The role of naïve T-cells in HIV-1 pathogenesis:
An emerging key player

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Abstract
Functional naïve T-cells are critical for an effective immune response to multiple pathogens. HIV leads to a significant reduction in CD4+ naïve T-cell number and impaired function and there is incomplete recovery following combination antiretroviral therapy (cART). Here we review the basic homeostatic mechanisms that maintain naïve CD4+ T-cells and discuss recent developments in understanding the impact of HIV infection on naïve CD4+ T-cells. Finally we review therapeutic interventions in HIV-infected individuals aimed at specifically enhancing recovery of naïve CD4+ T-cells.

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1. Introduction

HIV infection is characterized by substantial depletion of CD4+ T-cells including recent thymic emigrants, naïve T-cells and memory T-cells. Following control of HIV replication with effective combination antiretroviral therapy (cART), CD4+ T-cells recover to normal levels, in most but not all patients, and impaired CD4+ T-cell recovery has been associated with non-AIDS events including cardiovascular disease, liver disease and malignancy [1–4]. Therefore despite the substantial reduction in morbidity and mortality from cART, life expectancy has still not returned to normal [5,6]. A detailed understanding of naïve CD4+ T-cell homeostasis is required in order to develop novel strategies to enhance the quality of immune reconstitution following cART.

2. Naïve T-cell homeostasis: insights from murine models

Naïve T-cells are characterized by the expression of surface markers CD45RA, CD27, CD28, CD62L, CCR7 and the IL-7 receptor [7,8]. Naïve T-cells exit the thymus following maturation and are enriched for T-cell receptor excision circles (TREC) and express the surface marker platelet endothelial cell adhesion molecule-1 (PECAM-1), also known as CD31 [9]. Naïve T-cells circulate between the blood and the lymphoid tissue driven by cell surface markers CD62L and CCR7 [10]. The number of naïve T-cells in blood remains relatively constant throughout adult life despite continuous stimulation with foreign antigens and a dramatic reduction in thymic output with age. Maintenance of naïve T-cells in adults is largely dependent on homeostatic proliferation which leads to a decrease in CD31 expression and TREC concentration resulting in an increase in the proportion of CD31- naïve T-cells with aging [9,11]. The mechanisms which drive homeostatic proliferation of naïve T-cells in humans are not fully understood, however extensive studies in murine models have demonstrated multiple factors are involved in both post-thymic survival and proliferation of naïve T-cells. Exposure to the cytokine IL-7 and contact with major histocompatibility complex (MHC) molecules presenting self-peptides through the T-cell receptor (TCR) within secondary lymphoid tissue are both critical for naïve T-cell homeostasis [12–15]. (See Fig. 1).

2.1. Homeostatic expansion

In lymphopenic murine models, adoptive transfer of naïve T-cells results in a biphasic increase in naïve T-cells with both rapid and slow proliferation phases. Rapid proliferation (or homeostatic expansion) was associated with conversion of naïve T-cells into an effector/memory phenotype [16,17]. There are diverse views on whether IL-7 is required for homeostatic expansion which is thought to be dependent on the strength of binding between antigen and the TCR. Proliferative responses following low-affinity binding, as seen with self-peptides, is thought to be more dependent on IL-7 [12,18] compared to high-affinity cognate antigen binding (reviewed in [19]).

Naïve T-cell homeostatic expansion has also been shown to be associated with exposure to microbial products. Following adoptive transfer of T-cells from immunocompetent BALB/cThy1.1 mice to mice with severe combined immunodeficiency (SCID) that have been raised in a germ-free environment, there was little naïve T-cell proliferation, activation or...
differentiation into a memory/effector phenotype suggesting that exposure to environmental pathogens may contribute to naïve T-cell expansion [20]. In addition, in mice who have had chemical ablation of the thymus, microbial products in plasma were elevated and there was an increase in chronic immune activation thought to be secondary to a reduction in circulating naïve T-cells and loss of gut integrity [21]. Therefore, naïve T-cell homeostatic expansion is influenced by exposure to environmental antigens and loss of naïve T-cells may in turn indirectly influence gut integrity. Given HIV infection is characterized by high levels of circulating lipopolysaccharide (LPS) and significant abnormalities in the gastrointestinal (GI) tract mucosa, these data may account for a significant change in naïve T-cell homeostasis in HIV infection [22–24].

2.2. Homeostatic proliferation

In addition to rapid proliferation, adoptive transfer of naïve T-cells also leads to slow proliferation (also called homeostatic proliferation) where the naïve phenotype is maintained and is dependent on IL-7 [16,17,25,26]. When naïve T-cells were adoptively transferred into IL-7-deficient mice, survival of these cells was significantly compromised [12]. Survival of naïve T-cells was also dependent upon ongoing contact with self-peptide through presentation by either MHC Class I or II and interaction with TCR of CD8+ and CD4+ naïve T-cells respectively [14,15,27,28].

A recent study suggested that the source of IL-7 also plays an important role in homeostatic proliferation of CD4+ naïve T-cells [29]. Following adoptive transfer of T-cells into lymphopenic mice deficient in recombination-activation gene-1 (Rag1−/−), CD8+ T-cells underwent proliferation in response to elevated peripheral IL-7 levels while proliferation of CD4+ naïve T-cells required the presence of dendritic cells (DCs) for both IL-7 and contact with MHC class II. The authors demonstrated that during lymphopenia, when IL-7 levels were elevated, a subset of DCs that express the IL-7 receptor α chain (CD127) down-regulate MHC class II by negative feedback [29]. This reduced the ability of the DC to interact with
the CD4+ naïve T-cell and therefore prevented proliferation. It is unknown whether similar mechanisms involving CD127-positive DCs exist in humans.

3. Naïve T-cell homeostasis, HIV infection and the impact of cART

3.1. Naïve T-cell number

Following HIV infection there is a significant decline in number and function of naïve CD4+ T-cells in the blood and lymph node compared to healthy individuals [30–34](summarized in Table 1). Collagen deposition in the paracortical T-cell zones of the lymphoid tissue also plays a critical role in limiting naïve T-cell homeostasis [36,37]. This resulted in restricted T-cell access to the fibroblastic reticular cell (FRC) network which is the major source of the survival factor IL7, resulting in apoptosis and depletion of naïve CD4+ T-cells. The loss of naïve T-cells in turn, removed a major source of lymphotxin-β, a survival factor for FRCs [38]. The resulting loss of FRCs and the loss of IL-7 produced by FRCs may thus perpetuate a vicious cycle of depletion of naïve CD4+ T-cells and the FRC network in the setting of untreated HIV infection.

HIV infection leads to significant depletion of total mucosal CD4+ T-cells in the GI tract which allow for migration of microbial products into the systemic circulation [22,24]. Microbial products, specifically LPS, can drive DC activation via ligation of toll like receptors (TLR) leading to elevated pro-inflammatory cytokines and enhanced T-cell proliferation [22,39,40]. Microbial products may also be responsible for enhanced naïve T-cell proliferation as demonstrated in mouse models but not yet confirmed in human studies [20,21]. More recently, the contribution of cytomegalovirus (CMV) co-infection to naïve T-cell proliferation and depletion has been explored. HIV-infected patients with strong CMV-specific T-cell responses had fewer naïve T-cells [41]. The authors suggested that the loss of naïve T cells could be due to movement of naïve T-cells into the large pool of CMV-specific memory T-cells leading to an adverse effect on total CD4+ T-cell immune recovery following cART.

Following initiation of cART, naïve T-cell proliferation decreases; thymic output increases and the total number of naïve T-cells significantly increases [30,32,33,35]. However, the numbers of naïve T-cells rarely reach normal levels in adults even after more than 7 years of cART [42]. In contrast, in HIV-infected children, naïve T-cells reached normal levels following cART, and recovery of thymic output was independent of age and time of initiation of cART [43]. A recent study showed that naïve T-cell count at initiation of cART could predict successful immune reconstitution [44]. In this case, immune reconstitution was defined as an increase of

| Table 1 | Naïve T-cell abnormalities in the setting of HIV infection. |
|---|---|---|
| Compartments assessed | Abnormality in naïve T-cells in HIV (measurement) | Reference |
| **Blood** | ↓ Numbers of naïve T-cells and RTE (CD45RA+, CD45RA+ CD62L+, TREC) | [30–32,34] |
| | ↑ CMV responses associated with reduced number of naïve T-cells | [41] |
| | ↑ Expansion proliferation (Ki67) | [31] |
| | ↓ Cell cycle entry and proliferation (Ki67, CFSE) | [48,49] |
| | ↑ Co-stimulatory response (CD27+ CD28) | [49] |
| | ↑ Signaling (Basal STAT5 phosphorylation) | [50,52] |
| | ↓ Survival (↓ Bcl-2) | [50] |
| | ↑ Phosphorylated STAT5 in cytoplasm | [52] |
| | ↑ Phosphorylated STAT5 nuclear localisation | [52] |
| **Lymph node** | ↓ Number of naïve T-cells (TREC) | [32] |
| | ↑ Number of naïve T-cells (CD45RA+ CD62L+, TREC) | [34] |
| | Collagen deposition in | | |
| | • Paracortical T zones | | |
| | • High endothelial venules | | |
| | • Fibroblastic reticular cell network | | |
| | ↑ Distance between IL-7 source and naïve T-cells (CD45RA+) | | |
| | ↓ Access to IL-7 | | |
| | ↑ Naïve T-cell apoptosis (TUNEL+) | | |
| **Gastro-intestinal tract** | ↓ CD4+ T cells (naïve, effector and memory) | | |
| | ↑ Microbial translocation (↑ LPS, 16s rDNA) | | |
| | ↑ Fibrosis | | |
100–200 CD4+ T-cells after 2 years on therapy. There is a need to further understand why recovery of naïve T-cells is impaired following cART. The role of age, nadir CD4 T-cell count or other biological factors that may enhance lymph node fibrosis or impair thymic output remains to be further explored.

3.2. Naïve T-cell function

Abnormalities in naïve.

3.2.1. Response to IL-7

The dramatic depletion of CD4+ T-cells in HIV infection is also associated with significant functional defects in naïve T-cells (summarised in Table 1). HIV infection is associated with increased systemic levels of IL-7, low surface expression of the α chain of the IL-7 receptor (IL7Rα), CD127, and impaired response to IL-7 [45–47]. Naïve T-cells from HIV-infected individuals had a reduced capacity to expand following stimulation with TCR ligation and IL-7 [48]. In addition, CD31+ naïve T-cells, a subset of naïve T-cells that have recently left the thymus and not undergone proliferation [9,11], failed to express the co-stimulatory molecules CD28 and CD27 after TCR stimulation [49]. Following TCR engagement of CD31+ naïve T-cells, there was an increase in co-stimulatory molecules but the cells were unable to enter cell cycle [49]. These data demonstrate discreet functional defects in response to IL-7 and TCR engagement in the setting of HIV infection, in addition to reduced total number of naïve T-cells.

IL-7 signaling which occurs through the STAT5 pathway has also been shown to be altered during HIV infection. The basal level of STAT5 phosphorylation was elevated in both naïve and memory CD4+ T-cell subsets in patients receiving cART, who were viremic and aviremic [50]. Expression of CD127 on naïve T-cells and the ability of IL-7 to bind to the IL-7R on naïve T-cells in patients on cART were similar to healthy controls [50]. Recent analysis of signaling pathways in CD4+ T-cells from viremic and aviremic patients also suggested that naïve CD4+ T-cells, in contrast to memory CD4+ T-cells, were able to sustain CD127-STAT5 signaling [51]. However, despite normal IL7 binding and sustained IL7R signaling in HIV-infected patients, the expression of the survival protein Bcl-2 was reduced in naïve T-cells from both viremic and cART-treated patients, consistent with a post-receptor block that prevented the induction of downstream pro-survival signals [50]. A study of viremic patients attributed this outcome to an increased accumulation of phosphorylated STAT5 within the cytoplasm due to the inability to enter the nucleus [52]. The multiple defects in signaling pathways important for survival of naïve and memory T-cells could therefore play an important role in limiting the capacity for CD4+ T-cell recovery following cART.

Following long-term cART in HIV-infected adults [53], and children [54], the concentration of IL-7 reduces to normal levels. One multivariate analysis showed that an increase in the mean CD4+ T-cell count in patients was associated with increased IL-7 responsiveness and this was independent of T-cell activation, CD4-1 expression, CCR5 expression and IL-7 plasma levels [55]. In addition, patients on suppressive cART with a complete immunological response (>500 CD4+ T-cells/mm³ ≥ 5 years after initiation of ART), demonstrated higher functional response to IL-7 (measured by STAT5 phosphorylation), which correlated with increased total and naïve T-cell levels compared to patients with incomplete responses (CD4 <500 cells/μl) [55]. Other studies have shown that defects in IL-7 responsiveness were only partially corrected with cART while levels of IL-7 remained elevated and IL-7 receptor signaling defects were still present in many patients following cART [50,56–58].

3.2.2. TCR diversity

TCR diversity is also altered in patient with HIV infection. TCR diversity is quantified by the length of the hypervariable CDR3 β regions of the TCR [59–62]. In healthy individuals the multiple CDR3 lengths follow a Gaussian distribution but in HIV infection this distribution is skewed and is associated with disease progression and accelerated loss of total CD4+ T-cells in both adults and children [59–62]. Translocation of microbial products as well as the HIV glycoprotein gp160 which can induce superantigen responses in specific Vβ groups can also lead to a skewed TCR repertoire [63]. In individuals who have a poor immunological response to cART (<20% increase in CD4+ T-cells from baseline or do not reach 200 cells/μl) there was an increased expansion in some Vβ groups and this was associated with reduced naïve T-cell number and thymic output [58]. Two longitudinal studies have shown that despite an increase in total CD4+ T-cell count on long-term cART, CD4+ T-cell subsets remain skewed [64,65].

3.2.3. Response to neo-antigens

A reduction in functional responses to new antigens and vaccinations has also been associated with late initiation of cART and was most likely due to skewing of the proportion of CD4+ T-cell populations and depletion of naïve T-cells [66]. Other studies have shown that despite increases in CD4+ T-cells to levels >500 CD4+ T-cells with long-term cART, responses to neoantigens through vaccinations were lower in HIV-infected individuals compared to healthy controls [67]. Lack of cellular response to neoantigens has also been observed in children and adolescents vaccinated during their first year on cART [68]. One possible explanation for these findings is a defect in naïve T-cell proliferation in response to neoantigen [69]. The implications of long-term impaired naïve T-cell recovery are unclear but it is highly likely that impaired naïve T-cell number and function will contribute to ongoing immune dysregulation in patients on cART.

4. HIV infection of naïve T-cells

One potential mechanism for depletion and impaired function of naïve T-cells is that HIV can directly infect naïve T-cells both in vivo and in vitro. However the extent of infection, the impact of HIV infection on function and the effect of cART on the reservoir of infected naive T-cells is less clear.

4.1. Infection of naïve T-cells in vitro

Naïve CD4+ T-cells are relatively resistant to HIV infection in vitro but in some conditions, direct infection can occur [70–72]. Infection of naïve T-cells with both CXCR4-using (X4) and CCR5-using (R5) viruses can lead to integration but the efficiency of infection of naive CD4+ T-cells was significantly lower.
than in resting memory CD4+ T-cells [70]. In vitro infection of naïve T-cells was associated with accumulation of partial or incomplete viral transcripts and delayed viral integration but the major block to direct infection of naïve T-cells appears to be decreased efficiency of viral fusion [70,73]. Viral fusion in naïve T-cells can be significantly enhanced in viruses that co-express vesicular stomatitis virus (VSV) and R5 envelope protein, but fusion was inefficient with VSV or R5 env protein alone [71]. The molecular mechanisms for this observation are currently unclear.

4.1.1. Role of chemokines
Our laboratory recently demonstrated that direct infection of resting CD4+ T-cells can be established following incubation with chemokines that bind to chemokine receptors highly expressed on the surface of resting T-cells, including CCL19 and CCL21 which both bind to CCR7 which is highly expressed on both naïve and memory T-cells [74,75]. However, these chemokine mediated changes only facilitated infection of resting memory and not naïve T-cells, [75]. Taken together these data demonstrate that there are clear differences in the ability of HIV to infect resting naïve and memory T-cells and that the major block to infection of naïve T-cells occurs early in the viral life cycle, most likely at the level of viral fusion.

4.1.2. Role of IL-7
The role of IL-7 in enhancing infection of naïve T-cells has also been explored [76–79]. Naïve T-cells become more susceptible to infection following culture with IL-7 [79]. Following treatment with IL-7, cord blood naïve T-cells and adult memory T-cells, but not adult naïve T-cells, enter G1 phase of cell cycle and become susceptible to infection with both VSV-pseudotyped HIV and wild type HIV [76–78]. Total resting CD4+ T-cells treated with IL-7 together with the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, led to no change in HIV infection suggesting that pathways other than PI3K may be involved [78].

4.1.3. Tissue microenvironment and abortive infection
The microenvironment may also have an important role in susceptibility of naïve T-cells to HIV infection. HIV infection of resting naïve CD4+ T-cells was significantly enhanced when infection was performed using tonsil lymphoid tissue ex vivo [80,81]. In contrast, when naïve CD4+ T-cells were purified from tonsil and cultured alone in vitro, HIV infection did not occur [80]. Differences between susceptibility to HIV infection of naïve T-cells from lymphoid tissue and those isolated from the blood may be due to differences in the expression of active and inactive forms of the cellular protein APOBEC3G [82].

When human lymphoid aggregated cultures (HLACs) from tonsil were infected with X4-using virus, incomplete reverse transcription products accumulated within resting CD4+ T-cells [72]. These incomplete transcripts triggered pro-apoptotic and pro-inflammatory cellular responses resulting in the death of resting CD4+ T-cells and may potentially explain the significant CD4+ T-cell loss in HIV infection [72]. These experiments however didn’t distinguish between memory and naïve T-cells, so it is currently unknown if this occurs in both T-cell subsets [72]. Enhanced apoptosis of resting CD4+ T-cells isolated from the blood and infected in vitro has also been described [83]. This newly described mechanism of accumulation of incomplete reverse transcripts and abortive infection could be an important contributing factor to naïve CD4+ T-cell depletion in HIV-infected patients.

4.1.4. DC-naïve T-cell interactions
DCs located within the lymphoid tissue may play a role in enhancing infection of naïve T-cells. One group has demonstrated that in vitro co-culture of naïve T-cells with DCs can significantly enhance infection of naïve CD4+ T-cells [84], while others have shown no effect of DCs [70]. A study using monocyte-derived DCs (MDDCs) explored the efficiency of MDDCs to transmit HIV to different T-cell subsets including naïve, central and effector memory subsets [84], The study showed that MDDC-mediated transmission of virus was dependent on virus co-receptor usage and co-receptor expression on the target T-cell. MDDCs were only able to transfer X4 strains to CD4+ naïve T-cells [84]. In other experiments, when resting T-cells were infected in the presence of DCs and then sorted into naïve and memory cells, infection of naïve T-cells was not enhanced by the presence of DCs [70]. Other groups have demonstrated that physical contact of total resting CD4+ T-cells with uninfected autologous immature-MDDCs increased infection of resting CD4+ T-cells [85]. This was not simply a result of a mixed lymphocytic reaction as, an increase in activation markers was only observed when a higher ratio of DC:T-cell was used [85].

Combined, these studies demonstrate that HIV infection of naïve T-cells requires additional signals, mediated by either cytokines such as IL-7 or cell-cell contact via DC-T-cell contact, and that these signals are more likely to be present within the endogenous microenvironment of lymphoid tissue.

4.2. Infection of thymocyte subsets in vitro
HIV infection of thymocyte subsets could be the main source of HIV-infected naïve T-cells in the periphery. All thymocyte populations predominately express high levels of CXCR4 and low levels of CCR5 making these cells more susceptible to infection with X4-using viruses [86,87]. However, HIV is able to enter thymocytes and undergo reverse transcription after infection with either X4 or R5-using viruses [88]. Our lab has demonstrated that thymic plasmacytoid dendritic cells play an important role in the infection of thymocytes through transfer of R5-using virus to both immature and mature thymocytes [89]. Various cytokines have differential effects on thymocyte infection. IL-7 alone or in combination with IL-4 increased expression of CXCR4 and enhanced infection with X4-using viruses [87,90]. Thymocytes cultured with IL-2 and IL-4 expanded, the expression of both co-receptors increased and thymocytes were more permissive to X4 and R5-using viruses [90]. In addition, mature thymocytes cultured with TNF-α and IL-7 were more likely to become infected as a result of IL-7 enhancing cell survival of mature cells [91].

4.3. Infection of naïve T-cells in vivo
4.3.1. Total and integrated HIV DNA
In contrast to findings in vitro, naïve T-cells are clearly infected with HIV in vivo, although at low levels compared with other T-cell subsets such as CD4+ memory T-cells [92–100];
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Accurate quantification of HIV in naïve T-cells in vivo can be complicated by the surface markers used to define naïve T-cells. If insufficient surface markers are used to define a naïve T-cells (<3 specific markers) [7] then it is possible that memory T-cell contamination may be contributing to the assessment of naïve T-cells [101–103]. In addition, many studies only assess total HIV DNA rather than integrated DNA which again may provide an over estimation of the number of infected naïve T-cells [92–97,99,100]. However, to further strengthen the case that naïve T-cells are indeed infected in vivo, replication competent virus has been detected in naïve T-cells from patients receiving cART [93,94,98,99].

There is conflicting data on whether specific naïve T-cell subsets are preferentially infected. In one study, there was a significantly higher concentration of HIV DNA in CD31+ naïve T-cells compared with the CD31+ naïve T-cells [92]. These findings suggested that infection of naïve T-cells did not occur in the thymus; but that infection potentially occurred following naïve T-cell homeostatic proliferation in the periphery. However, the samples in this study were fixed prior to sorting and this could have had an effect on the efficiency of HIV DNA detection [92]. In a more recent longitudinal study of patients initiating cART, our group showed that both CD31+ and CD31− naïve T-cell subsets were infected at similar frequency [95]. In addition we showed that the contribution of naïve T-cells to the total reservoir increased following cART [95]. Overall, even though the absolute number of infected naïve T-cells in patients on cART is significantly smaller than infected memory T-cells, naïve T-cells expand significantly following cART and therefore may represent a significant persistent reservoir in patients on cART.

A more recent cross-sectional study looking at both viremic and aviremic patients showed that both CD31+ and CD31− naïve T-cell subsets were infected with HIV at similar frequencies and in some patients, infected naïve T-cells made a substantial contribution to the overall pool of infected T-cells [96]. This study also showed that in CD31+ naïve T-cells from both viremic and aviremic patients, the concentration of HIV DNA positively correlated with IL-7 plasma levels, suggesting a possible function of IL-7 in the infection of naïve T-cells.

### 4.3.2. Viral compartmentalization

In order to better understand the origin of infection of naïve T-cells, several groups have examined the phylogenetic relationship between virus isolated from naïve T-cell subsets, memory cells and plasma. We recently showed that there was no compartmentalisation of virus envelope sequences in CD31+ and CD31− naïve and memory T-cell subsets from patients prior to and following cART [95]. Similar findings have been described in another study using sorted samples from patients naïve to cART [93]. In a smaller cohort of aviremic patients on cART for seven years, compartmentalization of virus in naïve T-cells was observed in two of the three patients studied [100]. In order to better understand the origin of infection of naïve T-cells, these studies should be performed in larger prospective studies of patients on long term cART.

### 4.3.3. Viral co-receptor usage

Both R5-using and X4 using virus has been isolated from naïve T-cells from HIV-infected patients [93–95,100]. In all of these studies naïve T-cells were predominantly infected with R5-using virus. However, two of the studies did detect some X4-using viruses within the naïve T-cell compartment [94,100].

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**Table 2** Detection of HIV infection of naïve T-cells in vivo. Studies that have assessed HIV infection of naïve T-cells in HIV-infected patients who are either on or off cART (upper rows without shading) or on cART (lower rows, gray shading) are shown.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patient Number (naïve/cART)</th>
<th>CD4+ cells/µl (Median)</th>
<th>Plasma HIV RNA copies/ml (Median)</th>
<th>Phenotype naïve T-cells</th>
<th>Total HIV DNA</th>
<th>Integrated HIV DNA</th>
<th>Replication competent virus</th>
<th>Compartmentalisationa</th>
</tr>
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<tbody>
<tr>
<td>[92]</td>
<td>22 (11/11)</td>
<td>526</td>
<td>15,989</td>
<td>CD11a10, CD45RO−CD57−</td>
<td>Yes (CD31 neg)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>[93] 13 (13/0)</td>
<td>610</td>
<td>17,090</td>
<td>CD27+ CD31+/−</td>
<td>Yes (total)</td>
<td>–</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>[94]</td>
<td>11 (4/7)</td>
<td>547</td>
<td>1,085</td>
<td>CD45RA+CD62L+</td>
<td>Yes (total)</td>
<td>Yes</td>
<td>–</td>
<td>No</td>
</tr>
<tr>
<td>[95]</td>
<td>10 (10/10)</td>
<td>143b</td>
<td>65,150b</td>
<td>CD45RO−CD28+</td>
<td>Yes (CD31 neg)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>[96]</td>
<td>26 (11/15)</td>
<td>561</td>
<td>36,541(viremic)</td>
<td>CD45RA+CCR7+</td>
<td>Yes (CD31+ = CD31 neg)</td>
<td>Yes CD31+ (10 cART− patients)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>[97]</td>
<td>102 (0/102)</td>
<td>271</td>
<td>6,908</td>
<td>CD45RA+CD62L+</td>
<td>Yes (total)</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>[98]</td>
<td>34 (0/34)</td>
<td>581</td>
<td>&lt;50</td>
<td>CD45RA+CCR7+</td>
<td>Yes (total)</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>[99]</td>
<td>11 (0/11)</td>
<td>582</td>
<td>&lt;20</td>
<td>CD45RA+CD62L+</td>
<td>Yes (total)</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>[100]</td>
<td>3 (0/3)</td>
<td>305</td>
<td>&lt;20</td>
<td>CD45RA+CD62L+</td>
<td>Yes (total)</td>
<td>–</td>
<td>Yes (2/3)</td>
<td>–</td>
</tr>
</tbody>
</table>

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a Not assessed.

b Phylogenetic analysis.

c Baseline sample at the beginning of longitudinal study.
still remains unclear how naïve T-cells become infected with R5-using viruses given the low expression of CCR5 on the surface of naïve T-cells. We have recently demonstrated in vitro that thymic plasmacytoid DC are able to efficiently transfer R5-using virus to both immature and mature thymocytes providing a potential mechanism to explain R5-using infection of naïve T-cells [89].

5. Therapeutic approaches to enhance naïve T-cell recovery

In addition to cART, there is now increasing interest to develop immunotherapeutic agents that specifically enhance CD4+ T-cell recovery, including naïve T-cells.

5.1. Exogenous IL-2 therapy

Administration of IL-2 to lymphopenic mice increased total CD4+ T-cells including naïve, resting memory and activated CD4+ T-cells [104]. Similar responses were seen in HIV-infected patients on cART who received IL-2 [105–107]. Both thymic output and peripheral homeostatic proliferation contributed to the increase in naïve CD4+ T-cells [104–109]. In patients treated with CART and IL-2 there was a reduction in apoptosis of total and naïve CD4+ T-cells [106,110].

Two large, randomized international clinical trials of IL-2 in patients on cART, Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT) [111] and Subcutaneous, Recombinant Human Interleukin-2 in HIV-Infected Patients With Low CD4+ Counts Receiving Active Antiretroviral Therapy (SILCAAT) [112] showed no additional clinical benefit following administration of IL-2 to patients receiving cART. These studies demonstrated that IL-2 led to a clear increase in total CD4+ T-cell counts, including naive CD4+ T-cells, but this did not lead to a reduction in opportunistic infections or mortality, showing that IL-2 therapy had no additional clinical benefit for patients on cART [111–113]. Similar findings were observed in the Study of ALdesleukin With and without Antiretroviral Therapy, (STALWART), which tested intermittent IL-2 combined with peri-cycle cART in HIV-infected individuals who were cART-naïve and asymptomatic [114]. These studies demonstrated that an increase in CD4+ T-cell number secondary to IL-2 did not translate to effective function and improved clinical outcomes and further work is needed to understand the biological basis for these findings.

5.2. Exogenous IL-7 therapy

5.2.1. Animal preclinical studies

Administration of IL-7 to SIV-infected and uninfected cynomolgus monkeys, baboons and macaques, induced proliferation of naïve CD4+ T-cells and a decline in the frequency of TREC with enhanced homeostatic proliferation [115–119]. Administration of IL-7 induced migration of T-cells to the lymph node where they entered cell cycle [120] and greater TCR diversity was observed consistent with enhanced thymic output [119]. Importantly, administration of IL-7 to SIV-infected macaques resulted in no increase in SIV viral load [116,117].

5.2.2. Human clinical trials: HIV-infected patients

There have been two completed trials of recombinant human (rh) IL-7 in cART-treated HIV-infected patients. In one study, patients with CD4+ T-cell counts 100–400 cells/μL and HIV RNA <50 copies/ml had eight subcutaneous injections three times per week over a 16 day period and two doses of rhIL-7 were examined [121]. There was a preferential increase in both naïve and central memory T-cells following rhIL-7. Increases in CD4+ naïve T-cells peaked at day 14 and started to decline 14 days after completion of rhIL-7. At the higher dose, four patients also experienced a transient increase in plasma HIV RNA [121].

The second trial was performed in HIV-infected patients with CD4+ T-cell counts >100 cells/μL, in patients with a viral load of <50 or 50–50,000 copies/ml HIV RNA [122]. A transient increase in HIV RNA was demonstrated in 6 out of 11 patients who had HIV RNA <50 copies/ml at baseline. On day 1 following rhIL-7, there was a significant decrease in circulating CD4+ and CD8+ T-cells. By day 14, the levels had increased but mainly consisted of central memory T-cells, with minimal increase in naïve T-cells. The number of proliferating naïve T-cells increased compared to baseline, however these were mainly CD8+ T-cells [122].

Aside from the effects of IL-7 on CD4+ T-cell number, IL-7 can also have an impact on latently infected cells and possibly co-receptor usage of virus in vivo. In both clinical trials of IL-7, some patients experienced a transient increase in plasma HIV RNA at the time of peak T-cell proliferation, which eventually returned to <50 copies/ml [121,122]. There was no significant increase in the concentration of cell-associated HIV DNA in either PBMC or CD4+ T-cells following IL-7 [121]. However, this study did not correct for the overall increase in total T-cell number following rhIL-7, meaning that the absolute number of infected cells (expressed as HIV copies/ml blood) may have increased as a result of proliferation of latently infected naive, central memory or transitional memory T-cells, as has recently been demonstrated ex vivo [98]. Therefore, it is possible that IL-7 may induce proliferation of both uninfected and latently infected naïve and memory T-cells and it remains unclear if IL-7 will expand or activate latently infected cells. Clinical trials are currently underway to test this (ERAMUNE; www.clinicaltrials.gov).

IL-7 may also potentially lead to a change in HIV co-receptor usage. Following long-term culture of HIV-infected PBMC with IL-7 in vitro, there was an increase in the expression of CCR4 on CD4+ T-cells, which led to a change in co-receptor usage from R5-using to X4-using virus [123]. The same group also suggested that increases in CCRX4 expression on CD4+ T-cells from HIV-infected patients could lead to the emergence of X4-using viruses [124]. Using IL-7 as an immunotherapy could potentially have the same effect in vivo although to date this has not been demonstrated.

5.2.3. IL-7 Receptor polymorphisms

It is possible that response to endogenous and exogenous rhIL-7 is dependent upon differences in the IL-7R which are determined by genetic polymorphisms. The IL-7R is composed of an α and common γ chain [125]. Four haplotypes of the gene coding for IL-7Rα (CD127) have been described in Caucasians [126]. These haplotypes are defined by single nucleotide polymorphisms located throughout the IL-7Rα gene and lead to alternative splicing of mRNA resulting in varying ratios of soluble and membrane bound CD127 [127–129]. Using a multivariate
analysis, we have recently shown that the IL-7Rα haplotype 2 was significantly associated with more rapid recovery of CD4+ T-cells >500 cells/μl following cART [53]. In addition, soluble CD127 (sCD127) levels in cART-treated HIV-infected patients were significantly lower in individuals who were homozygous for haplotype 2 than non-haplotype 2 carriers. A potential explanation for these findings is that high levels of sCD127 “mops” up circulating IL-7 allowing for less efficient IL-7-related cell signaling and impaired CD4+ T-cell recovery. Evaluation of these and other polymorphisms should be performed in other patient cohorts. In addition these polymorphisms may potentially be important in determining how effectively a patient responds to exogenous IL-7, although these studies have not been performed to date.

5.3. Other novel therapeutic approaches

Other immunotherapies like Growth Hormone (GH), Keratinocyte Growth Factor (KGF), and androgen blockade which are known to improve thymopoiesis and thymic architecture regeneration are also being considered for evaluation in HIV-infected patients. Other approaches could also include anti-fibrotic agents that could potentially reduce lymph node fibrosis [38].

A randomized control trial of GH in a cohort of 46 HIV-infected patients receiving cART showed promising results with significant increases in thymopoiesis as measured by TREC concentration [130]. Studies in macaques have shown that KGF targets epithelial cells, improves both naïve T-cell numbers and TCR diversity [131] and also activates the production of IL-7 within the thymus [132]. At puberty when sex steroid levels increase, the thymus decreases in size and thymic output significantly reduces. Therefore androgen blockade may improve thymic output of HIV patients as shown following castration in mouse models [133]. In addition, hypogonadal men have been shown to have an increased number of RTE and following treatment with androgens, the levels of RTE decline [134]. Sex steroid blockade using luteinizing-hormone releasing hormone antagonists is currently used in the treatment of prostate cancer [133] and has also been trialed in patients undergoing stem cell transplantations [135]. In both of these scenarios, thymic function and production of naïve T-cells were enhanced. No trials have yet been performed in the setting of HIV infection.

6. Outstanding research questions

Many outstanding research questions remain about naïve T-cells and HIV infection (Table 3). Most of our detailed understanding of the mechanisms of naïve T-cell homeostasis is derived from mouse models. The effects of self-peptide and microbial translocation in driving enhanced naïve T-cell proliferation should be further explored in ex vivo human models. New interventions targeting immune reconstitution like exogenous IL-7 have shown promise in clinical trials. However, a major challenge for the field is to determine how best to evaluate efficacy of immunomodulators such as IL-7 given the observation of enhanced CD4+ T-cell recovery but no improvement in clinical endpoints in the recent IL-2 studies. The efficacy of adjunct immunomodulatory therapy with cART can no longer be evaluated on the basis of CD4+ T-cell number alone and large randomised trials with clinical endpoints with better surrogate markers of immune function other than the number of CD4+ T-cells alone will be needed.

The varying responses in immune reconstitution between individuals may potentially be predicted by genetic differences and larger genome wide association studies similar to those recently conducted on HIV controllers [136] need be conducted to assess the determinants of CD4+ T-cell reconstitution. These studies will be important in identifying novel therapeutic targets to enhance immune recovery.

7. Conclusion

Successful immune reconstitution requires an increase in total CD4+ T-cells as well as the recovery of CD4+ naïve T-cell number and function. Understanding the multiple factors involved in naïve T-cell homeostasis and proliferation will allow for the development of new strategies to enhance immune reconstitution.

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Conflict of interest statement

The author(s) declare that there are no conflicts of interest.

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<td>Are naïve T-cells infected in all patients or only a select proportion?</td>
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The role of naïve T-cells in HIV-1 pathogenesis: An emerging key player


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