transmission of HIV-1. It has recently been shown that the majority of mucosal transmission events are the result of a single virus, referred to as a transmitted founder virus (T/F) [116, 117]. Several studies have been conducted on T/F viruses from clade B [118] and clade C [117, 119] demonstrating that they generally do not infect macrophages well. There have been very few T/F viruses characterized, so these observations will need to be verified using additional T/F viruses.

1-7 Myeloid Dendritic Cells and HIV-1:

There are two major types of dendritic cells, myeloid (mDC) and plasmacytoid (pDC). Langerhans cells are also a type of dendritic cell found in the dermis. The mDCs bridge the innate and adaptive immune responses, and play a significant role in antigen presentation. They are readily found in immune and mucosal tissue and are one of the first cell types to encounter HIV during transmission [120]. Both immature and mature mDCs are susceptible to infection by HIV-1 [121-123]. These mDCs are known to express CD4, CCR5, and CXCR4, although they express these receptors at relatively low levels [124-126]. R5-tropic virus infects mDCs significantly better than X4-tropic virus, possibly due to a generally higher level of CCR5 expression on these cells compared to CXCR4 [121]. HIV-1 replication in mDCs is generally significantly less than what is observed in CD4+ T-cells, probably due to several factors, including low levels of receptor and co-receptor, significant degradation of internalized HIV in

endosomal compartments [110], and the expression of host restriction factors, such as SAMHD1.

Due to the small amount of mDCs able to be isolated from donors, monocyte-derived dendritic cells (MDDCs) are often used in vitro to study DC interactions with HIV. Monocytes isolated from peripheral blood differentiate into MDDCs after being stimulated in culture by IL-4 and granulocyte-macrophage colony stimulating factor (GM-CSF) [127]. Similar to myeloid DCs, they express CD11c, MHC class II molecules, and c-type lectin receptors such as DC specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) [128]. They may bind HIV virions more through C-type lectins than through CD4 [126]. MDDCs, however, do not completely recapitulate DC subsets found in vivo.

mDCs and MDDCs both efficiently transmit HIV-1 to CD4+ T-cells. They are able to bind HIV-1 via several receptors including DC-SIGN, langerin, mannose receptor, CD4, and others [129]. After binding virus, immature dendritic cells traffic to lymphoid tissues, where they mature and stimulate naïve T-cells via MHC class II antigen presentation [130]. Chemotaxis to lymphoid tissue brings DCs carrying HIV into contact with CD4+ T-cells, facilitating trans-infection of T-cells. Mature DCs transfer virus to target cells more efficiently than immature DCs [123] via an infectious synapse, a junction formed between DCs and T-cells. In addition to transmitting virus via the infectious synapse, DCs can also take up virus into endosomes, and facilitate trans-infection via these cellular compartments [131]. mDCs can also transfer virus in cis, presenting de novo

virus to target cells for infection, despite the low incidence of direct infection [132].

1-8 Scope of Thesis

The findings presented in this thesis demonstrate that position 153 in the V1 loop modulates macrophage tropism in several primary isolates. In several clades, a glutamate at position 153 (E153) is well conserved. It was observed that a glycine at the same position facilitated greater macrophage tropism, and that by changing this residue to a glutamate (G153E), this ability to infect macrophages was abrogated. When the reverse change was made, E153G, this substitution was able to impart an ability to infect macrophages. It will be demonstrated that these changes to position 153 cause shifts in sensitivity to the V3 MAb 447-52D and soluble CD4, while only modestly affecting sensitivity to the broadly neutralizing antibody b12. These changes in sensitivity indicate that these substitutions at position 153 cause a rearrangement of the V3 loop that allows low CD4 usage. The significant changes in sensitivity to sCD4 and minimal changes in sensitivity to b12 further indicate that residue 153 confers an increased ability for gp120 to undergo conformational changes after initially interacting with CD4.

In addition to individual determinants, data presented in this thesis demonstrate that R5 non-macrophage tropic envelopes do not confer an enhanced tropism for CD4+ T-cells. Using a panel of envelopes from nine patients, data will demonstrate that envelopes that are able to exploit the low

levels of CD4 on macrophages are also able to do the same on dendritic cells, although less efficiently. Non-macrophage tropic envelopes predominate in immune tissue, yet these non-mac tropic envelopes are unable to infect CD4+ T-cells directly as well as the mac-tropic Envs. In the context of DCs transmitting these non-mac tropic isolates, they have no advantage over mac-tropic envelopes. With respect to tropism and stimulation of an immune response, there does not appear to be any advantage to non-mac tropic Envs despite their prevalence throughout the course of disease. These findings help to further elucidate the role of mac-tropic and non-mac tropic Envs in the context of HIV-1 infection.

# CHAPTER II: MATERIALS AND METHODS

## 2-1 Patient envelopes:

HIV-1 clade B envelopes amplified from pediatric subject P1114, as well as NA118 LN27, NA420 LN40, NA20 LN8, and JRCSF, have been described previously [89, 91, 98]. The non-macrophage-tropic LN40 envelope was chimeric carrying the gp41 sequence of NA420 B33 to confer optimal infectivity [91, 98]. The Indian clade C envelope, 25925-2, was obtained from the NIH AIDS Research and Reference Reagent Program [133]. The clade D envelope, CMT19 S531, was amplified from a Cameroon semen sample, whereas the clade B envelope, FL5-2-209 was amplified from a frontal lobe autopsy sample of an AIDS patient with HIV-associated dementia that was obtained from the National NeuroAIDS Tissue Consortium. Envelopes derived from patients 7766, 10017, 6568, and CA110 were also obtained from tissue obtained from the National NeuroAIDS Tissue Consortium (refer to table 4-1). Patient 43 Envs were amplified from the blood and semen of an adult patient [98]. All transmitted/founder viruses used were obtained from the NIH AIDS Research and Reference Reagent Program [116]. Envs described herein as macrophage tropic are those envelopes better able to exploit low levels of CD4 on the target cell for infection. Envelopes were expressed from pSVIIIenv or from pcDNA3.1TOPO for the production of pseudovirions.

## 2-2 Cell cultures:

293T cells were used to prepare Env-containing (Env+) pseudovirions by transfection. $Env^+$ pseudovirions were titered on HeLa TZM-bl cells, which were also used for HIV-1 neutralizations. HeLa TZM-bl cells express high levels of CD4 and CCR5, and possess β-galactosidase and luciferase reporter genes under the control of the HIV long terminal repeat [134]. 293T and HeLa TZM-bl cells were cultured in Dulbecco modified Eagle medium (DMEM) with 4% fetal bovine serum (FBS) and gentamicin (10µg/mL).

Primary macrophages and monocyte derived dendritic cells (MDDCs) were prepared from elutriated monocytes (provided by the University of Massachusetts Medical School Center for AIDS Research) or by adherence from buffy coats. Briefly, 0.5 ml of elutriated monocytes ($5x10^5$/ml) was plated in each well of 48-well cell culture dishes and cultured in 10% human plasma in DMEM for 5 to 7 days before infection.

Alternatively, $5x10^7$ peripheral blood mononuclear cells (PBMC) from a buffy coat (Research Blood Components LLC, Brighton, MA) were plated into 14-cm bacterial culture dishes for 3 h before extensively washing away nonadherent cells, culturing overnight, and repeating the washes. The adhered monocytes were then cultured for 5 to 7 days in 10% human plasma in DMEM before treatment with EDTA and transfer to 48-well tissue culture dishes the day prior to infection [92]. MDDCs were obtained from adhered monocytes by culturing in XVIVO15 media (Lonzo) with 100ng/mL granulocyte-macrophage colony stimulating factor (GMC-SF) and 40ng/mL IL-4 (Peprotech) for 5-7 days in 75ml

cell-culture flasks [127]. MDDCs were scraped from the flask for use in assays after incubating with EDTA for 7 minutes at 37°C.

Primary PBMCs were isolated from whole human blood or buffy coats by Ficoll- Paque separation. Red blood cells were lysed with an Ammonium Chloride solution, and cells were then washed twice with sterile PBS and cultured in Roswell Park Memorial Institute 1640 medium (RPMI) with 10% fetal bovine serum and IL-2 (5ng/mL, Roche Inc.). PBMCs were stimulated with 5 µg/ml Phytohemagglutinin. CD4+ T-cells were isolated from stimulated PBMCs using Stem Cell negative selection for CD4+ T-cells.

CD4+ T-cells used in the $\alpha 4\beta 7$ experimentation were derived from PBMCs cultured in 10% RPMI, and activated with the OKT3 MAb to CD3 (1 µg/ml) (eBioscience), IL-2 (20 IU/ml) (Roche), and retinoic acid (10 nM) (Sigma Inc.). CD4+ T-cells were negatively selected, washed with PBS, and stained with an anti-human $\beta 7$ antibody (eBioscience) at 4°C for 1 hr. They were then washed with PBS and sorted into $\beta 7+$ and $\beta 7-$ populations using a BD FACSAria. Prior to use in any assays, they were cultured overnight post-sorting in 10% RPMI, IL-2, and retinoic acid (the same concentrations).

Jurkat E6-1 cells stably express integrin $\alpha 4$ [135]. Jurkat E6-1 cells stably transfected with either pCXbst-CCR5, or pCXbst-CCR5 and pCX4pur-ITGB7c were obtained from H. Gottlinger. They were cultured in 10% FBS RPMI with 0.5 µg/ml puromycin (driving expression of integrin β7) and 5 µg/ml blasticidin (driving expression of CCR5).

2-3 Preparation of Env+ pseudovirions and Replication Competent Virus**:**

Portions (1.25 µg) of pSVIIIenv or pcDNA3.1TOPO plasmids carrying complete *env* sequences were cotransfected by using calcium phosphate into 293T cells (Profection mammalian transfection kit, Promega), together with 1.25 µg of pNL43 that carried a premature stop codon in the envelope (Env+) [105, 107]. In the case of GFP reporter Env+ pseudovirions, the pHIvec2.GFP [136] vector was also transfected at a ratio of 2:1:1 to the pNL4.3 *env*- and the envelope carrying vector. Media (4% FBS DMEM) was changed 8-18 hrs post-transfection. Pseudovirions were harvested 48 h post-transfection, clarified by low-speed centrifugation, aliquoted into 0.5-ml portions, and snap-frozen in liquid nitrogen. 3 µg of DNA for each infectious clone was transfected into 293T cells using the same method described above, along with 1.25 µg VSV-G.

2-4 Infectivity assays**:**

Infectivity assays were carried out as described previously [105, 107]. Primary macrophages were treated with 100 µl of DEAE dextran (10 µg/ml) prior to infection before adding an equal volume of serially diluted Env+ pseudovirions and spinoculation for 30 min in a benchtop centrifuge [137]. Growth medium containing 10% human plasma was then added. After 7 days of incubation, the macrophages were fixed in cold methanol-acetone (1:1), washed, and immunostained for p24 with the MAbs 38:96K and EF7 (UK Centre for AIDS Research), followed by an anti-mouse IgG-β-galactosidase conjugate and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) substrate (0.5mg of X-

Gal/ml, 3 mM potassium ferricyanide, 3 mM potassium ferricyanide, 1mM magnesium chloride). DEAE dextran and spinoculation enhance virus infectivity by ~20-fold by increasing attachment [137]. Infection according to this procedure does not bypass the requirement of CD4 and CCR5 for infection, which thus remains sensitive to entry inhibitors. Thus, macrophage infection conferred by envelopes described here was completely inhibited by maraviroc (data not shown). HeLa TZM-BL cells were plated at 0.5 ml per well ($5x10^5$ cells/ml) in 48-well dishes the day prior to infection and infected. After 72 h, the HeLa TZM-BL cells were fixed in 0.5% glutaraldehyde in phosphate-buffered saline, and β-galactosidase–X-Gal substrate was added.

Infected macrophages and HeLa TZM-BL cells stain blue for β-galactosidase. This β –gal expression is driven by the HIV-1 LTR promoter in TZM-BLs, and is thereby dependent upon Tat expression [138]. Since Env+ pseudovirions are only capable of a single round of infection, stained infected cells are recorded as focus-forming units (FFU). The macrophage infectivity data presented were derived from FFU values averaged from two experiments using cells from different donors. Envs are determined to be macrophage tropic if they are able to exploit the low levels of CD4 on macrophages for infection. This is a qualitative determination.

PBMCs and CD4+ T-cells were infected with GFP reporter Env+ pseudovirus by titrating the virus on the cells in a 96-well plate (130,000 cells/well). 100 µL of viral supernatant was added to each well, serially diluted,

and infection was quantified 2 days post infection by counting GFP-positive cells using a fluorescent microscope. Jurkat E6-1 cells and β7 sorted CD4+ T-cells were infected in the same manner.

2-5 Trans infection assay**:**

Autologous PBMCs/CD4+ T-cells and MDDCs were derived from whole blood or a buffy coat. After MDDCs were cultured as described, they were treated with EDTA, scraped, and aliquoted into 96-well PCR plate (90,000 cells/well). 100 µL of viral supernatant was added, and cells were incubated for 1 hr at 37°C. Cells were then washed twice with PBS, and co-cultured with autologous PBMCs/CD4+ T-cells, which were cultured as described previously. Infectivity was quantified 2 days post infection by counting GFP+ cells. Only PBMCs/CD4+ T-cells were counted, as infected MDDCs do not express the GFP reporter gene until 3 days post infection.

2-6 Inhibition and neutralization assays**:**

Inhibition and neutralization assays were carried out as described previously using HeLa TZM-BL cells as target cells [97, 105, 107]. Neutralization was measured as a reduction in β-galactosidase reporter gene expression after infection of HeLa TZM-BL cells with MAb-treated pseudovirions. 200 µL of HeLa TZM-BL ($5\times10^4$ cells/ml) was added to each well in 96-well luminescence plates (Corning Inc.) 1 day prior to neutralization assays. 200 FFU of pseudovirions was incubated with twofold dilutions of the respective inhibitor or antibody for 2 hrs at 37°C. 50 µL of this mixture was incubated with HeLa TZM-BL cells at

37°C overnight before being removed, and 200 µL of 4% DMEM was added. Seventy-two hours after incubation the residual infectivity was estimated by measuring the β-galactosidase activity using a luminescent readout. The luminescence reaction was initiated by first removing the medium in each well and adding 100 µL of Beta-Glo (Promega Inc.) with 100 µL of DMEM (no phenol red) per well. Luminescence was measured 30 min later.

2-7 Analysis of V1 loop phylogeny**:**

HIV-1 V1 loop sequences were downloaded from the HIV sequence database at Los Alamos and aligned by using CLUSTAL W2, and the frequencies of different amino acids at residue 153 were evaluated.

2-8 Immunostaining for Fluorescent Microscopy**:**

MDDCs were washed once with PBS, and then incubated with respective antibodies from BD Biosciences for 1 hr at 37°C. Cells were then washed with PBS, and placed in 10% FBS-RPMI media. CD11c staining was imaged using the appropriate phycoerythrin filter.

2-9 ELISAs:

HIV-1 gp120 ELISA kit (Advanced Bioscience Laboratories inc.) was used on HIV-1 viral supernatant to quantify gp120 levels. p24 ELISA kit (Advanced Bioscience Laboratories Inc.) was used to quantify amount of virus captured by MDDCs. MDDCs were incubated with viral supernatant for 1 hr, washed twice with PBS and lysed in the provided disruption buffer. This lysate was used in the p24 ELISA. The IFNα ELISA kit (Mabtech Inc.) was used to quantify IFNα in the

supernatant of the infected MDDCs four days post infection.

2-10 Flow Cytometry Analysis**:**

Uninfected MDDCs were treated with Versene, scraped, and washed with 2% PBS 7 days after isolation from blood. All antibodies used were incubated with cells for 1 hr at 4°C, then washed with PBS, and analyzed on a BD FACSCalibur. 7-AAD was used to gate on live cells, and all compensation was performed using Flowjo (Tree Star, Inc.).

# CHAPTER III: A CONSERVED DETERMINANT IN THE V1 LOOP OF HIV-1 MODULATES THE V3 LOOP TO PRIME LOW CD4 USE AND MACROPHAGE INFECTION

## 3-1 Introduction:

HIV-1 fusion and entry into cells is triggered by sequential interactions between the viral envelope glycoprotein with CD4 and a seven-transmembrane coreceptor. HIV-1 R5 viruses efficiently infect primary CD4+ memory T cells that express CCR5. However, as was previously described, these viruses confer widely divergent abilities to infect primary macrophages [90, 91, 96-98]. In contrast, many envelopes from immune tissue such as lymph nodes, infected macrophages very inefficiently [98]. Macrophage infectivity correlated with the capacity to infect indicator cell lines via low levels of CD4 [91, 96, 98, 139], an observation that reflects the lower expression of CD4 on macrophages compared to T cells [140-142]. Consistent with this observation, envelope determinants that control macrophage tropism have been mapped to residues within or proximal to the CD4 binding site [98, 104, 105]. Thus, Dunfee et al. reported that an asparagine at residue 283 in the C2 part of the CD4bs was associated with brain envelopes and HIV-associated dementia [104]. N283 also conferred increased levels of macrophage infectivity when introduced into R5 envelopes where residue 283 is usually T or I [104, 105]. N283 was reported to confer an increased affinity of gp120 for CD4, probably because the asparagine may facilitate the formation of a hydrogen bond with Q40 on CD4 [104]. Nevertheless, N283 only partially associates with macrophage infectivity. Other envelope

determinants that likely affect exposure of CD4 contact residues have also been identified [105], and non-macrophage tropic envelopes possessing N283 have also been amplified form immune tissue [91, 98, 108]. Determinants on the flanks of the CD4 binding loop were identified, which probably modulate exposure of the adjacent and conserved CD4 contact residues. The CD4 binding loop is likely to be the main part of the CD4bs that is exposed on the native envelope trimer and an early contact for CD4 [143]. Of note, the identified CD4 binding loop flank residues also shifted sensitivity to the glycan-specific monoclonal antibody (MAb), 2G12, indicating that movement of proximal glycans could be one mechanism involved in the exposure of CD4 contact residues [144].

The CD4 binding site is also a major target for vaccine development aimed at eliciting neutralizing antibodies [145]. It is therefore important to fully understand how this site is exposed or protected during viral evolution and how this affects tropism, neutralization sensitivity, and other envelope properties. Here, closely related HIV-1 envelopes from a pediatric subject in late disease are described that differ profoundly in their capacity to infect primary macrophages. A single amino acid determinant in the V1 loop is identified that confers dramatic shifts in macrophage tropism, as well as affecting sensitivity to the V3 loop MAb, 447-52D, sCD4, and the CD4bs MAb, b12. These data are consistent with a model where the V1 determinant controls the conformation or exposure of the V3 loop, which in turn primes the envelope for low CD4 use and macrophage infection.

3-2 HIV-1 envelopes amplified from the plasma of a pediatric HIV-1 subject vary in their capacities to confer macrophage infection:

Several HIV-1 envelopes were previously described that had been amplified by PCR from the plasma of a pediatric AIDS patient, P1114 [98]. These envelopes were amplified when the patient was asymptomatic in the A2 stage of disease (in 1995 to 1996), as well as after the patient had progressed to symptomatic disease in C3 (in 1998) using the CDC categories (http://www.aids-ed.org/aidsetc?page_cm-105_disease). Single round pseudovirions carrying each envelope were titrated in infectivity assays on HeLa TZM-BL cells and monocyte-derived macrophages. All of the Envs conferred high infectivity titers on HeLa TZM-BL cells, which express high levels of CD4 and CCR5. However, only two Envs from the C3 stage of infection (C98-15 and C98-18) conferred significant infection of primary macrophages (Fig. 3-1).

3-3 Specific envelope residues in the V1V2 loops that segregate with macrophage infectivity**:**

The five envelopes from the C3 stage of infection were very closely related in sequence. Two distinct amino acid changes in the V1V2 loops were the only differences over the entire gp160 sequence that segregated with macrophage infectivity (Fig. 3-2). Figure 3-3 shows a sequence alignment for the V1V2 loops of these envelopes. Env 98-15 is the most macrophage-tropic envelope and carries E153G and D167N substitutions in V1 and V2, respectively.

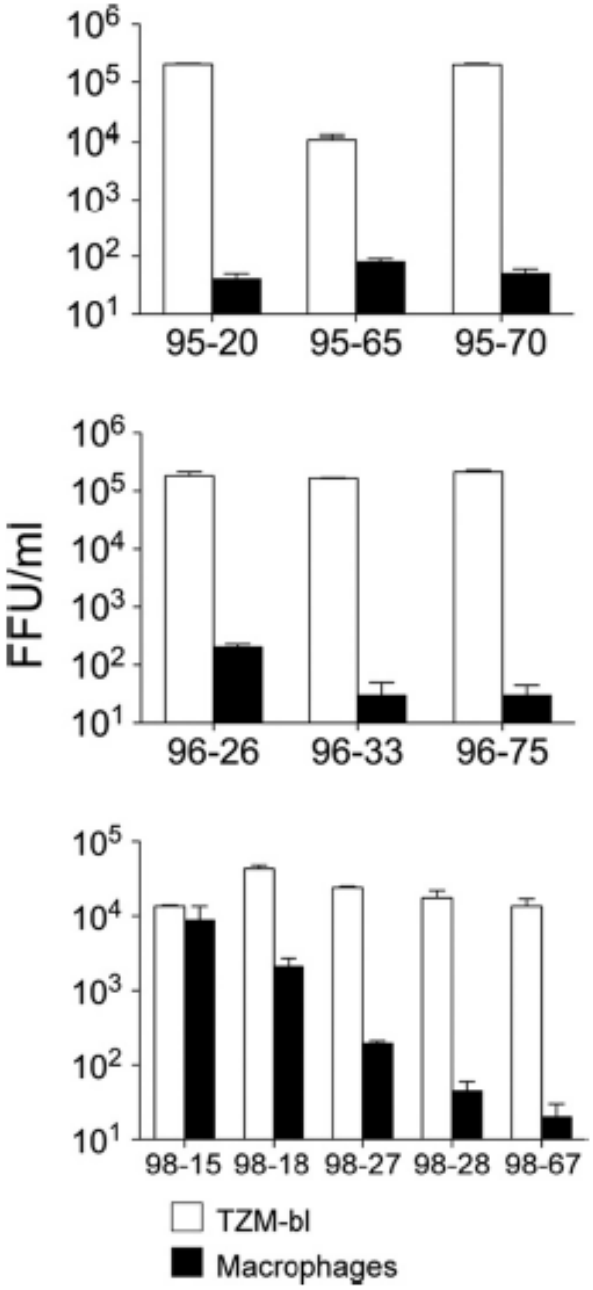



FIG. 3-1. Macrophage tropic R5 viruses detected in late-stage disease of a pediatric AIDS patient. R5 envelopes were amplified from three plasma samples of a pediatric AIDS patient P1114 in 1995, 1996 (disease stage A2), and 1998 (disease stage C3). Single-round Env pseudovirions were prepared and used to infect HeLa TZM-BL cells and monocyte-derived macrophages. All envelopes exhibited high infectivity titers on TZM-BL cells. Two of the envelopes, 98-15 and 98-18, exhibited significant infection on macrophages.

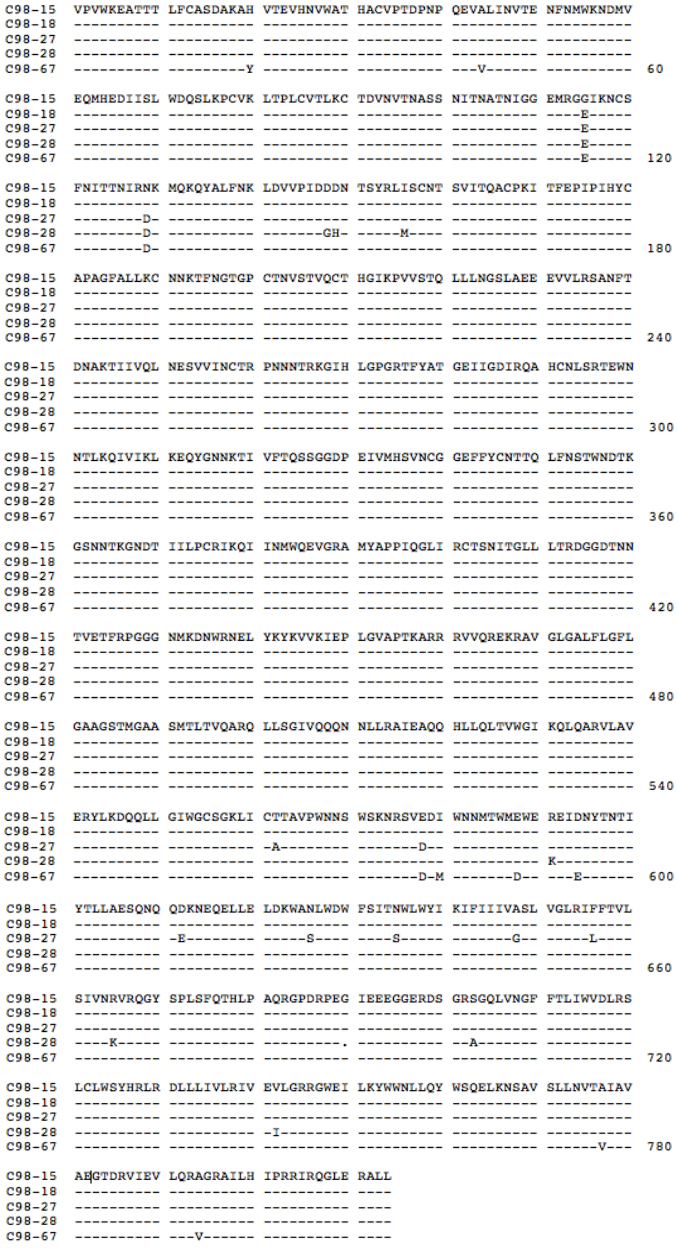



FIG. 3-2. gp160 alignment of pediatric AIDS patient P1114. The gp160 sequences of envelopes isolated from P1114, including 98-15, 98-18, 98-27, 98-28, and 98-67. There are slight differences between these envs, but the only polymorphisms corresponding to phenotypic changes are found in the V1/V2 region of gp160. The numbering used in this alignment is not relative to HXB2 numbering. The non-macrophage-tropic envelopes all have E114 and D129 (E153 and D167 relative to HXB2 numbering). These residues are the only candidates that could explain the differences in macrophage infectivity between these envelopes.

Env 98-18 confers more modest levels of macrophage infectivity and possesses only the D167N change. The non-macrophage-tropic envelopes all have E153 and D167. These residues are the only candidates that could explain the differences in macrophage infectivity between these envelopes.

3-4 Macrophage infectivity of envelopes mutated at residues 153 and 167:

To determine whether the two polymorphisms in the V1/V2 region of these envelopes were responsible for the differences in macrophage infectivity, point mutations were made by site-directed mutagenesis and infectivity evaluated on TZM-BL cells and macrophages. A G153E substitution in 98-15 abolished macrophage tropism, which is consistent with the requirement of G153 for macrophage infectivity. In contrast, an N167D substitution in 98-15 had no effect on macrophage infectivity, while a combination of G153E and N167D substitutions conferred the same loss of macrophage infectivity as the G153E substitution alone (Fig. 3-4).

The reciprocal substitutions were then made in the non-macrophage tropic envelopes, 98-27 and 98-28. For both of these envelopes, E153G caused a drastic increase in macrophage infectivity, approximately equal to the level of the 98-15 wild-type (wt) envelope. In contrast, D167N did not confer any significant increase in macrophage infectivity for 98-27 and 98-28. For 98-18, E153G enhanced the modest levels of macrophage infection conferred by this envelope to levels similar to 98-15, whereas N167D abrogated macrophage infection.

Taken together, these results indicate that G153 is the major determinant

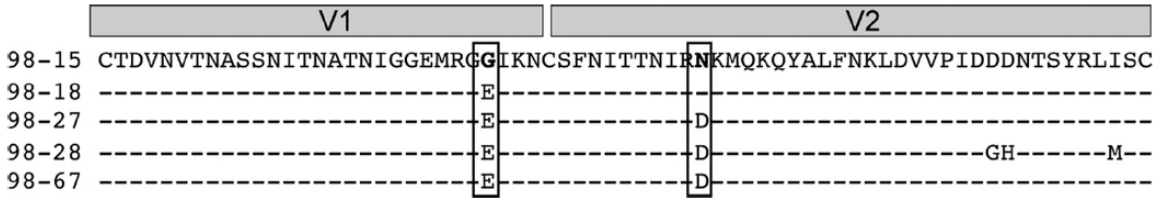



FIG. 3-3. Specific envelope residues in the V1V2 loops segregate with macrophage infectivity. Aligned V1V2 sequences from C3 disease stage envelopes show that E153 and D167 in boxes are found in non-macrophage-tropic envelopes. 98-15 is the only envelope carrying G153 and is the most macrophage tropic. Both 98-15 and 98-18 have N167, but 98-18, which has intermediate macrophage infectivity, lacks G153.

responsible for the macrophage-tropic phenotype observed for the C98-15 envelope, while N167 conferred only modest effects on macrophage infectivity for 98-18.

3-5 Residue E153 is conserved across HIV-1 clades:

The frequencies of E and G at position 153 were then evaluated for other HIV-1 clade B and non-B clades. In clades A, B, and C, residue E153 is highly prevalent, a situation similar to the non-macrophage-tropic *env*s evaluated in the present study, whereas G153 is less common (Table 3-1). Interestingly, G153 is more prevalent in clades D and CRF02-AG, while for clade G and CRF01-AE, E153 was less prevalent but was not frequently substituted with a G. The conservation of E153 is also apparent in the review by Zolla-Pazner and Cardozo [146].

3-6 The E153G substitution confers macrophage infectivity for heterologous clade B envelopes:

Since E153 is relatively conserved, it was next investigated whether the E153G substitution conferred macrophage infectivity for heterologous *env*s or was limited to the pediatric envelopes described here. I introduced the E153G substitution into four heterologous clade B R5 envelopes that carry E153 in the V1 loop (NA20 LN8, NA118 LN27, NA420 LN40, and JRCSF) and that are non-macrophage-tropic. For two of the envelopes, LN27 and JRCSF [89, 91], the E153G substitution conferred substantial levels of infectivity for macrophages (Fig. 3-5). The effects of the reverse G153E substitution into a highly

TABLE 3-1. Conservation of V1 loop residue 153

| Clade or CRF | Prevalence (%)[a] at amino acid: | | Total no. of sequences |
|---|---|---|---|
| | E153 | G153 | |
| Clades | | | |
| A | 74.22 | 10.59 | 1,699 |
| B | 89.40 | 3.86 | 17,721 |
| C | 79.55 | 3.42 | 4,968 |
| D | 12.43 | 23.98 | 1,593 |
| G | 22.38 | 1.23 | 648 |
| CRF | | | |
| CRF01_AE | 32.92 | 2.97 | 1,953 |
| CRF02_AG | 33.84 | 28.24 | 1,179 |

[a] Residue E153 is conserved across clades. V1 sequences were obtained from the HIV database (http://www.hiv.lanl.gov/content/index) for clades A, B, C, D, G, AE, and 02AG. These sequences were aligned using CLUSTAL W2 and manually corrected. E153 is highly conserved across clades A, B, and C. G153 is most prevalent in clades D and 02AG.

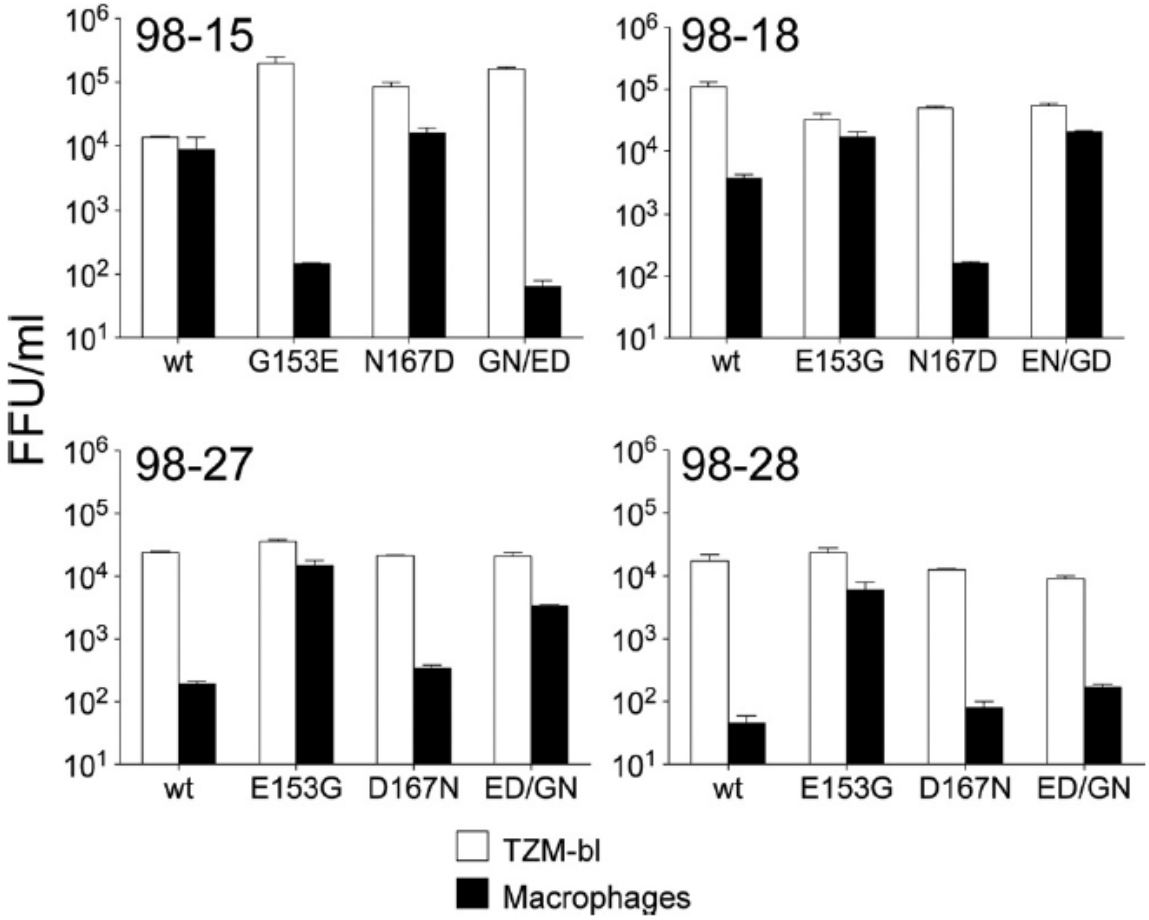



FIG. 3-4. V1 loop residue 153 modulates macrophage tropism. A G153E substitution in macrophage-tropic R5 C98-15 reduced macrophage infectivity by at least a 100-fold, while N167D had only a modest effect (top, left panel). For 98-18 (top, right panel), E153G enhanced modest macrophage infectivity to levels similar to 98-15 wt, whereas N167D abrogated macrophage infection to levels close to background. For non-macrophage-tropic envelopes (98-27 and 98-28; bottom panels), an E153G substitution conferred 100-fold increase in macrophage infectivity. In contrast, a D167N substitution had only modest effects on these two envelopes. Double substitutions at residues 153 and 167 conferred phenotypes similar to the single change at residue 153, although this was less evident for 98-28. These data show that residue 153 alone modulates macrophage infectivity.

macrophage-tropic brain-derived envelope, FL5-2-209, that naturally carried a G at residue 153, was then investigated. However, this change had no effect on the capacity of FL5-2-209 to infect macrophages (Fig. 3-5). Finally, E153G substitutions into clade C (25925-2) and D (CMT19 S531) envelopes were made. However, these envelopes remained non-macrophage-tropic. In summary, substitution of V1 loop residue E153 with G conferred macrophage tropism for two of four heterologous clade B envelopes tested.

3-7 Substitutions in V1 loop residue 153 affect sensitivity to neutralizing antibodies and entry inhibitors:

(i) MAb b12 and sCD4

The capacity of HIV-1 R5 envelopes to infect macrophages correlated with infection of indicator cell lines via low levels of CD4 [91, 96, 98, 139]. The ability to use low levels of CD4 for infection was shown previously to be due to changes within or proximal to the CD4bs [104, 105], which presumably confer an increased envelope avidity for cell surface CD4. Such changes in the use of CD4 are usually evident in shifts in the sensitivity of envelopes to inhibition by soluble CD4 and the CD4bs MAb, b12, with sensitivity to b12 a reasonable surrogate for the exposure of the CD4bs on many clade B envelopes. However, residue 153 is in the V1 loop, which has been proposed to sit at the apex of the envelope trimer away from the CD4bs [56]. It was tested whether substitutions at residue 153 affected sensitivity to sCD4 and b12. For the non-macrophage-tropic envelopes where E153G substitutions increased macrophage tropism (98-

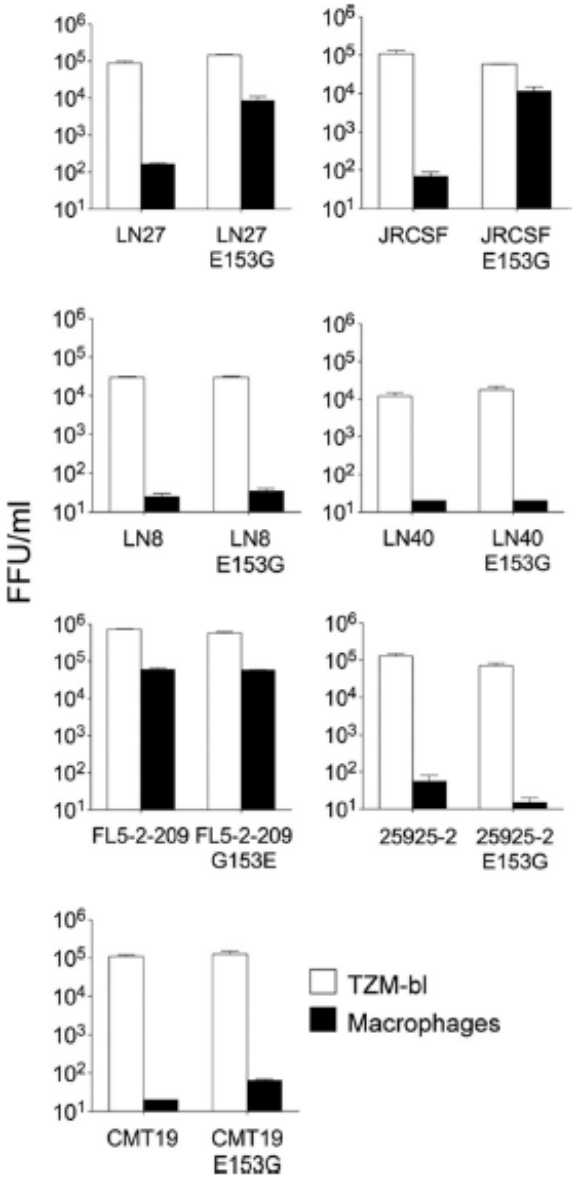



FIG. 3-5. V1 loop residue 153 modulates macrophage tropism for two of seven heterologous R5 envelopes. The E153G substitution was introduced into four heterologous, non-macrophage-tropic clade B R5 envelopes. For two (LN27 and JRCSF; top panels), the substitution conferred at least a 100-fold increase in macrophage infectivity. In contrast, E153G had no effect on the lack of macrophage infection conferred by LN8 and LN40 (second panels down). The reverse substitution G153E in the highly macrophage-tropic FL5-2-209 also had no effect on the high levels of macrophage tropism conferred by this envelope (third panel down, left). Finally, E153G substitutions in clade C (25925-2) (third panel down, right) and clade D (CMT19 S531) (bottom panel) envelopes also failed to affect their lack of macrophage infection. E153G substitutions thus conferred macrophage tropism for two of seven heterologous HIV-1 R5 envelopes.

27, 98-28, LN27, and JRCSF), there were dramatic increases in sensitivity to sCD4 reflected in decreased 50% inhibitory concentrations (IC50s) of 50- to 100-fold (Fig. 3-6 A, left panels). We also noted more modest shifts in b12 sensitivity for these envelopes. Thus, for envelopes with E153G, IC50 shifts of around 10-fold were observed for b12 inhibition. For the macrophage-tropic envelope 98-15, the reverse G153E substitution (which abrogated macrophage infection) conferred decreased sensitivity to sCD4 with an increased IC50 of about 50-fold observed but only a minimal shift in b12 sensitivity at best. Nevertheless, these changes in sCD4 and b12 sensitivity are consistent with effects on the CD4bs mediated by residue 153 in the V1 loop.

The effects of the E153G substitution on sCD4 and b12 sensitivity for envelopes that failed to switch tropism were then investigated. For LN8 and LN40, E153G had no significant effect on sCD4 or b12 sensitivity (Fig. 3-6 B). However, for both the clade C (25925-2) and clade D (CMT19 S531) envelopes, E153G increased their sensitivity to sCD4, although there was no change for b12. The clade D envelope (CMT19 S531) stayed resistant to b12, and this may reflect the reduced crossreactivity of b12 for this clade [147]. Interestingly, the reverse G153E substitution in the highly macrophage-tropic FL5-2-209 envelope, which naturally carries G153, conferred substantial decreases in sensitivity to sCD4 and b12, even though the mutant envelope still conferred high levels of macrophage infection.

Together, these results show that the V1 loop residue at 153 frequently

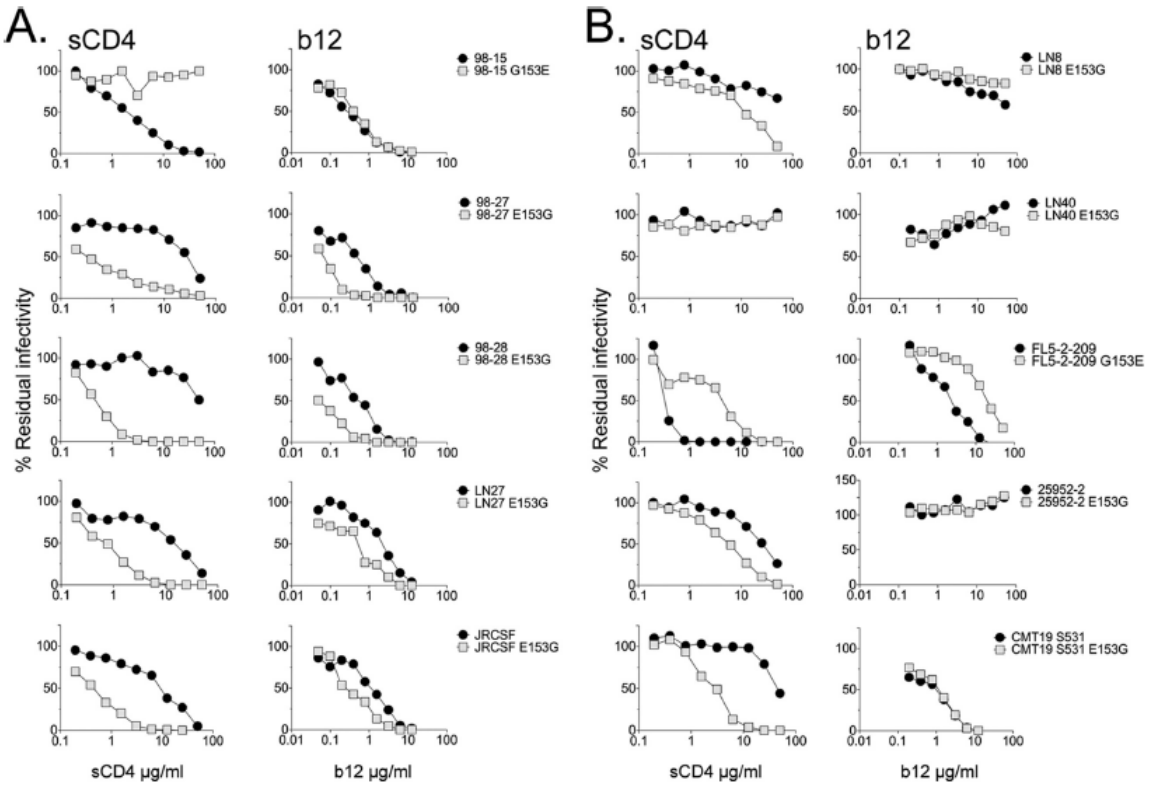



FIG. 3-6. Substitutions at residue 153 in the V1 loop modulate sensitivity to sCD4 and b12 in addition to macrophage tropism. (A) The five R5 envelopes that switched macrophage tropism following E153G (C98-27, C98-28, LN27, and JRCSF) or G153E (C98-15) substitutions showed changes in sensitivity to sCD4 and to b12. (B) The five R5 envelopes that failed to switch macrophage tropism following E153G (LN8, LN40, 25925, and CMT19S531) or G153E (FL5-2-209) substitutions were also tested for changes in sensitivity to sCD4 and b12. Despite no change in tropism, changes in sensitivity to these reagents were detected for some but not all of the envelopes. Nonetheless, consistent changes in sCD4 and b12 sensitivity were associated with changes in macrophage tropism.

confers alterations in the CD4bs affecting sensitivity to sCD4 and b12. Of note, changes in sCD4 sensitivity sometimes occurred in the absence of detectable changes in macrophage tropism. Nevertheless, envelopes that did switch tropism generally showed the largest changes in sensitivity to sCD4 and b12.

(ii) The V3 loop-specific MAb, 447-52D**:**

Dramatic changes in sensitivity to the V3 loop MAb, 447-52D, were also observed for envelopes that switched macrophage tropism after substitution at residue 153. For the non-macrophage-tropic envelopes where E153G conferred macrophage infection, there were large increases in sensitivity to 447-52D with at least 100-fold decreases in IC50s (Fig. 3-7). In contrast, for 98-15, G153 conferred resistance to 447-52D to go alongside the abrogation of macrophage infection (Fig. 3-7).

For LN8 and LN40, the E153G mutant envelopes remained resistant to 447-52D consistent with the lack of an effect on their non-macrophage tropism. For the macrophage-tropic brain Env FL5-2-209, G153E conferred resistance to 447-52D even though it had no effect on macrophage infectivity. Overall, these observations indicate an intriguing role for the V3 loop in R5 macrophage tropism that could be associated with effects on the CD4bs or in altered interactions with CD4 or CCR5. These possibilities are addressed below.

(iii) Other entry inhibitors and neutralizing antibodies**:**

The same envelopes and mutants were also tested for sensitivity to neutralizing MAbs and inhibitors that target different envelope sites or functions.

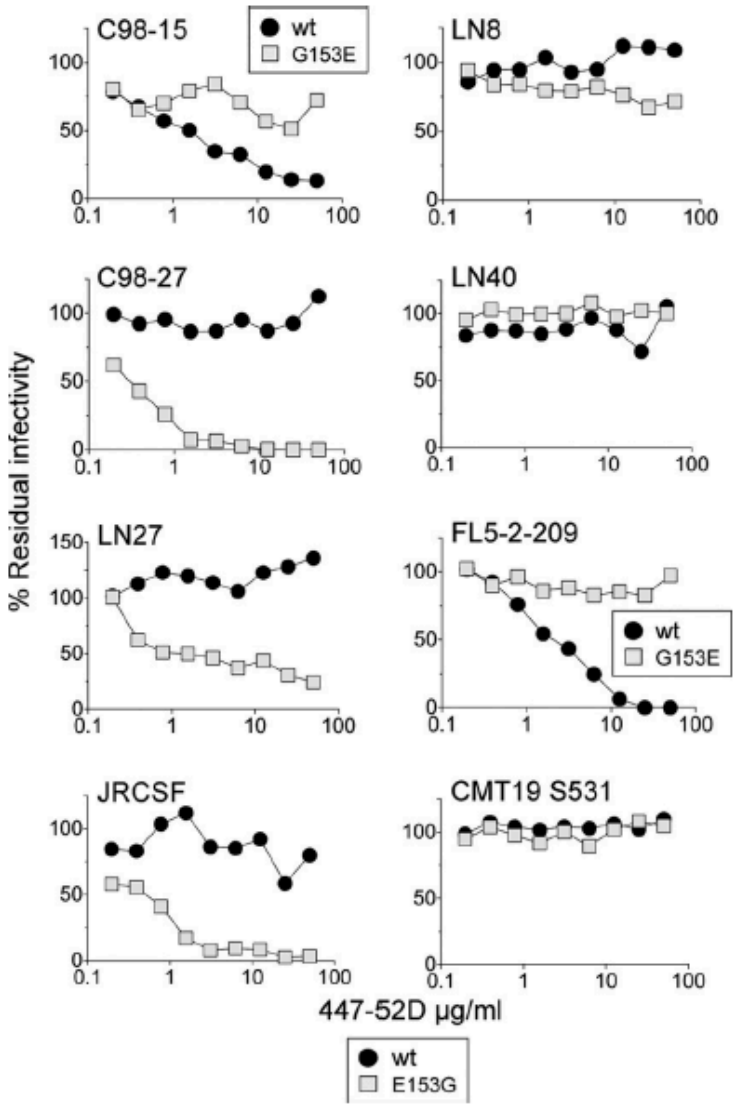



FIG. 3-7. Sensitivity to the V3 loop-specific MAb, 447-52D, is modulated by substitutions at residue 153 and associated with switches in macrophage tropism. For R5 envelopes with altered macrophage tropism following E153G (C98-27, LN27, and JRCSF) or G153E (C98-15) substitutions, there were substantial changes in sensitivity to the V3 loop mAb 447-52D (left panels). For three envelopes that failed to switch tropism following E153G substitution, there were no changes in 447-52D sensitivity. However, FL5-2-209 showed decreased sensitivity to 447-52D following the reverse substitution, even though this change failed to affect high levels of macrophage infectivity (right panels). In summary, substantial changes in sensitivity to the V3 loop MAb, 447-52D, were associated with changes in macrophage tropism following substitutions at V1 loop residue 153.

Table 2 shows IC50s for envelopes tested. Overall, E153G and G153E had no substantial effect on sensitivity to the glycan-specific MAbs 2G12, 17b, maraviroc, 2F5, or T20. These observations indicate that the substitutions at residue 153 affect the CD4bs but do not confer a more global effect on other envelope functions, including interactions with CCR5 (maraviroc) or gp41 conformational changes required for fusion (2F5 and T20), nor do they result in increased exposure of the CD4i epitope (17b). Of note, the lack of change in maraviroc sensitivity suggests that alterations in the V3 loop that confer shifts on 447-52D sensitivity do not impact on the efficiency of Env-CCR5 interactions, although this needs to be verified through further experimentation.

3-8 Residue 153 is proximal to conserved V1/V2 determinants that are targeted by broadly active neutralizing MAbs: PG9 and PG16**:**

Two human MAbs, PG9 and PG16, that were derived from a clade A-infected African donor were reported [148]. These MAbs are able to neutralize pseudovirions expressing a wide range of diverse envelopes [148]. They preferentially bind to the trimeric envelope and target an epitope that involves determinants in the V2 and V3 loops [148]. The proximity of the V2 determinants to V1 residue 153 in the V1 loop and the implication of the V3 loop in macrophage tropism prompted us to test whether envelopes carrying E153G or G153E substitutions differed in sensitivity to PG9 or PG16. Some shifts in sensitivity to PG9 and PG16 were noted (Table 3-2). However, these were mainly modest and did not segregate with macrophage infectivity.

TABLE 3-2. Sensitivity of R5 envelopes and their corresponding mutants with V1 loop residue 153 substitutions to various entry inhibitors and neutralizing antibodies

| Envelope | $IC_{50}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 17b (μg/ml) | 2G12 (μg/ml) | Maraviroc (nM) | 2F5 (μg/ml) | T20 (nM) | PG9 (μg/ml) | PG16 (μg/ml) |
| 98-15 | >50 | 1.05 | 5.89 | 4 | 40.59 | 0.056 | 0.0049 |
| 98-15 G153E | >50 | 1.16 | 10.2 | 3 | 65.48 | 0.02 | 0.007 |
| 98-27 | >50 | 2.55 | 12.9 | 7.41 | 58.86 | 0.05 | 0.02 |
| 98-27 E153G | >50 | 1.5 | 10.0 | 1.78 | 61.62 | 0.09 | 0.02 |
| 98-28 | >50 | 0.88 | 1.72 | 2.03 | 74.89 | <0.006 | <0.006 |
| 98-28 E153G | >50 | 1.08 | 0.92 | 3.60 | 33.43 | 0.01 | <0.006 |
| JRCSF | >50 | 1.5 | 5.12 | 7.11 | 16.62 | <0.006 | <0.006 |
| JRCSF E153G | >50 | 1.1 | 3.99 | 6.33 | 28.54 | 0.02 | <0.006 |
| LN27 | >50 | 0.59 | 3.66 | 8.2 | 32.87 | >1.56 | >1.56 |
| LN27 E153G | >50 | 0.17 | 0.72 | 5.5 | 28.41 | >1.56 | >1.56 |
| LN8 | 24 | 3.15 | 2.0 | 6.34 | 208.2 | 0.06 | 0.04 |
| LN8 E153G | 24 | 3.77 | 2.82 | 8.09 | 278.1 | >1.56 | >1.56 |
| LN40 | >50 | 1.97 | 2.01 | 2.33 | 42.37 | >1.56 | >1.56 |
| LN40 E153G | >50 | 3.53 | 2.28 | 0.84 | 97.00 | >1.56 | >1.56 |
| FL5-2-209 | >50 | >50 | 0.47 | >50 | 115.50 | >1.56 | >1.56 |
| FL5-2-209 G153E | 37.5 | >50 | 0.38 | >50 | 131.20 | >1.56 | 0.22 |
| 25925-2 cl.22 | 20 | >50 | 0.29 | >50 | 70.00 | 0.04 | 0.01 |
| 25925-2 cl.22 E153G | 23 | >50 | 0.38 | >50 | 125.40 | 0.08 | 0.008 |
| CMT 19S531 | >50 | >50 | 0.68 | 13.22 | 39.00 | 0.06 | 0.01 |
| CMT 19S531 E153G | >50 | >50 | 0.53 | 8.63 | 40.00 | 0.03 | 0.008 |

3-9 DISCUSSION:

Here it is shown that a single amino acid substitution in the V1 loop modulates macrophage tropism for heterologous HIV-1 clade B R5 envelopes. The structures of the V1 and V2 loops have recently been solved bound to the MAb PG9 [48], although their structure in the context of gp120 is still not resolved. Various studies showed that the V1 and V2 loops influenced the binding of CD4bs MAbs, suggesting that these loops may help to shield the CD4bs [149-152]. Electron tomographic structures, including a recent high resolution structure of the trimeric envelope, strongly suggest that the bulk of the V1 and V2 loops sit on the apex of the trimeric envelope and thus some distance from the CD4bs [56]. This data is consistent with a model where the V1 loop determinant affects the ability of the V1/V2 and V3 loops to separate and expose the V3 loop in response to CD4 binding. The increased efficiency of V1/V2 conformational shifts increases the ability of the envelope to exploit low CD4 use for macrophage infection. Moreover, it is striking that V1 residue 153 control of the V3 loop and CD4bs was active for envelopes derived from three of six clade B-infected subjects. This mechanism is thus likely to be operative for up to 50% of clade B envelopes.

Previously several determinants have been reported to have major effects on macrophage infection by HIV-1 R5 envelopes. These include residue 283 in the CD4bs [98, 104, 105] and residues adjacent to the CD4 contact residues on the CD4 binding loop [105]. Thus, macrophage tropism can be modulated by

changes within the CD4bs, which directly impact Env-CD4 affinity, or by changes that indirectly affect the CD4bs, presumably by affecting the exposure CD4 contact residues. The identification of a distal V1 loop determinant that also confers macrophage tropism is striking and indicates that multiple envelope sites may have the potential to modulate macrophage tropism.

The change from a charged glutamic acid (E) to the uncharged glycine could have profound effect on the interaction of the V1 loop with other envelope residues due to the loss of potential ionic bonds. The initial interpretation was that the V1 determinant acted by affecting the exposure of CD4 contact residues. However, shifts in b12 sensitivity (an indicator of CD4bs exposure) following substitutions at residue 153 ranged from extremely minor to modest at best and were substantially smaller than for sCD4 or 447-52D. It is therefore unlikely that the effects of V1 residue 153 result entirely from increased exposure of CD4bs residues. Thus, changes in the capacity of the envelope to react to suboptimal levels of cell surface CD4 (without changes in Env-CD4 affinity) and trigger conformational changes and viral entry should be considered as an alternative mechanism (see below).

For envelopes where the substitution in V1 did not affect tropism, changes in sCD4, b12, and 447-52D sensitivity were noted for some but not other envelopes. For envelopes that showed no change in sCD4, 447-52D, and b12 sensitivity, it is possible that a putative connection between the V1 and V3 loops is absent or held by alternative mechanisms, so that residue 153 substitutions

have no effect on the CD4bs. Alternatively, other determinants that affect the CD4bs and macrophage tropism may simply override any effects conferred by changes in the V1 loop determinant. This latter possibility could apply to the highly macrophage-tropic brain envelope FL5-2-209. This envelope was initially highly sensitive to sCD4, 447-52D, and b12 (IC50s of 0.34, 2.0, and 2.5 µg/ml, respectively) but became substantially more resistant (IC50s of 7.5, 50, and 11.8 µg/ml, respectively) when the G153E substitution was introduced, even though no changes in macrophage infection were observed. It is thus likely that alternative envelope residues confer macrophage infectivity and simply override the effects of V1 residue 153. It is noteworthy that an E153G substitution was previously reported to be responsible for increased syncytium formation following infection of brain microglial cultures by an HIV-1 R5 variant selected *in vitro* [153].

The C98-18 envelope conferred modest levels of macrophage infection compared to the highly macrophage-tropic C98-15 and the non-macrophage-tropic P1114 envelopes. C98-18 carries the D167N substitution in the V2 loop but not the V1 loop E153G substitution present in the C98-15 envelope. An N167D substitution in C98-18 abrogated the modest levels of macrophage infection confirming the role of this residue (Fig. 3-4). In addition, N167D conferred an increase in the sensitivity of C98-18 to sCD4, reducing the IC50 from 26.62 to 8.19 µg/ml, but had no affect on the resistance to the V3 loop MAb, 447-52D (data not shown). These observations are curious since substitutions at

residue 167 had no effect on the macrophage infectivity of the other P1114 envelopes tested. Presumably, there are other determinants in the P1114 envelopes that influence whether residue 167 affects macrophage infectivity. Regardless, the data indicate that V2 residue 167 is only a minor player in the macrophage tropism of the P1114 envelopes.

Other studies have also reported single or limited substitutions in the envelope that affect macrophage infectivity and/or sensitivity to neutralizing antibodies. Lynch et al. reported that the introduction of a leucine residue to replace a highly conserved isoleucine (I309) in the V3 loop of clade C envelopes affected the exposure of the CD4bs and conferred an increase in macrophage replication, although this was modest [154]. Li et al. described an N197Q substitution proximal to V2, which removed a potential N-linked glycosylation site. N197Q conferred increases in sensitivity to sCD4 and b12. However, N197Q also conferred sensitivity to 17b, a CD4i MAb, indicating that more substantial structural changes had resulted that exposed sites involved in coreceptor binding [155]. Similarly, Zhu et al. reported on an adjacent substitution in the β3 strand, T198P [156], while O'Rourke et al. described a D179N substitution in the LDV motif of V2 [150], both of which conferred global neutralization sensitivity and exposure of the 17b epitope. Some of these substitutions may affect the interactions of the gp120 subunits on the trimer, perhaps altering the position of the V1V2 loops, which may interact at the apex. Such changes may also result in triggering some of the conformational changes (usually associated with CD4

binding) and exposure of CD4i epitopes.

Limited changes in the extracellular segment of gp41 have also been shown to affect neutralization sensitivity. Thus, two amino acid changes in the conserved HR1 and MPER regions in gp41 together conferred increased sensitivity to sCD4, broadly neutralizing gp120 and gp41 MAbs, and to human HIV-1+ plasmas [157]. In addition, Shen et al. recently reported an L669S substitution in the MPER that conferred increased sensitivity to a range of gp41- and gp120-specific neutralizing MAbs. Presumably, these substitutions also act indirectly by influencing the arrangement of gp120 subunits on the trimer and thus affecting their sensitivity to neutralizing antibodies.

In contrast, the effects of V1 residue 153 are unique in conferring large shifts in envelope phenotype that are focused specifically on the V3 loop and CD4bs without major effects on other envelope sites, as estimated by envelope sensitivity to inhibitors that targeted other stages of HIV-1 entry, including Env-CCR5 interactions and gp41 conformational changes. All envelopes also remained relatively resistant to 17b, a CD4i MAb, indicating that extensive conformational changes that are usually associated with gp120-CD4 interactions had not occurred. The changes affected by V1 residue 153 thus contrast with the substitutions described above [155].

These experiments indicate that V1 residue 153 has strong affects on the V3 loop and the CD4bs, which are only partially explained by increased exposure of the CD4 contact residues. On the unliganded envelope, the V3 loop may sit

close enough to restrict the approach of both CD4 and b12 [56, 158]. It is also tempting to envisage that residue 153 modulates a gp120 conformational change to form a structure intermediate between the unliganded envelope and the CD4-bound form. Such an intermediate may be partially activated for the conformational changes required to form the coreceptor binding site and thus require less robust interactions with fewer CD4 molecules to trigger fusion and infection.

The determinant at residue 153 is proximal to sites in the V2 loop that are targeted by the recently described MAbs PG9 and PG16 [148]. In fact, these MAbs target determinants in both the V2 and the V3 loops. Since the substitutions in V1 described here also had effects on the V3 loop, we hypothesized that they would impact on PG9 and PG16 sensitivity. However, only modest effects on PG9 and PG16 sensitivity were observed. This result illustrates the robustness of the PG9 and PG16 epitope and is a reassuring result for vaccine research focused on this epitope.

The conservation of glutamic acid at position 153 across several clades is intriguing and implies that this site plays an important functional role for the envelope. Our data suggest that this role may involve an interaction with the V3 loop, which in turn affects the CD4bs. The decreased frequency of E153 among clade D and CRF02_AG envelopes and increased frequency of G153 is also striking. Whether the higher prevalence of G153 associates with increased macrophage tropism for these clades is not known. An E153G substitution for

the one clade D envelope that we tested (CMT19 S531) had no affect on macrophage tropism. However, a more extensive survey would be needed to establish an influence of G153 on clade D or CRF02_AG envelopes. Of note, clade D viruses have been associated with faster disease progression [159-163] and a higher frequency of dementia [164]. In addition, CXCR4 tropism was common among CRF02_AG viruses, a sign of a mature HIV epidemic [165]. It is thus possible that an increased frequency of G153 may simply reflect a high prevalence of R5 viruses with increased macrophage tropism late in disease [99-101].

In summary, we describe here a novel determinant in the V1 loop that modulates macrophage tropism conferred by HIV-1 R5 envelopes. The V1 loop determinant appears to control the exposure or conformation of the V3 loop, which in turn impacts the CD4bs to prime the envelope for low CD4 use and macrophage infection. Our data also have relevance for vaccines that aim to target the CD4bs.

# CHAPTER IV: HIV-1 R5 NON-MAC TROPIC ENVELOPE GLYCOPROTEINS DO NOT CONFER AN ENHANCED TROPISM FOR CD4+ T-CELLS COMPARED TO HIGHLY MAC-TROPIC VARIANTS

## 4-1 Introduction:

As described previously, HIV-1 viruses using CCR5 (R5) vary extensively in their ability to infect macrophages. Thus, macrophage-tropic R5 strains can exploit the low levels of cell surface CD4 on macrophages for infection, while non-macrophage-tropic R5 viruses that require high levels of CD4 for infection cannot [91, 98, 104]. Determinants that modulate R5 macrophage infection have been mapped to residues within or proximal to the CD4 binding site of gp120 [104, 105, 139], although as described previously here, a single substitution of a conserved V1 loop residue modulated macrophage infection for some but not all R5 envelopes [166]. Amongst R5 viruses, non-macrophage tropic strains comprise the majority of transmitted or founder viruses [117, 119, 167] and are predominant in immune tissue [91, 98] even at late stages of disease when macrophage-tropic variants can be increasingly detected in blood [99-101] or brain tissue [108]. Nonetheless, the contribution of non-macrophage-tropic and macrophage-tropic R5 viruses to HIV-1 pathogenesis of HIV-1 remains relatively uncharacterized.

The predominance of non-macrophage-tropic R5 viruses in immune tissue strongly indicates that they have a selective advantage over more macrophage-

tropic variants. However, it is unclear how this advantage is manifested. Previous data by Peters PJ et al. showed that infection of primary CD4+ T-cells conferred by macrophage-tropic R5 envelopes was at least as efficient as for non-macrophage-tropic counterparts [168], while both mac-tropic and non-mac-tropic Env+ pseudovirions show similar sensitivities to heterologous HIV-1+ human sera (P. Clapham, unpublished observations) [157].

Like macrophages, mDCs express only low levels of CD4 [127]. Nonetheless, immature mDCs were reported to be more susceptible to infection compared to mature DCs [123, 169], even though they express SAMHD1, a post-entry HIV-1 restriction also expressed in monocytes [73, 74]. Myeloid DCs also efficiently capture HIV via interactions with DC-SIGN or other mechanisms [170, 171] and then present and transfer infectious virus particles to CD4+ T-cells during DC: T-cell interactions [172-174]. Nevertheless, the role of myeloid dendritic cell (mDC) infection and trans-infection in HIV-1 disease progression is unclear.

Here, I investigated whether mDCs are sensitive to infection mediated by pseudovirions carrying macrophage-tropic and non-macrophage-tropic R5 envelopes (including early founder and acute stage envelopes [116]) and whether they influence infection of CD4+ T-cell infection via their capacity to present virions via cis and trans mechanisms. It is demonstrated here that only mac-tropic envelopes could confer infection of MDDCs. In contrast, efficient infection of CD4+ T-cells was observed for both mac-tropic and non-mac-tropic

R5 Envs, although infection by mac-tropic Envs was consistently highest. Nevertheless, equivalent infection of CD4+ T-cells by non-mac-tropic and mac-tropic R5 envelopes was observed if virions were first captured and presented by mDCs. The data presented here reveal that trans-infection (via capture and presentation of virions by DCs) is critical in conferring maximal infection of CD4+ T-cells by non-macrophage-tropic R5 viruses and is likely to be an important mechanism for such strains to become predominant in immune tissue.

## Results

### 4-2 Envelope+ pseudovirions for infectivity assays:

A panel of well-characterized Envs was selected for investigation (Table 4-1). The panel included Env pairs from separate individual subjects, where each pair comprised one that is highly mac-tropic and one that is non-mac-tropic. Several of these individuals were AIDS subjects with dementia or other neurological complications [108]. One set of Envs was derived from a chronic stage pediatric patient described in the previous chapter. P43 Envs were derived from the blood and semen of an acutely infected individual. Most of these Envs have been previously characterized with respect to their macrophage tropism and were selected for this study based upon these previous results [97]. Env+ pseudovirions carrying a GFP reporter gene were prepared for infectivity assays described throughout this study. We first tested for infection of HeLa TZM-bl cells and macrophages. GFP+ cells were counted 2 days post-

Table 4-1 Patients and Envelopes used in pseudotype studies.

| Patient | Disease Stage | Macrophage Tropic | | | Non-Macrophage Tropic | | |
|---|---|---|---|---|---|---|---|
| | | Env | Env Origin | Envelope ID | Env | Env Origin | Envelope ID |
| Chronic Stage Patients | | | | | | | |
| NA20 | AIDS | B59 | Brain | | LN8 | Lymph Node | |
| | | B501 | Brain | | | | |
| NA420 | AIDS | B33 | Brain | | LN40 | Lymph Node | |
| JR | AIDS | JRFL | Frontal Lobe | | JRCSF | Cerebrospinal fluid | |
| P1114 | AIDS | 98-15 | Plasma | | 98-27 | Plasma | |
| P7766 | AIDS | FL19 | Frontal Lobe | 7766 FL19-56-66 | SP13 | Spleen | 7766 SP13-33-41 |
| P10017 | AIDS | FL1 | Frontal Lobe | 10017 FL9-1-2 | SP2 | Spleen | 10017 SP10-9-65 |
| P6568 | AIDS | FL1 | Frontal Lobe | 6568 FL11-1-249 | SP1 | Spleen | 6568 SP6-11-9 |
| CA110 | AIDS | OC1 | Occipital Lobe | CA110 OC58-11-57 | SP4 | Spleen | CA110 SP52-22-83 |
| Acute Stage Patients | | | | | | | |
| P43 | Acute | 380.4 | Semen | | 378.2 | Blood | |
| Transmitted/Founder Sequences | | | | | | | |
| 3T | Acute | | | | 3T | Plasma | p1054.TC4.1499 |
| 6T | Acute | | | | 6T | Plasma | p63358.p3.4013 |
| 15T | Acute | | | | 15T | Plasma | p700010040.C9.4520 |
| 19T | Acute | | | | 19T | Plasma | pPRB958_06.TB1.4305 |

infection for HeLa TZM-bl cells and 5-7 days post infection for macrophages.

Both non-mac-tropic and mac-tropic Envs conferred strong infection of HeLa TZM-bl cells that express high levels of CD4 and CCR5. Nonetheless, mac-tropic Envs consistently conferred between 5- and 20-fold higher infectivity titers compared to non-mac-tropic Envs (Fig. 4-1 A). All of those envelopes previously determined to be mac-tropic conferred high levels of infectivity for macrophages as expected with infectivity titers that were several orders of magnitude higher than for non-mac-tropic Envs (Fig. 4-1 B). Since the non-macrophage-tropic R5 Envs conferred lower levels of infectivity on HeLa TZM-bl cells that carry high levels of CD4 and CCR5 [134], we measured the levels of gp120 present in each pseudovirion preparations to establish whether differences in envelope expression levels might be an explanation. However, no significant difference between the amount of gp120 present in non-mac-tropic and mac-tropic Env+ pseudovirion preparations was detected (Fig. 4-1 C).

4-3 Infection of MDDCs by pseudovirions expressing mac-tropic and non-mac-tropic R5 Envs:

We next investigated whether myeloid dendritic cells (mDCs) were susceptible to infection by mac-tropic or non-mac-tropic Env+ pseudovirions. mDCs are derived from the same cell lineage as monocytes and macrophages and like macrophages, express low levels of CD4 [127]. However, CCR5

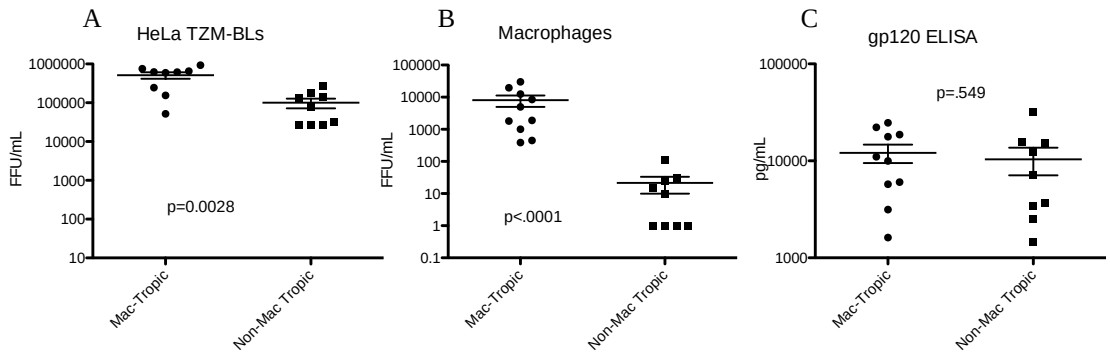



FIG. 4-1. Envelopes selected from the same patient differ significantly in their ability to infect macrophages. R5 envelopes were amplified from the patients detailed in table 3 and used to generate GFP reporter env+ pseudovirions. (A) The macrophage tropic isolates had slightly higher infectivity on HeLa TZM-bl cells than the non-macrophage tropic isolates. All were able to significantly infect the TZM-bls. (B) Mac-tropic isolates infect monocyte-derived macrophages around 1000-fold better than the non-mac-tropic isolates. These are mean values from three separate donors. (C) There is no significant difference between the amount of gp120 detected in the viral supernatants between mac and non-mac tropic env+ pseudovirions.

expression on mDCs was also reported to be low [141, 175]. Myeloid DCs express SAMDH1, an HIV restriction factor that is also expressed in monocytes [73, 74]. The low levels of CD4 and CCR5, as well as SAMHD1 expression has led to a consensus view that these cells are relatively insensitive to HIV-1 infection *in vivo*. In this study, we prepared mDCs from blood monocytes using standard (IL-4, GM-CSF) differentiation protocols [127]. These monocyte-derived dendritic cells express DC-SIGN [128]. Flow cytometry analysis showed that MDDCs expressed CD11c, an integrin marker for DCs, but were around 90% negative for the monocyte marker CD14 (Fig. 4-2 A). The dendritic cell specific maturation marker CD83 shows that the LPS-matured DCs are distinct from the immature MDDCs. There is a small amount of CD4 expression on these MDDCs, consistent with what has been previously reported (Fig. 4-2 B). The levels of CCR5 are very low, while there is a slightly higher level of CXCR4 expression on these cells. In addition, the morphology of MDDCs by light microscopy was typical for DCs, and they immunostain positive for CD11c (Fig. 4-2 C).

We tested infection of MDDCs with our panel of GFP-reporter Env+ pseudovirions. MDDCs were susceptible to mac-tropic Envs (with the exception of 98-15) (Fig. 4-3), but were approximately 10-20 times less sensitive compared to macrophages. Moreover, all of the non-mac tropic Envs including the founder/acute stage envelopes conferred background or very weak infection of

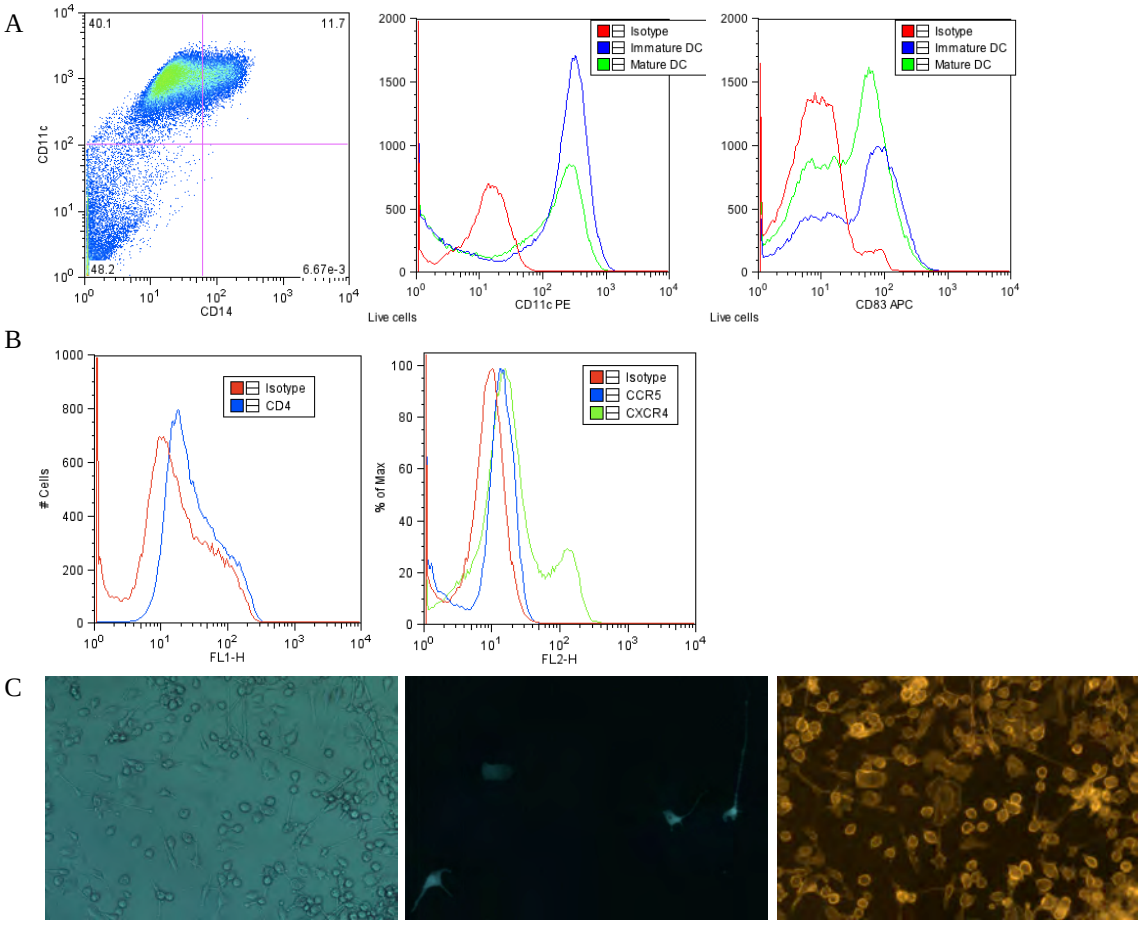



FIG. 4-2. Characterization of MDDCs. Monocytes were differentiated for 7 days in GMCSF + IL-4. MDDCs were stained and analyzed by flow cytometry. (A) The majority of the cells express CD11c, while 11.7% still express CD14. Autologous LPS-matured DCs were stained for CD11c and CD83. (B) MDDCs express low amounts of CD4, and low amounts of CCR5. They do express slightly higher amounts of CXCR4 than CCR5. (C) From left to right, phase contrast, MDDCs infected with JRFL GFP-reporter Env+ pseudovirions, and the same MDDCs immunostained with CD11c.

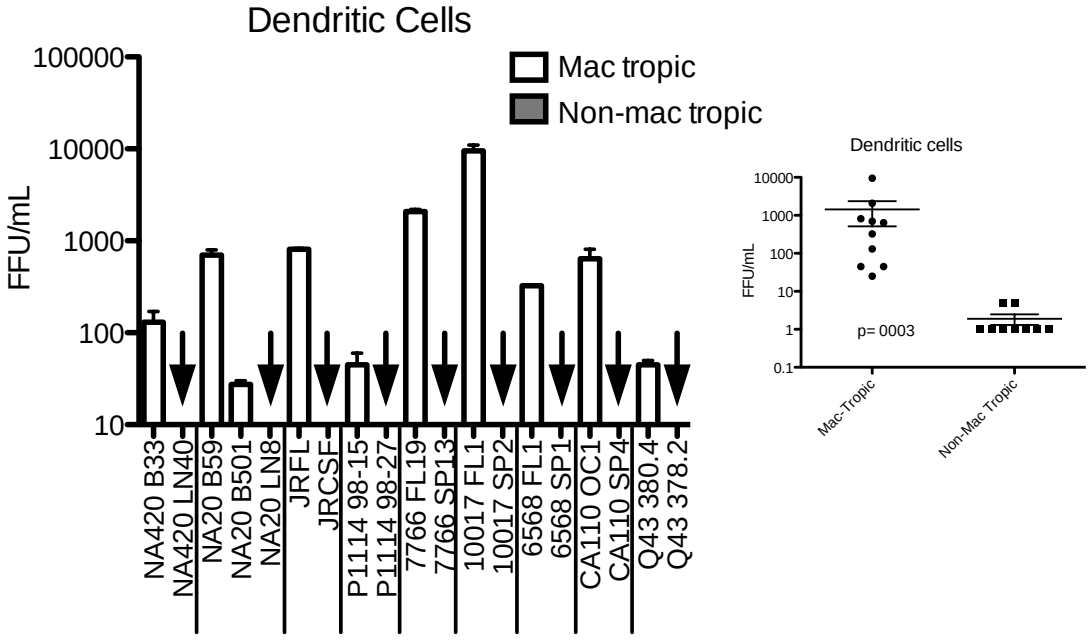



FIG. 4-3. Infection of MDDCs by pseudovirions expressing mac-tropic and non-mac-tropic R5 envelopes. Monocytes were differentiated for 7 days in GMCSF + IL-4. Env+ pseudovirions were used to infect MDDCs and infection was quantified 5-7 days post infection. On the left are the titers of the Envs used. On the right is a comparison between mac-tropic and non-mac tropic Envs on MDDCs. Only Mac-tropic Envs are capable of infecting MDDCs. These are mean values from three separate donors. P values were generated using the Mann-Whitney T-test.

the MDDCs, with only JRCSF and 7766 SP envelopes conferring detectable infection (not shown in Fig. 4-3).

4-4 LPS matured MDDCs support infection by mac-tropic but not non-mac-tropic Env+ pseudovirions:

Previous studies showed that immature DCs are more sensitive to infection than mature or activated DCs [123]. To determine if the maturation state of MDDCs plays a role in infection by mac-tropic and non-mac tropic Envs, we prepared MDDCs treated with 100 ng/ml of LPS. These matured MDDCs expressed significant levels of CD83 (46.9% positive for CD83, MFI 64.4), a marker of myeloid DC maturation, and formed a distinctive morphology by light microscopy (Fig. 4-2 A). However, the MDDCs not treated with LPS used in Fig. 4-2 also expressed significant levels of CD83 (51.2% positive for CD83, MFI 76.1). These non-LPS treated MDDCs may have been inadvertantly subjected to stimuli caused maturation. Other donors have shown 9.9% of non-LPS treated MDDCs positive for CD83 (MFI of 26.7). Further flow cytometry analysis will need to be done to definitively demonstrate a maturation difference upon LPS treatment, although a morphological difference was apparent in all donors. I then infected these LPS treated MDDCs alongside autologous non-LPS treated MDDCs shown in Fig. 4-4. Mac-tropic R5 Envs conferred similar levels of infection on LPS treated and untreated DCs. Infection of both populations of MDDCs by pseudovirions carrying non-mac-tropic Envs was either at background levels or extremely weak.

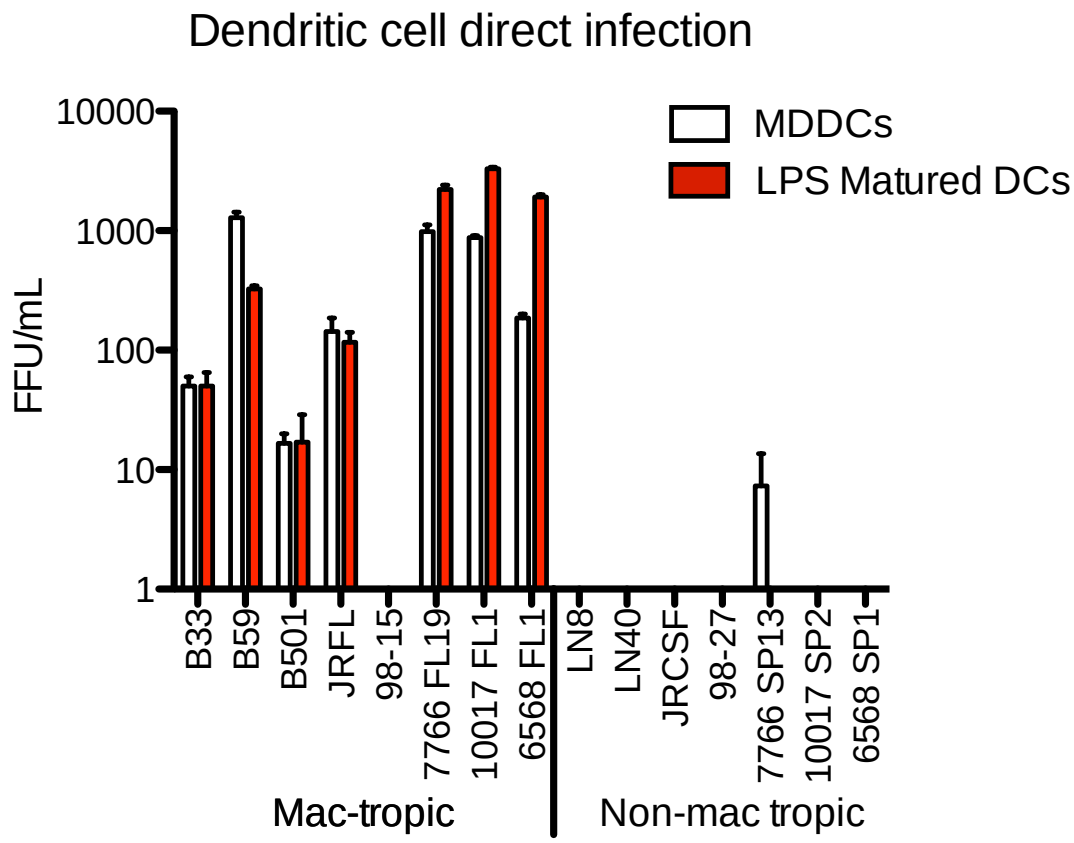



FIG. 4-4. Mac tropic Envs infect both mature and immature MDDCs. Monocytes were differentiated for 7 days in GMCSF + IL-4, and either allowed to remain naïve or treated with LPS. Env+ pseudovirions were used to infect MDDCs and infection was quantified 5-7 days post infection. On the left are the mac-tropic Envs, and on the right are the non-mac tropic Envs. Only Mac-tropic Envs are capable of infecting MDDCs, and they infect both mature and immature MDDCs similarly. These are mean values from two separate donors.

4-5 Infection of PBMCs and CD4+ T-cells by mac-tropic and non-mac-tropic R5 Env+ pseudovirions:

Non-mac-tropic R5 variants are predominant in immune tissue, where CD4+ T-cells expressing high levels of CD4 are presumably targeted [98]. I next investigated whether non-mac-tropic R5 Envs have an increased ability to confer infection of PBMCs or CD4+ T-cells infection compared to mac-tropic Envs. PBMCs and autologous purified CD4+ T-cells obtained from three separate donors were stimulated with PHA and IL-2 and tested for their sensitivity to infection 2 days later. Both mac-tropic and non-mac-tropic R5 Envs conferred efficient infection of both PBMCs and CD4+ T-cells. However, mac-tropic R5 Envs displayed a trend to higher infectivity titers for both cell cultures compared to non-mac-tropic R5 Envs (Fig. 4-5). Although results for individual donors were not significant, they become highly significant if all the data are combined across donors ($p<0.0001$). This result confirms previous observations made with just three mac-tropic and 3 non-mac-tropic R5 envelopes in the context of replication competent viruses [98].

4-6 MDDCs enable efficient T-cell infection by non-mac-tropic envelopes:

DCs capture HIV-1 particles and present them to CD4+ T-cells, thereby increasing the efficiency of infection via virological synapses [172, 176, 177]. It was first evaluated whether non-macrophage-tropic or macrophage-tropic Envs were preferentially transmitted by MDDCs, and whether MDDCs that were first treated with Env+ pseudovirions could confer efficient infection of autologous

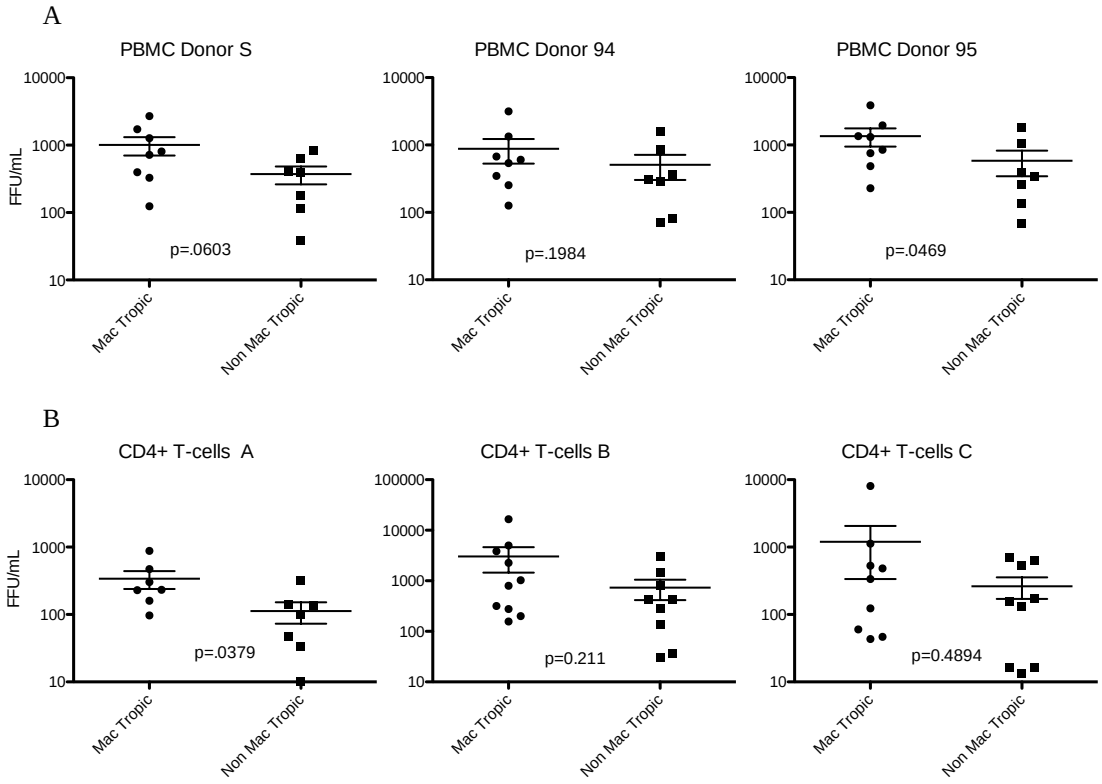



FIG. 4-5. Macrophage tropic envelopes trend to better directly infect PBMCs and CD4+ T-cells. 100µL of GFP-reporter viral supernatant was titrated on PBMCs or enriched CD4+ T-cells, and infectivity was quantified 2dpi. (A) Three separate donors were used to generate PBMC cultures. Macrophage tropic Envs infected PBMCs slightly better than their non-macrophage tropic counterparts. (B) The same trend of mac-tropic Envs better infecting PBMCs is seen using CD4+ T-cells with three separate donors. P values were generated using the Mann-Whitney T-test.

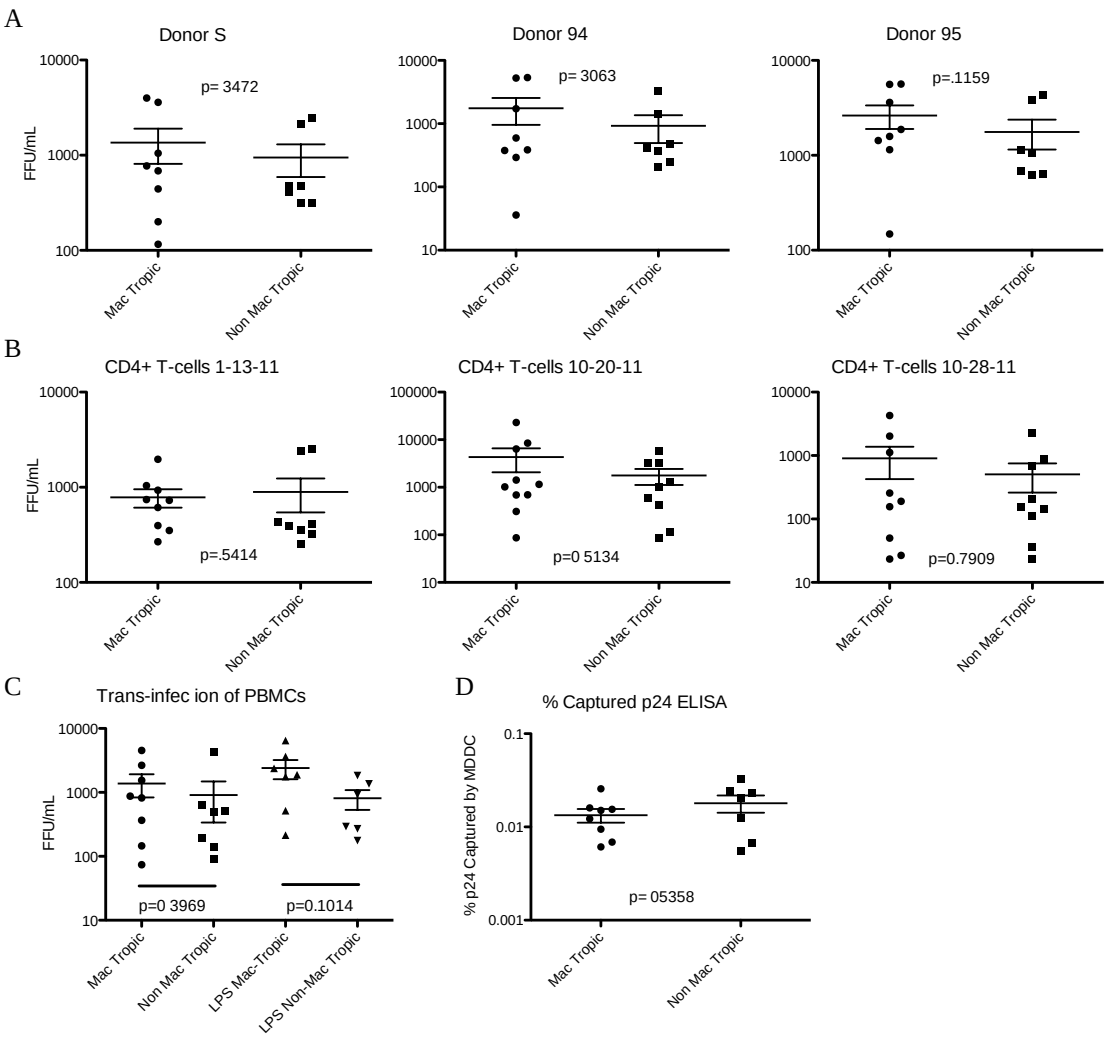



FIG. 4-6. Trans-infection of CD4+ T-cells is as efficient for non-macrophage tropic envs as for macrophage tropic envs. 100µL of GFP-reporter viral supernatant was incubated with MDDCs for 1 hr. The MDDCs were then washed twice and co-cultured with autologous target cells, which were assayed for infectivity 2 days post co-culture. (A) Three separate donors were used to generate PBMC cultures. Non-macrophage tropic Envs infected PBMCs as well as macrophage tropic Envs. (B) CD4+ T-cells from three separate donors were as readily infected by non-macrophage tropic Envs as by mac-tropic Envs. (C) Trans-infection of PBMCs was done using LPS-matured MDDCs. (D) To determine the amount of virus captured, MDDCs were incubated with viral supernatant for 1 hr, then washed twice and used in a p24 ELISA. No significant differences were observed. P values were generated using the Mann-Whitney T-test.

PBMCs or CD4+ T-cells. MDDCs were treated with virus for one hour before washing and co-culturing with autologous PHA, IL-2 stimulated PBMCs or CD4+ T-cells. In this infectivity system both mac-tropic and non-mac-tropic Envs conferred efficient and similar (not significantly different) infection of both PBMCs and CD4+ T-cells (Fig. 4-6 A-B). To determine if the maturation state of the MDDCs played a significant role in this trans infection, MDDCs were first activated with LPS, and used in the same experiment. These matured MDDCs yielded similar levels of trans-infection as those not treated with LPS (Fig. 4-6 C).

In order to address whether or not mac-tropism effects virion capture by the MDDCs, and thereby has an effect on the subsequent transmission of the captured virions, the amount of virus that had bound was then estimated by measuring the amount of cell associated p24 antigen by ELISA. MDDCs were incubated with virus and washed as before. There was no significant difference in the amount of p24 bound to MDDCs between macrophage-tropic and non-macrophage-tropic Env+ pseudovirions (Fig. 4-6 D). Env- controls were used in this assay for each variant used, and generally exhibited an 8-fold decrease in virus capture by MDDCs (results not shown).

4-7 The effect of $\alpha 4\beta 7$-integrin expression on CD4+ T-cells:

Recently, there has been a focus on the potential importance of $\alpha 4\beta 7$-integrin in the establishment of HIV-1 infection and the initial steps involved in

transmission. The α4β7 integrin has been shown to play a role in gut-homing for CD4+ T-cells [178, 179], as well as localizing on the cell surface with both CD4 and CCR5 [180], and is known to be present in abundance in the genital mucosa on CD4+ T-cells [181]. α4β7 interacts directly with HIV-1 gp120 via a conserved LDV motif found in the V2 loop [182]. Taken together, this implies there may be a significant role for α4β7 and its interaction with gp120 in determining which HIV-1 variants are preferentially spread across the genital mucosa and the gut wall. The distinct conformation of mac tropic and non-mac tropic Envs, in addition to their differing interactions with CD4, could lead to divergent requirements for their interactions with α4β7. First, using Jurkat cells, a leukemic T-cell line [183], which express CD4, CCR5, CXCR4, α4, and are either β7+ or β7-, these cells were infected by the macrophage tropic and non-macrophage tropic Env+ GFP reporter pseudovirions using the *env*s detailed in table 4.1. The macrophage tropic Envs infected both α4β7+ and α4β7- Jurkat cells better than non-macrophage tropic Envs (Fig. 4-7). When comparing overall infectivity of the α4β7+ and α4β7- cells, there was no difference between them, indicating that α4β7 may not play a role in Env tropism in this *in vitro* system.

The Jurkat cells may not adequately represent how these Envs infect primary human cells. To test this, α4β7+ and α4β7- CD4+ T-cells populations were isolated and infected directly with Env+ GFP-reporter pseudovirions. Two days post infection, GFP positive cells were quantified. α4β7+ and α4β7- CD4+

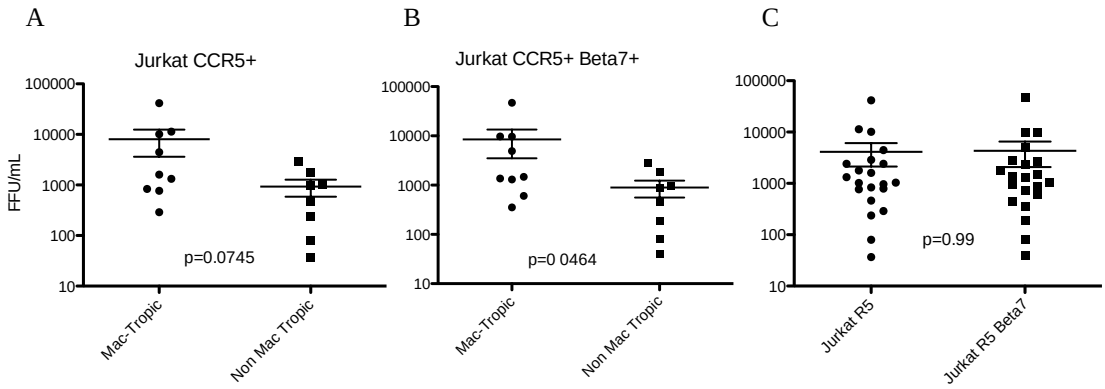



FIG. 4-7. Macrophage tropic Envs infect Jurkat cells more efficiently than non-macrophage tropic Envs. R5 Envs described previously were used to generate GFP reporter pseudovirions. These were then titrated onto a T-cell line, Jurkat E6-1 cells. (A) Jurkat cells expressing CD4 and CCR5 were used to titrate pseudovirus. 2 days post infection, infectivity was quantified. Mac-tropic Envs infected these cells more efficiently than non-mac tropic Envs. (B) Jurkat cells expressing α4β7 in addition to CD4 and CCR5 were used in the same assay as in (A), and the same result was achieved. (C) There is no difference in sensitivity between Jurkat cells that express and do not express α4β7 with the envelopes used in this study. P values were generated using the Mann-Whitney T-test.

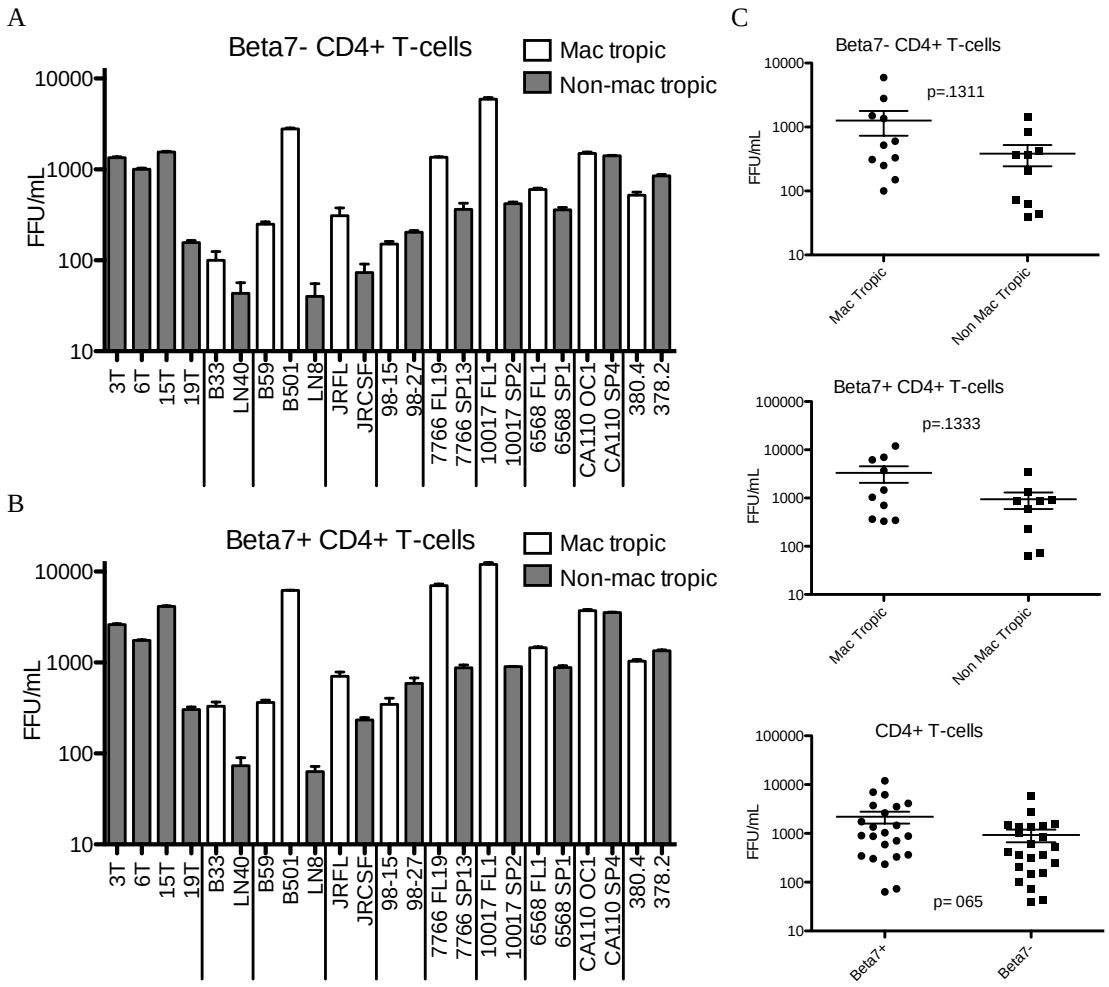


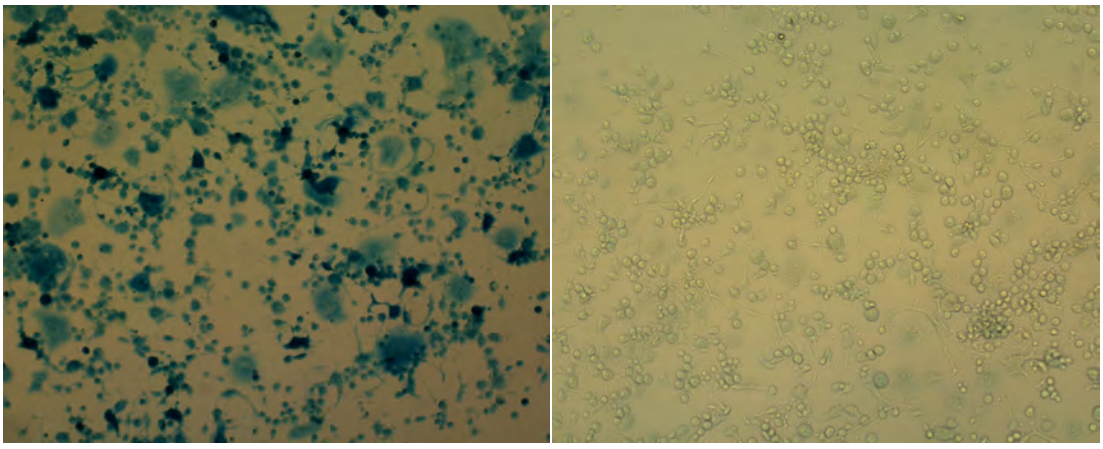

FIG. 4-8. Infection of β7+ CD4+ T-cells by macrophage tropic and non-macrophage tropic envs. 100µL of GFP-reporter viral supernatant titrated on β7+ CD4+ T-cells sorted by flow cytommetry and infectivity was quantified 2dpi. (A) No significant difference was observed between macrophage tropic and non-macrophage tropic envs on β7- CD4+ T-cells. The four transmitted-founder envs used infected the sorted cells efficiently. (B) β7+ CD4+ T-cells were similarly infected regardless of macrophage tropism. (C) The same pattern of infectivity is observed on β7- and β7+ CD4+ T-cells between mac and non-mac tropic envs, although β7+ cells were more susceptible to infection. Data presented here is from a single experiment, representative of several conducted. P values were generated using the Mann-Whitney T-test.

T-cells were both efficiently infected by both mac and non-mac tropic envelopes (Fig. 4-8 A-B), and no significant difference is seen with respect to macrophage tropism. Mac-tropic Envs confer higher infection on CD4+ T-cells compared to non-mac tropic Envs regardless of the presence of the integrin $\alpha 4\beta 7$ (Fig. 4-8 C). Overall, $\beta 7$+ cells were more susceptible to infection than $\beta 7$- cells (Fig. 4-8 C), as has been reported previously [180].

4-8 The innate immune response of MDDCs does not affect trans infection of CD4+ T-cells by non-mac tropic Envs

Non-macrophage tropic R5 envelopes are frequently predominant in immune tissue late in disease [91, 98, 108]. However the data presented here do not show that they carry a replicative advantage for CD4+ T-cells. It has been suggested that HIV-1 avoids infection of mDCs so as to not induce an innate immune response, such as type I interferon release. Here, it was evaluated whether highly mac-tropic envelopes induced type I interferon production following infection of MDDCs. MDDCs are readily infected, whether they are mature or immature, by highly mac-tropic Env+ pseudovirions. Pseudovirions do not produce a viable infection (they do not code for Env and carry T-cell line adapted HIV-1 genes), and they may interact with the DC in a different manner from replication competent viruses, potentially stimulating different innate immune responses. In the context of an *in vivo* infection, the DCs may also generate a significant immune response that may affect their ability to transmit virus to other target cells. To address this, replication competent virus was used

A



B

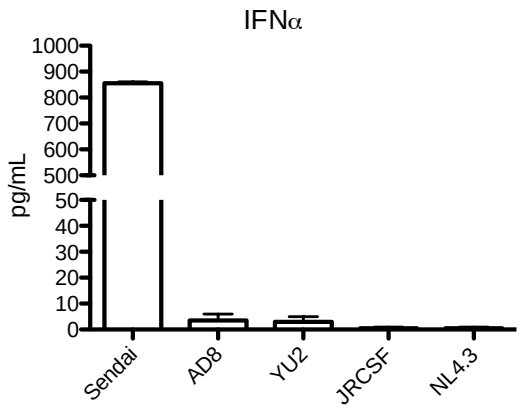



FIG. 4-9. Infection of MDDCs with replication competent virus does not induce IFN production. Replication competent full-length molecular clones were pseudotyped with VSV-G, and used to directly infect MDDCs. The supernatant from these infected MDDCs was measured by ELISA for IFNα. (A) On the left are MDDCs infected with VSV-G AD8 and on the right are mock-infected MDDCs. Both were immunostained for p24. (B) The supernatant from these infected MDDCs was measured by ELISA for IFNα, 4 days post infection. AD8 and YU2 supernatants were at the very bottom of the limit of detection, while JRCSF and NL4.3 supernatants had no detectable IFNα. Data shown are from a single experiment representative of several.

to directly infect MDDCs. These full-length molecular clones were also pseudotyped with VSV-G protein in order to facilitate maximum virus entry. Four days post infection, cellular supernatant was used from these infected MDDCs to measure type I IFN. There was virtually no type I IFN response in the infected MDDCs (Fig. 4-9).

4-9 DISCUSSION:

Non mac-tropic R5 viruses predominate in immune tissue, even in late stage disease of HAD subjects, when highly mac-tropic variants can be found in the brain [108]. These non-mac tropic viruses must have some advantage that allows them to predominate in immune tissue, or conversely there may be a disadvantage to mac-tropic viruses in immune tissue. In the *in vitro* experiments conducted here with mac-tropic and non-mac tropic Envs, the mac-tropic Envs directly infected DCs and CD4+ T-cells better than the non-mac tropic Envs, and are at least equivalent when trans-infected via MDDCs. I also evaluated whether or not the integrin $\alpha 4\beta 7$ conferred an advantage for non-mac tropic Envs. Although $\beta 7$+ cells were more susceptible to infection than $\beta 7$-, there was no advantage for non-mac tropic viruses using this integrin. In fact, none of these assays demonstrated an advantage for non-mac tropic Envs. Yet mac-tropic virus does not seem to predominate in immune tissue throughout the majority of infection, nor is it preferentially transmitted. Given that these experiments demonstrate that macrophage tropic Envs facilitate more efficient infection of dendritic cells, it is possible that they may stimulate a greater immune response,

acting to curtail their replication in CD4+ T-cells. At least for type I interferon, this does not appear to be the case.

It has been shown before that dendritic cells can be infected by HIV-1, and that immature DCs were significantly more susceptible to infection than mature DCs [123, 169]. The immature MDDCs used in this study were susceptible to infection, but only by R5 Envs, similar to what has been observed previously with macrophages [91, 97, 98, 168]. The isolate most often used in DC infection assays reported previously was BaL [121, 184], which is known to be a highly mac-tropic Env. The non-mac tropic R5 Envs tested here most likely were unable to utilize the low CD4 levels of these MDDCs, indicating that the term macrophage tropism can also refer to dendritic cells. The DCs were significantly less susceptible to infection than were macrophages, possibly due to the post-entry restriction of SAMHD1 and APOBEC3A [73, 74, 185], which were not alleviated in these assays.

Mature dendritic cells have been generally regarded as refractory to infection with HIV-1, but here they were as susceptible to infection by mac-tropic strains as are immature MDDCs. During the course of HIV infection, increased bacterial translocation across the intestinal lumen occurs, thereby increasing plasma LPS levels [186]. This can potentially cause systemic activation of DCs, which might indicate that HIV-1 has a greater chance of interacting with mature DCs over the course of infection than immature DCs. A previous study demonstrated that mature DCs were more resistant to viral fusion [187], however

this study utilized lab adapted virus. Had mac-tropic primary isolated virus been used, the ability to exploit the very low levels of receptor and co-receptor may have overcame this fusion resistance.

In addition to infecting DCs, mac-tropic Envs are able to infect CD4+ T-cells very efficiently. Previously, CXCR4 tropic viruses were described as T-tropic viruses, until it was shown that CCR5 using viruses can readily infect these cells [87, 88]. The non-mac tropic Envs infect CD4+ T-cells efficiently, which is expected since these were derived from immune tissue, where CD4+ T-cells are major targets for infection. The mac-tropic Envs are derived from mostly neural sites and are adapted to utilize the low levels of CD4 found on myeloid cells. CD4+ T-cells express significantly higher levels of CD4 on the cellular surface than do macrophages or dendritic cells, which might explain why they are more efficiently infected by these mac-tropic isolates that use CD4 more proficiently than non-mac tropic isolates.

Mac-tropic Envs infected CD4+ T-cells more efficiently than non-mac tropic Envs, however in the context of a trans-infection, both types of Envs infected CD4+ T-cells similarly. Trans infection of CD4+ T-cells facilitated by DCs has been described, stemming from the transfer of virions internalized by the DC [177]. These mac tropic Envs interact more efficiently with CD4, which could act to either bind the virus to the DC more efficiently or more effectively present the virus at the site of infectious synapse formation. However, this interaction with CD4 by mac tropic viruses did not result in more virus particles

(measured by p24) being captured (Fig. 4-6 D), consistent with alternative mechanisms of HIV-1 attachment, such as DC-SIGN and others [188, 189].

Recently, it has been suggested that a subset of CD4+ T-cells expressing the integrin $\alpha4\beta7$ could play an important role in HIV-1 infections. Mac-tropic Envs infected this subset of T-cells more efficiently than non-mac tropic Envs, which was no different from CD4+ T-cells unsorted for $\alpha4\beta7$ expression. Overall, the $\alpha4\beta7$+ T-cell subset was more efficiently infected, which is in accordance with previous findings [180], regardless of macrophage tropism. The determinants for gp120 interaction with $\alpha4\beta7$ may not be related to determinants for macrophage tropism. In light of recent findings regarding the role of transmitted founder variants and $\alpha4\beta7$ [190], several non-mac tropic T/F Envs were used to infect $\alpha4\beta7$+ CD4+ T-cells, and exhibited the same infectivity pattern as the other mac and non-mac Envs used. Overall, this data suggests that interaction of gp120 with $\alpha4\beta7$ is distinct from macrophage tropism.

In light of macrophage tropic isolates ability to exploit the low levels of CD4 on DCs for infection, these mac-tropic Envs may stimulate a greater innate immune response than non-mac-tropic Envs. Using replication competent, VSVG-pseudotyped virus, virtually no type-I IFN response was generated by infected MDDCs. A very slight response can be detected in the case of mac-tropic viruses, but most likely does not account for the ability of non-macrophage tropic virus to persist in immune tissue during the course of disease progression.

In summary, macrophage tropic Envs are able to facilitate infection of both mature and immature MDDCs, due to their ability to exploit low levels of CD4 for infection. CD4+ T-cells are also more efficiently infected by mac tropic envelopes, most likely due to their efficient interaction with CD4 as well, without preferential use of $\alpha 4\beta 7$. Together, these data show that mac tropic Envs confer more efficient (or equivalent) infection of all CD4+ cell types tested. The predominance of non-mac tropic R5 Envs in immune tissue is therefore not due to an enhanced tropism for DCs or CD4+ T-cells present there.

# CHAPTER V: DISCUSSION

## 5-1 Summary of Results:

My data demonstrate that a conserved determinant in the V1 loop of gp120 can modulate macrophage tropism. Making the glutamate to glycine change at position 153 can impart the ability to utilize low levels of CD4 for infection and macrophage tropism. This change can also cause an enhanced sensitivity to the V3 mAb 447-52D, as well as sCD4. Taken together, these data indicate that E153G causes a shift in the position of the V3 loop that in turn facilitates an enhanced ability to undergo conformational changes upon interacting with CD4. This greater propensity or flexibility for undergoing the conformational change to the CD4-bound form of gp120 enables these envelopes to utilize the low levels of CD4 on macrophages for infection.

The ability to utilize low levels of CD4 for infection of macrophages was also shown here to expand to mDCs, regardless of their maturation state. Macrophage tropism extends to other myeloid lineage cells, while non-macrophage tropic Envs do not have an increased tropism for CD4+ T-cells compared to mac-tropic Envs. Non-macrophage tropic Envs, largely isolated from immune tissue, infect CD4+ T-cells directly less efficiently than mac-tropic Envs. MDDCs transmission to CD4+ T-cells occurs irrespective of the degree of macrophage tropism, demonstrated by the transmission experiments presented here. Jurkat cells and CD4+ T-cells expressing $\alpha 4\beta 7$ are more permissive to infection, but macrophage tropism does not affect the interaction of gp120 with

$\alpha 4\beta 7$. Lastly, mac-topic viruses do not stimulate a significant innate immune response via DCs. Taken together, these data indicate there is no significant beneficial advantage to non-mac tropic Envs over mac-tropic Envs with respect to CD4+ T-cell infectivity and innate immune stimulation.

5-2 Residue 153 and Tropism:

Determinants have been identified for R5 macrophage tropism in a number of regions of gp120 [98, 104, 105], but not before in the V1 loop. It is striking that in several heterologous *env*s residue 153 is able to drastically alter sensitivity to a V3-loop antibody and sCD4, as well as the ability to exploit low levels of CD4 for infection. From a structural perspective, this makes sense in light of recent structural determinations of the V1/V2 loops [48]. Several studies indicate that V1/V2 shields and interacts with V3 [151, 191]. The presence of a charged residue, specifically a glutamate, at position 153, the base of the V1 loop, may more stably shield the V3 loop by facilitating a polar interaction, whereas a glycine may allow greater mobility of the V1 loop. This greater mobility and weaker interaction with V3 would explain the greater neutralization sensitivity to 447-52D, as well as a greater sensitivity to sCD4. Changes resulting from mutation at position 153 are very focused and specific, indicating that a major structural change is not occurring in the context of the trimer. Further analysis of this V1/V2-V3 loop interaction may help to show what conformational changes occur following CD4 binding.

Understanding the interrelationship between V1/V2, V3, and CD4 binding will contribute to a greater understanding of how to target this site to prevent binding and to develop effective vaccines to the CD4 binding site. In light of recent data stemming from the RV144 vaccine trial, indicating a correlation with V1/V2 antibodies and protection, understanding the structural implications of residue 153 may contribute to better antigen design [192]. The V1/V2 epitope may be significantly important in contributing to the adaptive immune response to HIV-1, especially if the interaction of V1/V2 with V3 affects how gp120 interacts with CD4. Macrophage tropic envelopes may be generally more susceptible to neutralization or inhibition by V1 and V3 directed antibodies, which could certainly help explain why they are not found in immune tissue during the course of disease progression. If non-mac tropic Envs are more resistant to V1 and V3 antibodies, then these Envs should be utilized as antigenic models for the development of antibodies and small molecule inhibitors.

These data show a potential interaction between V1/V2 and V3. This interaction may result in altered interaction with the co-receptors for HIV-1, CCR5 and CXCR4, due to theV3 loop being the deterministic site of co-receptor binding [193]. Although no significant change in sensitivity to maraviroc was observed (Table 3-2), this alone may not explain altered co-receptor interaction since maraviroc is a small molecule inhibitor directed towards CCR5 usage that binds CCR5 to inhibit its interaction with gp120 [194]. Inhibitors directed towards the sites of CCR5 and gp120 interaction may better elucidate how macrophage

tropism affects CCR5 usage. A higher sensitivity to V3 loop antibodies by mac tropic Envs may correlate to a greater exposure of the V3 loop in these Envs. The experiments presented here may help support experiments that suggest mac tropic Envs are better able to utilize low levels of CCR5 [111], or even CXCR4. This may be contributed to through a mechanism whereby the charge or structure of V1 affects co-receptor interaction with gp120 through V3.

5-3 Mac-tropic and Non-Mac tropic envelopes and tropism:

Non-mac tropic Envs predominate in immune tissue throughout infection, yet the data presented here demonstrate they do not have any advantage infecting CD4+ T-cells. Their predominance in immune tissue may stem from their inability to utilize low levels of CD4 for infection. This trait may translate to a decreased sensitivity to CD4bs antibodies, leading to less neutralization and antibody-dependent cell-mediated cytotoxicity and viral inhibition (ADCC and ADCVI) than mac tropic Envs. CD4bs antibodies are known to be present in the host at all stages of infection [195, 196], and although they may not be present in large quantities, they are most likely constantly exerting selective pressure on viral quasi-species, particularly those present in immune tissue [197]. The greater sensitivity to CD4bs antibodies of mac-tropic Envs may explain their apparent relegation to more immune privileged sites. Despite being capable of more efficiently infecting CD4+ target cells, this increased efficiency may result in greater stimulation of ADCC and ADCVI. While there doesn't appear to be any difference between the level of immune activation of the mac-tropic and non-mac

tropic viruses, it may not be immune activation that distinguishes between these two types of virus, but immune detection.

Dendritic cells are susceptible to macrophage tropic viruses in these experiments. Interestingly, despite not alleviating the post-entry restriction present in myeloid lineage cells described earlier in this thesis, efficient infection of these cells by mac tropic Envs is accomplished. The specific mechanisms that permit this infection are not known, although there may be something specific to mac tropic gp120 that makes it less susceptible to this post-entry restriction by SAMHD1. Further experimentation is needed to determine if there is a difference in the behavior of mac tropic and non-mac tropic variants post-entry. Additionally, upon infecting MDDCs with VSV-G pseudotyped replication competent HIV-1, a very minimal type I IFN response is observed. This is contrary to what is seen by others after treating these MDDCs with SIV virus-like particles in order to introduce Vpx to alleviate SAMHD1 restriction [198]. This IFN response may be dependent upon Vpx, which is not a protein encoded by HIV-1. The MDDCs that were treated with Vpx may stimulate an innate immune response because they are treated with antigens from two different pathogens, namely SIV and HIV-1. This lack of an innate immune response in the HIV-1 infected MDDCs presented herein requires further investigation to determine the difference in innate immune response observed.

5-4 Future Studies:

Experiments detailed here identified residue 153 in the V1 loop as modulating macrophage tropism. The presence of a glutamate at position 153 is conserved across clades A, B, and C (Table 3-1), yet most experiments shown here focus on R5 clade B Envs. Several heterologous envelopes are identified here that are sensitive to the E153G change with respect to macrophage tropism. Dementia has been studied in many cases of clade B infection, and it has been argued that instances of neuropathogenesis are even less severe in clade C than in clade B [199]. This is consistent with E153 being associated with non-mac tropic Envs, and potentially slower disease progression. It was further determined that the presence of a glycine at position 153 was prevalent in clade D. The potential link between HIV-associated dementia and macrophage tropism has been discussed, but in light of this potential link, it is interesting that there are reports of clade D being associated with higher incidences of dementia [164], as well as faster disease progession [159-163]. It would be interesting to determine if G153 is associated with neurotropic viruses across clades, as well as HIV-1 associated neurological conditions. G153 could be consistent with faster rates of disease progression and a macrophage-tropic phenotype in clade D isolates, as well as other clades.

This E153G change causes an increased sensitivity to 447-52D, a V3-loop antibody, as well as sCD4. This suggests that the presence of a glycine at position 153 facilitates a shift in the position of the V3 loop. This V3 loop conformational change may result in a more open conformation, providing

greater accessibility to CD4, or it may permit an easier transition from the unliganded structure of the trimeric glycoprotein spike to the CD4-bound conformation. This change may be the result of the charge change within V1 resulting from the absence of a glutamate. In order to determine specifically the structural implications of this position, obtaining highly resolved structures of the trimeric glycoprotein spike in complex with 447-52D or CD4 with varying amino acids at postion 153 would shed light on the conformational effect position 153 imparts, if any.

In addition to further exploring the effects of specific determinants, the effects of HIV-1 infection on mDCs and MDDCs needs further investigation. The type I IFN response was evaluated here, and found to be minimally stimulated in the context of these experiments. It may be the case that there are other cytokines or chemokines that are more relevant in the case of HIV-1 infection. IL-10 is a known immunoregulatory cytokine, and there have been several reports regarding how it is upregulated upon infection and co-culture with T-cells [200, 201]. It may be the case that mac-tropic envelopes elicit greater production of immunosuppressive cytokines due to their ability to infect MDDCs. Utilization of multiplex cytokine analysis may help to shed light on the effect HIV-1 infection has on this. In addition to evaluating differential innate immune response, evaluating the potential difference in the ability to stimulate ADCC and ADCVI may show that mac-tropic virus is more readily detectable and more easily dispatched by the humoral immune system than are non-mac tropic viruses.

## 5-5 Conclusion:

This thesis identifies a determinant of macrophage tropism in the V1 loop. This determinant appears to change the conformation of the V3 loop and facilitate a more efficient change when gp120 encounters CD4. Understanding how the V1/V2 and the V3 loop contribute to the accessibility of the CD4 binding site will greatly contribute to antigen and vaccine design. The role of macrophage tropic virus in the context of infection is still unclear. Non-macrophage tropic virus persists throughout the course of infection, and appears to be preferentially transmitted; yet it does not seem to have a distinct advantage for infecting distinct cell types. Overall, understanding macrophage tropism, from the level of determinants to its role in pathogenesis, may contribute to the development of novel therapeutics.

## CHAPTER VI: REFERENCES


1. *Pneumocystis pneumonia--Los Angeles.* MMWR Morb Mortal Wkly Rep, 1981. **30**(21): p. 250-2.
2. Barre-Sinoussi, F., J.C. Chermann, F. Rey, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier, *Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 868-71.
3. Popovic, M., M.G. Sarngadharan, E. Read, and R.C. Gallo, *Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS.* Science, 1984. **224**(4648): p. 497-500.
4. Coffin, J., A. Haase, J.A. Levy, L. Montagnier, S. Oroszlan, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and et al., *Human immunodeficiency viruses.* Science, 1986. **232**(4751): p. 697.
5. Mansky, L.M. and H.M. Temin, *Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase.* J Virol, 1995. **69**(8): p. 5087-94.
6. Jetzt, A.E., H. Yu, G.J. Klarmann, Y. Ron, B.D. Preston, and J.P. Dougherty, *High rate of recombination throughout the human immunodeficiency virus type 1 genome.* J Virol, 2000. **74**(3): p. 1234-40.

7. Perelson, A.S., A.U. Neumann, M. Markowitz, J.M. Leonard, and D.D. Ho, *HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time.* Science, 1996. **271**(5255): p. 1582-6.

8. Plantier, J.C., M. Leoz, J.E. Dickerson, F. De Oliveira, F. Cordonnier, V. Lemee, F. Damond, D.L. Robertson, and F. Simon, *A new human immunodeficiency virus derived from gorillas.* Nat Med, 2009. **15**(8): p. 871-2.

9. Stebbing, J. and G. Moyle, *The clades of HIV: their origins and clinical significance.* AIDS Rev, 2003. **5**(4): p. 205-13.

10. Clapham, P.R. and A. McKnight, *Cell surface receptors, virus entry and tropism of primate lentiviruses.* J Gen Virol, 2002. **83**(Pt 8): p. 1809-29.

11. Miyauchi, K., Y. Kim, O. Latinovic, V. Morozov, and G.B. Melikyan, *HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes.* Cell, 2009. **137**(3): p. 433-44.

12. McDonald, D., M.A. Vodicka, G. Lucero, T.M. Svitkina, G.G. Borisy, M. Emerman, and T.J. Hope, *Visualization of the intracellular behavior of HIV in living cells.* J Cell Biol, 2002. **159**(3): p. 441-52.

13. Warrilow, D. and D. Harrich, *HIV-1 replication from after cell entry to the nuclear periphery.* Curr HIV Res, 2007. **5**(3): p. 293-9.

14. Fassati, A. and S.P. Goff, *Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1.* J Virol, 2001. **75**(8): p. 3626-35.

15. Iordanskiy, S., R. Berro, M. Altieri, F. Kashanchi, and M. Bukrinsky, *Intracytoplasmic maturation of the human immunodeficiency virus type 1 reverse transcription complexes determines their capacity to integrate into chromatin.* Retrovirology, 2006. **3**: p. 4.

16. Karn, J. and C.M. Stoltzfus, *Transcriptional and Posttranscriptional Regulation of HIV-1 Gene Expression.* Cold Spring Harb Perspect Med, 2012. **2**(2): p. a006916.

17. Tazi, J., N. Bakkour, V. Marchand, L. Ayadi, A. Aboufirassi, and C. Branlant, *Alternative splicing: regulation of HIV-1 multiplication as a target for therapeutic action.* FEBS J, 2010. **277**(4): p. 867-76.

18. Swanstrom, R. and J.W. Wills, *Synthesis, Assembly, and Processing of Viral Proteins*, in *Retroviruses*, J.M. Coffin, S.H. Hughes, and H.E. Varmus, Editors. 1997: Cold Spring Harbor (NY).

19. Perlman, M. and M.D. Resh, *Identification of an intracellular trafficking and assembly pathway for HIV-1 gag.* Traffic, 2006. **7**(6): p. 731-45.

20. Wyatt, R. and J. Sodroski, *The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens.* Science, 1998. **280**(5371): p. 1884-8.

21. Strack, B., A. Calistri, S. Craig, E. Popova, and H.G. Gottlinger, *AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding.* Cell, 2003. **114**(6): p. 689-99.

22. Hill, M., G. Tachedjian, and J. Mak, *The packaging and maturation of the HIV-1 Pol proteins.* Curr HIV Res, 2005. **3**(1): p. 73-85.

23. Turner, B.G. and M.F. Summers, *Structural biology of HIV.* J Mol Biol, 1999. **285**(1): p. 1-32.

24. Navia, M.A., P.M. Fitzgerald, B.M. McKeever, C.T. Leu, J.C. Heimbach, W.K. Herber, I.S. Sigal, P.L. Darke, and J.P. Springer, *Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1.* Nature, 1989. **337**(6208): p. 615-20.

25. Louis, J.M., R. Ishima, D.A. Torchia, and I.T. Weber, *HIV-1 protease: structure, dynamics, and inhibition.* Adv Pharmacol, 2007. **55**: p. 261-98.

26. Gotte, M., X. Li, and M.A. Wainberg, *HIV-1 reverse transcription: a brief overview focused on structure-function relationships among molecules involved in initiation of the reaction.* Arch Biochem Biophys, 1999. **365**(2): p. 199-210.

27. Castro, H.C., N.I. Loureiro, M. Pujol-Luz, A.M. Souza, M.G. Albuquerque, D.O. Santos, L.M. Cabral, I.C. Frugulhetti, and C.R. Rodrigues, *HIV-1 reverse transcriptase: a therapeutical target in the spotlight.* Curr Med Chem, 2006. **13**(3): p. 313-24.

28. Zeinalipour-Loizidou, E., C. Nicolaou, A. Nicolaides, and L.G. Kostrikis, *HIV-1 integrase: from biology to chemotherapeutics.* Curr HIV Res, 2007. **5**(4): p. 365-88.

29. Semenova, E.A., C. Marchand, and Y. Pommier, *HIV-1 integrase inhibitors: update and perspectives.* Adv Pharmacol, 2008. **56**: p. 199-228.

30. Romani, B., S. Engelbrecht, and R.H. Glashoff, *Functions of Tat: the versatile protein of human immunodeficiency virus type 1.* J Gen Virol, 2010. **91**(Pt 1): p. 1-12.

31. Pollard, V.W. and M.H. Malim, *The HIV-1 Rev protein.* Annu Rev Microbiol, 1998. **52**: p. 491-532.

32. Wissing, S., N.L. Galloway, and W.C. Greene, *HIV-1 Vif versus the APOBEC3 cytidine deaminases: an intracellular duel between pathogen and host restriction factors.* Mol Aspects Med, 2010. **31**(5): p. 383-97.

33. Dube, M., M.G. Bego, C. Paquay, and E.A. Cohen, *Modulation of HIV-1-host interaction: role of the Vpu accessory protein.* Retrovirology, 2010. **7**: p. 114.

34. Kondo, E. and H.G. Gottlinger, *A conserved LXXLF sequence is the major determinant in p6gag required for the incorporation of human immunodeficiency virus type 1 Vpr.* J Virol, 1996. **70**(1): p. 159-64.

35. Kogan, M. and J. Rappaport, *HIV-1 accessory protein Vpr: relevance in the pathogenesis of HIV and potential for therapeutic intervention.* Retrovirology, 2011. **8**: p. 25.

36. Laguette, N., C. Bregnard, S. Benichou, and S. Basmaciogullari, *Human immunodeficiency virus (HIV) type-1, HIV-2 and simian immunodeficiency virus Nef proteins.* Mol Aspects Med, 2010. **31**(5): p. 418-33.

37. Foster, J.L. and J.V. Garcia, *HIV-1 Nef: at the crossroads.* Retrovirology, 2008. **5**: p. 84.

38. Freed, E.O., *HIV-1 gag proteins: diverse functions in the virus life cycle.* Virology, 1998. **251**(1): p. 1-15.

39. Orenstein, J.M., M.S. Meltzer, T. Phipps, and H.E. Gendelman, *Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study.* J Virol, 1988. **62**(8): p. 2578-86.

40. Welsch, S., F. Groot, H.G. Krausslich, O.T. Keppler, and Q.J. Sattentau, *Architecture and regulation of the HIV-1 assembly and holding compartment in macrophages.* J Virol, 2011. **85**(15): p. 7922-7.

41. Stremlau, M., M. Perron, M. Lee, Y. Li, B. Song, H. Javanbakht, F. Diaz-Griffero, D.J. Anderson, W.I. Sundquist, and J. Sodroski, *Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor.* Proc Natl Acad Sci U S A, 2006. **103**(14): p. 5514-9.

42. Darlix, J.L., J. Godet, R. Ivanyi-Nagy, P. Fosse, O. Mauffret, and Y. Mely, *Flexible nature and specific functions of the HIV-1 nucleocapsid protein.* J Mol Biol, 2011. **410**(4): p. 565-81.

43. Huang, M., J.M. Orenstein, M.A. Martin, and E.O. Freed, *p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease.* J Virol, 1995. **69**(11): p. 6810-8.

44. Chu, H., J.J. Wang, and P. Spearman, *Human immunodeficiency virus type-1 gag and host vesicular trafficking pathways.* Curr Top Microbiol Immunol, 2009. **339**: p. 67-84.

45. Bernstein, H.B., S.P. Tucker, E. Hunter, J.S. Schutzbach, and R.W. Compans, *Human immunodeficiency virus type 1 envelope glycoprotein is modified by O-linked oligosaccharides.* J Virol, 1994. **68**(1): p. 463-8.

46. Freed, E.O., D.J. Myers, and R. Risser, *Mutational analysis of the cleavage sequence of the human immunodeficiency virus type 1 envelope glycoprotein precursor gp160.* J Virol, 1989. **63**(11): p. 4670-5.

47. Raska, M. and J. Novak, *Involvement of envelope-glycoprotein glycans in HIV-1 biology and infection.* Arch Immunol Ther Exp (Warsz), 2010. **58**(3): p. 191-208.

48. McLellan, J.S., M. Pancera, C. Carrico, J. Gorman, J.P. Julien, R. Khayat, R. Louder, R. Pejchal, M. Sastry, K. Dai, S. O'Dell, N. Patel, S. Shahzad-ul-Hussan, Y. Yang, B. Zhang, T. Zhou, J. Zhu, J.C. Boyington, G.Y. Chuang, D. Diwanji, I. Georgiev, Y.D. Kwon, D. Lee, M.K. Louder, S. Moquin, S.D. Schmidt, Z.Y. Yang, M. Bonsignori, J.A. Crump, S.H. Kapiga, N.E. Sam, B.F. Haynes, D.R. Burton, W.C. Koff, L.M. Walker, S. Phogat, R. Wyatt, J. Orwenyo, L.X. Wang, J. Arthos, C.A. Bewley, J.R. Mascola, G.J. Nabel, W.R. Schief, A.B. Ward, I.A. Wilson, and P.D. Kwong, *Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9.* Nature, 2011. **480**(7377): p. 336-43.

49. Pancera, M., S. Majeed, Y.E. Ban, L. Chen, C.C. Huang, L. Kong, Y.D. Kwon, J. Stuckey, T. Zhou, J.E. Robinson, W.R. Schief, J. Sodroski, R. Wyatt, and P.D. Kwong, *Structure of HIV-1 gp120 with gp41-interactive region reveals layered envelope architecture and basis of conformational mobility.* Proc Natl Acad Sci U S A, 2010. **107**(3): p. 1166-71.

50. Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski, *Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 receptor binding.* J Virol, 1990. **64**(12): p. 5701-7.

51. Da, L.T., J.M. Quan, and Y.D. Wu, *Understanding of the bridging sheet formation of HIV-1 glycoprotein gp120.* J Phys Chem B, 2009. **113**(43): p. 14536-43.

52. Freed, E.O., D.J. Myers, and R. Risser, *Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain.* J Virol, 1991. **65**(1): p. 190-4.

53. Hwang, S.S., T.J. Boyle, H.K. Lyerly, and B.R. Cullen, *Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1.* Science, 1991. **253**(5015): p. 71-4.

54. Shioda, T., J.A. Levy, and C. Cheng-Mayer, *Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene.* Nature, 1991. **349**(6305): p. 167-9.

55. Rizzuto, C. and J. Sodroski, *Fine definition of a conserved CCR5-binding region on the human immunodeficiency virus type 1 glycoprotein 120.* AIDS Res Hum Retroviruses, 2000. **16**(8): p. 741-9.

56. Liu, J., A. Bartesaghi, M.J. Borgnia, G. Sapiro, and S. Subramaniam, *Molecular architecture of native HIV-1 gp120 trimers.* Nature, 2008. **455**(7209): p. 109-13.

57. White, T.A., A. Bartesaghi, M.J. Borgnia, M.J. de la Cruz, R. Nandwani, J.A. Hoxie, J.W. Bess, J.D. Lifson, J.L. Milne, and S. Subramaniam, *Three-dimensional structures of soluble CD4-bound states of trimeric simian immunodeficiency virus envelope glycoproteins determined by using cryo-electron tomography.* J Virol, 2011. **85**(23): p. 12114-23.

58. Roux, K.H. and K.A. Taylor, *AIDS virus envelope spike structure.* Curr Opin Struct Biol, 2007. **17**(2): p. 244-52.

59. Checkley, M.A., B.G. Luttge, and E.O. Freed, *HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation.* J Mol Biol, 2011. **410**(4): p. 582-608.

60. Boge, M., S. Wyss, J.S. Bonifacino, and M. Thali, *A membrane-proximal tyrosine-based signal mediates internalization of the HIV-1 envelope glycoprotein via interaction with the AP-2 clathrin adaptor.* J Biol Chem, 1998. **273**(25): p. 15773-8.

61. Schief, W.R., Y.E. Ban, and L. Stamatatos, *Challenges for structure-based HIV vaccine design.* Curr Opin HIV AIDS, 2009. **4**(5): p. 431-40.

62. Brenchley, J.M., T.W. Schacker, L.E. Ruff, D.A. Price, J.H. Taylor, G.J. Beilman, P.L. Nguyen, A. Khoruts, M. Larson, A.T. Haase, and D.C. Douek, *CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract.* J Exp Med, 2004. **200**(6): p. 749-59.

63. Lindback, S., A.C. Karlsson, J. Mittler, A. Blaxhult, M. Carlsson, G. Briheim, A. Sonnerborg, and H. Gaines, *Viral dynamics in primary HIV-1 infection. Karolinska Institutet Primary HIV Infection Study Group.* AIDS, 2000. **14**(15): p. 2283-91.

64. Borrow, P., H. Lewicki, B.H. Hahn, G.M. Shaw, and M.B. Oldstone, *Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection.* J Virol, 1994. **68**(9): p. 6103-10.

65. Ogg, G.S., X. Jin, S. Bonhoeffer, P.R. Dunbar, M.A. Nowak, S. Monard, J.P. Segal, Y. Cao, S.L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D.D. Ho, D.F. Nixon, and A.J. McMichael, *Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA.* Science, 1998. **279**(5359): p. 2103-6.

66. Ho, D.D., A.U. Neumann, A.S. Perelson, W. Chen, J.M. Leonard, and M. Markowitz, *Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection.* Nature, 1995. **373**(6510): p. 123-6.

67. El-Far, M., R. Halwani, E. Said, L. Trautmann, M. Doroudchi, L. Janbazian, S. Fonseca, J. van Grevenynghe, B. Yassine-Diab, R.P. Sekaly, and E.K. Haddad, *T-cell exhaustion in HIV infection.* Curr HIV/AIDS Rep, 2008. **5**(1): p. 13-9.

68. Poignard, P., R. Sabbe, G.R. Picchio, M. Wang, R.J. Gulizia, H. Katinger, P.W. Parren, D.E. Mosier, and D.R. Burton, *Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo.* Immunity, 1999. **10**(4): p. 431-8.

69. Masur, H., F.P. Ognibene, R. Yarchoan, J.H. Shelhamer, B.F. Baird, W. Travis, A.F. Suffredini, L. Deyton, J.A. Kovacs, J. Falloon, and et al., *CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection.* Ann Intern Med, 1989. **111**(3): p. 223-31.

70. Moore, J.P., S.G. Kitchen, P. Pugach, and J.A. Zack, *The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection.* AIDS Res Hum Retroviruses, 2004. **20**(1): p. 111-26.

71. Regoes, R.R. and S. Bonhoeffer, *The HIV coreceptor switch: a population dynamical perspective.* Trends Microbiol, 2005. **13**(6): p. 269-77.

72. Ho, D.D., *Time to hit HIV, early and hard.* N Engl J Med, 1995. **333**(7): p. 450-1.

73. Hrecka, K., C. Hao, M. Gierszewska, S.K. Swanson, M. Kesik-Brodacka, S. Srivastava, L. Florens, M.P. Washburn, and J. Skowronski, *Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein.* Nature, 2011. **474**(7353): p. 658-61.

74. Laguette, N., B. Sobhian, N. Casartelli, M. Ringeard, C. Chable-Bessia, E. Segeral, A. Yatim, S. Emiliani, O. Schwartz, and M. Benkirane, *SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx.* Nature, 2011. **474**(7353): p. 654-7.

75. Peng, G., K.J. Lei, W. Jin, T. Greenwell-Wild, and S.M. Wahl, *Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity.* J Exp Med, 2006. **203**(1): p. 41-6.

76. Yan, N., A.D. Regalado-Magdos, B. Stiggelbout, M.A. Lee-Kirsch, and J. Lieberman, *The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1.* Nat Immunol, 2010. **11**(11): p. 1005-13.

77. Alter, G., M.P. Martin, N. Teigen, W.H. Carr, T.J. Suscovich, A. Schneidewind, H. Streeck, M. Waring, A. Meier, C. Brander, J.D. Lifson, T.M. Allen, M. Carrington, and M. Altfeld, *Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes.* J Exp Med, 2007. **204**(12): p. 3027-36.

78. Alter, G., S. Rihn, K. Walter, A. Nolting, M. Martin, E.S. Rosenberg, J.S. Miller, M. Carrington, and M. Altfeld, *HLA class I subtype-dependent*

*expansion of KIR3DS1+ and KIR3DL1+ NK cells during acute human immunodeficiency virus type 1 infection.* J Virol, 2009. **83**(13): p. 6798-805.

79. Lakshmanan, V., G. Alter, M. Altfeld, and N. Bhardwaj, *Biology of plasmacytoid dendritic cells and natural killer cells in HIV-1 infection.* Curr Opin HIV AIDS, 2007. **2**(3): p. 189-200.

80. Baker, B.M., B.L. Block, A.C. Rothchild, and B.D. Walker, *Elite control of HIV infection: implications for vaccine design.* Expert Opin Biol Ther, 2009. **9**(1): p. 55-69.

81. Girard, M.P., S. Osmanov, O.M. Assossou, and M.P. Kieny, *Human immunodeficiency virus (HIV) immunopathogenesis and vaccine development: a review.* Vaccine, 2011. **29**(37): p. 6191-218.

82. Tomaras, G.D., N.L. Yates, P. Liu, L. Qin, G.G. Fouda, L.L. Chavez, A.C. Decamp, R.J. Parks, V.C. Ashley, J.T. Lucas, M. Cohen, J. Eron, C.B. Hicks, H.X. Liao, S.G. Self, G. Landucci, D.N. Forthal, K.J. Weinhold, B.F. Keele, B.H. Hahn, M.L. Greenberg, L. Morris, S.S. Karim, W.A. Blattner, D.C. Montefiori, G.M. Shaw, A.S. Perelson, and B.F. Haynes, *Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia.* J Virol, 2008. **82**(24): p. 12449-63.

83. Forthal, D.N. and C. Moog, *Fc receptor-mediated antiviral antibodies.* Curr Opin HIV AIDS, 2009. **4**(5): p. 388-93.

84. Tersmette, M., R.E. de Goede, B.J. Al, I.N. Winkel, R.A. Gruters, H.T. Cuypers, H.G. Huisman, and F. Miedema, *Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex.* J Virol, 1988. **62**(6): p. 2026-32.

85. Gartner, S., P. Markovits, D.M. Markovitz, M.H. Kaplan, R.C. Gallo, and M. Popovic, *The role of mononuclear phagocytes in HTLV-III/LAV infection.* Science, 1986. **233**(4760): p. 215-9.

86. Alkhatib, G., C. Combadiere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy, and E.A. Berger, *CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1.* Science, 1996. **272**(5270): p. 1955-8.

87. Berger, E.A., R.W. Doms, E.M. Fenyo, B.T. Korber, D.R. Littman, J.P. Moore, Q.J. Sattentau, H. Schuitemaker, J. Sodroski, and R.A. Weiss, *A new classification for HIV-1.* Nature, 1998. **391**(6664): p. 240.

88. Goodenow, M.M. and R.G. Collman, *HIV-1 coreceptor preference is distinct from target cell tropism: a dual-parameter nomenclature to define viral phenotypes.* J Leukoc Biol, 2006. **80**(5): p. 965-72.

89. Koyanagi, Y., S. Miles, R.T. Mitsuyasu, J.E. Merrill, H.V. Vinters, and I.S. Chen, *Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms.* Science, 1987. **236**(4803): p. 819-22.

90. Gorry, P.R., G. Bristol, J.A. Zack, K. Ritola, R. Swanstrom, C.J. Birch, J.E. Bell, N. Bannert, K. Crawford, H. Wang, D. Schols, E. De Clercq, K. Kunstman, S.M. Wolinsky, and D. Gabuzda, *Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity.* J Virol, 2001. **75**(21): p. 10073-89.

91. Peters, P.J., J. Bhattacharya, S. Hibbitts, M.T. Dittmar, G. Simmons, J. Bell, P. Simmonds, and P.R. Clapham, *Biological analysis of human immunodeficiency virus type 1 R5 envelopes amplified from brain and lymph node tissues of AIDS patients with neuropathology reveals two distinct tropism phenotypes and identifies envelopes in the brain that confer an enhanced tropism and fusigenicity for macrophages.* J Virol, 2004. **78**(13): p. 6915-26.

92. Simmons, G., D. Wilkinson, J.D. Reeves, M.T. Dittmar, S. Beddows, J. Weber, G. Carnegie, U. Desselberger, P.W. Gray, R.A. Weiss, and P.R. Clapham, *Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry.* J Virol, 1996. **70**(12): p. 8355-60.

93. Carrington, M., M. Dean, M.P. Martin, and S.J. O'Brien, *Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences.* Hum Mol Genet, 1999. **8**(10): p. 1939-45.

94. Cavarelli, M. and G. Scarlatti, *Phenotype variation in human immunodeficiency virus type 1 transmission and disease progression.* Dis Markers, 2009. **27**(3): p. 121-36.

95. Dejucq, N., G. Simmons, and P.R. Clapham, *Expanded tropism of primary human immunodeficiency virus type 1 R5 strains to CD4(+) T-cell lines determined by the capacity to exploit low concentrations of CCR5.* J Virol, 1999. **73**(9): p. 7842-7.

96. Gorry, P.R., J. Taylor, G.H. Holm, A. Mehle, T. Morgan, M. Cayabyab, M. Farzan, H. Wang, J.E. Bell, K. Kunstman, J.P. Moore, S.M. Wolinsky, and D. Gabuzda, *Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate.* J Virol, 2002. **76**(12): p. 6277-92.

97. Peters, P.J., M.J. Duenas-Decamp, W.M. Sullivan, R. Brown, C. Ankghuambom, K. Luzuriaga, J. Robinson, D.R. Burton, J. Bell, P. Simmonds, J. Ball, and P.R. Clapham, *Variation in HIV-1 R5 macrophage-tropism correlates with sensitivity to reagents that block envelope: CD4 interactions but not with sensitivity to other entry inhibitors.* Retrovirology, 2008. **5**: p. 5.

98. Peters, P.J., W.M. Sullivan, M.J. Duenas-Decamp, J. Bhattacharya, C. Ankghuambom, R. Brown, K. Luzuriaga, J. Bell, P. Simmonds, J. Ball, and P.R. Clapham, *Non-macrophage-tropic human immunodeficiency virus type 1 R5 envelopes predominate in blood, lymph nodes, and semen: implications for transmission and pathogenesis.* J Virol, 2006. **80**(13): p. 6324-32.

99. Gray, L., J. Sterjovski, M. Churchill, P. Ellery, N. Nasr, S.R. Lewin, S.M. Crowe, S.L. Wesselingh, A.L. Cunningham, and P.R. Gorry, *Uncoupling coreceptor usage of human immunodeficiency virus type 1 (HIV-1) from macrophage tropism reveals biological properties of CCR5-restricted HIV-1 isolates from patients with acquired immunodeficiency syndrome.* Virology, 2005. **337**(2): p. 384-98.

100. Li, S., J. Juarez, M. Alali, D. Dwyer, R. Collman, A. Cunningham, and H.M. Naif, *Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates.* J Virol, 1999. **73**(12): p. 9741-55.

101. Tuttle, D.L., C.B. Anders, M.J. Aquino-De Jesus, P.P. Poole, S.L. Lamers, D.R. Briggs, S.M. Pomeroy, L. Alexander, K.W. Peden, W.A. Andiman, J.W. Sleasman, and M.M. Goodenow, *Increased replication of non-syncytium-inducing HIV type 1 isolates in monocyte-derived macrophages*

*is linked to advanced disease in infected children.* AIDS Res Hum Retroviruses, 2002. **18**(5): p. 353-62.

102. Thomas, E.R., R.L. Dunfee, J. Stanton, D. Bogdan, J. Taylor, K. Kunstman, J.E. Bell, S.M. Wolinsky, and D. Gabuzda, *Macrophage entry mediated by HIV Envs from brain and lymphoid tissues is determined by the capacity to use low CD4 levels and overall efficiency of fusion.* Virology, 2007. **360**(1): p. 105-19.

103. Dunfee, R.L., E.R. Thomas, and D. Gabuzda, *Enhanced macrophage tropism of HIV in brain and lymphoid tissues is associated with sensitivity to the broadly neutralizing CD4 binding site antibody b12.* Retrovirology, 2009. **6**: p. 69.

104. Dunfee, R.L., E.R. Thomas, P.R. Gorry, J. Wang, J. Taylor, K. Kunstman, S.M. Wolinsky, and D. Gabuzda, *The HIV Env variant N283 enhances macrophage tropism and is associated with brain infection and dementia.* Proc Natl Acad Sci U S A, 2006. **103**(41): p. 15160-5.

105. Duenas-Decamp, M.J., P.J. Peters, D. Burton, and P.R. Clapham, *Determinants flanking the CD4 binding loop modulate macrophage tropism of human immunodeficiency virus type 1 R5 envelopes.* J Virol, 2009. **83**(6): p. 2575-83.

106. Dunfee, R.L., E.R. Thomas, J. Wang, K. Kunstman, S.M. Wolinsky, and D. Gabuzda, *Loss of the N-linked glycosylation site at position 386 in the HIV*

*envelope V4 region enhances macrophage tropism and is associated with dementia.* Virology, 2007. **367**(1): p. 222-34.

107. Duenas-Decamp, M.J., P. Peters, D. Burton, and P.R. Clapham, *Natural resistance of human immunodeficiency virus type 1 to the CD4bs antibody b12 conferred by a glycan and an arginine residue close to the CD4 binding loop.* J Virol, 2008. **82**(12): p. 5807-14.
108. Gonzalez-Perez, M.P., O.J. O'Connell, R. Lin, W.M. Sullivan, J. Bell, P. Simmonds, and P.R. Clapham, *Independent evolution of macrophage-tropism and increased charge between HIV-1 R5 envelopes present in brain and immune tissue.* Retrovirology, 2012. **9**(1): p. 20.
109. Grossman, Z., M. Meier-Schellersheim, W.E. Paul, and L.J. Picker, *Pathogenesis of HIV infection: what the virus spares is as important as what it destroys.* Nat Med, 2006. **12**(3): p. 289-95.
110. Cashin, K., M. Roche, J. Sterjovski, A. Ellett, L.R. Gray, A.L. Cunningham, P.A. Ramsland, M.J. Churchill, and P.R. Gorry, *Alternative coreceptor requirements for efficient CCR5- and CXCR4-mediated HIV-1 entry into macrophages.* J Virol, 2011. **85**(20): p. 10699-709.
111. Sterjovski, J., M. Roche, M.J. Churchill, A. Ellett, W. Farrugia, L.R. Gray, D. Cowley, P. Poumbourios, B. Lee, S.L. Wesselingh, A.L. Cunningham, P.A. Ramsland, and P.R. Gorry, *An altered and more efficient mechanism of CCR5 engagement contributes to macrophage tropism of CCR5-using HIV-1 envelopes.* Virology, 2010. **404**(2): p. 269-78.

112. Bell, J.E., A. Busuttil, J.W. Ironside, S. Rebus, Y.K. Donaldson, P. Simmonds, and J.F. Peutherer, *Human immunodeficiency virus and the brain: investigation of virus load and neuropathologic changes in pre-AIDS subjects.* J Infect Dis, 1993. **168**(4): p. 818-24.

113. Thieblemont, N., L. Weiss, H.M. Sadeghi, C. Estcourt, and N. Haeffner-Cavaillon, *CD14lowCD16high: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection.* Eur J Immunol, 1995. **25**(12): p. 3418-24.

114. Ancuta, P., P. Autissier, A. Wurcel, T. Zaman, D. Stone, and D. Gabuzda, *CD16+ monocyte-derived macrophages activate resting T cells for HIV infection by producing CCR3 and CCR4 ligands.* J Immunol, 2006. **176**(10): p. 5760-71.

115. Clay, C.C., D.S. Rodrigues, Y.S. Ho, B.A. Fallert, K. Janatpour, T.A. Reinhart, and U. Esser, *Neuroinvasion of fluorescein-positive monocytes in acute simian immunodeficiency virus infection.* J Virol, 2007. **81**(21): p. 12040-8.

116. Keele, B.F., E.E. Giorgi, J.F. Salazar-Gonzalez, J.M. Decker, K.T. Pham, M.G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J.L. Kirchherr, F. Gao, J.A. Anderson, L.H. Ping, R. Swanstrom, G.D. Tomaras, W.A. Blattner, P.A. Goepfert, J.M. Kilby, M.S. Saag, E.L. Delwart, M.P. Busch, M.S. Cohen, D.C. Montefiori, B.F. Haynes, B. Gaschen, G.S. Athreya, H.Y. Lee, N. Wood, C. Seoighe, A.S. Perelson, T.

Bhattacharya, B.T. Korber, B.H. Hahn, and G.M. Shaw, *Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection.* Proc Natl Acad Sci U S A, 2008. **105**(21): p. 7552-7.

117. Salazar-Gonzalez, J.F., M.G. Salazar, B.F. Keele, G.H. Learn, E.E. Giorgi, H. Li, J.M. Decker, S. Wang, J. Baalwa, M.H. Kraus, N.F. Parrish, K.S. Shaw, M.B. Guffey, K.J. Bar, K.L. Davis, C. Ochsenbauer-Jambor, J.C. Kappes, M.S. Saag, M.S. Cohen, J. Mulenga, C.A. Derdeyn, S. Allen, E. Hunter, M. Markowitz, P. Hraber, A.S. Perelson, T. Bhattacharya, B.F. Haynes, B.T. Korber, B.H. Hahn, and G.M. Shaw, *Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection.* J Exp Med, 2009. **206**(6): p. 1273-89.

118. Li, H., K.J. Bar, S. Wang, J.M. Decker, Y. Chen, C. Sun, J.F. Salazar-Gonzalez, M.G. Salazar, G.H. Learn, C.J. Morgan, J.E. Schumacher, P. Hraber, E.E. Giorgi, T. Bhattacharya, B.T. Korber, A.S. Perelson, J.J. Eron, M.S. Cohen, C.B. Hicks, B.F. Haynes, M. Markowitz, B.F. Keele, B.H. Hahn, and G.M. Shaw, *High Multiplicity Infection by HIV-1 in Men Who Have Sex with Men.* PLoS Pathog, 2010. **6**(5): p. e1000890.

119. Isaacman-Beck, J., E.A. Hermann, Y. Yi, S.J. Ratcliffe, J. Mulenga, S. Allen, E. Hunter, C.A. Derdeyn, and R.G. Collman, *Heterosexual transmission of human immunodeficiency virus type 1 subtype C:*

*Macrophage tropism, alternative coreceptor use, and the molecular anatomy of CCR5 utilization.* J Virol, 2009. **83**(16): p. 8208-20.

120. Hladik, F. and M.J. McElrath, *Setting the stage: host invasion by HIV.* Nat Rev Immunol, 2008. **8**(6): p. 447-57.

121. Smed-Sorensen, A., K. Lore, J. Vasudevan, M.K. Louder, J. Andersson, J.R. Mascola, A.L. Spetz, and R.A. Koup, *Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells.* J Virol, 2005. **79**(14): p. 8861-9.

122. Blauvelt, A., H. Asada, M.W. Saville, V. Klaus-Kovtun, D.J. Altman, R. Yarchoan, and S.I. Katz, *Productive infection of dendritic cells by HIV-1 and their ability to capture virus are mediated through separate pathways.* J Clin Invest, 1997. **100**(8): p. 2043-53.

123. Granelli-Piperno, A., E. Delgado, V. Finkel, W. Paxton, and R.M. Steinman, *Immature dendritic cells selectively replicate macrophagetropic (M-tropic) human immunodeficiency virus type 1, while mature cells efficiently transmit both M- and T-tropic virus to T cells.* J Virol, 1998. **72**(4): p. 2733-7.

124. Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojsov, N. Bhardwaj, I. Clark-Lewis, M. Baggiolini, and R.M. Steinman, *Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors.* J Exp Med, 1996. **184**(6): p. 2433-8.

125. Rubbert, A., C. Combadiere, M. Ostrowski, J. Arthos, M. Dybul, E. Machado, M.A. Cohn, J.A. Hoxie, P.M. Murphy, A.S. Fauci, and D. Weissman, *Dendritic cells express multiple chemokine receptors used as coreceptors for HIV entry.* J Immunol, 1998. **160**(8): p. 3933-41.

126. Turville, S.G., J. Arthos, K.M. Donald, G. Lynch, H. Naif, G. Clark, D. Hart, and A.L. Cunningham, *HIV gp120 receptors on human dendritic cells.* Blood, 2001. **98**(8): p. 2482-8.

127. Sallusto, F. and A. Lanzavecchia, *Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha.* J Exp Med, 1994. **179**(4): p. 1109-18.

128. Geijtenbeek, T.B., R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, G.J. Adema, Y. van Kooyk, and C.G. Figdor, *Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses.* Cell, 2000. **100**(5): p. 575-85.

129. Wu, L. and V.N. KewalRamani, *Dendritic-cell interactions with HIV: infection and viral dissemination.* Nat Rev Immunol, 2006. **6**(11): p. 859-68.

130. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, and K. Palucka, *Immunobiology of dendritic cells.* Annu Rev Immunol, 2000. **18**: p. 767-811.

131. Wiley, R.D. and S. Gummuluru, *Immature dendritic cell-derived exosomes can mediate HIV-1 trans infection.* Proc Natl Acad Sci U S A, 2006. **103**(3): p. 738-43.

132. Nobile, C., C. Petit, A. Moris, K. Skrabal, J.P. Abastado, F. Mammano, and O. Schwartz, *Covert human immunodeficiency virus replication in dendritic cells and in DC-SIGN-expressing cells promotes long-term transmission to lymphocytes.* J Virol, 2005. **79**(9): p. 5386-99.

133. Kulkarni, S.S., A. Lapedes, H. Tang, S. Gnanakaran, M.G. Daniels, M. Zhang, T. Bhattacharya, M. Li, V.R. Polonis, F.E. McCutchan, L. Morris, D. Ellenberger, S.T. Butera, R.C. Bollinger, B.T. Korber, R.S. Paranjape, and D.C. Montefiori, *Highly complex neutralization determinants on a monophyletic lineage of newly transmitted subtype C HIV-1 Env clones from India.* Virology, 2009. **385**(2): p. 505-20.

134. Platt, E.J., K. Wehrly, S.E. Kuhmann, B. Chesebro, and D. Kabat, *Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1.* J Virol, 1998. **72**(4): p. 2855-64.

135. Hyduk, S.J. and M.I. Cybulsky, *Alpha 4 integrin signaling activates phosphatidylinositol 3-kinase and stimulates T cell adhesion to intercellular adhesion molecule-1 to a similar extent as CD3, but induces a distinct rearrangement of the actin cytoskeleton.* J Immunol, 2002. **168**(2): p. 696-704.

136. Hofmann, W., D. Schubert, J. LaBonte, L. Munson, S. Gibson, J. Scammell, P. Ferrigno, and J. Sodroski, *Species-specific, postentry barriers to primate immunodeficiency virus infection.* J Virol, 1999. **73**(12): p. 10020-8.

137. O'Doherty, U., W.J. Swiggard, and M.H. Malim, *Human immunodeficiency virus type 1 spinoculation enhances infection through virus binding.* J Virol, 2000. **74**(21): p. 10074-80.

138. Wei, X., J.M. Decker, H. Liu, Z. Zhang, R.B. Arani, J.M. Kilby, M.S. Saag, X. Wu, G.M. Shaw, and J.C. Kappes, *Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy.* Antimicrob Agents Chemother, 2002. **46**(6): p. 1896-905.

139. Martin-Garcia, J., W. Cao, A. Varela-Rohena, M.L. Plassmeyer, and F. Gonzalez-Scarano, *HIV-1 tropism for the central nervous system: Brain-derived envelope glycoproteins with lower CD4 dependence and reduced sensitivity to a fusion inhibitor.* Virology, 2006. **346**(1): p. 169-79.

140. Bannert, N., D. Schenten, S. Craig, and J. Sodroski, *The level of CD4 expression limits infection of primary rhesus monkey macrophages by a T-tropic simian immunodeficiency virus and macrophagetropic human immunodeficiency viruses.* J Virol, 2000. **74**(23): p. 10984-93.

141. Lee, B., M. Sharron, L.J. Montaner, D. Weissman, and R.W. Doms, *Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets,*

*dendritic cells, and differentially conditioned monocyte-derived macrophages.* Proc Natl Acad Sci U S A, 1999. **96**(9): p. 5215-20.

142. Mori, K., M. Rosenzweig, and R.C. Desrosiers, *Mechanisms for adaptation of simian immunodeficiency virus to replication in alveolar macrophages.* J Virol, 2000. **74**(22): p. 10852-9.

143. Chen, B., E.M. Vogan, H. Gong, J.J. Skehel, D.C. Wiley, and S.C. Harrison, *Structure of an unliganded simian immunodeficiency virus gp120 core.* Nature, 2005. **433**(7028): p. 834-41.

144. Duenas-Decamp, M.J., P.J. Peters, A. Repik, T. Musich, M.P. Gonzalez-Perez, C. Caron, R. Brown, J. Ball, and P.R. Clapham, *Variation in the biological properties of HIV-1 R5 envelopes: implications of envelope structure, transmission and pathogenesis.* Future Virol, 2010. **5**(4): p. 435-451.

145. Montefiori, D.C. and J.R. Mascola, *Neutralizing antibodies against HIV-1: can we elicit them with vaccines and how much do we need?* Curr Opin HIV AIDS, 2009. **4**(5): p. 347-51.

146. Zolla-Pazner, S. and T. Cardozo, *Structure-function relationships of HIV-1 envelope sequence-variable regions refocus vaccine design.* Nat Rev Immunol, 2010. **10**(7): p. 527-35.

147. Binley, J.M., T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C.J. Petropoulos, and D.R. Burton, *Comprehensive cross-clade neutralization analysis of a panel*

*of anti-human immunodeficiency virus type 1 monoclonal antibodies.* J Virol, 2004. **78**(23): p. 13232-52.

148. Walker, L.M., S.K. Phogat, P.Y. Chan-Hui, D. Wagner, P. Phung, J.L. Goss, T. Wrin, M.D. Simek, S. Fling, J.L. Mitcham, J.K. Lehrman, F.H. Priddy, O.A. Olsen, S.M. Frey, P.W. Hammond, S. Kaminsky, T. Zamb, M. Moyle, W.C. Koff, P. Poignard, and D.R. Burton, *Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target.* Science, 2009. **326**(5950): p. 285-9.

149. Ly, A. and L. Stamatatos, *V2 loop glycosylation of the human immunodeficiency virus type 1 SF162 envelope facilitates interaction of this protein with CD4 and CCR5 receptors and protects the virus from neutralization by anti-V3 loop and anti-CD4 binding site antibodies.* J Virol, 2000. **74**(15): p. 6769-76.

150. O'Rourke, S.M., B. Schweighardt, P. Phung, D.P. Fonseca, K. Terry, T. Wrin, F. Sinangil, and P.W. Berman, *Mutation at a single position in the V2 domain of the HIV-1 envelope protein confers neutralization sensitivity to a highly neutralization-resistant virus.* J Virol, 2010. **84**(21): p. 11200-9.

151. Pinter, A., W.J. Honnen, Y. He, M.K. Gorny, S. Zolla-Pazner, and S.C. Kayman, *The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary human immunodeficiency virus type 1 isolates to neutralization by antibodies commonly induced upon infection.* J Virol, 2004. **78**(10): p. 5205-15.

152. Ye, Y., Z.H. Si, J.P. Moore, and J. Sodroski, *Association of structural changes in the V2 and V3 loops of the gp120 envelope glycoprotein with acquisition of neutralization resistance in a simian-human immunodeficiency virus passaged in vivo.* J Virol, 2000. **74**(24): p. 11955-62.

153. Shieh, J.T., J. Martin, G. Baltuch, M.H. Malim, and F. Gonzalez-Scarano, *Determinants of syncytium formation in microglia by human immunodeficiency virus type 1: role of the V1/V2 domains.* J Virol, 2000. **74**(2): p. 693-701.

154. Lynch, R.M., R. Rong, B. Li, T. Shen, W. Honnen, J. Mulenga, S. Allen, A. Pinter, S. Gnanakaran, and C.A. Derdeyn, *Subtype-specific conservation of isoleucine 309 in the envelope V3 domain is linked to immune evasion in subtype C HIV-1 infection.* Virology, 2010. **404**(1): p. 59-70.

155. Li, Y., B. Cleveland, I. Klots, B. Travis, B.A. Richardson, D. Anderson, D. Montefiori, P. Polacino, and S.L. Hu, *Removal of a single N-linked glycan in human immunodeficiency virus type 1 gp120 results in an enhanced ability to induce neutralizing antibody responses.* J Virol, 2008. **82**(2): p. 638-51.

156. Zhu, C.B., L. Zhu, S. Holz-Smith, T.J. Matthews, and C.H. Chen, *The role of the third beta strand in gp120 conformation and neutralization sensitivity of the HIV-1 primary isolate DH012.* Proc Natl Acad Sci U S A, 2001. **98**(26): p. 15227-32.

157. Blish, C.A., M.A. Nguyen, and J. Overbaugh, *Enhancing exposure of HIV-1 neutralization epitopes through mutations in gp41.* PLoS Med, 2008. **5**(1): p. e9.

158. Chen, L., Y.D. Kwon, T. Zhou, X. Wu, S. O'Dell, L. Cavacini, A.J. Hessell, M. Pancera, M. Tang, L. Xu, Z.Y. Yang, M.Y. Zhang, J. Arthos, D.R. Burton, D.S. Dimitrov, G.J. Nabel, M.R. Posner, J. Sodroski, R. Wyatt, J.R. Mascola, and P.D. Kwong, *Structural basis of immune evasion at the site of CD4 attachment on HIV-1 gp120.* Science, 2009. **326**(5956): p. 1123-7.

159. Baeten, J.M., B. Chohan, L. Lavreys, V. Chohan, R.S. McClelland, L. Certain, K. Mandaliya, W. Jaoko, and J. Overbaugh, *HIV-1 subtype D infection is associated with faster disease progression than subtype A in spite of similar plasma HIV-1 loads.* J Infect Dis, 2007. **195**(8): p. 1177-80.

160. Easterbrook, P.J., M. Smith, J. Mullen, S. O'Shea, I. Chrystie, A. de Ruiter, I.D. Tatt, A.M. Geretti, and M. Zuckerman, *Impact of HIV-1 viral subtype on disease progression and response to antiretroviral therapy.* J Int AIDS Soc, 2010. **13**: p. 4.

161. Kaleebu, P., N. French, C. Mahe, D. Yirrell, C. Watera, F. Lyagoba, J. Nakiyingi, A. Rutebemberwa, D. Morgan, J. Weber, C. Gilks, and J. Whitworth, *Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda.* J Infect Dis, 2002. **185**(9): p. 1244-50.

162. Kiwanuka, N., M. Robb, O. Laeyendecker, G. Kigozi, F. Wabwire-Mangen, F.E. Makumbi, F. Nalugoda, J. Kagaayi, M. Eller, L.A. Eller, D. Serwadda, N.K. Sewankambo, S.J. Reynolds, T.C. Quinn, R.H. Gray, M.J. Wawer, and C.C. Whalen, *HIV-1 viral subtype differences in the rate of CD4+ T-cell decline among HIV seroincident antiretroviral naive persons in Rakai district, Uganda.* J Acquir Immune Defic Syndr, 2010. **54**(2): p. 180-4.

163. Vasan, A., B. Renjifo, E. Hertzmark, B. Chaplin, G. Msamanga, M. Essex, W. Fawzi, and D. Hunter, *Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype.* Clin Infect Dis, 2006. **42**(6): p. 843-52.

164. Sacktor, N., N. Nakasujja, R.L. Skolasky, M. Rezapour, K. Robertson, S. Musisi, E. Katabira, A. Ronald, D.B. Clifford, O. Laeyendecker, and T.C. Quinn, *HIV subtype D is associated with dementia, compared with subtype A, in immunosuppressed individuals at risk of cognitive impairment in Kampala, Uganda.* Clin Infect Dis, 2009. **49**(5): p. 780-6.

165. Esbjornsson, J., F. Mansson, W. Martinez-Arias, E. Vincic, A.J. Biague, Z.J. da Silva, E.M. Fenyo, H. Norrgren, and P. Medstrand, *Frequent CXCR4 tropism of HIV-1 subtype A and CRF02_AG during late-stage disease--indication of an evolving epidemic in West Africa.* Retrovirology, 2010. **7**: p. 23.

166. Musich, T., P.J. Peters, M.J. Duenas-Decamp, M.P. Gonzalez-Perez, J. Robinson, S. Zolla-Pazner, J.K. Ball, K. Luzuriaga, and P.R. Clapham, *A*

*conserved determinant in the V1 loop of HIV-1 modulates the V3 loop to prime low CD4 use and macrophage infection.* J Virol, 2011. **85**(5): p. 2397-405.

167. Alexander, M., R. Lynch, J. Mulenga, S. Allen, C.A. Derdeyn, and E. Hunter, *Donor and recipient envs from heterosexual human immunodeficiency virus subtype C transmission pairs require high receptor levels for entry.* J Virol, 2010. **84**(8): p. 4100-4.
168. Peters, P.J., M.J. Duenas-Decamp, W.M. Sullivan, and P.R. Clapham, *Variation of macrophage tropism among HIV-1 R5 envelopes in brain and other tissues.* J Neuroimmune Pharmacol, 2007. **2**(1): p. 32-41.
169. Bakri, Y., C. Schiffer, V. Zennou, P. Charneau, E. Kahn, A. Benjouad, J.C. Gluckman, and B. Canque, *The maturation of dendritic cells results in postintegration inhibition of HIV-1 replication.* J Immunol, 2001. **166**(6): p. 3780-8.
170. Geijtenbeek, T.B., D.S. Kwon, R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, J. Middel, I.L. Cornelissen, H.S. Nottet, V.N. KewalRamani, D.R. Littman, C.G. Figdor, and Y. van Kooyk, *DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells.* Cell, 2000. **100**(5): p. 587-97.
171. de Witte, L., M. Bobardt, U. Chatterji, G. Degeest, G. David, T.B. Geijtenbeek, and P. Gallay, *Syndecan-3 is a dendritic cell-specific*

*attachment receptor for HIV-1.* Proc Natl Acad Sci U S A, 2007. **104**(49): p. 19464-9.

172. Cameron, P.U., P.S. Freudenthal, J.M. Barker, S. Gezelter, K. Inaba, and R.M. Steinman, *Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells.* Science, 1992. **257**(5068): p. 383-7.
173. Pinchuk, L.M., P.S. Polacino, M.B. Agy, S.J. Klaus, and E.A. Clark, *Cell-cell interactions regulate dendritic cell-dependent HIV-1 production in CD4+ T lymphocytes.* Adv Exp Med Biol, 1995. **378**: p. 461-3.
174. Tsunetsugu-Yokota, Y., S. Yasuda, A. Sugimoto, T. Yagi, M. Azuma, H. Yagita, K. Akagawa, and T. Takemori, *Efficient virus transmission from dendritic cells to CD4+ T cells in response to antigen depends on close contact through adhesion molecules.* Virology, 1997. **239**(2): p. 259-68.
175. Delgado, E., V. Finkel, M. Baggiolini, C.R. Mackay, R.M. Steinman, and A. Granelli-Piperno, *Mature dendritic cells respond to SDF-1, but not to several beta-chemokines.* Immunobiology, 1998. **198**(5): p. 490-500.
176. Pope, M., M.G. Betjes, N. Romani, H. Hirmand, P.U. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R.M. Steinman, *Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1.* Cell, 1994. **78**(3): p. 389-98.

177. Boggiano, C., N. Manel, and D.R. Littman, *Dendritic cell-mediated trans-enhancement of human immunodeficiency virus type 1 infectivity is independent of DC-SIGN.* J Virol, 2007. **81**(5): p. 2519-23.

178. von Andrian, U.H. and C.R. Mackay, *T-cell function and migration. Two sides of the same coin.* N Engl J Med, 2000. **343**(14): p. 1020-34.

179. Wagner, N., J. Lohler, E.J. Kunkel, K. Ley, E. Leung, G. Krissansen, K. Rajewsky, and W. Muller, *Critical role for beta7 integrins in formation of the gut-associated lymphoid tissue.* Nature, 1996. **382**(6589): p. 366-70.

180. Cicala, C., E. Martinelli, J.P. McNally, D.J. Goode, R. Gopaul, J. Hiatt, K. Jelicic, S. Kottilil, K. Macleod, A. O'Shea, N. Patel, D. Van Ryk, D. Wei, M. Pascuccio, L. Yi, L. McKinnon, P. Izulla, J. Kimani, R. Kaul, A.S. Fauci, and J. Arthos, *The integrin alpha4beta7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1.* Proc Natl Acad Sci U S A, 2009. **106**(49): p. 20877-82.

181. Cicala, C., J. Arthos, and A.S. Fauci, *HIV-1 envelope, integrins and co-receptor use in mucosal transmission of HIV.* J Transl Med, 2011. **9 Suppl 1**: p. S2.

182. Arthos, J., C. Cicala, E. Martinelli, K. Macleod, D. Van Ryk, D. Wei, Z. Xiao, T.D. Veenstra, T.P. Conrad, R.A. Lempicki, S. McLaughlin, M. Pascuccio, R. Gopaul, J. McNally, C.C. Cruz, N. Censoplano, E. Chung, K.N. Reitano, S. Kottilil, D.J. Goode, and A.S. Fauci, *HIV-1 envelope*

*protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells.* Nat Immunol, 2008. **9**(3): p. 301-9.

183. Schneider, U., H.U. Schwenk, and G. Bornkamm, *Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma.* Int J Cancer, 1977. **19**(5): p. 621-6.

184. Granelli-Piperno, A., I. Shimeliovich, M. Pack, C. Trumpfheller, and R.M. Steinman, *HIV-1 selectively infects a subset of nonmaturing BDCA1-positive dendritic cells in human blood.* J Immunol, 2006. **176**(2): p. 991-8.

185. Berger, G., S. Durand, G. Fargier, X.N. Nguyen, S. Cordeil, S. Bouaziz, D. Muriaux, J.L. Darlix, and A. Cimarelli, *APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells.* PLoS Pathog, 2011. **7**(9): p. e1002221.

186. Brenchley, J.M., D.A. Price, T.W. Schacker, T.E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B.R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J.N. Martin, F.M. Hecht, L.J. Picker, M.M. Lederman, S.G. Deeks, and D.C. Douek, *Microbial translocation is a cause of systemic immune activation in chronic HIV infection.* Nat Med, 2006. **12**(12): p. 1365-71.

187. Cavrois, M., J. Neidleman, J.F. Kreisberg, D. Fenard, C. Callebaut, and W.C. Greene, *Human immunodeficiency virus fusion to dendritic cells declines as cells mature.* J Virol, 2006. **80**(4): p. 1992-9.

188. Geijtenbeek, T.B. and Y. van Kooyk, *DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission.* Curr Top Microbiol Immunol, 2003. **276**: p. 31-54.

189. Izquierdo-Useros, N., J. Blanco, I. Erkizia, M.T. Fernandez-Figueras, F.E. Borras, M. Naranjo-Gomez, M. Bofill, L. Ruiz, B. Clotet, and J. Martinez-Picado, *Maturation of blood-derived dendritic cells enhances human immunodeficiency virus type 1 capture and transmission.* J Virol, 2007. **81**(14): p. 7559-70.

190. Nawaz, F., C. Cicala, D. Van Ryk, K.E. Block, K. Jelicic, J.P. McNally, O. Ogundare, M. Pascuccio, N. Patel, D. Wei, A.S. Fauci, and J. Arthos, *The genotype of early-transmitting HIV gp120s promotes alpha (4) beta(7)-reactivity, revealing alpha (4) beta(7) +/CD4+ T cells as key targets in mucosal transmission.* PLoS Pathog, 2011. **7**(2): p. e1001301.

191. Rusert, P., A. Krarup, C. Magnus, O.F. Brandenberg, J. Weber, A.K. Ehlert, R.R. Regoes, H.F. Gunthard, and A. Trkola, *Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimer against cross-neutralizing antibodies.* J Exp Med, 2011. **208**(7): p. 1419-33.

192. Haynes, B.F., P.B. Gilbert, M.J. McElrath, S. Zolla-Pazner, G.D. Tomaras, S.M. Alam, D.T. Evans, D.C. Montefiori, C. Karnasuta, R. Sutthent, H.X. Liao, A.L. DeVico, G.K. Lewis, C. Williams, A. Pinter, Y. Fong, H. Janes, A. DeCamp, Y. Huang, M. Rao, E. Billings, N. Karasavvas, M.L. Robb, V.

Ngauy, M.S. de Souza, R. Paris, G. Ferrari, R.T. Bailer, K.A. Soderberg, C. Andrews, P.W. Berman, N. Frahm, S.C. De Rosa, M.D. Alpert, N.L. Yates, X. Shen, R.A. Koup, P. Pitisuttithum, J. Kaewkungwal, S. Nitayaphan, S. Rerks-Ngarm, N.L. Michael, and J.H. Kim, *Immune-correlates analysis of an HIV-1 vaccine efficacy trial.* N Engl J Med, 2012. **366**(14): p. 1275-86.

193. Hartley, O., P.J. Klasse, Q.J. Sattentau, and J.P. Moore, *V3: HIV's switch-hitter.* AIDS Res Hum Retroviruses, 2005. **21**(2): p. 171-89.

194. Fatkenheuer, G., A.L. Pozniak, M.A. Johnson, A. Plettenberg, S. Staszewski, A.I. Hoepelman, M.S. Saag, F.D. Goebel, J.K. Rockstroh, B.J. Dezube, T.M. Jenkins, C. Medhurst, J.F. Sullivan, C. Ridgway, S. Abel, I.T. James, M. Youle, and E. van der Ryst, *Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1.* Nat Med, 2005. **11**(11): p. 1170-2.

195. Li, Y., S.A. Migueles, B. Welcher, K. Svehla, A. Phogat, M.K. Louder, X. Wu, G.M. Shaw, M. Connors, R.T. Wyatt, and J.R. Mascola, *Broad HIV-1 neutralization mediated by CD4-binding site antibodies.* Nat Med, 2007. **13**(9): p. 1032-4.

196. Scheid, J.F., H. Mouquet, B. Ueberheide, R. Diskin, F. Klein, T.Y. Oliveira, J. Pietzsch, D. Fenyo, A. Abadir, K. Velinzon, A. Hurley, S. Myung, F. Boulad, P. Poignard, D.R. Burton, F. Pereyra, D.D. Ho, B.D. Walker, M.S. Seaman, P.J. Bjorkman, B.T. Chait, and M.C. Nussenzweig, *Sequence*

*and structural convergence of broad and potent HIV antibodies that mimic CD4 binding.* Science, 2011. **333**(6049): p. 1633-7.

197. Wu, X., C. Wang, S. O'Dell, Y. Li, B.F. Keele, Z. Yang, H. Imamichi, N. Doria-Rose, J.A. Hoxie, M. Connors, G.M. Shaw, R.T. Wyatt, and J.R. Mascola, *Selection Pressure on HIV-1 Envelope by Broadly Neutralizing Antibodies to the Conserved CD4-Binding Site.* J Virol, 2012. **86**(10): p. 5844-56.
198. Manel, N., B. Hogstad, Y. Wang, D.E. Levy, D. Unutmaz, and D.R. Littman, *A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells.* Nature, 2010. **467**(7312): p. 214-7.
199. Rao, V.R., A.R. Sas, E.A. Eugenin, N.B. Siddappa, H. Bimonte-Nelson, J.W. Berman, U. Ranga, W.R. Tyor, and V.R. Prasad, *HIV-1 clade-specific differences in the induction of neuropathogenesis.* J Neurosci, 2008. **28**(40): p. 10010-6.
200. Granelli-Piperno, A., A. Golebiowska, C. Trumpfheller, F.P. Siegal, and R.M. Steinman, *HIV-1-infected monocyte-derived dendritic cells do not undergo maturation but can elicit IL-10 production and T cell regulation.* Proc Natl Acad Sci U S A, 2004. **101**(20): p. 7669-74.
201. Brockman, M.A., D.S. Kwon, D.P. Tighe, D.F. Pavlik, P.C. Rosato, J. Sela, F. Porichis, S. Le Gall, M.T. Waring, K. Moss, H. Jessen, F. Pereyra, D.G. Kavanagh, B.D. Walker, and D.E. Kaufmann, *IL-10 is up-regulated in*

*multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells.* Blood, 2009. **114**(2): p. 346-56.