Increased production of IL-7 accompanies HIV-1–mediated T-cell depletion: implications for T-cell homeostasis

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We hypothesized that HIV-1–mediated T-cell loss might induce the production of factors that are capable of stimulating lymphocyte development and expansion. Here we perform cross-sectional (*n* = 168) and longitudinal (*n* = 11) analyses showing that increased circulating levels of interleukin (IL)-7 are strongly associated with CD4+ T lymphopenia in HIV-1 disease. Using immunohistochemistry with quantitative image analysis, we demonstrate that IL-7 is produced by dendritic-like cells within peripheral lymphoid tissues and that IL-7 production by these cells is greatly increased in lymphocyte-depleted tissues. We propose that IL-7 production increases as part of a homeostatic response to T-cell depletion.

HIV-1 infection results in profound immunodeficiency marked by increased peripheral T-cell destruction, decreased T-cell production, and defects in cell-mediated immunity[1,2]. We previously reported that abundant thymic tissue could be detected by computed tomography (CT) in a large fraction of HIV-1-seropositive adults[3]. Independent of age, thymic enlargement was significantly associated with increased numbers of circulating naive (CD45RA+CD62L+) CD4+ T cells. Because sort-purified cells of this phenotype are more likely to bear T-cell receptor (TCR) excision circles (evidence of recent intrathymic TCR rearrangements)[4–6], this association indicates that the thymic tissue visualized by CT is functional and not simply an inflammatory infiltrate[7]. These observations support the hypothesis that thymic function may be enhanced in some HIV-1–infected adults and indicate that the T-cell loss incurred during the course of HIV-1 disease might trigger a compensatory feedback loop which facilitates T-cell production.

Homeostasis within the T-cell compartment might be regulated by production of one or more factors that stimulate the differentiation, survival and/or expansion of T cells. Although such factors have not been linked to T-cell homeostasis in humans, known precedents exist for feedback regulation of other hematopoietic lineages. Erythropoietin is produced in the kidney and exerts its function upon the bone marrow, inhibiting the apoptosis of erythroid progenitor cells and stimulating their growth and differentiation[8]. Likewise, thrombopoietin is produced by the liver and functions to promote the differentiation of megakaryocytes within the bone marrow[9]. A similar feedback loop might connect the peripheral lymphoid compartment with the bone marrow or thymus, regulating homeostasis in the T-lymphoid lineage.

Various cytokines and hormones have been identified as positive regulators of thymopoiesis in rodents. Stem cell factor (SCF) and Flt-3 ligand stimulate thymopoiesis by acting on early hematopoietic progenitors[10]. Growth hormone and insulin-like growth factor (IGF)-1 are potent stimulators of thymopoiesis which have been associated with thymic regeneration in aging rodents[11,12] and acceleration of immune reconstitution after bone marrow transplantation in mice[13]. Interleukin (IL)-7 is a non-redundant cytokine essential for thymopoiesis. In murine models, disruption of IL-7 function results in massive reduction of lymphocytes within the thymus and peripheral lymphoid tissues[14,15]. IL-7 stimulates thymopoiesis by providing a crucial survival signal to early thymocyte progenitors[16–18]. In murine studies, administration of IL-7 mobilizes pluripotent hematopoietic stem cells from the bone marrow into the peripheral circulation[19] and also enhances T-cell reconstitution in myeloablated mice[20,21]. Additionally, IL-7 stimulates the proliferation and cytotoxic function of mature T cells in the periphery[22,23]. We hypothesized that HIV-1 induced lymphopenia might induce increased production of one or more of these potential stimulators of thymopoiesis.

We report here that increased circulating levels of IL-7 are strongly correlated with CD4+ T-cell loss in HIV-1–seropositive subjects. Thus, increased production of IL-7 may occur in response to lymphopenia. We also show production of IL-7 by dendritic-like cells within peripheral lymph nodes and an increase in IL-7 production within lymphocyte-depleted peripheral lymphoid tissues. We hypothesize that HIV-1–mediated T-cell depletion may be 'sensed' in the periphery by lymphoid stromal cells, and that these cells may then produce IL-7 to stimulate T-cell differentiation, survival and/or expansion. Finally, we show that higher IL-7 levels are associated with increased HIV-1 viral load. Given the capacity of IL-7 to augment HIV-1 replication *in vitro* [24,25], we consider a role for IL-7 in HIV-1 disease progression.

Table 1 Cohort characteristics[a]

Cohort	n	Age (y)	Total CD4 (cells/μl)	Total CD8 (cells/μl)	HIV Viral Load (log_{10} copies/ml)[b]	Plasma IL-7 (pg/ml)
HIV+ Cohort	168	40.3	270	750	4.4	2.12
		[26–68]	[4–1,020]	[78–2,403]	[<1.7– >5.7]	[0.28–39.7]
CD4 ≤ 270	84	41	143	616	4.7	3.36
		[27–67]	[4–270]	[78–2279]	[<1.7– >5.7]	[0.53–39.7]
CD4 > 270	84	39	403	879	3.7	1.48
		[26–56]	[271–1,020]	[311–2,403]	[<1.7–5.4]	[0.29–17.4]
Untreated[c]	65	38	226	656	4.8	2.57
		[26–59]	[4–685]	[78–2,360]	[2.5– >5.7]	[0.35 – 39.7]
Non - PI ARV[d]	33	42	221	813	4.7	2.11
		[30 - 68]	[15 - 644]	[230 - 1,883]	[3.7 – >5.7]	[0.60 – 17.9]
Successful PI ARV[e]	32	38	442	849	<2.7	1.22
		[28 - 56]	[72 - 1,020]	[321 – 2,260]	[<2.7]	[0.29 – 28.1]
Unsuccessful PI ARV[f]	32	42	258	861	4.4	2.88
		[29 - 53]	[23 – 878]	[200 - 2,403]	[3.1 – >5.7]	[0.78 – 10.2]
Former PI ARV[g]	6	45	165	714	4.5	4.04
		[36 - 52]	[19 - 273]	[413 - 1,886]	[2.1 – >5.7]	[0.94 – 9.79]

[a] Reported as median values; range shown in brackets.[b] Adjusted viral load determined as described in Methods section. [c] No exposure to ARVs.[d] At least 1 ARV, no exposure to PI therapy.[e] At least 3 ARVs, including at least 1 PI. HIV viral load (pre-adjusted value) < 500 copies/ml. On therapy ≥ 3 mo.[f] At least 3 ARVs, including at least 1 PI. HIV viral load (pre-adjusted value) > 500 copies/ml. On therapy ≥ 3 mo.[g] Prior exposure to PI-based therapy. At time of study, off all ARV at least 6 mo.

Cross-sectional analysis

We performed a pilot study of a small cohort (n = 19) of HIV-1-seropositive individuals, in which we investigated the relationship between parameters of immune function (such as thymus size and naive T-cell counts) and circulating levels of various potential positive and negative regulators of thymopoiesis, including IL-7, SCF, Flt-3 ligand, IL-2, IGF-1, thyroid stimulating hormone, testosterone and glucocorticoids. We found a strong inverse correlation between IL-7 levels and both total CD4+ T cell-count (ρ = –0.62; P = 0.013) and naive CD4+ T-cell count (ρ = –0.69; P = 0.001). No similar correlations were noted with any of the other cytokines or hormones studied (Napolitano, unpublished data).

We extended our study to a cohort of 168 HIV-1 infected adults (Table 1). The cohort was divided into two groups defined by the median CD4+ T-cell count: those with early/intermediate disease (CD4 > 270 cells/μl) and those with advanced disease (CD4 ≤ 270 cells/μl). 39% of subjects were untreated; 20% were taking antiretroviral (ARV) therapy which did not include an HIV-1 protease inhibitor (PI); 19% were taking PI-containing combination ARV (PI-ARV) with successful suppression of viremia; and 19% were taking PI-ARV with an incomplete virologic response. Circulating IL-7 determinations were compared with simultaneous measurements of thymic index (TI), circulating lymphocyte subsets that included B-cell and natural killer (NK)-cell analysis, viral load and complete blood count with differential. As observed[3], thymic tissue (n = 47) was positively correlated with increased circulating naïve CD4+ T cells (ρ = 0.40; P = 0.005) but not with circulating memory/effector T cells (ρ = 0.12; P = 0.46).

We previously reported a correlation between circulating IL-7 levels and CD4+ T lymphopenia. Univariate analysis (Table 2) showed that IL-7 levels were increased in the setting of lower T-cell counts, including both the CD4+ and CD8+ T-cell subpopulations. Higher IL-7 levels were strongly associated with depletion of both memory/effector and naive T cells. Additionally, increased IL-7 levels were associated with higher viral load. There was an inverse correlation between IL-7 levels and counts of red blood cells and neutrophils, but these associations were less significant and likely due to their association with advanced HIV-1 disease since they did not remain significant in multivariate analysis. Only 28% (n = 47) of the cohort underwent CT imaging of the thymus. Although there was a trend toward increased IL-7 levels in subjects with lower TI, this did not reach statistical significance (ρ = –0.25; P = 0.08). Weak correlations were seen between IL-7 and both B-cell and NK-cell counts but these also did not reach statistical significance. There was no correlation between IL-7 level and age. ARV status did not alter the observed correlations, although the relationships were more difficult to appreciate among subjects virologically suppressed

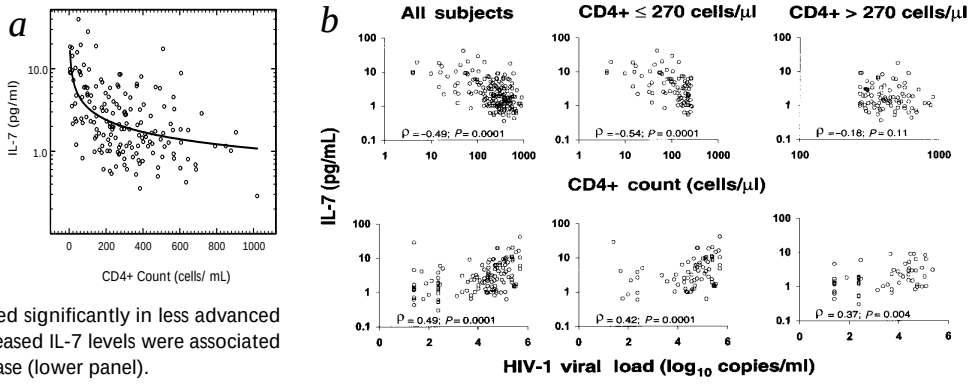

Fig. 1 Changes in IL-7, CD4+ T cell count, and viral load over the course of HIV-1 disease progression. ***a***, IL-7 increases logarithmically as the CD4+ T cell count decreases below the normal range. The line represents the least-squares fit of the model: log_{10}(IL-7) = 1.52 – 0.49 × log_{10}(CD4). The model R^2 is 0.29 (P = 0.0001). ***b***, In subjects with advanced HIV-1 disease (CD4 ≤ 270), increased circulating levels of IL-7 were strongly correlated with CD4+ T lymphopenia. The correlation weakened significantly in less advanced disease (CD4 > 270) (upper panel). Increased IL-7 levels were associated with higher viral load in all stages of disease (lower panel).

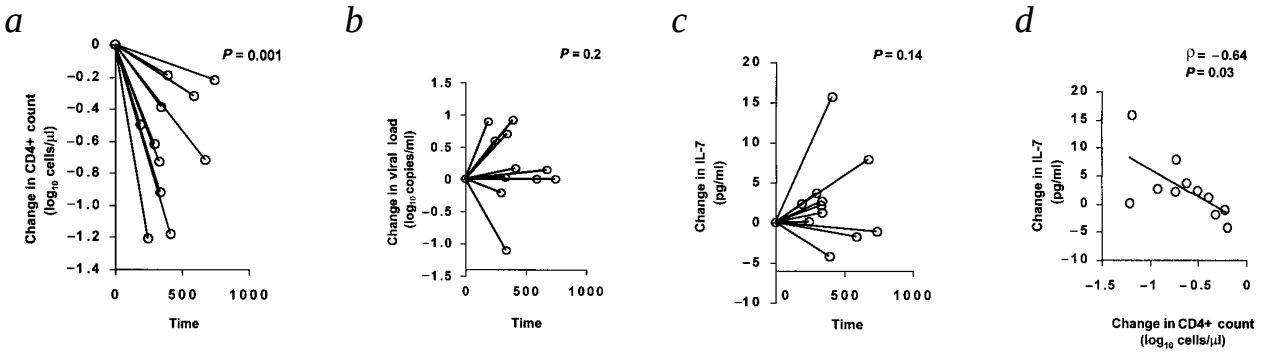

Fig. 2 Longitudinal analysis showing changes in 11 subjects followed for 6–25 mo. ***a***, There was a significant decline in CD4+ T cell count of the cohort over the course of observation ($P = 0.001$). ***b***, There was not a significant increase or decrease in the viral load of the cohort ($P = 0.2$). ***c***, There was a trend towards increased IL-7 levels ($P = 0.14$). ***d***, IL-7 increased significantly in proportion to the decline in CD4+ T cells ($\rho = -0.64$; $P = 0.03$).

on PI-ARV. We attributed these differences to the higher CD4+ T-cell counts and uniformly low viral loads of these subjects.

In multivariate analysis (Table 2), only lower CD4+ T-cell counts ($P = 0.0001$, partial $R^2 = 0.33$) and higher plasma viral RNA ($P = 0.002$, partial $R^2 = 0.07$) were independently associated with higher IL-7 levels. The associations between CD4+ count, viral load and IL-7 were not significantly nor substantially affected by other variables including non-CD4+ lineages, percent naive T cells, age, ARV treatment and cohort of origin.

Association of IL-7 levels with HIV-1 disease progression

We analyzed circulating IL-7 levels with respect to the stage of HIV-1 disease. IL-7 levels rose in a logarithmic fashion as the CD4+ T-cell count fell below normal levels and higher IL-7 levels were seen predominantly in those subjects with lower CD4+ T-cell counts (Fig. 1*a*). The CD4 data were subsequently log-transformed to create linear relationships (Fig. 1*b*). Using the median CD4+ T-cell count (270 cells/μl) to divide the cohort into two groups, bivariate analysis showed a stronger correlation of increased IL-7 with CD4+ T lymphopenia in subjects with more advanced disease ($\rho = -0.54$; $P = 0.0001$) in comparison with those with early/intermediate disease ($\rho = -0.18$; $P = 0.11$) (Fig. 1*b*, upper panel). In contrast, the correlation between higher levels of IL-7 and increased viral load was present in subjects with both lower ($\rho = 0.42$; $P = 0.0001$) and higher ($\rho = 0.37$; $P = 0.004$) CD4+ T-cell counts (Fig. 1*b*, lower panel). The association between increased IL-7 levels and increased viral load persisted in all stages of disease, regardless of the parameters chosen for CD4+ T-cell stratification.

Table 2 Cross-sectional analysis of circulating IL-7 levels in HIV-1-infected subjects

Univariate Analysis	ρ	***P* value**	***n***
Total CD4 count	(–) 0.49	0.0001	168
Viral load	(+) 0.49	0.0001	133
Memory CD4 Count	(–) 0.48	0.0001	157
Naive CD4 count	(–) 0.46	0.0001	157
T-cell count	(–) 0.44	0.0001	130
Total lymphocyte count	(–) 0.39	0.0001	168
Total CD8 count	(–) 0.35	0.0001	167
Naive CD8 count	(–) 0.32	0.0001	165
Memory CD8 count	(–) 0.26	0.0005	165
Thymic index	(–) 0.25	0.08	47
Neutrophil count	(–) 0.20	0.009	165
Red blood cell count	(–) 0.19	0.02	158
NK-cell count	(–) 0.15	0.09	129
B-cell count	(–) 0.15	0.09	129
Age	(–) 0.02	0.7	156
Platelet count	(–) 0.01	0.9	166
Multivariate Analysis	**Partial R^2**	***P* value**	***n***
Total CD4 count	0.33	0.0001	132
Viral load	0.07	0.002	132

Longitudinal analysis

To better relate these cross-sectional studies to disease progression, 11 HIV-1–seropositive subjects were studied over 6–25 months (Fig. 2). Five of the subjects were untreated and six were taking non-PI ARV during the period of observation. The median CD4+ T-cell count of the cohort decreased from 311 to 70 cells/μl ($P = 0.001$) (Fig. 2*a*) and the median viral load increased from 44,000 to 92,500 RNA copies/ml ($P = 0.2$) (Fig. 2*b*). Over this same period, the median plasma IL-7 increased from 2.31 to 4.52 pg/ml ($P = 0.14$) (Fig. 2*c*). Consistent with the findings of the cross-sectional study, the increase in IL-7 levels was significantly associated with a decrease in the CD4+ T cell count ($\log_{10}$ cells/μl) ($\rho = -0.64$; $P = 0.03$) (Fig. 2*d*). Changes in IL-7 were not, however, significantly associated with changes in viral load ($\rho = -0.41$; $P = 0.21$) (data not shown).

Production of IL-7 within peripheral lymph nodes

We investigated whether lymphocyte-depleted peripheral lymph nodes of HIV-1 infected patients might be producing IL-7. We performed immunohistochemical analysis of IL-7 production at the single-cell level within peripheral lymph nodes from patients with advanced HIV-1 disease (Fig. 3*a*). These specimens exhibited lymphocyte depletion as assessed by hematoxylin and eosin (H&E) staining and quantitative image analysis[26] (Table 3). In all of the specimens examined, and in multiple sections, there was distinct staining of IL-7–producing cells. Most of the IL-7–staining cells appeared non-lymphoid in morphology. These cells were characterized by large, irregular cytoplasm and some featured cytoplasmic processes that were ramified into the interstitium (Fig. 3*a*, inset). Phenotypic markers of the IL-7 producing cells were positive for S100b (66%), CD68 (60%) and CD83 (20%), consistent with dendritic-cell or macrophage lineage (data not shown). Most of the IL-7–producing cells were located within the parafollicular T-cell regions and were immediately surrounded by CD3+ cells (data not shown). Examination of

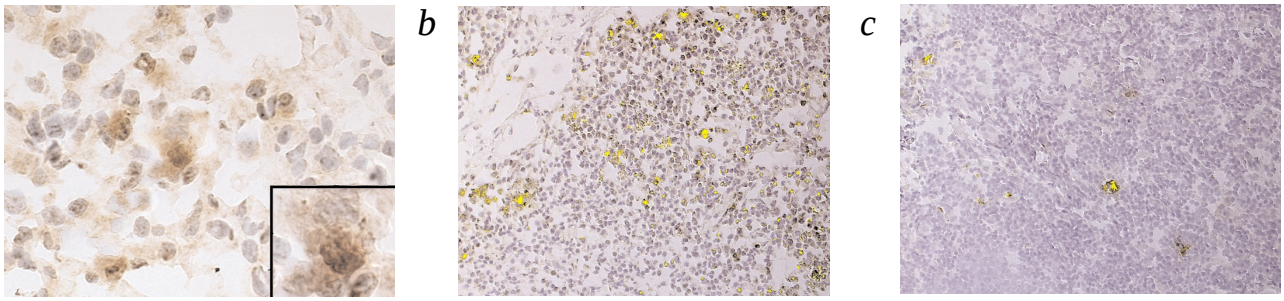

Fig.3 Production of IL-7 within peripheral lymphoid tissue. ***a***, Immunohistochemical detection of IL-7 production within a peripheral lymph node from a representative patient with AIDS (magnification: ×400). Stromal cells stain specifically for the presence of IL-7 (see insets; magnification: ×1000). IL-7 positive cells were stained brown by DAB while total nucleated cells were stained blue by hematoxylin. IL-7 positive cells, most of which stain positively for S100b and CD68, likely represent either dendritic cells or macrophages. ***b*** and ***c***, Quantitative image analysis of IL-7 production within peripheral lymphoid tissues. Representative sections are shown from depleted (*b*) and hyperplastic (*c*) specimens (magnification: ×100). IL-7 positive cells are stained brown. IL-7 signal intensity was digitalized and is represented by overlying yellow pixels. Production of IL-7 is increased in the depleted tissues as evidenced by a higher number of IL-7 staining cells as well as an increased intensity of IL-7 staining per cell (see Table 3).

lymphoid tissue from HIV-1–seronegative individuals also revealed IL-7 production within dendritic-like cells with similar phenotypic and morphological characteristics.

Increased IL-7 production within depleted HIV-1 lymphoid tissues

We examined the possibility that IL-7 production might be increased in the setting of lymphocyte depletion. Immunohistochemistry with quantitative image analysis was performed on 12 peripheral lymphoid specimens (Table 3). Six specimens, taken from individuals with AIDS, demonstrated moderate to severe lymphocyte depletion by both total cell number (median of 45.4% nucleated cells per total tissue area) and CD3 staining (data not shown). The remaining six tissues were hyperplastic (median of 74.3% nucleated cells per total lymphoid area). Representative sections of depleted (Fig. 3*b*) and hyperplastic (Fig. 3*c*) lymph nodes are displayed. IL-7 positive cells are stained brown and the staining intensity is represented by overlying yellow pixels. An increase in IL-7 production within the depleted lymph node is evidenced by an increase in both the number of IL-7 positive cells and the staining intensity of the IL-7 positive area. Data from all 12 specimens were compiled (Table 3). Production of IL-7 was significantly higher in the depleted tissues (median 1.56% IL-7 positive area within total cell area) when compared to the hyperplastic tissues (median 0.12% IL-7 positive area within total cell area) ($P = 0.004$). In addition, measurement of the intensity of IL-7 positive staining by quantitative image analysis revealed a significantly higher intensity in samples from the depleted specimens, indicating that each cell in the depleted tissue was making more IL-7 than those in the hyperplastic tissues (median 1.46 versus 0.25 intensity units/mm^2 respectively) ($P = 0.004$). There was a strong inverse correlation of cellularity with both % IL-7 positive area ($R^2 = -0.70$; $P = 0.020$) and IL-7 intensity ($R^2 = -0.71$; $P = 0.018$) (Spearman correlation).

Table 3 IL-7 production within peripheral lymphoid tissues.

Tissue source	Cellularity (% nucleated cells / total lymphoid area)	IL-7 positive area (% IL-7 positive cell area / total cell area)	IL-7 intensity (intensity units/mm^2 of IL-7 positive area)
Depleted			
Lymph node[a]	46.6	1.81	1.35
Lymph node[b]	56.1	2.68	3.25
Lymph node	47.1	0.97	0.89
Lymph node	41.5	1.86	0.88
Lymph node	33.0	1.47	1.63
Lymph node	48.2	0.57	0.75
Median	45.4 *	1.56 *	1.46*
Hyperplastic			
Tonsil	76.8	0.02	0.04
Lymph node[c]	84.7	0.18	0.65
Lymph node	66.3	0.07	0.19
Lymph node	74.2	0.15	0.25
Tonsil	67.6	0.13	0.15
Tonsil	76.3	0.17	0.23
Median	74.3	0.12	0.25

[a] Displayed in Fig. 3*b*.[b] Displayed in Fig. 3*a*.[c] Displayed in Fig. 3*c***P* = 0.004 compared with hyperplastic tissue (Mann-Whitney U)

Discussion

The processes which regulate T-cell homeostasis are not well understood, but the homeostatic response likely consists of both thymus-dependent and thymus-independent pathways[27–29]. Though the interplay between the thymus and the peripheral lymphoid compartment has been investigated in rodents[30,31], no studies have addressed the homeostatic response to chronic lymphocyte depletion in humans.

Our findings demonstrate an increase in both production of IL-7 and in circulating levels of IL-7 in the setting of HIV-1–induced lymphopenia. IL-7 has been shown to enhance thymopoiesis and to induce the expansion of mature T lymphocytes, and these survival signals seem to be specifically directed to the lymphoid lineage[14,17,22,23,32]. We propose that IL-7 production is increased as part of a compensatory feedback loop that enhances T-cell differentiation, survival and/or expansion, and consider that this may occur as part of a homeostatic response to HIV-1-mediated T-cell depletion. This hypothesis is supported

by the detection of elevated IL-7 levels in other lymphopenic conditions (for example, severe combined immunodeficiency syndrome, acute lymphocytic leukemia, chemotherapy-induced lymphopenia and idiopathic lymphopenia) and by the normalization of IL-7 levels when lymphopenia resolves (Mackall, pers. comm. and ref. 33).

Other non-exclusive explanations may account for the association between circulating levels of IL-7 and CD4+ T lymphopenia. Because the IL-7 receptor is expressed on mature T cells, circulating levels of IL-7 may be higher when the CD4+ T-cell count is lower simply because fewer receptors are available. We believe that this is unlikely, however: in our cohort with advanced disease (CD4 count ≤ 270 cells/μl), the CD8+ T-cell count was almost twice that of normal subjects (Table 1 and control data, not shown), making it less likely that there was a large drop in the number of available IL-7 receptors.

Another possibility is that IL-7 levels may rise in response to HIV-1 related processes other than (or in addition to) CD4+ T lymphopenia. For instance, increased IL-7 levels may be induced by opportunistic infection. Though theoretically possible, it probably is not a factor in our cohort since all of our participants underwent clinical screening during their study visits. We cannot exclude the presence of subclinical infections in this group, but we saw no signs or symptoms of infection. Alternatively, the production and secretion of IL-7 may be stimulated by the generalized state of immune activation that accompanies late stage HIV-1 disease. The presence, however, of elevated IL-7 levels in individuals with non-HIV-related lymphopenia (Mackall, pers. comm. and ref 33) makes it improbable that the increased IL-7 levels seen in our cohort are secondary to an HIV-related phenomenon such as cytokine dysregulation.

We favor the hypothesis that production (and circulating levels) of IL-7 increase as part of a homeostatic response to lymphopenia. The finding of increased IL-7 levels in the setting of lymphopenia from a variety of etiologies (Mackall, pers. comm. and ref. 33) supports this hypothesis. We also postulate that T-cell homeostasis might be regulated by non-lymphoid stromal cells which are able to 'sense' T-cell depletion in the periphery, possibly via interactions with adhesion molecules and/or other products made by T cells (cytokines for example). In this respect, interactions between hematolymphoid progenitors and bone marrow stromal cells have been shown to be responsible for the induction of IL-7 secretion by bone marrow stroma[34,35]. We speculate that similar interactions may occur between circulating T cells and stromal cells residing within peripheral lymphoid organs. When T cells are depleted from these organs, the 'sensor' stromal cells might be induced to produce IL-7. IL-7 could then act upon thymic, extrathymic or both sites of T lymphopoiesis to stimulate *de novo* T-cell production, or it could act locally within peripheral lymphoid tissues to induce expansion of mature T cells.

IL-7 production has been detected within stromal cells of the bone marrow and thymus[36] and also within mucosal lymphoid tissue[37,38]; however, IL-7 synthesis within peripheral lymph nodes has not been reported. Here we have demonstrated that IL-7 is produced by dendritic-like cells within peripheral lymph nodes (Fig. 3). Furthermore, we have shown that IL-7 production is significantly increased within lymphocyte depleted tissues (Table 3, Figs. 3*b* and *c*). These findings, which support our hypothesis that increased IL-7 production by lymphoid stromal cells occurs in response to lymphopenia, have important implications for our understanding of T-cell homeostasis. Though our data suggest a significant role for IL-7 in the maintenance of T-cell homeostasis, we cannot conclude that IL-7 is the sole factor involved in this process. Thus, IL-7 may work alone or in conjunction with other factors, such as IL-15, which has recently been shown to play an important role in memory T-cell expansion[39,40]. Additional studies are underway to further examine the relationship between IL-7 production and lymphopenia, focusing in particular on the possible role of additional positive and negative regulators of T lymphopoiesis[41].

Although there is a strong correlation between increased IL-7 levels and CD4+ T lymphopenia in our cohort, some individuals with advanced lymphopenia do not have increased circulating IL-7 levels. This heterogeneity is not unexpected since chronic HIV-1 disease is accompanied by infection and eventual destruction of lymphoid stromal cells[42]. Thus, the capacity to produce IL-7 may be significantly diminished in certain individuals with late-stage HIV-1 infection. Though our immunohistochemical analysis demonstrates that IL-7 production remains at least partially intact within peripheral lymphoid tissues of some patients with advanced HIV-1 disease, this may not be the case for all individuals. We might expect to see improvement in immune parameters (such as thymic index and CD4+ T cell count) in those individuals who are capable of producing higher levels of IL-7. In fact, this finding might be predicted by murine models where IL-7 facilitates immune reconstitution in the setting of immunodeficiency[20,21]. If present, however, this effect may be difficult to demonstrate in our cohort due to the presence of ongoing HIV-1 replication. This is particularly true in the setting of advanced disease where the immunodestructive effects of HIV-1 may overwhelm compensatory increases in positive regulators of T-cell production or expansion. If so, it may be possible to shift this balance more towards increased T-cell production if HIV-1 replication is controlled by use of effective antiretroviral therapy.

We have shown that increased IL-7 levels are associated with increased viral load in all stages of HIV-1 disease (Fig. 1*b*, lower panel). IL-7 has been reported to augment *in vitro* HIV-1 infection of both thymocytes and peripheral blood mononuclear cells[24,25]. Thus, our findings imply that higher circulating levels of IL-7 may drive viral replication *in vivo* in those who are infected with HIV-1. Accordingly, higher baseline levels of IL-7 in early disease may increase viral burden and lead to faster CD4+ T-cell depletion over time[43]. Moreover, a physiological feedback loop involving IL-7 could not only drive *de novo* T-cell production but also accelerate HIV-1–mediated T-cell loss via IL-7 enhancement of viral replication. Recent studies from our laboratory confirm that IL-7 accelerates HIV-1 infection *in vivo* in the SCID-hu Thy/Liv mouse (Napolitano, unpublished observations). These results have important implications, particularly if IL-7 is to be considered as a form of immunomodulatory therapy during HIV-1 infection.

We have demonstrated a strong and independent correlation between circulating IL-7 levels and CD4+ T lymphopenia in HIV-1–infected individuals using both cross-sectional and longitudinal analyses. We have also observed that IL-7 is produced within dendritic-like cells of lymph nodes within HIV-1 infected individuals and that IL-7 production by these cells is significantly increased in the setting of lymphocyte depletion. We propose that these cells may increase production of IL-7 after 'sensing' T-lymphocyte depletion and that this occurs as part of a homeostatic response to T-cell depletion. Finally, we have found that higher circulating levels of IL-7 are associated with increased HIV-1 viral load, suggesting that IL-7 may enhance HIV-1 replication *in vivo* and lead to

increased viral burden and accelerated disease progression. Our data support further investigation into the role of IL-7 in T-cell homeostasis and into the role of IL-7 in the progression and treatment of HIV-1 disease.

Methods

Human subjects. HIV-1-seropositive patients were participants in studies of immune function at University of California at San Francisco or at Stanford University between 1993 and 1999. Initially, 170 patients were selected based upon the availability of T-cell subset analyses, CBC with differential, and frozen plasma specimens for IL-7 determinations. Two patients were excluded due to a recent change (< 3 mo) in ARV therapy. All studies were approved by the University of California Committee on Human Research or by the Stanford University Panel on Human Subjects in Medical Research.

Thymus measurement. Measurements of residual thymic tissue were performed using CT scan images as previously described[3]. Only CT scans performed within 6 mo (mean = 1.5 mo) of phlebotomy were included in the analysis.

Flow cytometry. Circulating naive and memory CD4+ and CD8+ T cells were measured by multiparameter flow cytometry on PBMCs using CD62L and CD45RA markers as previously described[3]. Multiparameter determinations of B lymphocytes (CD45+CD19+CD3–) and NK cells (CD45+CD56+CD3–) were performed similarly.

Clinical labs. CBC with differential and routine chemistries were performed at the clinical laboratories of San Francisco General Hospital or Stanford Medical Center.

Viral load analysis. HIV-1 viral load was determined by Chiron (Emeryville, California) bDNA 2.0, Chiron bDNA 3.0, or Roche PCR. Results from bDNA 2.0 assays were multiplied by 1.75 to adjust for systematic differences between the assays[44].

IL-7 determinations. Non-heparinized plasma specimens (in ACD or EDTA) were stored at ≤ -70 °C. Aliquots were thawed and analyzed in duplicate using a commercially available, high sensitivity immunoassay which detects both free IL-7 and IL-7 bound to carrier proteins or soluble IL-7 receptors (R&D Systems QuantikineHS IL-7 Immunoassay Kit, Minneapolis Minnesota). Analysis was performed on a blood specimen drawn at the same time as those specimens taken for flow cytometry, CBC with differential and viral load analysis. According to the manufacturer, the mean IL-7 level in healthy human adult plasma is 2.8 pg/ml for ACD plasma and 2.2 pg/ml for EDTA plasma using this assay (range 0.66–7.8 for ACD plasma; 0.66–9.2 for EDTA plasma, $n = 38$).

Immunohistochemistry. Tissue was obtained during autopsy or surgery, immediately immersed in OCT, flash frozen in liquid nitrogen, and stored at –70°C until immunohistochemical staining was performed. The sections were cut to 8 μm, fixed in 2% formaldehyde (Sigma, Sweden) and permeabilized with 0.1% saponin (Riedel-de Haen, Seelze, Germany). Following peroxidase quenching, immunohistochemistry was performed using specific monoclonal antibody against human IL-7 (Rat IgG2a BVD10-11C10, PharMingen, San Diego, California) and the Vectastain Elite ABC kit (Vector Labs, Burlingame, California) according to manufacturer's recommendation. Diaminobenzidine (DAB) was used as substrate with hematoxylin counter stain. IL-7 expressing cells were quantified using computerized *in situ* image analysis as described[26,45]. Control staining with normal rat serum or secondary isotype-specific antibodies (Vector) showed virtually no non-specific staining. Digital images of stained samples were transferred from a DMR-X microscope (Leica, Wetzlar, Germany) into a computerized image analysis system, Quantimet 550IW (Leica, Cambridge, United Kingdom), which allowed detection of 16.7 million different colors. Percentage of IL-7 producing area was derived by dividing the total IL-7–positive cell area by the total area of nucleated hematoxylin positive cells (mucosal epithelium and crypts were excluded from total nucleated cell area in the tonsil specimens). To calculate the total IL-7 positive area, several fields (mean=10 fields; mean tissue area= $1.4 \times 10^6 \mu m^2$) encompassing the entire area of the biopsy were assessed for positive IL-7 signal (defined by specific red, green and blue levels of the digital image; each color contains 256 levels). All samples were analyzed at the same threshold values. The frequency of positively-stained cells and the accumulated intensity of the positive staining signal were measured in a semiquantitative way by a specialized software program[46], and results were expressed as percentage positive area and total positive intensity of total tissue area. Limiting dilution of cDNA-transfected cells injected into control tissue indicated a sensitivity of the assay of at least 1 positive cell/1000 events[45]. To calculate the percentage positive cells and cellularity, the positive and negative cells in the digital image were counted by a special computer program (Ola Norén, Leica, Kista, Sweden). Cellularity was given as number of cells/total tissue area. Two-color staining for the phenotype of IL-7 expressing cells was performed using commercially available antibodies against CD3 (Becton Dickinson Immunocytometry Systems, San Jose, California) and CD20, CD83, CD68 (all from DAKO, Carpinteria, California) and S100b (SH-B1, Sigma) at concentrations optimized on control tissues[26]. Cell surface staining was performed before IL-7 staining. Tissues analyzed in Table 3 were obtained from a different set of individuals than those described in Table 1.

Statistical analysis. Statistical analysis of the cross-sectional and longitudinal studies was performed using SAS version 6.12 software (SAS Institute, Cary, North Carolina). Spearman rank correlation coefficients were calculated to assess associations between any two continuous variables prior to any data transformations. The longitudinal analysis was performed by calculating the change in $\log_{10}$ transformations of IL-7, CD4+ T-cell count and viral load from baseline for each study subject and testing for significant changes over time using the Wilcoxon Signed-Rank test. Multivariate linear regression was performed after $\log_{10}$ transformation of IL-7 in order to normalize the variance of the dependent variable. Predictor variables, including CD4+ T-cell count and viral load, were $\log_{10}$ transformed whenever necessary to transform non-linear relationships with IL-7 into linear relationships. Predictor variables were considered for stepwise inclusion in the multivariate model if associations with IL-7 were observed in bivariate analysis ($P < 0.15$) or if the predictor variable was related to thymopoiesis (age for example). Thymus score was not considered in multivariate analysis because data were available for only 28% of the subjects. After inclusion of CD4+ T-cell count and viral load in the multivariate model, no other variable was independently associated ($P < 0.15$) with IL-7. To determine if the associations between CD4+ T-cell count, viral load and IL-7 could be affected by interactions with age, other cell lineages, treatment or cohort of origin, these variables were added as main effects and interaction terms to the multivariate model predicting IL-7 based on CD4+ T-cell count and viral load. The number of subjects varies between analyses because of missing data. No adjustment for multiple comparisons was performed. Statistical analysis of IL-7 production within peripheral lymphoid tissues (Table 3) was performed with StatView version 5.0 software (SAS Institute).

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1. Hellerstein, M. *et al.* Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nature Med.* **5**, 83–89 (1999).
2. Ho, D.D. *et al.* Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**, 123–126 (1995).
3. McCune, J.M. *et al.* High prevalence of thymic tissue in adults with HIV-1 infection. *J. Clin. Invest.* **101**, 2301–2308 (1998).
4. Douek, D.C. *et al.* Changes in thymic function with age and during the treatment of HIV infection. *Nature* **396**, 690–695 (1998).
5. Poulin, J.-F. *et al.* Direct evidence for thymic function in adult humans. *J. Exp. Med.* **190**, 479–486 (1999).
6. Zhang, L. *et al.* Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. *J. Exp. Med.* **190**, 725–732 (1999).
7. Haynes, B.F. *et al.* Analysis of the adult thymus in reconstitution of T lymphocytes in HIV-1 infection. *J. Clin. Invest.* **103**, 453–460 (1999).
8. Silva, M. *et al.* Erythropoietin can promote erythroid progenitor survival by repressing apoptosis through Bcl-X_L and Bcl-2. *Blood* **88**, 1576–1582 (1996).
9. Kato, T. *et al.* Native thrombopoietin: structure and function. *Stem Cells* **16**, 322–328 (1998).
10. Lyman, S. & Jacobsen, S.E.W. c-Kit ligand and Flt-3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. *Blood* **91**, 1101–1134 (1998).
11. Kelley, K.W. *et al.* GH3 pituitary adenoma cells can reverse thymic aging in rats. *Proc. Natl. Acad. Sci. USA* **83**, 5663–5667 (1986).
12. Bar-Dayan, Y. & Small, M. Effect of bovine growth hormone administration on the pattern of thymic involution in mice. *Thymus* **23**, 95–101 (1994).
13. Beschorner, W.E. *et al.* Enhancement of thymic recovery after cyclosporine by recombinant human growth hormone and insulin-like growth factor I. *Transplantation* **52**, 879–884 (1991).
14. Peschon, J.J. *et al.* Early lymphocyte expansion is severely impaired in interleukin-7 receptor-deficient mice. *J. Exp. Med.* **180**, 1955–1960 (1994).
15. von Freeden-Jeffry, U. *et al.* Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* **181**, 1519–1526 (1995).
16. Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R. & Weissman, I.L. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell* **89**, 1033–1041 (1997).
17. Di Santo, J. *et al.* The common cytokine receptor γ chain and the pre-T-cell receptor provide independent but critically overlapping signals in early α/β T-cell development. *J. Exp. Med.* **189**, 563–573 (1999).
18. Pallard, C. *et al.* Distinct roles of the phosophatidylinositol 3-kinase and STAT5 pathways in IL-7-mediated development of human thymocyte precursors. *Immunity* **10**, 525–535 (1999).
19. Grzegorzewski, K.J. *et al.* Mobilization of long-term reconstituting hematopoietic stem cells in mice by recombinant human interleukin 7. *J. Exp. Med.* **181**, 369–374 (1995).
20. Morrissey, P.J. *et al.* Administration of IL-7 to mice with cyclophosphamide-induced lymphopenia accelerates lymphocyte repopulation. *J. Immunol.* **146**, 1547–1552 (1991).
21. Bolotin, E., Smogorzewska, M., Smith, S., Widmer, M. & Weinberg, K. Enhancement of thymopoiesis after bone marrow transplant by in vivo interleukin-7. *Blood* **88**, 1887–1894 (1996).
22. Chazen, G.D., Pereira, G.M., LeGros, G., Gillis, S. & Shevach, E.M. IL-7 is a T-cell growth factor. *Proc. Nat. Acad. Sci. USA* **86**, 5923–5927 (1989).
23. Hickman, C.J., Crim, J.A., Mostowski, H.S. & Siegel, J.P. Regulation of human cytotoxic T lymphocyte development by IL-7. *J. Immunol.* **145**, 2415–2420 (1990).
24. Smithgall, M.D., Wong, J.G.P., Critchett, K.E. & Haffar, O.K. IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. *J. Immunol.* **156**, 2324–2330 (1996).
25. Chene, L. *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.* **73**, 7533–7542 (1999).
26. Andersson, J. *et al.* Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. *AIDS* **12**, 123–129 (1998).
27. Mackall, C.L., Hakim, F.T. & Gress, R.E. Restoration of T-cell homeostasis after T-cell depletion. *Semin. Immunol.* **9**, 339–346 (1997).
28. Tanchot, C., Rosado, M.M., Agenes, F., Frietas, A.A. & Rocha, B. Lymphocyte homeostasis. *Semin. Immunol.* **9**, 331–337 (1997).
29. Goldrath, A. & Bevan, M.J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402**, 255–262 (1999).
30. Berzins, S.P., Boyd, R.L. & Miller, J.F.A.P. The role of the thymus and recent thymic migrants in the maintenance of the adult peripheral lymphocyte pool. *J. Exp. Med.* **187**, 1339–1348 (1998).
31. Gabor, M.J., Scollay, R. & Godfrey, D.I. Thymic T cell export is not influenced by the peripheral T cell pool. *Eur. J. Immunol.* **27**, 2986–2993 (1997).
32. Puel, A., Ziegler, S.F., Buckley, R.H. & Leonard, W.J. Defective IL7R expression in T (–) B (+) NK (+) severe combined immunodeficiency. *Nature Genet.* **20**, 394–397 (1998).
33. Bolotin, E., Annett, G., Parkman, R. & Weinberg, K. Serum levels of IL-7 in bone marrow transplant recipients: relationship to clinical characteristics and lymphocyte count. *Bone Marrow Transplant.* **23**, 783–788 (1999).
34. Ryan, D.H. & Tang, J. Regulation of human B cell lymphopoiesis by adhesion molecules and cytokines. *Leuk. Lymphoma* **17**, 375–389 (1995).
35. Tang, J. *et al.* TGF-beta down-regulates stromal IL-7 secretion and inhibits proliferation of human B cell precursors. *J. Immunol.* **159**, 117–125 (1997).
36. Komschlies, K., Grzegorzewski, K. & Wiltrout, R.H. Diverse immunological and hematological effects of interleukin 7: implications for clinical application. *J. Leukoc. Biol* **58**, 623–633 (1995).
37. Kroncke, R., Loppnow, H., Flad, H.D. & Gerdes, J. Human follicular dendritic cells and vascular cells produce interleukin-7: a potential role for interleukin-7 in the germinal center reaction. *Eur. J. Immunol.* **26**, 2541–2544 (1996).
38. Watanabe, M. *et al.* Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. *J. Clin. Invest.* **95**, 2945–2953 (1995).
39. Lodolce, J.P. *et al.* IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* **9**, 669–676 (1998).
40. Ku, C.C., Murakami, M., Sakamoto, A., Kappler, J. & Marrack, P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. *Science.* **288**, 675–678 (2000).
41. McCune, J.M. Thymic function in HIV-1 disease. *Semin. Immunol.* **9**, 397–404 (1997).
42. Haase, A.T. Population biology of HIV-1 infection: Viral and CD4+ T cell demographics and dynamics in lymphatic tissues. *Annu. Rev. Immunol.* **17**, 625–656 (1999).
43. Mellors, J.W. *et al.* Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* **272**, 1167–1170 (1996).
44. Elbeik, T. *et al.* Quantitative and cost comparison of ultrasensitive human immunodeficiency virus type 1 RNA viral load assays: Bayer bDNA Quantiflex versions 3.0 and 2.0 and Roach PCR Amplicor Monitor version 1.5 *J Clin Microbiol* 38, 1113-1120 (2000)
45. Litton, M. *et al.* Tumor therapy with antibody targeted superantigens generates a dichotomy between local and systemic immune responses. *Am. J. Pathol.* **150**, 1607–1618 (1997).
46. Bjork, L., Fehniger, T., Andersson, U., Andersson, J. Computerized assessment of production of multiple human cytokines at the single cell level using image analysis. *J. Leukoc. Biol.* **59**, 287–295 (1996).