AIDS Rev 2001; 3: 67-74

Alternative Targets of Productive HIV Infection: Role of CD4 Up-Regulation on Susceptibility of Cells to HIV Infection

Lena Al-Harthi and Alan Landay

Department of Immunology/Microbiology at Rush-Presbyterian St. Luke's Medical Center, Rush University, Chicago, IL, USA

Abstract

The hallmark of HIV infection is the progressive depletion of its main cellular target, the CD4+ T cells. Considerable evidence, however, indicate that cell types that classically do not express CD4 may also be active sites of HIV infection. These "non-conventional" targets for HIV infection may play a role in the pathogenesis of HIV, especially at late stage of the disease, as the primary CD4+ cellular targets are depleted and these alternative cell types may play a more dominant role in the propagation of the virus. Numerous cell types support HIV entry but fail to produce virus progeny. Therefore, virus entry may not always translate into reverse transcription, integration, and ultimately productive replication. In this review, we will concentrate on productive HIV infection, unless otherwise stated, of lymphoid and non-lymphoid cells that are not classically associated with HIV. We also describe the role of CD4 protein up-regulation on the cell surface of otherwise CD4+ cells, promoting their permissiveness to HIV infection.

Key words

HIV. *Naïve* T cells. Lymphoid cells. Non-lymphoid cells. B cells. NKT. CD8+ T cells.

Introduction

Much attention has focused on cells bearing the CD4 molecule and chemokine co-receptors as the primary targets of HIV infection. However, accumulating data indicates that the CD4 molecule can be up-regulated on lymphoid cells altering their susceptibility to HIV. This review will focus on the role of CD4 up-regulation on susceptibility to HIV infection. We will also discuss and review HIV infection of cell types of lymphoid and non-lymphoid origin other than CD4+ T helper cells. First, however, we will present current analysis of the primary targets of HIV infection.

Correspondence to:
Lena Al-Harthi
Rush Presbyterian St. Luke's Medical Center
Department of Immunology/Microbiology.
1653 West Congress Parkway, Rm. 1577 JSC
Chicago, IL 60612, USA

Primary Targets of HIV infection

The main targets of HIV infection are CD4+ T cells and cells of the myeloid lineage, which consist of monocyte/macrophages (reviewed in[49]) and dendritic cells, including the mucosal epithelial dendritic cells known as Langerhan cells[14,22,41,47].

HIV infection of memory/primed and naïve T cells

Although the mechanism is still unidentified, primed/activated cells are known to support the

productive infection of HIV while *naïve*/unprimed T cells support viral entry[9,60] but not productive infection[67]. *Naïve* cells respond poorly to recall antigens and secrete a limited cytokine profile consisting mainly of IL-2. They are designated as CD45RA+, producing all three alternatively spliced exons. On the other hand, memory cells respond to recall antigens, produce an expanded cytokine profile, and are designated as CD45RO+, expressing the smallest isoform of CD45[18,72,82]. Although CD45RA/CD45RO are extensively used to distinguish between *naïve* (RA+) and memory/primed (RO+) T cells, the presence of CD45RA+RO+ T cell populations[37,44,81] especially during the $S/G_2/M$ stage of the cell cycle[45], reversion of RO+ memory cells to RA+ *naïve* T cells in humans[54,61] and rats[8], and the cellular proliferation and expansion of CD45RA+ T cells without the acquisition of the CD45RO phenotype[74,84,85,89], collectively render the distinction between primed/memory and *naïve* T cells on the sole criteria of RA/RO expression inadequate.

Initial studies indicated that *naïve* T cells harbor replication competent HIV[9,60] but require an additional signal such as mitogenic stimulation to support HIV productive infection[67]. Infected *naïve* cell populations have also been identified in HIV+ individuals[9,60] but it is unclear if such *in vivo naïve* T cells were once primed cells that reverted to a *naïve* phenotype[54,61] or are truly *naïve* T cells that are infected *in vivo*. Recent data from ex vivo lymphoid histocultures point to HIV productive infection of *naïve* T cells[35]. The infected *naïve* T cells were in the G0/G1A phase of the cell cycle, indicating that HIV may not require active cell replication as previously thought for productive infection. Cells at the G1B cell cycle phase or greater promote higher degree of HIV p24 production, presumably via higher level of deoxyribonucleotides present for reverse transcription[79] or that actively replicating cells may express certain cellular factor(s) that may allow for the stabilization of the HIV pre-integration complex. A recent report has shown that cells that express HLA-DR, an MHC-II molecule up-regulated on activated T cells, are associated with higher level of HIV replication than parental cells lacking HLA-DR[70-71].

We have determined that interleukin (IL)-7 pretreatment of *naïve* (CD45RA+CD45RO-) cells prior to HIV infection can induce the productive HIV infection of *naïve* T cells, while maintaining their CD45RA+CD45RO- phenotype (unpublished data). The virions produced from these *naïve* T cells were also infectious. IL-7 treatment of *naïve* T cells also up-regulated CXCR4 but not CCR5 expression (unpublished data). Recently, few studies have reported an inverse relationship between IL-7 serum levels and CD4+ T cell count[16,27]. This inverse relationship was explained by the role that IL-7 may have on T cell homeostasis, whereby as T cell counts are reduced due to HIV-mediated pathogenesis, IL-7 levels are increased to attempt to overcome this depletion. However, as CD4 counts increase post-HAART, IL-7 serum levels returns to normal levels[27]. Although IL-7 may still contribute to T cell homeostasis, our finding that IL-7 induces HIV replication in *naïve* T cells coupled with a previous report that IL-7 is a critical factor produced by thymic stromal cells that mediates HIV replication in thymocytes[13] suggest that this inverse relationship between IL-7 levels and CD4 counts may also be due to IL-7 mediated enhanced viral load, leading to direct or indirect CD4 loss.

HIV infection of monocyte/macrophages and dendritic cells:

Although monocyte/macrophages support the productive infection of HIV, they are not affected by the cytopathic effect of HIV and hence are believed to constitute a source of chronic HIV infection. HIV productive or non-productive infection of dendritic cells is regulated by whether the dendritic cells are immature or mature, respectively[5]. Based on the SIV model, dendritic cells appear to constitute an initial target for primary sexual HIV infection[40,76]. Although dendritic cells are infected by HIV, these cells play a major role in virus transmission to susceptible cells. Recently, a dendritic cell receptor (DC-SIGN) has been implicated in efficiently binding HIV and transmitting the virus to T cells[33]. A DC-SIGN homologue (DC-SIGNR) expressed on sinusoidal endothelial liver cells, endothelial lymph node cells, and placental villi may play a role in HIV transmission to cells in lymph node as well as in vertical transmission of HIV[62,75].

CD4 expression on CD4 negative cells: A mechanism of promoting susceptibility to HIV infection

Lessons learned from efforts to develop the mouse model to study HIV pathogenesis indicated that CD4 expression alone is not sufficient to induce HIV entry. Chemokine co-receptors, belonging to the family of G protein-coupled seven-transmembrane-domain proteins (CXCR4, CCR1, CCR2a/b, CCR3 and CCR5), are essential for HIV fusion. The cellular tropism of HIV is dictated by the expression of CD4 and chemokine co-receptors. Given that HIV utilizes CD4 as a receptor to gain entry to target cells, expression of CD4 along with one of the chemokine co-receptors (CCR3, CCR5, CXCR4) on target cells is critical for HIV infection. CD4 is expressed on T-helper cells and a subset of monocyte/macrophages. The CD4 molecule can also be expressed on CD4 negative T cells, rendering these cells permissive to HIV infection. To date, it appears that two possible mechanisms exist for CD4 expression on CD4 negative T cells: 1) *de novo* synthesis and cell surface expression of the CD4 molecule post-cellular activation, and 2) CD4 antigen transfer via microvesicle formation.

De novo synthesis and cell surface expression of the CD4 molecule on lymphocytes (CD8 and B cells)

The expression of the CD4 or CD8 co-receptors classically distinguishes T helper from cytotoxic T

cells, respectively. However, in the periphery, 3-5% of normal T cells express both CD4 and CD8 on their surface. During thymic T cell development, a double positive (CD4+CD8+) T cell stage exits. However, it is unlikely that these peripheral CD4+CD8+ T cells are prematurely released from the thymus given their lack of CD1a expression, which is a marker of premature thymic T cells[80]. To date, a number of studies have shown that under the appropriate conditions CD4 can be up-regulated on the surface of CD8+ T cells. Specifically, CD3/CD28 co-stimulation[43,80], superantigen (Staphylococcal enterotoxin B, SEB)[24,80] stimulation, or allogeneic dendritic cell interaction[94] induces the *de novo* expression of CD4 as demonstrated by elevation in CD4 mRNA43 and protein expression on CD8+ T cells[24,43,80,94]. Both *naïve* and memory CD8+ T cells can express CD4 upon stimulation. However, the purified *naïve* CD8+ population is more responsive to CD4 induction[43]. In all of these studies, while the percent induction of CD4 expression on CD8+ T cells was substantial, the intensity of CD4 expression as evaluated by flow cytometry, was not as intense as that observed on single positive CD4+ T cells, reflecting a "dim" expression of CD4 on CD8+ T cells (Figure 1). We will refer to these cells as $CD4^{dim}CD8^{bright}$ cells in this review.

$CD4^{dim}CD8^{bright}$ T cells appear to be phenotypically distinct from CD8+ T cells that do not up-regulate CD4 expression[80]. These cells express significantly higher levels of CD95 (Fas receptor and a marker for primed cells), CD25 (IL-2 receptor α chain), CD38 (activation marker), CD69 (an early marker of T cell activation), CD28 (receptor for co-stimulatory signal), and CD45RO (marker of primed/memory T cells) than their CD8+CD4- counterparts[80]. $CD4^{dim}CD8^{bright}$ T cells are also predominately TCR $\alpha\beta$ cells[24,80]. The finding that $CD4^{dim}CD8^{bright}$ T cells are an activated phenotype of CD8+ T cells suggests that CD4 up-regulation on CD8+ T cells may function as an additional marker to identify activated CD8+ T cells, however, currently no data exists regarding the role that $CD4^{dim}CD8^{bright}$ T cells may play in normal T cell biology and in HIV disease.

In recent years, a model has emerged of CD4+ T cell help for effective CD8+ T cell immune response. According to this model, CD4 and CD8 T cells must recognize the same antigen presented on the same antigen presenting cell (APC). In this manner, CD4 cells must secret short-range cytokines to activate CD8+ T cells[15,42]. Having CD4 and CD8 expression on the same cell may allow for CD4 and CD8 co-recognition of the antigen being presented on the same APC. Additionally, the finding that the induced CD4 protein on activated CD8+ T cells is linked to the Src family protein tyrosine kinase $p56^{lck}$ (Lck)[24], a kinase that is required for the CD4-mediated signal transduction cascade, indicates that CD4 may be functional and may provide the necessary T-helper function without a requirement for an adjacent CD4+ T cells. Recently, CD8+ T cells, with HIV specificity, were genetically engineered to express CD4ζ molecule[55]. The rationale for this approach was to generate HIV-specific CD8+ T cells that provide their own helper function via the expression of the CD4 molecule. These engineered CD4+CD8+ cells homed preferentially to rectal tissue where enhanced reduction in rectal viral load was detected among CD4+CD8+ transfused HIV+ patients[55]. This recent finding points to a functional role for the CD4 molecule on CD8+ T cells.

We have shown that $CD4^{dim}CD8^{bright}$ T cells express both CXCR4 and CCR5 (unpublished data) and others have reported that $CD4^{dim}CD8^{bright}$ T cells are susceptible to infection by both CCR5-dependent[94] and CXCR4-dependant HIV strains[43]. We have examined approximately 200 HIV+ patients for the presence of $CD4^{dim}CD8^{bright}$ T cells and found

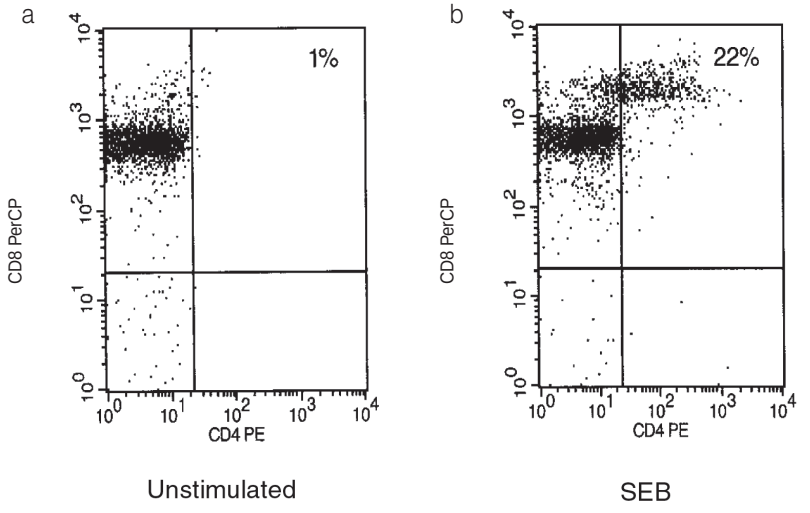

Fig. 1. *$CD4^{dim}CD8^{bright}$ cells: A flow cytometric representation of the dim expression of CD4 on CD8+ T cells. These cells were generated by isolating purified CD8+ T cells and stimulating the cells with SEB for six days (b). Unstimulated CD8+ T cells are shown (a). The cells were then stained with CD3 fluorescein isothiocyanate (FITC): CD4 phycoerythrin (PE): CD8 peridinin chlorophyll protein (PerCP) conjugated antibodies and analyzed using flow cytometry.*

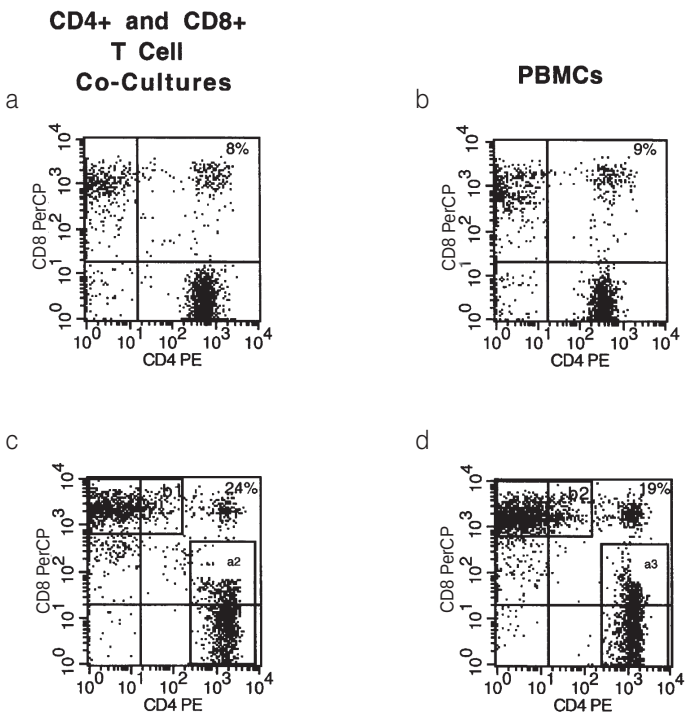

Fig. 2. *Generation of $CD4^{dim}CD8^{bright}$ and $CD4^{bright}CD8^{dim}$ T cells: These cells were generated by purifying CD4 and CD8 T cells and co-culturing the cells together in the presence of PHA (b) or by PHA-stimulating PBMCs (d). Unstimulated cultures are shown in (a) and (c), respectively. Gating is shown as indicated in the figure.*

levels of $CD4^{dim}CD8^{bright}$ T cell expression greater than the normal 3-5% range in only 5 patients. Given that infection of these cells *in vivo* can lead to the down-regulation of CD4 and that no information exists on the stability of this phenotype *in vivo*, we were not surprised by this low frequency of detection of these cells in HIV+ patients (Sullivan et al, unpublished data).

$CD4^{dim}CD8^{bright}$ cells may play a role in HIV pathogenesis. HIV infection is associated with a progressive loss of CD4+ T cells. Induction of CD4 on CD8+ T cells may be a normal physiological response and might play a role in maintaining "helper" responses in HIV infection. On one hand, CD4 induction on CD8+ T cells may compensate for the functional loss of CD4+ T cells, especially since some individuals with low CD4 counts do not experience opportunistic infections. On the other hand, CD4 expression on CD8+ T cells may also be detrimental because CD8+ T cells will become susceptible to virus replication. CD8+ T cell loss is often associated with advanced HIV disease and infection of CD8+ T cells may be a possible mechanism for this depletion. Additionally, as CD4 cells are progressively lost in advanced HIV disease, these $CD4^{dim}CD8^{bright}$ T cells may support the replication of CXCR4 viruses, possibly playing a role in the switch from CCR5 to CXCR4 tropic HIV strains observed in late HIV disease.

B cells can also be induced to express CD4. Upon CD40-CD40L engagement, B cells up-regulate CD4 on their surface[56] *in vivo*. It is estimated that 10% of peripheral blood B cells from healthy donors express CD4, CXCR4, and CCR5[26]. CD4+ B cells (CD4+CD19+) represent an activated phenotype. Unprimed B cells express CD4mRNA and not CD4 protein, suggesting a post-transcriptional block to CD4 expression in these unprimed B[96]. Activated CD4+CD19+ cells are susceptible to productive *in vitro* HIV infection by both T-tropic and M-tropic strains of HIV[26,56]. Although *in vivo*, complement and anti-HIV antibodies may enhance B cell HIV infection[36]; the presence of infected CD4+CD19+ cells *in vivo* is undetermined.

CD4 expression on non-T/ non B-lymphocytes (NKT) and eosinophils

The CD4 molecule is also expressed on other cells, including NKT and eosinophils. Unlike NK cells, NKT cells are characterized by the expression of NK-associated molecules along with an intermediate expression of the T cell receptor (TCR) and either CD4 (CD4+NK), CD8 (CD8+NK), or neither molecule (CD4-CD8-NK). Human NKT TCR is limited to Vα24JαQVβ11 repertoire. In mice, the equivalent recombination (TCR Vα14Jα281Vβ8.2) is abundant in CD4+ NKT cells but CD8+ NKT cells have a more diverse repertoire (reviewed in[34]). NKT cells play a role in immunoregulatory cytokine production (IL-4, IL-12, INFγ, TNFα), lytic activity, and even immunosuppression of tumor cells[57,83]. To date, it is undetermined if NKT cells can be infected by HIV. However, given that some NKT cells express CD4 and providing that they also co-express one of the chemokine co-receptors, NKT cells could be viable targets for HIV infection. Studies evaluating the role of NKT cells in normal T and/or NK cell im-

munology and even in HIV disease has been hampered by the low frequency of these cells in the blood. NKT cells constitute only 0.1- 0.5% of total peripheral blood lymphocytes (reviewed in[34]). The recent report of *ex vivo* expansion of NKT through the use of alpha-galactosylceramide KRN7000 loaded monocyte-derived dendritic cells[88], which present this antigen in the context of CD1d to NKT cells, should pave the way for studies to evaluate the susceptibility of CD4+ NKT cells to HIV infection *in vitro* and *in vivo*.

During the maturation process of eosinophils, these cells express higher levels of CD4, which declines as eosinophils mature[25,46]. Low level of CD4 expression on mature peripheral eosinophils still supports HIV productive infection[91]. Infectivity assays utilizing CD4+ eosinophilic cell line (AML14.3D10) indicate that these cells are infected by T-tropic but not M-tropic HIV, even though this particular cell line is positive for both CXCR4 and CCR5[95]. HIV provirus in eosinophils was also detected in 11% of a small cohort of HIV+ patients[19]. Given that eosinophils are abundant in the gastrointestinal and the urinary tract, HIV infection of eosinophils may confer HIV access to these target tissues, which may contribute to some of the reported dysregulation associated with HIV tropism in these sites.

CD4 antigen transfer via microvesicle formation as a possible mechanism promoting cellular susceptibility to HIV infection

Thus far we have introduced the concept of *de novo* CD4 expression on CD4 negative cells, allowing for HIV infection. An interesting yet understudied concept is concerning the role of antigen transfer in HIV pathogenesis. Considerable data indicate that microvesicle, also referred to as microparticles or exosomes, are spontaneously released from cells. These microvesicles are 0.1-2 µm in size and contain cytoplasmic components packaged within the plasma membrane of the original cell and often express host cell surface proteins[2,7,97]. Microvesicles may also be functional. In one example, microvesicles formed from B cells were able to present antigen and activate T cells[66]. Microvesicles may also be able to transfer cell surface proteins from one cell to another. One study in particular provided functional relevance to this transfer in the context of HIV. Mack et al[48] demonstrated that microvesicles transferred from CCR5+ to CCR5- cells rendered the recipient cells susceptible to M-tropic HIV infection. This study in particular is critical in highlighting the role that antigen transfer may play in providing additional targets for HIV infection. In our experience, co-culturing of CD4 and CD8 T cells, with or without PHA stimulation, also leads to the generation of both $CD4^{dim}CD8^{bright}$ (CD8 cells that express CD4), and $CD8^{dim}CD4^{bright}$ (CD4 cells that express CD8) cells[80]. The role of antigen transfer in the generation of these phenotypes cannot be ruled out. Close interaction between CD8+ T cells and CD4+ T cells in hyper-immune activated backdrop in HIV+ patients may lead to the expression of these phenotypes that will add to the complexity of potential cellular targets for HIV infection.

CD4-independent HIV infection

Infection of lymphoid cells

We previously described the role that CD4 up-regulation on CD8+ T cells may have in HIV pathogenesis. Evidence of HIV infection of CD4-CD8+ T cells are also slowly emerging[20,45,52,95]. HIV clones isolated from naturally infected CD4-CD8+ T cells *in vitro* productively infected CD4-CD8+ primary and CD4-CD8+ T cell clones, independent of CD4, CXCR4, or CCR5 usage[95]. Blocking of the CD8 co-receptor in these studies also blocked HIV infection[95], demonstrating that the CD8 molecule in some occasions can be used as a receptor for HIV, although it is still unclear if this adaptation is common *in vivo*, an artifact of the cloning process to isolate these HIV strains, or a unique characteristic of these HIV clones. *In vivo*, HIV provirus was also detected in CD4-CD8+ T cells[45]. Three possible scenarios exist for *in vivo* detection of HIV provirus in CD4-CD8+ T cells: 1) the initial infection could have been in CD8+ T that up-regulated the CD4 molecule (CD4+CD8+) but that HIV infection led to the down-regulation of the CD4 molecule. 2) Infection of lymphocyte at the double positive stage in the thymus or earlier[77], leading to harboring of the provirus as the cells mature and exist into the periphery[10]. This scenario maybe supported by the predominant presence of HIV provirus in CD45RA+ T cells and not CD45RO+ T cells[51]. However, it is unclear from this study if the RA+ CD8+ T cells were also RO+ cells, which is characteristic of a primed phenotype. 3) Genuine infection of CD4-CD8+ T cells through a CD4-independent mechanism. Further studies need to be performed to distinguish between these three pathways. NK cells lacking CD4 expression can be non-productively infected by HIV[73]. NK cell function is reported to be impaired in HIV disease but normalizes after viral suppression[90], which is most probably due to HIV-mediated immune dysregulation rather than direct cytopathic effect of HIV on NK cells. Recently a subpopulation of NK cells, identified as CD3-CD4+TCR-CD56+, was reported to be positive for HIV proviral DNA *in vivo*[86]. Because this subpopulation is TCR-, they cannot be characterized as NKT cells. Additionally, it is unclear if the infection of this NK subpopulation is CD4 dependent or independent.

Infection of non-lymphoid cells

HIV infection of non-lymphoid cells, such as neutrophils, hepatocytes, renal cells, brain cells, and possibly even gametes has also been documented. Neutrophils can be a target for HIV infection, as illustrated by the presence of HIV DNA in both *in vitro* and *in vivo* studies[29,31]. Neutrophils in late stage of HIV disease are often altered in their LPS-induced

cytokine production[32] as well as in transmigration studies across an epithelial barrier[39], which according to one study appear to normalize after highly active antiretroviral therapy[50]. Basophils express CCR3 but no data exists to date on their susceptibility to HIV infection[21].

HIV infection of renal cells may explain the reported HIV-associated renal failure. Transgenic model of HIV associated nephropathy (HIVAN) demonstrated HIV infection of glomerular and tubular epithelial cells[6] and HIV provirus was detected from kidney biopsies of HIV+ patients with HIVAN[11,17]. Hepatic cells may also be a source of HIV. In one study, a hepatoma cell line was productively infected by HIV in a CD4-independent mechanism[12].

Fifty percent of HIV infected patients not undergoing antiretroviral therapy develop HIV-associated central nervous system dysfunction. The underlying mechanism is still elusive. HIV predominantly replicates in the macrophage/microglial lineage of brain cells[1,92]. However, brain autopsy of HIV+ patients provided evidence for HIV p24 and HIV DNA in astrocytes and brain endothelial cells[1,69]. HIV p24 was not detected in oligodendrocytes[87] but HIV DNA was found in these cells[1]. Oligodendrocytes cells, however, were positive for HIV Tat, supporting the concept that Tat may be secreted from infected cells to uninfected cells, where it may play a role in brain cell cytotoxicity[23,87]. *in vitro* HIV infection of astrocytes is initially productive but then persists as a latent infection[53], suggesting that brain cells provide a reservoir for HIV.

HIV infection of cells of the male and female reproductive system can negatively impact the transmission of HIV both sexually and from mother to child. HIV integration into the genome of the germ line may conceivably allow for HIV transmission from one generation to the next. Studies evaluating HIV infection of sperm cells have been discordant[58,63,64]. Limited *in situ* hybridization, electron microscopy, and PCR studies have implicated viral particles and/or HIV DNA in sperms[3,4,58]. An alternative receptor (GalAAG) for HIV was even implicated for HIV entry into sperms[4]. Other studies, however, demonstrated that motile spermatozoa or immature sperms do not harbor proviral DNA and that only contaminating T cells and macrophages in sperm cell preparations are the source of HIV infected cells in semen[64,65]. Given the few studies that have been conducted to evaluate the susceptibility of sperms to HIV infection, no definitive conclusion(s) can be made on the ability of HIV to enter and replicate in sperm cells. Oocytes, on the other hand, are negative for CD4, CXCR4, CCR5 and even GalAAC and are resistant to HIV infection, according to one study[4].

Concluding Remarks

Up-regulation of the CD4 molecule on classically CD4 negative cells represent one mode of HIV productive infection of otherwise non-permissive cells. The significance of this up-regulation in normal cell function is unknown. It is interesting to note that CD4 is also constitutively expressed on a subset of monocyte/macrophages. The role of this constitutive CD4 expression on cells that do not require antigen recognition in the context of MHC-II representation is a puzzle. Does CD4 have another yet unidentified function? This question remains to be answered. Additionally, productive HIV replication may not be a prerequisite for transmission to T cells. Many cell types bind HIV and transmit the virus to susceptible targets. While the role of dendritic cells[68,78] and follicular dendritic cells[28,38] is well established in the transmission of HIV to T cells, recently red blood cells, neutrophils, and even platelets were shown to efficiently bind HIV and transmit the virus to T cells[59]. These different pathways of HIV infection and even transmission to susceptible targets indicate that HIV is far more ubiquitous than initially thought at the beginning of the HIV epidemic.

Acknowledgements

Part of the data presented is supported by a grant from the American Foundation for AIDS Research (amFAR # 02634-26-RGI & 02682-28-RGI). We thank Dr. Joan Siegel (Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA) for critically reading the manuscript and for valuable suggestions.

References

1. An S, Groves M, Giometto B, Beckett A, Scaravilli F. Detection and localisation of HIV-1 DNA and RNA in fixed adult AIDS brain by polymerase chain reaction/in situ hybridisation technique. Acta Neuropathol (Berl) 1999; 98: 481-7.
2. Armstrong M, Storch J, Dainiak N. Structurally distinct plasma membrane regions give rise to extracellular membrane vesicles in normal and transformed lymphocytes. Biochim Biophys Acta 1988; 946: 106-12.
3. Baccetti B, Benedetto A, Burrini A *et al.* HIV-particles in spermatozoa of patients with AIDS and their transfer into the oocyte. J Cell Biol 1994; 127: 903-14.
4. Baccetti B, Benedetto A, Collodel G *et al*. The debate on the presence of HIV-1 in human gametes. J Reprod Immunol 1998; 41: 41-67.
5. Bakri Y, Schiffer C, Zennou V *et al.* The maturation of dendritic cells results in postintegration inhibition of HIV-1 replication. J Immunol 2001; 166: 3780-8.
6. Barisoni L, Bruggeman L, Mundel P, D'Agati V, Klotman P. HIV-1 induces renal epithelial dedifferentiation in a transgenic model of HIV-associated nephropathy. Kidney Int 2000; 58: 173-81.
7. Beaudoin A, Grondin G. Shedding of vesicular material from the cell surface of eukaryotic cells: different cellular phenomena. Biochim Biophys Acta 1991; 1071: 203-19.
8. Bell E, Sparshott S. Interconversion of CD45R subsets of CD4 T cells *in vivo* [see comments]. Nature 1990; 348: 163-6.
9. Blaak H, van't Wout A, Brouwer M *et al*. *in vivo* HIV-1 infection of CD45RA(+)CD4(+) T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4(+) T cell decline. Proc Natl Acad Sci 2000; 97: 1269-74.
10. Brooks D, Kitchen S, Kitchen C, Scripture-Adams D, Zack J. Generation of HIV latency during thymopoiesis. Nat Med 2001; 7: 459-64.
11. Bruggeman L, Ross M, Tanji N *et al.* Renal epithelium is a previously unrecognized site of HIV-1 infection. J Am Soc Nephrol 2000; 11: 2079-87.

12. Cao Y, Friedman-Kien A, Huang Y *et al.* CD4-independent, productive human immunodeficiency virus type 1 infection of hepatoma cell lines *in vitro*. J Virol 1990; 64: 2553-9.
13. Chene L, Nugeyre M, Guillemard E *et al*. Thymocyte-thymic epithelial cell interaction leads to high-level replication of human immunodeficiency virus exclusively in mature CD4(+) CD8(-) CD3(+) thymocytes: a critical role for tumor necrosis factor and interleukin-7. J Virol 1999; 73: 7533-42.
14. Chou L, Epstein J, Cassol S, West D, He W, Firth J. Oral mucosal Langerhans' cells as target, effector and vector in HIV infection. J Oral Pathol Med 2000; 29: 394-402.
15. Clarke S. Leukoc Biol 2000 May. The critical role of CD40/CD40L in the CD4-dependent generation of CD8+ T cell immunity J 2000; 67: 607-14.
16. Clerici M, Saresella M, Colombo F *et al.* T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood 2000; 96: 3866-71.
17. Cohen A, Sun N, Shapshak P, Imagawa D. Demonstration of human immunodeficiency virus in renal epithelium in HIV-associated nephropathy. Mod Pathol 1989; 2: 125-8.
18. Constant S, Zain M, West J *et al*. Are primed CD4+ T lymphocytes different from unprimed cells? Eur J Immunol 1994; 24: 1073-9.
19. Conway B, Baskar P, Bechtel L *et al.* Eosinophils as host cells for HIV-1. Arch Virol 1992; 127: 373-7.
20. De Maria A, Colombini S, Schnittman S, Moretta L. CD8+ cytolytic T lymphocytes become infected *in vitro* in the process of killing HIV-1-infected target cells. Eur J Immunol 1994; 24: 531-6.
21. De Paulis A, De Palma R, Di Gioia L *et al.* Tat protein is an HIV-1-encoded beta-chemokine homolog that promotes migration and up-regulates CCR3 expression on human Fc epsilon RI+ cells. J Immunol 2000; 165: 7171-9.
22. Dezutter-Dambuyant C. *in vivo* and *in vitro* infection of human Langerhans cells by HIV-1. Adv Exp Med Biol 1995; 378: 447-51.
23. Ensoli B, Buonaguro L, Barillari G *et al.* Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. J Virol 1993; 67: 277-87.
24. Flamand L, Crowley R, Lusso P *et al*. Activation of CD8+ T lymphocytes through the T cell receptor turns on CD4 gene expression: implications for HIV pathogenesis. Proc Natl Acad Sci 1998; 95: 3111-6.
25. Freedman A, Gibson F, Fleming S, Spry C, Griffin G. Human immunodeficiency virus infection of eosinophils in human bone marrow cultures. J Exp Med 1991; 174: 1661-4.
26. Fritsch L, Marechal V, Schneider V *et al.* Production of HIV-1 by human B cells infected *in vitro*: characterization of an EBV genome-negative B cell line chronically synthetizing a low level of HIV-1 after infection. Virology 1998; 244: 542-51.
27. Fry T, Connick E, Landay A *et al.* A potential role of IL-7 in T-cell homeostasis in HIV infected patients. In: Program and abstracts of the 7th CROI. January 30-February 2, 2000; Chicago, IL. Abstract LB1.
28. Fujiwara M, Tsunoda R, Shigeta S, Yokota T, Baba M. Human follicular dendritic cells remain uninfected and capture human immunodeficiency virus type 1 through CD54-CD11a interaction. J Virol 1999; 73: 3603-7.
29. Gabrilovich D, Vasilev V, Nosikov V *et al.* [The presence of HIV DNA in the neutrophils of persons infected with the human immunodeficiency virus and the clinical parallels of this phenomenon]. Zh Mikrobiol Epidemiol Immunobiol 1995; 27: 52-5.
30. Gabrilovich D, Vassilev V, Nosikov V *et al*. Clinical significance of HIV DNA in polymorphonuclear neutrophils from patients with HIV infection. J Acquir Immune Defic Syndr 1993; 6: 587-91.
31. Gabrilovich D, Vassilev V, Nosikov V *et al*. Presence of HIV DNA in neutrophils: relationship to their functional activity. AIDS 1992; 6: 134-6.
32. Gasperini S, Zambello R, Agostini C *et al.* Impaired cytokine production by neutrophils isolated from patients with AIDS. AIDS 1998; 12: 373-9.
33. Geijtenbeek T, Kwon D, Torensma R *et al.* DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000; 100: 587-97.
34. Godfrey D, Hammond K, Poulton L, Smyth M, Baxter A. NKT cells: facts, functions and fallacies. Immunol Today 2000; 21: 573-83.
35. Goldsmith M, Penn M, Eckstein D *et al*. Determinants of HIV-1 pathogenesis in lymphoid tissue. 8th CROI, 2001 [abstract S18].
36. Gras G, Richard Y, Roques P, Olivier R, Dormont D. Complement and virus-specific antibody-dependent infection of normal B lymphocytes by human immunodeficiency virus type 1. Blood 1993; 81: 1808-18.
37. Hamann D, Baars P, Hooibrink B, Van Lier R. Heterogeneity of the human CD4+ T-cell population: two distinct CD4+ T- cell subsets characterized by coexpression of CD45RA and CD45RO isoforms. Blood 1996; 88: 3513-21.
38. Heath S, Tew J, Szakal A, Burton G. Follicular dendritic cells and human immunodeficiency virus infectivity. Nature 1995; 377: 740-4.
39. Hofman P, Fischer F, Far D *et al.* Impairment of HIV polymorphonuclear leukocyte transmigration across T84 cell monolayers: an alternative mechanisms for increased intestinal bacterial infections in AIDS? Eur Cytokine Netw 1999; 10: 373-82.
40. Hu J, Gardner M, Miller C. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. J Virol 2000; 74: 6087-95.
41. Kawamura T, Qualbani M, Thomas E, Orenstein J, Blauvelt A. Low levels of productive HIV infection in Langerhans cell-like dendritic cells differentiated in the presence of TGF-beta1 and increased viral replication with CD40 ligand-induced maturation. Eur J Immunol 2001; 31: 360-8.
42. Keene J, Forman J. Helper activity is required for the *in vivo* generation of cytotoxic T lymphocytes. J Exp Med 1999; 155: 768-82.
43. Kitchen S, Korin Y, Roth M, Landay A, Zack J. Costimulation of *naïve* CD8(+) lymphocytes induces CD4 expression and allows human immunodeficiency virus type 1 infection. J Virol 1998; 72: 9054-60.
44. LaSalle J, Hafler D. The coexpression of CD45RA and CD45RO isoforms on T cells during the S/G2/M stages of cell cycle. Cell Immunol 1991; 138: 197-206.
45. Livingstone W, Moore M, Innes D, Bell J, Simmonds P. Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. Edinburgh Heterosexual Transmission Study Group. Lancet 1996; 348: 649-54.
46. Lucey D, Dorsky D, Nicholson-Weller A, Weller P. Human eosinophils express CD4 protein and bind human immunodeficiency virus 1 gp120. J Exp Med 1989; 169: 327-32.
47. Ludewig B, Gelderblom H, Becker Y, Schafer A, Pauli G. Transmission of HIV-1 from productively infected mature Langerhans cells to primary CD4+ T lymphocytes results in altered T cell responses with enhanced production of IFN-gamma and IL-10. Virology 1996; 215: 51-60.
48. Mack M, Kleinschmidt A, Bruhl H *et al.* Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. Nat Med 2000; 6: 769-75.
49. Martin J, Bandres J. Cells of the monocyte-macrophage lineage and pathogenesis of HIV-1 infection. J Acquir Immune Def Syndr 1999; 22: 413-29.
50. Mastroianni C, Lichtner M, Mengoni F *et al.* Improvement in neutrophil and monocyte function during highly active antiretroviral treatment of HIV-1-infected patients. AIDS 1999; 13: 883-90.
51. McBreen S, Imlach S, Shirafuji T *et al.* Infection of the CD45ra(+) (*naïve*) subset of peripheral cd8(+) lymphocytes by human immunodeficiency virus type 1 *in vivo*. J Virol 2001; 75: 4091-102.
52. Mercure L, Phaneuf D, Wainberg M. Detection of unintegrated human immunodeficiency virus type 1 DNA in persistently infected CD8+ cells. J Gen Virol 1993; 74: 2077-83.
53. Messam C, Major E. Stages of restricted HIV-1 infection in astrocyte cultures derived from human fetal brain tissue. J Neurovirol 2000; 6 (suppl) 1: S90-4.
54. Michie C, McLean A, Alcock C, Beverley P. Lifespan of human lymphocyte subsets defined by CD45 isoforms. Nature 1992; 360: 264-5.

55. Mitsuyasu R, Anton P, Deeks S *et al.* Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood 2000; 96: 785-93.
56. Moir S, Lapointe R, Malaspina A *et al.* CD40-Mediated induction of CD4 and CXCR4 on B lymphocytes correlates with restricted susceptibility to human immunodeficiency virus type 1 infection: potential role of B lymphocytes as a viral reservoir. J Virol 1999; 73: 7972-80.
57. Moodycliffe A, Nghiem D, Clydesdale G, Ullrich S. Immune suppression and skin cancer development: regulation by NKT cells. Nat Immunol 2000; 1: 521-5.
58. Muciaccia B, Filippini A, Ziparo E *et al*. Testicular germ cells of HIV-seropositive asymptomatic men are infected by the virus. J Reprod Immunol 1998; 41: 81-93.
59. Olinger G, Saifuddin M, Spear G. CD4-Negative cells bind human immunodeficiency virus type 1 and efficiently transfer virus to T cells. J Virol 2000; 74: 8550-7.
60. Ostrowski M, Chun T, Justement S *et al.* Both memory and CD45RA+/CD62L+ *naïve* CD4(+) T cells are infected in human immunodeficiency virus type 1-infected individuals. J Virol 1999; 73: 6430-5.
61. Pilling D, Akbar A, Bacon P, Salmon M. CD4+ CD45RA+ T cells from adults respond to recall antigens after CD28 ligation. Int Immunol 1996; 8: 1737-42.
62. Pohlmann S, Soilleux E, Baribaud F *et al.* DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. Proc Natl Acad Sci 2001; 98: 2670-5.
63. Pudney J, Nguyen H, Xu C, Anderson D. Microscopic evidence against HIV-1 infection of germ cells or attachment to sperm. J Reprod Immunol 1999; 44: 57-77.
64. Quayle A, Xu C, Mayer K, Anderson D. T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. J Infect Dis 1997; 176: 960-8.
65. Quayle A, Xu C, Tucker L, Anderson D. The case against an association between HIV-1 and sperm: molecular evidence. J Reprod Immunol 1998; 41: 127-36.
66. Raposo G, Nijman H, Stoorvogel W *et al.* B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996; 183: 1161-72.
67. Roederer M, Raju P, Mitra D, Herzenberg L. HIV does not replicate in *naïve* CD4 T cells stimulated with CD3/CD28. J Clin Invest 1997; 99: 1555-64.
68. Rowland-Jones S. HIV: The deadly passenger in dendritic cells. Curr Biol 1999; 9: R248-50.
69. Sabri F, Tresoldi E, Di Stefano M *et al.* Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors. Virology 1999; 264: 370-84.
70. Saifuddin M, Roebuck K, Chang C, Ting J, Spear G. Cutting edge: activation of HIV-1 transcription by the MHC class II transactivator. J Immunol 2000; 164: 3941-5.
71. Saifuddin M, Spear G, Chang C, Roebuck K. Expression of MHC class II in T cells is associated with increased HIV-1 expression. Clin Exp Immunol 2000; 121: 324-31.
72. Salmon M, Kitas G, Bacon P. Production of lymphokine mRNA by CD45R+ and CD45R- helper T cells from human peripheral blood and by human CD4+ T cell clones. J Immunol 1989; 143: 907-12.
73. Scott-Algara D, Vuillier F, Cayota A *et al. in vitro* non-productive infection of purified natural killer cells by the BRU isolate of the human immunodeficiency virus type 1. J Gen Virol 1993; 74: 725-31.
74. Soares M, Borthwick N, Maini M, Janossy G, Salmon M, Akbar A. IL-7-dependent extrathymic expansion of CD45RA+ T cells enables preservation of a *naïve* repertoire. J Immunol 1998; 161: 5909-17.
75. Soilleux E, Barten R, Trowsdale J. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J Immunol 2000; 165: 2937-42.
76. Spira A, Marx P, Patterson B *et al.* Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 1996; 183: 215-25.
77. Stanley S, McCune J, Kaneshima H *et al.* Human immunodeficiency virus infection of the human thymus and disruption of the thymic microenvironment in the SCID-hu mouse. J Exp Med 1993; 178: 1151-63.
78. Steinman R. DC-SIGN: a guide to some mysteries of dendritic cells. Cell 2000; 100: 491-4.
79. Stevenson M. Highlights of the 8th CROI, part 1. Topics in HIV medicine 2001; 9: 7.
80. Sullivan Y, Landay A, Zack J, Kitchen S, Al-Harhi L. Upregulation of CD4 on CD8+ T cells: $CD4^{dim}CD8^{bright}$ cells constitute an activated phenotype of CD8+ T cells. Immunology 2001 (in press).
81. Summers K, JL O, Hart D. Co-expression of the CD45RA and CD45RO antigens on T lymphocytes in chronic arthritis. Clin Exp Immunol 1994; 97: 39-44.
82. Swain S, Bradley L, Croft M *et al.* Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. Immunol Rev 1991; 123: 115-44.
83. Terabe M, Matsui S, Noben-Trauth N *et al.* NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. Nat Immunol 2000; 1: 515-20.
84. Unutmaz D, Baldoni F, Abrignani S. Human *naïve* T cells activated by cytokines differentiate into a split phenotype with functional features intermediate between *naïve* and memory T cells. Int Immunol 1995; 7: 1417-24.
85. Unutmaz D, Pileri P, Abrignani S. Antigen-independent activation of *naïve* and memory resting T cells by a cytokine combination. J Exp Med 1994; 180: 1159-64.
86. Valentin A, Rosati M, Patenaude D *et al*. Natural killer cells are targets for HIV-1 infection *in vivo*: Use of Real-Time PCR for the quantification of cell-associtaed viral load and cellular compartments. 8th CROI, 2001 [abstract 505].
87. Valle L, Croul S, Morgello S, Amini S, Rappaport J, Khalili K. Detection of HIV-1 Tat and JCV capsid protein, VP1, in AIDS brain with progressive multifocal leukoencephalopathy. J Neurovirol 2000; 6: 221-8.
88. Van der Vliet H, Nishi N, Koezuka Y *et al.* Potent expansion of human natural killer T cells using alpha-galactosylceramide (KRN7000)-loaded monocyte-derived dendritic cells, cultured in the presence of IL-7 and IL-15. J Immunol Methods 2001; 247: 61-72.
89. Webb L, Foxwell B, Feldmann M. Putative role for interleukin-7 in the maintenance of the recirculating *naïve* CD4+ T-cell pool. Immunology 1999; 98: 400-5.
90. Weber K, Meyer D, Grosse V, Stoll M, Schmidt R, Heiken H. Reconstitution of NK cell activity in HIV-1 infected individuals receiving antiretroviral therapy. Immunobiology 2000; 202: 172-8.
91. Weller P, Marshall W, Lucey D *et al*. Infection, apoptosis, and killing of mature human eosinophils by human immunodeficiency virus-1. Am J Respir Cell Mol Biol 1995; 13: 610-20.
92. Wiley C, Achim C, Christopherson C *et al.* HIV mediates a productive infection of the brain. AIDS 1999; 13: 2055-9.
93. Wooley D, Peterson K, Taylor R, Paul C, Baumann M. 2000. Strain-dependent productive infection of a unique eosinophilic cell line by human immunodeficiency virus type 1. AIDS Res Hum Retroviruses 2000; 16: 1405-15.
94. Yang L, Riley J, Carroll R *et al.* Productive infection of neonatal CD8+ T lymphocytes by HIV-1. J Exp Med 1998; 187: 1139-44.
95. Zhang J, Gupta A, Dave R *et al*. Isolation of primary HIV-1 that target CD8+ T lymphocytes using CD8 as a receptor. Nat Med 2001; 7: 65-72.
96. Zhang R, Henderson E. CD4 mRNA expression in CD19-positive B cells and its suppression by the Epstein-Barr virus. Virology 1994; 198: 709-13.
97. Zwaal R, Schroit A. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. Blood 1997; 89: 1121-32.