HIV-1 driven regulatory T cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS

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Short title: Tissue Treg affect HIV disease progression

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ABSTRACT

Regulatory T cells (T_{reg}) accumulate in the lymphoid tissues of HIV-infected individuals, contributing to the inability of the immune system to control virus replication. We investigate here T_{reg} numbers and functional markers (FOXP3, CTLA-4, IDO and TGF-β1) in lymphoid tissues from untreated infected hosts with progressive or non-progressive disease (HIV-infected humans and SIV-infected macaques). We found that increased numbers of FOXP3+ T cells as well as increased expression of T_{reg}-associated functional markers were detected only during progressive disease. Such increases were not correlated with immune activation. Importantly, a high perforin/FOXP3 ratio was associated with non-progressive disease, suggesting that the immune control of virus replication represents a balance between cell-mediated immune responses and T_{reg}-mediated counter-regulation of such responses. Furthermore, using an in vitro model of T_{reg}-HIV interactions, we showed that exposure of T_{reg} to HIV selectively promoted their survival via a CD4-gp120 dependent pathway, thus providing an underlying mechanism for the accumulation of T_{reg} in infected hosts with active viral replication. Considered together, our findings imply that therapeutic manipulation of T_{reg} number and/or function could improve immune control of HIV infection.
INTRODUCTION

Defects in cell-mediated immunity (CMI) are important in chronic human immunodeficiency virus (HIV) infection. Failure of the immune system to clear infected cells results in high viral load and disease progression. Hallmarks of HIV-associated defects in CMI include: impaired or absent CD4+ T cell responses; inefficient CD8+ T cell activity; and dysregulation of antigen-presenting cells (APC). The majority of simian immunodeficiency virus (SIV)-infected macaques display similar features, although immunodeficiency generally develops more rapidly than in HIV-infected humans.

However, viral replication is controlled in a minority of HIV-infected humans and SIV-infected macaques, resulting in a non-progressive phenotype. Many non-progressors maintain vigorous and broadly-directed HIV-specific cytotoxic T-cell (CTL) and CD8-mediated HIV suppressive activity. These findings imply a central role for CMI in the immunological control of HIV and SIV infection. Nonetheless, the molecular bases for the progressive impairment of CMI in most infected hosts remain largely unknown.

Regulatory T cells (T\text{reg}), a specialized subset of CD4+ T cells, were reported to suppress effector T cell responses in chronic infections, including retroviral infections. T\text{reg} can suppress the function of APC and of CD4+ and CD8+ effector T cells. T\text{reg} were proposed to suppress protective anti-HIV CMI based on the finding that in vitro depletion of T\text{reg} from circulating leukocytes of HIV-infected subjects resulted in increased anti-HIV T cell responses. We and others have shown that T\text{reg} accumulate in the lymphoid tissue (LT) of HIV-infected individuals with active viral replication, and of acutely SIV-infected macaques, their number correlating inversely with the incidence of SIV-specific CD8+ T cells. However, the mechanisms underlying such accumulation, and its consequences on immune control of HIV/SIV infection, are unknown. Herein, we show that direct interactions between T\text{reg} and HIV promote T\text{reg} survival, which in turn may lead to their accumulation in LT of
chronically-infected hosts, where they are involved in reduced CMI activity and consequently in disease progression.
METHODS

Human subjects

Lymphoid tonsillar biopsies were obtained from 6 treatment-naïve adults with chronic, progressing HIV-1 infection (HIVprog) and from 5 untreated adults with non-progressing infection (HIVnp), all recruited at the Karolinska Institute, Stockholm, Sweden. The criteria for inclusion in the HIVnp group were: (1) treatment-naïve; and (2) documented minimum of 5 years of infection with low viral load and stable CD4 counts. Those criteria predict a non-progressing or slow-progressing disease 22. Lymphoid tonsillar biopsies were also obtained from 5 uninfected adults undergoing routine tonsillectomy due to mild sleep apnea syndrome (Karolinska Institute). Demographic and clinical data are presented in Table 1 and Table 2. All biopsies from HIVprog, but none from HIVnp, were graded as follicular hyperplasia, which is prognostic of disease progression 23. The biopsies were snap-frozen and cryopreserved in OCT (Sakura, Torrance, CA). In addition, leukophoresis products were obtained from uninfected donors (Department of Blood Transfusion, NIH, Bethesda, MD). Informed consent was obtained from all subjects and protocols were approved by the Institutional Review Boards of the participating institutions.

Macaques

Sixteen colony-bred Indian rhesus macaques (Macaca mulata) were obtained from Covance Research Products (Alice, TX) and handled in accordance with the standards of the American Association for the Accreditation of Laboratory Animal Care. Fourteen of these macaques were infected with SIVmac251 for two years prior to lymph node excision (see Table 3). SIVmac251 RNA was quantified in plasma by nucleic acid sequence-based amplification (detection limit: 2x10^3 RNA copies/ml). Two animals were uninfected. The protocol was approved by the NIH animal review board. Whole axillary lymph nodes were mechanically disrupted and
mononuclear cells were isolated by density centrifugation (Ficoll-Hypaque, Amersham, Piscataway, NJ).

**Determination of mRNA expression in macaque LT**

Total RNA was extracted from macaque LT mononuclear cells using the guanidium thiocyanate-phenol-chloroform method, modified for TRIzol (Invitrogen, Carlsbad, CA). RNA was reverse-transcribed using random hexanucleotide primers and oligo dT, and Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI). Quantification of FOXP3, IFN-γ, granzyme B, perforin, CTLA-4, IDO, TGF-β, IL-10, CD4 and GAPDH mRNA was performed by real-time PCR (ABI Prism 7900HT, Foster City, CA) using a SYBR green PCR mix (Qiagen, Valencia, CA) and specific primers designed from the macaque gene sequences. Results are presented as relative units (RU) obtained by normalizing the target gene mRNA with either the corresponding CD4 or GAPDH mRNA values.

**Determination of mRNA expression in human cells and tissues**

Total RNA was extracted from cells and human LT using RNAeasy mini kits, following manufacturer’s instruction (Qiagen), as described. RNA was reverse-transcribed using superscript reverse transcriptase (Invitrogen) and random hexanucleotide primers. RT product was amplified using real-time PCR, performed in a Light-Cycler (Roche, Indianapolis, IN) using a SYBR green PCR kit (Roche) and specific primers for the analyzed human genes.

**Flow cytometry analysis of T<sub>reg</sub> markers**

Cells were stained with anti-CD25-APC (M-A251, BD PharMingen, San Diego, CA) or anti-GITR-PE (110416, R&D Systems, Minneapolis, MN) after incubation with human IgG (10µg/ml; Sigma, St. Louis, MO) to block Fc receptors. Cells were then stained with anti-FOXP3-FITC (PCH101, eBiosciences, San Diego, CA), according to eBiosciences’s instructions. In another tube, anti-GITR-PE was omitted and anti-CTLA-4-PE (14D3,
eBiosciences) was added at the same time as anti-FOXP3 Ab. The appropriate isotype-matched control Abs were used to define positivity. Marker expression was analyzed using a FACSCalibur and the CellQuest software (BD). A minimum of 10,000 cells/tube was analyzed.

**Immunohistochemistry and confocal analysis of human LT**

Eight µm thick biopsy samples were fixed in 2% formaldehyde, and blocked for endogenous biotin (Vector Laboratories, Burlingame, CA). Anti-human CTLA-4, CD4, CD8 (BD Pharmingen), CD25 (Pelicluster, Netherlands), CD69 (DakoCytomation, Denmark), FOXP3 (Ab2481, Novus Biologicals, Littleton, CO), perforin and granzyme B (Endogen, Rockford, IL), and irrelevant isotype matched Ab were used. The mean size of the scanned area per section was 2.1 X 10^6 µm². Double and single positive stained cells were quantified in 15 high-power fields using the Qwin 550 software and a confocal microscope (Leica TCS SP2 AOBS). A blinded investigator carried out image analyses.

**In vitro exposure to inactivated HIV**

CD4⁺ T cells were purified from the blood of HIV-uninfected donors by negative selection (CD4 T cell isolation KitII human, Miltenyi, Auburn, CA). Cells were cultured in presence of aldrithiol-2-inactivated HIV-1 (AT-2 HIV), kindly provided by Dr. J. Lifson (AIDS Vaccine Program, SAIC Frederick Inc., Frederick, MD). AT-2 HIV was used at the concentration of 1 µg HIV p24^gag^ equivalent/ml, which corresponds to a gp120 concentration of ~1nM. Microvesicles, isolated from uninfected cultures of CEM X 174 cells following the same procedures as those used to prepare AT-2 HIV, were used as negative control. These vesicles control for the effect of AT-2 treatment, as well as the effect of cellular proteins budding from vesicles. Microvesicles were added at a concentration that provided an equal amount of total protein as AT-2 HIV. In most experiments, HIVMN was used. In some
experiments, HIVADA was used at the same concentration as HIVMN.

Two x 10^6 total CD4^+ T cells were cultured in complete RPMI medium supplemented with 2% human AB^+ serum in presence of AT-2 HIV or microvesicles. At d1, IL-2 (20 UI/ml, AIDS Research and Reference Reagent Program, NIH) was added to every well. Cells were prepared for mRNA extraction or FACS analysis of T_{reg} markers at different times after culture initiation. In some experiments, CD4^+CD25^+ T cells were deleted from total CD4^+ cells (CD25 purification kit, Miltenyi). Purity of >90% was routinely achieved, as assessed by FACS analysis. Cells were exposed to AT-2 HIV as described for total CD4^+ T cells.

In some experiments, soluble CD4-IgG (sCD4, which encompasses the CD4 N-terminal 183 amino-acid residues 29, 10 µg/ml, AIDS Research and Reference Reagent Program), was added to AT-2 HIV-exposed CD4^+ T cells.

In some experiments, purified CD4^+ T cells were exposed to anti-CD4 Ab, which recognizes the domain 1 of the CD4 molecule (clone QS4120), or to an isotype-matched control Ab (Ancell, Bayport, MN, both at 10 µg/ml) for 1h at +4°C, washed, and then cultured as described for AT-2 HIV-exposed CD4^+ T cells.

**Determination of T_{reg} apoptosis**

CD4^+CD25^+ cells, purified by positive selection (CD25 purification kit, Miltenyi, purity of >90%), were exposed to AT-2 HIV or microvesicles, as described for total CD4^+ T cells. At d5, cells were stained for surface expression of annexin V, following Pharmingen’s protocol.

**In vitro T_{reg} suppressive activity**

Purified CD4^+CD25^+ cells were exposed to AT-2 HIV or microvesicles. At d5, cells were washed 3 times to remove unbound virus or microvesicles, live cells were counted and mixed at a ratio of 1:1 with autologous effector CD4^+CD25^- T cells (5 x 10^5/well). Effector cells had been labeled with 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE).
(Invitrogen; 1.25 µM for 5 min, followed by several washes). Since, in our experience, the
PHA-induced proliferation of highly purified CD4+ T cells is not optimal but is enhanced by
addition of APC, autologous irradiated elutriated monocytes (5 x 10⁴/well) were added to all
cultures. Effector T cells were stimulated with PHA (PHA-M, 2 µg/ml; Sigma) for 3 days in
presence or absence of T_{reg}. Effector T cells were also cultured with APC alone, as a negative
control. CFSE levels were determined by FACS, acquiring a minimum of 20,000 labeled cells.

Statistical analysis
Gene expression in biopsies was statistically analysed using Mann-Whitney U tests.
Percentages of positive cells were assessed by ordinary least-squares regression after logit
transformation of the data. Correlations were determined with Spearman’s rank correlation. In
vitro expression of T_{reg} markers was analyzed using paired t-tests. A two-tailed \( p < 0.05 \) was
considered to be significant.
RESULTS

Non-progressors exhibit low FOXP3 expression in lymphoid tissues.

We and others have previously shown that active replicating HIV and SIV infection is associated with the accumulation of $T_{reg}$ in LTs, the major site of virus replication. To determine whether such accumulation has any effect on HIV disease progression, we compared FOXP3 protein expression at the single-cell level in the tonsils of 5 HIVnp and 6 HIVprog (see Table 1 and Table 2 for description of clinical characteristics). Tissues from 5 HIV-uninfected adults were also analyzed. Strikingly, the number of FOXP3$^+$ cells was 5-fold lower in HIVnp than in HIVprog, but similar to uninfected subjects (Fig. 1a-c). Similar significant differences were observed when FOXP3 mRNA levels were quantified in the same tissues (Fig. 1d). Importantly, FOXP3 expression in HIVprog was not directly associated with immune activation, since the percentage of FOXP3$^+$ and CD69$^+$ cells were not correlated in HIV-infected subjects ($p=0.98$) (Fig. 1f).

To validate our findings from HIV-infected individuals in the macaque model, we tested axillary lymph nodes (LNs) from 14 SIV-infected macaques (Table 3). Seven of these macaques were non-progressors and had persistently undetectable viral loads (SIVnp), whereas the remaining 7 exhibited progressive infection and persistent viremia (SIVprog). LNs were also collected in 2 uninfected macaques. A trend towards lower FOXP3 mRNA expression (approximately 3-fold) was also observed in the LTs from SIVnp, compared to SIVprog (Fig. 1e). These findings suggest that extensive $T_{reg}$ accumulation within LTs is a feature of chronic, progressive HIV or SIV infection, but not of non-progressive infection.

Non-progressors exhibit low expression of markers of $T_{reg}$ function in lymphoid tissues

We tested whether the low FOXP3 expression in HIVnp and SIVnp was accompanied by low expression of molecules associated with $T_{reg}$ function, such as CTLA-4. HIVprog showed a
trend to higher CTLA-4 than HIVnp (approximately 2-fold, \( p=0.09 \)) and uninfected controls (Fig. 2a). Similar results were obtained after analysis of CTLA-4 protein expression, which was 6-fold higher in HIVprog than in HIVnp (data not shown). The majority (>85%) of FOXP3\(^+\) cells in the LTs of the different groups co-expressed CTLA-4 (data not shown).

Because CTLA-4-expressing \( T_{\text{reg}} \) were shown to induce the tryptophan-catabolizing enzyme indoleamine 2-dioxygenase (IDO) by APC\(^30\), we analyzed IDO mRNA in LT. IDO levels in HIVnp were lower than in HIVprog (\( p=0.02 \)), but comparable to uninfected controls (Fig. 2b). These results were confirmed in SIV-infected macaques, SIVnp expressing lower levels of CTLA-4 (\( p=0.02 \)) and a trend towards decreased IDO (8-fold decrease; \( p=0.2 \)) mRNA than SIVprog (Fig. 2e, f).

Suppressive cytokines, particularly TGF-\( \beta 1 \) and IL-10, are also implicated in \( T_{\text{reg}} \)-mediated suppression. In human LTs, TGF-\( \beta 1 \) mRNA expression was lower in HIVnp than in HIVprog (\( p=0.004 \); Fig. 2c) and comparable to uninfected controls. SIVnp also exhibited lower TGF-\( \beta 1 \) expression compared to SIVprog (Fig. 2g). In contrast, expression of IL-10 mRNA was equally up-regulated in both HIV- and SIV-infected hosts, compared to uninfected hosts (Fig. 2d, h). Therefore, low FOXP3 levels in LTs from non-progressor subjects were associated with concomitant low levels of CTLA-4, IDO and TGF-\( \beta 1 \), but not of IL-10.

**Plasma viral load inversely correlates with expression of CD25 by FOXP3\(^+\) T cells.**

CD25 has been widely used to define \( T_{\text{reg}} \) in both human and murine systems. Therefore, we tested whether CD25 and FOXP3 were co-expressed in the different subject populations. Co-expression of the two markers varied considerably among the different groups, with medians of 19.4%, 44% and 75% of FOXP3\(^+\) cells also being CD25\(^+\) in HIVprog, HIVnp and uninfected donors, respectively (Fig. 3a, b). Importantly, the percentage of FOXP3\(^+\)CD25\(^+\)
cells inversely correlated with plasma viral load in HIV-infected subjects (Fig. 3c). These results suggest that T_{reg} that may have expanded/accumulated in LTs during HIV infection down-regulate CD25 expression in vivo.

**T_{reg} accumulation in progressor hosts interferes with CMI.**

To test whether accumulation of T_{reg} in LT interferes with efficient CMI, we determined CD8^{+} T cell numbers and expression of CMI effector molecules in the same LT biopsies. CD8 numbers in HIVnp were increased compared to uninfected controls but similar to HIVprog (Fig. 4a). We also quantified the mRNA expression of CMI effector cytokine IFN-γ, and the CTL-associated molecules perforin and granzyme B. LTs from both HIVnp and HIVprog expressed more IFN-γ compared to uninfected controls (Fig. 4b). In addition, we observed a trend toward increased granzyme B and perforin expression in both HIVnp and HIVprog compared to uninfected controls (Fig. 4c, d). The same parameters were assessed in macaques. IFN-γ and granzyme B mRNA expression was ~4-5-fold higher in SIVnp and SIVprog compared to uninfected macaques (Fig. 4e, g). In contrast, significantly higher perforin mRNA levels were measured in LTs from SIVnp than SIVprog (p=0.04) (Fig. 4f). Strikingly, a high perforin/FOXP3 ratio was associated with non-progressive disease in both humans and macaques (Fig. 4h, i). Collectively, these data suggest that control of virus replication depends on preferential activation of CMI in the absence of accumulating T_{reg} within the LTs.

**HIV induces T_{reg} accumulation through HIVgp120-CD4 interactions.**

To determine whether HIV plays a direct role in T_{reg} accumulation in LTs of infected hosts, we developed an in vitro model, in which CD4^{+} T cells from uninfected donors were exposed to inactivated HIV. We used inactivated virions because less than 1% of HIV virions are demonstrably infectious; therefore, noninfectious interactions between HIV and CD4 occur at
high frequency in HIV-infected individuals \(^2,^31\). We used aldri thiol-2 (AT-2)-inactivated HIV-1, which is reverse transcription deficient, but retains the structural and functional integrity of the envelope \(^25\). Microvesicles prepared from AT-2-treated uninfected cells were used as control. FOXP3 mRNA levels were significantly increased in AT-2 HIV-exposed CD4\(^+\) T cells on d3 and d5 (Fig. 5a). Increased percentages of cells expressing FOXP3 protein were detected at the same times (Fig. 5b). Exposure to AT-2 HIV also increased expression of CTLA-4, GITR and CD25 on d3 (not shown) and d5 (Fig. 5b). Interestingly, most of the HIV-exposed FOXP3\(^+\) cells also expressed CTLA-4 (75.6\(\pm\)6.6\%) and CD25 (71.1\(\pm\)3.2\%), but not GITR (43.8\(\pm\)6.1\%), on d5, suggesting that these molecules may be differently regulated. Because all T\(_{\text{reg}}\) markers can be expressed in activated non-T\(_{\text{reg}}\), including transient expression of FOXP3 \(^32\), we determined the mRNA expression of the activation marker CD69 in AT-2 HIV-and microvesicle-exposed cells. CD69 expression was not increased on d3 or d5 (\(p=0.39\) and 0.67, respectively, Fig. 5c), suggesting that increased expression of T\(_{\text{reg}}\) markers was not due to immune activation.

We then determined whether HIV gp120-CD4 interactions were involved in increased FOXP3 expression. Soluble CD4-IgG (sCD4) almost completely abrogated HIV-mediated increases in FOXP3 expression (Fig. 5d). Accordingly, heat treatment, which promotes dissociation of gp120 from the virions \(^33\), abolished the effect of AT-2 HIV on FOXP3 expression (data not shown). No difference was observed between the X4 MN and R5 ADA strains in their ability to induce FOXP3 at d5 (fold-induction of 8.4\(\pm\)2.3 and 7.1\(\pm\)1.9 for MN and ADA, respectively, 3 subjects, \(p=0.27\), suggesting a major role for CD4-gp120 interactions. Finally, CD4\(^+\) T cells exposed to an anti-CD4 Ab that binds the same domain of the CD4 molecule as HIV gp120 exhibited higher FOXP3 mRNA expression at d3 than isotype-exposed cells (Fig. 5e), suggesting that CD4 engagement is sufficient to induce FOXP3 up-regulation.
**HIV decreases apoptosis in CD25^+ cells.**

We investigated whether HIV-mediated increased FOXP3 was due to the induction of FOXP3 in non-T_{reg} or the survival/expansion of preexisting T_{reg}. FOXP3 mRNA levels were increased in total CD4^+ T cells but not in CD25^- cells following AT-2 HIV exposure (Fig. 6a), arguing against the induction of FOXP3 in non-T_{reg}. We then determined whether increased FOXP3 expression was due to increased survival of HIV-exposed T_{reg}, by analyzing the level of apoptosis in purified T_{reg} exposed to AT-2 HIV or microvesicles. At d5, the proportion of annexin V^+ cells was ~2-fold lower in HIV-exposed T_{reg} (27.5±4.9%), compared to microvesicle-exposed T_{reg} (58.9±3.2%; p=0.03, 3 donors, Fig. 6b). These data strongly suggest that HIV exposure results in increased survival of pre-existing T_{reg}, and not in induction of FOXP3 in non-T_{reg}.

TGF-β1 has been described as a cytokine involved in T_{reg} survival/expansion. Therefore, we determined TGF-β1 mRNA levels in AT-2 HIV- and vesicle-exposed total CD4^+ T cell cultures. No increase in TGF-β1 expression was found in AT-2 HIV-exposed T cells at any of the studied time points (d1, d3 and d5 post-exposure; data not shown), suggesting that TGF-β1 induction does not account for enhanced survival of in vitro HIV-exposed T_{reg}.

**HIV-exposed T_{reg} maintain strong suppressive activity.**

HIV-exposed T_{reg} maintained strong suppressive activity, on a per cell basis, as evidenced by their capacity to inhibit PHA-stimulated effector cell division, significantly reducing the percentage of CFSE-low effector cells after PHA stimulation from 66.7±2.5% to 35.6±3.6% (p=0.05, paired t-test; Fig. 7). AT-2 HIV and microvesicle-exposed T_{reg} were equally suppressive (paired t-test; p=0.8; Fig. 7). These data suggest that HIV-exposed T_{reg} remain functional and could hamper in vivo effector immune responses.
DISCUSSION

A growing body of evidence demonstrates that T<sub>reg</sub> interfere with protective immune responses in diverse chronic infections. Our data uniquely demonstrate that immune-mediated control of viral replication is associated with low numbers of LT FOXP3<sup>+</sup>CD4<sup>+</sup> T cells, whereas uncontrolled active viral replication is associated with high numbers of these cells in LT. Of note, T<sub>reg</sub> accumulation in tissues where active infectious processes occur was also documented in experimental models of viral or parasitic infections<sup>35</sup>, as well as in human infections<sup>36-38</sup>. An important question is whether T<sub>reg</sub> accumulation in LT represents a feedback mechanism to limit immune-mediated damage, in the context of massive immune activation. In our study, the number of CD69<sup>+</sup> T cells, used as a marker reflecting the activation status of the LT, was not correlated with the number of FOXP3<sup>+</sup> cells present in the same tissues (Fig. 1). Furthermore, similar increases in IFN-γ mRNA expression were observed in LT from progressors and non-progressors, despite the fact that T<sub>reg</sub> accumulation only occurred in progressors. Therefore, the persistence of numerous T<sub>reg</sub> in LT of progressor hosts cannot solely be interpreted as a direct consequence of persistent immune activation during the chronic phase of HIV infection. Our in vitro data provide an alternative mechanism, namely that HIV-CD4 interactions can directly drive T<sub>reg</sub> accumulation through increased T<sub>reg</sub> survival, explaining why infected hosts with low viral loads exhibited low number of FOXP3<sup>+</sup> cells.

Such HIV-mediated effect on T<sub>reg</sub> is likely to involve HIV gp120, as sCD4 blocked FOXP3 induction. Furthermore, engagement of CD4 appears sufficient to drive the process. HIV gp120 is present at high concentrations in LT<sup>39,40</sup> and circulates in the blood of HIV-infected donors on virions and as a free protein<sup>41</sup>. Although the actual concentrations of gp120 in LT are essentially unknown, they are predicted to be several log higher than in plasma, thus justifying the dose of AT-2 HIV used in this study, which is higher than the
reported gp120 plasma levels. Of note, both the X4 HIV
MN and the R5 HIV ADA induced increases in FOXP3 expression. Therefore, the mechanisms described here are not likely to be limited by coreceptor usage.

Our data strongly suggest the hypothesis that CD4-gp120 interactions mediate increased survival of Treg, and not conversion of non-Treg into Treg, based on two findings: depletion of CD4+CD25+ T cells before exposure to HIV abolished increased FOXP3 expression; and decreased percentage of apoptotic cells was found in HIV-exposed Treg compared to control Treg (Fig. 6). The mechanisms underlying such increased survival are not identified but could implicate gp120-mediated induction of the MAP kinase Erk, which in turn would lead to Bad phosphorylation on Ser112. Interestingly, it was recently shown that, in the presence of IL-2, human Treg survive better than effector cells, this effect being mediated by induction of Bcl-X(L) expression and Bad phosphorylation. This finding suggests a potential synergic effect of IL-2 and HIV in our in vitro model. TGF-β1 has been described as promoting Treg survival/expansion in vivo, as well as the conversion of non-Treg into Treg in vitro. However, the fact that no significant induction of TGF-β1 was detected in in vitro HIV-exposed CD4+ T cells argues against TGF-β1 as an important survival mechanism in our model.

Importantly, our data show that the functionality of HIV-exposed Treg was not altered, on a per cell basis, in agreement with the fact that circulating Treg purified from most HIV-infected patients are functional. The only qualitative difference between Treg from progressor, non-progressor and uninfected individuals was the low co-expression of CD25 by FOXP3+ cells in the progressors. Such data are reminiscent of murine models, in which in vivo expansion of Treg led to functional Treg expressing low levels of CD25, but high levels of CTLA-4 and FoxP3. Taken together, our data suggest that the major difference between progressor and non-progressor individuals is quantitative (i.e. the number of Treg), and not
In contrast to our results, increased cell death of *in vitro* HIV-infected T\(_{\text{reg}}\), compared to non-T\(_{\text{reg}}\) (4 vs. 1.5% d3 post-infection) was reported\(^5\). However, the fact that high numbers of T\(_{\text{reg}}\) in LT were still found 2 weeks after peak SIV infection argues against massive killing of T\(_{\text{reg}}\) by virus infection\(^2\). Furthermore, the recent report of T\(_{\text{reg}}\) accumulation in the gut of untreated HIV-infected patients\(^5\) also argues against increased killing of T\(_{\text{reg}}\) in compartments in which viral replication is raging. In addition to the direct survival signals given through HIVgp120-CD4 interactions, TGF-β1 could also promote T\(_{\text{reg}}\) survival *in vivo*\(^5\). Interestingly, we found increased levels of TGF-β1 in LT from HIVprog compared to HIVnp and uninfected individuals, although the cellular source of such elevated TGF-β was not determined and could be either HIV-infected cells or multiple uninfected cell types activated by the associated inflammatory response\(^2,5\). In contrast, it is difficult to predict the effect of infectious virus on T\(_{\text{reg}}\). FOXP3 over-expression in HEK293T and purified CD4\(^+\) T cells results in suppression of HIV-1 promoter transcription\(^5\). It is therefore possible that T\(_{\text{reg}}\) exposed to infectious HIV undergo limited HIV-1 transcription, which would protect them from HIV-mediated cell death *in vivo*. More work is clearly needed to address the role of different viral factors in the dynamics of T\(_{\text{reg}}\) in HIV infection.

As markers associated with T\(_{\text{reg}}\) activity can be up-regulated briefly during the activation of non-T\(_{\text{reg}}\), it is possible that *in vitro* and *in vivo* up-regulation of FOXP3, GITR and CTLA-4 may also derive from the presence of activated non-T\(_{\text{reg}}\). However, our current and previous results do not favor this possibility because FOXP3\(^+\) cells in the tonsils of HIV-infected individuals did not express CD69\(^2\) and increased *in vitro* expression of all those markers was not accompanied with concomitant expression of CD69 (Fig. 5) arguing against increased FOXP3 and CTLA-4 *in vivo* and *in vitro* as markers of non-T\(_{\text{reg}}\) activation. Furthermore, it was recently reported that although FOXP3 expression is increased in non-T\(_{\text{reg}}\) following anti-
CD3/CD28 activation, such induction is temporary, peaking at 48h\(^5\), which makes it concomitant to CD69 up-regulation. It should also be noted that several studies have not found increased FOXP3 expression in activated human CD4\(^+\)CD25\(^-\) cells\(^36,37,48\).

A key question that remains unanswered is how T\(_{\text{reg}}\) mediate immune suppression during HIV infection. CTLA-4 is expressed by a majority of T\(_{\text{reg}}\) in HIV-infected individuals, and has been implicated in the mediation of their suppressive activity. T\(_{\text{reg}}\) may act directly on effector T cells through binding of CTLA-4 to B7 molecules on activated effectors; alternatively, CTLA-4\(^+\) T\(_{\text{reg}}\) induce increased expression of the tryptophan-catabolizing enzyme IDO in APCs\(^15\). In agreement with an involvement of the latter pathway, higher IDO expression was evident in progressors compared to non-progressors (Fig. 2). The central role of T\(_{\text{reg}}\)-APC interactions in T\(_{\text{reg}}\) function is supported by the fact that in vivo persistent T\(_{\text{reg}}\)-APC interactions precede the inhibition of effector cells and, conversely, no direct T\(_{\text{reg}}\)-effector cell contacts are visualized\(^5\). In contrast, T\(_{\text{reg}}\)-mediated suppression also occurs in APC-free conditions\(^5\), indicating that T\(_{\text{reg}}\) function may extend beyond APCs. FOXP3\(^+\) T\(_{\text{reg}}\) themselves express high levels of IDO during acute SIV infection\(^21\). However, our results do not support this hypothesis in chronic HIV infection, since most IDO\(^+\) cells in LT also expressed APC markers\(^20\). Although the suppressive activity of circulating T\(_{\text{reg}}\) from HIV-infected patients was reported to be independent of TGF-\(\beta\)1 or IL-10\(^17,18\), those cytokines may play a role in vivo, as shown in several murine models\(^52,58,59\) and in human malaria\(^60\). TGF-\(\beta\)1 was overexpressed in HIVprog group, indicating a potential role for TGF-\(\beta\)1 suppressive effect. In contrast, IL-10 levels were comparable in progressors and non-progressors.

Similar expansion of CD8\(^+\) lymphocytes and increased IFN-\(\gamma\) expression were found in the LT of progressors and non-progressors. In contrast, significantly increased perforin expression was found only in LT of SIVnp. These results are in agreement with a previous
study, showing that HIV-specific CD8+ T cells from non-progressors, but not from progressors, produce perforin upon restimulation. However, the same difference was not apparent in the studied HIV-infected individuals. This may be due to the fact that most HIVnp included in our study exhibited persistently undetectable viremia (<50 copies/ml) whereas the threshold of detection of SIV viremia was higher (<2,000 copies/ml). Supporting that argument, the individual with the highest perforin expression (~10-fold up-regulation compared to median expression in uninfected controls) was a HIVnp with detectable viral load at the time of the biopsy. Strikingly, the ratio of expression of perforin to FOXP3 was clearly higher in non-progressors than in progressors. Since T_{reg} interfere with the generation of perforin-expressing CTL in both murine Friend virus and human hepatitis C infection, accumulation of T_{reg} in LT could constitute an underlying mechanism for inefficient CD8 function in progressor hosts, and consequently, a mechanism involved in progressive disease. In line with this finding, a high intra-tumor CD8/T_{reg} ratio is a strong predictor for favorable prognosis in ovarian cancer patients. Decreased perforin expression in HIVprog was reported to be more specific of HIV-specific CD8+ T cells, compared to CMV-specific cells, but not to EBV-specific specific cells. These data argue against a T_{reg}-mediated effect, since T_{reg} have been described to act in an antigen-independent manner. However, in vitro depletion of T_{reg} from the PBMC of HIV-infected patients showed a more pronounced effect on IFN-γ expression by HIV-specific CD8+ T cells, compared to that of CMV-specific cells. These data suggest the existence of subtle differences in the susceptibility of different CD8+ subsets and/or of different CD8-mediated functions, to T_{reg} activity, which needs to be carefully examined.

It should be noted that our data do not provide an answer to the important question of whether low numbers of T_{reg} in the lymphoid organs of non-progressor hosts are directly implicated in the differential outcome of infection, or are a consequence of low levels of viral
replication. Addressing such question will necessitate a prospective longitudinal study of the numbers of T_{reg} in lymphoid organs of infected hosts, as well as in vivo studies in which the number/function of T_{reg} are manipulated at different stages of infection. Also of note, although our in situ analyses reveal clear differences in the level of expression of T_{reg} markers in relation to HIV disease, they do not constitute a definitive demonstration of the suppressive role of these cells in vivo. This will require detailed functional analyses of LT T_{reg} from patients at different stages of HIV infection.

In summary, our data suggest a model in which interactions with HIV drive the accumulation of functional T_{reg} in LT. In turn, increased numbers of these cells negatively affects the immune control of virus replication, providing an underlying mechanism for the relationship between T_{reg} numbers and disease prognosis. These findings may have potential for the development of novel therapeutic approaches aimed at enhancing immune function in HIV-infected patients through the manipulation of T_{reg} numbers and/or function.
ACKNOWLEDGEMENTS

The authors thank Dr. J.D. Lifson and J. Bess for providing AT-2 HIV preparations, and Dr. L. Wahl for providing purified cell populations from HIV-uninfected donors. We also thank Drs. C.L. Karp and H.G. Ljunggren for their critical reading of this manuscript. We also thank all the patients involved in this study. We are also grateful to Drs. A. Sönnerborg and C. Broström (Infectious Disease Clinic, Karolinska University Hospital) for their involvement in the recruitment and follow-up of the included patients.
REFERENCES

18. Kinter A, Hennessey M, Bell A, et al. CD25+CD4+ regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4+ and CD8+ HIV-
42. Klasse PJ, Moore JP. Is there enough gp120 in the body fluids of HIV-1-infected individuals to have biologically significant effects? Virology. 2004;323:1-8.
Table 1: Clinical characteristics of included subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Age</th>
<th>CD4(^1)</th>
<th>VL(^2)</th>
<th>Treatment</th>
<th>Stage(^3)</th>
<th>Duration(^4)</th>
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<td>M</td>
<td>35</td>
<td>470</td>
<td>1,800</td>
<td>None</td>
<td>A1</td>
<td>&gt;2 years</td>
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<td>28</td>
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</tbody>
</table>

\(^1\) CD4 counts, expressed in cells/mm\(^3\).

\(^2\) Plasma viral load (VL), in copies/ml.

\(^3\) Disease stage, according to CDC classification. In addition, all biopsies from the HIVprog, but none from the HIVnp, were graded as follicular hyperplasia, which is prognostic of disease progression\(^23\).

\(^4\) Documented duration of HIV-1 infection, defined as years of known seropositivity.
Although his CD4 counts was relatively high and his VL was low at the time of biopsy, HIVprog 1 did not meet the definition of the non-progressors i.e. stable CD4 counts and low VL for a minimum of 5 years. Moreover, subsequent follow-up of HIVprog 1 has shown a pattern towards progression, with a steady VL increase (7,600 and 22,000 copies/ml at 3 and 5.5 years post-biopsy, respectively).
Table 2: Extended clinical follow-up of HIVnp.

<table>
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<th>Subjects</th>
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<th>Biopsy</th>
<th>Post-biopsy</th>
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<td>(2 yrs)(^3)</td>
<td>(2 yrs)</td>
<td>(1 yr)(^3)</td>
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<td>510</td>
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<td>(3 yrs)</td>
<td>(3 yrs)</td>
<td>(3 yrs)</td>
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<td>760</td>
</tr>
<tr>
<td></td>
<td>(1 yr)</td>
<td>(1 yr)</td>
<td>(4 yrs)</td>
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<tr>
<td>HIVnp 4</td>
<td>600</td>
<td>200</td>
<td>920</td>
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<tr>
<td></td>
<td>(2 yrs)</td>
<td>(2 yrs)</td>
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<td>HIVnp 5</td>
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<td>620</td>
</tr>
<tr>
<td></td>
<td>(2 yrs)</td>
<td></td>
<td>(5 yrs)</td>
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</table>

\(^1\) CD4 counts, in cells/mm\(^3\).

\(^2\) Plasma viral load (VL), in copies/ml.

\(^3\) Number of years prior- or post-biopsy when data were collected.

\(^4\) N/A: not available. This individual was put on HAART shortly after the biopsy was obtained, because she became pregnant. She did not have further follow-up.
Table 3: Viral loads at euthanasia.

<table>
<thead>
<tr>
<th>Animal#</th>
<th>VL ¹</th>
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<tr>
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<tr>
<td>SIVnp</td>
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</tr>
<tr>
<td>883</td>
<td>&lt;2,000</td>
<td></td>
</tr>
<tr>
<td>884</td>
<td>&lt;2,000</td>
<td></td>
</tr>
<tr>
<td>207</td>
<td>&lt;2,000</td>
<td></td>
</tr>
<tr>
<td>916</td>
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<td></td>
</tr>
<tr>
<td>299</td>
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<tr>
<td>878</td>
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</tr>
<tr>
<td>879</td>
<td>&lt;2,000</td>
<td></td>
</tr>
<tr>
<td>SIVprog</td>
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<td>911</td>
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<tr>
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<tr>
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<tr>
<td>173</td>
<td>380,647 ²</td>
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<tr>
<td>909</td>
<td>3,603</td>
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</tbody>
</table>

¹ VL expressed in copies/ml

² VL measured 12 weeks before euthanasia
FIGURE LEGENDS

Figure 1: Low expression of FOXP3 in LT from HIVnp and SIVnp. (a,b,c)
Photomicrograph of FOXP3 protein expressing cells (brown) in LT from a representative HIVprog (n=6) (a), HIVnp (n=5) (b) and uninfected (n=5) (c). Cell nuclei were counterstained in blue, the micron bar in bottom right corner indicates 20µm. (d) **FOXP3 mRNA expression was significantly decreased in LT of HIVnp.** Results are expressed in RU after normalization on CD4 mRNA. Similar results were obtained when FOXP3 was normalized on GAPDH expression (p=0.004, not shown). Horizontal bars within boxes correspond to the median; box limits correspond to the 25th and 75th percentiles; vertical lines indicate range. (e) **Median FOXP3 mRNA expression was decreased in LT from 7 SIVnp, compared to that in 7 SIVprog.** Results are expressed in RU after normalization on CD4 mRNA. (f) **Percentages of CD69+ and FoxP3+ cells were not correlated in LT of HIV-infected individuals** (r= 0.012, p=0.98; Spearman correlation).

Figure 2: Low expression of mediators associated with Treg function in LT of HIVnp and SIVnp. mRNA levels in tissues from human individuals (a-d) and macaques (e-h) are expressed in RU after normalization on GAPDH mRNA.

Figure 3: Plasma viral load is negatively correlated with expression of CD25 by FOXP3+ T cells. (a,b) High magnification confocal micrograph of a FOXP3 (green) and CD25 (red) double positive cell (a) and a FOXP3 (green) single positive cell (b). Microbar in bottom right corner indicates 10µm. (c) Plasma viral load was negatively correlated to the expression of CD25 by FOXP3+ T cells (r= -0.96, p=0.003; Spearman correlation). This characterization was done in 7 HIV-infected patients across a wide span of viral loads (5 HIVprog and 2 HIVnp were included in the analysis).
**Figure 4: T_{reg} accumulation in progressor hosts interferes with CMI.** (a) Significant increase of percentages of CD8^{+} T cells in LT of 6 HIVprog and 5 HIVnp compared to 5 uninfected controls (b-d) Expression of IFN-γ (b), perforin (c), and granzyme B (d) mRNA. Results are expressed in RU after normalization on GAPDH mRNA. Phenotyping of LT perforin and granzyme B-expressing cells confirmed that they were CD3^{+} and CD8^{+} (>95%; data not shown). (e-g) Expression of IFN-γ (e), perforin (f), and granzyme B (g) mRNA in LT of SIVprog, SIVnp and uninfected animals. Results are expressed in RU after normalization on GAPDH mRNA. (h, i) Increased ratio of perforin to FOXP3 mRNA expression in non-progressors compared with progressors (Mann-Whitney test).

**Figure 5: HIV directly induces accumulation of functional T_{reg} through gp120-CD4 interactions.** (a) Exposure of CD4^{+} T cells to AT-2 HIV increases FOXP3 mRNA expression. CD4^{+} T cells were cultured in presence of either AT-2 HIV_{MN} or control microvesicles (ves). FOXP3 mRNA RU were calculated after normalization on CD4 mRNA levels. CD4 mRNA levels were not affected by AT-2 HIV exposure (results not shown). Results are expressed as the mean ± SE of data obtained in 7 donors. Asterisks indicate a significant difference by paired t-test (*: p < 0.05, **: p < 0.005). (b) Increased expression of T_{reg} markers in AT-2 HIV-exposed T cells. Percentage of cells expressing each marker was determined by FACS. Results represent the mean ± SE of data obtained in 4 donors. Similar results were obtained at d3 (not shown). (c) AT-2 HIV exposure does not induce CD69 up-regulation. Results represent the mean ± SE of data obtained in 4 donors. (d) sCD4 abrogates AT-2 HIV-mediated FOXP3 increased expression. Results represent the mean ± SE of data obtained in 5 donors at d5. (e) CD4 engagement by anti-CD4 Ab induces
increased FOXP3 mRNA expression. Results represent the mean ± SE of data obtained in 3 donors.

Figure 6: (a) Depletion of CD25+ T cells before HIV exposure abrogates FOXP3 induction. CD4+ T cells from 4 individuals were either left unseparated (total) or depleted of CD25+ cells (depl) by negative selection, and were cultured in presence of AT-2 HIV or microvesicles. FOXP3 mRNA expression was determined at d5. Similar results were obtained at d3 (not shown). (f) Exposure of CD4+CD25+ T cells to AT-2 HIV decreases the number of apoptotic cells. Purified CD4+CD25+ T cells were cultured in presence of AT-2 HIV, or microvesicles for 5 days. Percentages of annexin V+ cells (determined in comparison to unstained controls) in one donor, representative of 3 donors, are shown.

Figure 7: HIV-exposed Treg maintain strong suppressive activity. Purified CD4+CD25+ T cells were cultured in presence of AT-2 HIV, or microvesicles. At d4, cells were mixed with CFSE-labeled autologous CD4+CD25- T cells, and stimulated with PHA for 3 days in presence of APC. Effector T cells were also cultured with PHA and APC (“PHA”), and with APC alone (“unstim”). Numbers indicate the percentages of effector cells that had undergone at least one division cycle. Results from one donor, representative of 3, are shown.
HIV-1 driven regulatory T cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS

Jakob Nilsson, Adriano Boasso, Paula A Velilla, Rui Zhang, Monica Vaccari, Genoveffa Franchini, Gene M Shearer, Jan Andersson and Claire Chougnet