Interferon-α differentially rescues CD4 and CD8 T cells from apoptosis in HIV infection

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Objective: To examine the effects of interferon-α (IFN-α) on T cell survival and activation in HIV infection.

Design: The effects of IFN-α on spontaneous apoptosis and CD38 expression among T cell subsets were determined *in vitro* and studied in relation to CD4 cell counts, plasma HIV RNA levels and the age of the subjects.

Methods: Peripheral blood mononuclear cells from 48 HIV-infected persons and 17 healthy donors were incubated *in vitro* overnight with or without the addition of IFN-α. Percentages of apoptotic cells (positive for annexin V) and CD38 cells were determined among T cell subsets.

Results: IFN-α inhibited spontaneous apoptosis of CD4 and CD8 T lymphocytes. This protective activity was impaired in CD4 T cells from HIV-infected persons. The reduced protection of IFN-α among CD4 cells from HIV-infected persons was not related to the percentages of activated (CD38 or CD45RO+CD38+) cells. Surprisingly, IFN-α induced CD38 expression among CD8 T cells from HIV-infected persons, and the magnitude of this effect was directly related to circulating CD4 T cell count. The CD8 T cell subset that expressed CD38 in response to IFN-α was defined as CD28 negative, CD62 ligand (CD62L) intermediate/negative.

Conclusions: Heightened expression of IFN-α in HIV infection may contribute to the phenotypic activation state that characterizes chronic infection while a diminished responsiveness of CD4 T cells to the protective effect of this cytokine may contribute to differential survival of CD4 and CD8 T cells in HIV disease.

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AIDS 2006, **20**:1379–1389

Keywords: CD38, T cells, interferon-α, apoptosis, HIV

Introduction

Persistent HIV infection results in the increased production of cytokines that regulate innate and adaptive immunity. Interferon-α (IFN-α) is one such cytokine detected at increased concentrations in serum of HIV-infected individuals [1–4]. This cytokine mediates many important biological activities including protection from viral infection [5–9], maturation of antigen-presenting cells [10–12], and regulation of proliferation and viability of T lymphocytes [13–18]. Despite its recognized importance in immune protection during acute infections, the role of IFN-α in chronic infections may be more complex, potentially even leading to detrimental effects associated with ongoing immune activation. The potential beneficial or detrimental consequences of continuous IFN-α production in HIV disease are unclear.

One aspect of IFN-α activity that may be especially important in HIV infection is the ability of this cytokine to regulate T cell survival. In experimental models, the effects of IFN-α on T cell viability appear to be complex. For example, IFN-α and IFN-β protect T cells from apoptosis in the setting of cytokine-deprivation [15,16,19].

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Received: 17 November 2005; accepted: 3 April 2006.

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Moreover, IFN-α protects naive T cells from apoptosis early after T cell receptor activation [18]. Nevertheless, IFN-α augments the expression of molecules involved in death receptor-mediated apoptosis in peripheral blood mononuclear cells (PBMC) from HIV-infected persons [20] and may actually increase apoptosis susceptibility in naive T cells that have progressed beyond the early phases of activation [18]. Thus, the effects of IFN-α on T cell viability will likely depend on the microenvironment and activation status of the cells.

It is possible that the effects of IFN-α on T cell viability and function may be different in HIV infection and in health because of the perturbations in T cell phenotypes that occur in HIV infection; these would include the increased proportions of circulating effector/memory cells [21–23], T cells with an activated phenotype [24–28], and T cells actively in the cell cycle [29–34]. Additionally, a finer characterization of the effects of IFN-α according to responder cell phenotype may help to clarify current uncertainties regarding the roles of IFN-α in T cell homeostasis and in HIV disease pathogenesis.

Spontaneous T cell apoptosis is elevated in HIV disease [35–37] but can be partially inhibited by the addition of cytokines such as interleukin-2 [36] or interleukin-15 [35]. IFN-α also can protect T cells from spontaneous apoptosis; however, these effects are diminished in HIV disease. Curiously, IFN-α induces CD38 expression, a marker of activation and disease progression in HIV disease [26,27,38], especially among CD8+CD28−CD62L−/intermediate T cells from HIV-infected persons. The CD8 responses to IFN-α (both induction of CD38 and rescue from spontaneous apoptosis) decline with disease progression. It is proposed that IFN-α may have some capacity to preserve T cell viability, but a decreased responsiveness to this cytokine perturbs this regulatory mechanism in HIV infection. The present study explores the effects of IFN-α on spontaneous apoptosis of T cells obtained from the peripheral blood of HIV-infected patients.

Methods

Cell culture and flow cytometry

Whole blood from 48 HIV-infected persons and 17 healthy adult controls was collected in heparin-coated tubes. Isolated PBMC were resuspended in RPMI 1640 (BioWhittaker, Walkersville, Maryland, USA) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mmol/l L-glutamine (BioWhittaker) and 10% fetal bovine serum (Sigma, St. Louis, Missouri, USA). PBMC were seeded at $1-2 \times 10^6$ cells/well in 24-well plates, incubated overnight in culture medium with or without recombinant IFN-α2a (rIFN-α; PBL, Piscacaway, New Jersey, USA), and examined by flow cytometry for coexpression of CD4, CD8, CD38 and surface phosphatidylserine (using the annexin V kit; BD Pharmingen, San Diego, California, USA). Most analyses included staining with anti-CD4–peridinin–chlorophyll *a* complex protein, anti-CD8–allophycocyanin (APC), anti-CD38–fluorescein isothiocyanate (FITC) and annexin V–phycoerythrin (PE). Some studies required anti-CD28–FITC, anti-CD62L–APC, anti-CD45RO–PE–cyanin 5 or anti-CD38–biotin with streptavidin-APC-cyanin 7 as a secondary reagent. Fluorochrome-matched mouse IgG_1 or mouse IgG_{2a} isotypes were used to establish background staining. Annexin V background was determined with unstained cells. Antibodies were purchased from BD Pharmingen.

The percentage apoptosis inhibition was calculated as 100 × [apoptotic cells in medium alone (%) − apoptotic cells in IFN-α-treated cultures (%)]/apoptosis in cells incubated in medium alone (%).

Subjects

The HIV-positive group were aged 21–62 years (median, 42); 30% were females and 66% were taking antiretroviral therapy. Plasma HIV RNA levels were > 400 copies/ml in 70% (median, 5667; range, 50–750 000). CD4 cell count ranged from 7 to 1416 cells/μl (median, 464). The healthy control donor group were aged 28–63 years (median, 42) and 59% were females. These studies were approved by institutional review committees.

Statistical methods

Differences across groups were determined by Student's *t*-tests, Mann–Whitney's *U*-test or Kruskal–Wallis' test, according to the number of groups and distribution of the data. The relationships between marker expression and continuous plasma HIV RNA levels, age or CD4 cell counts were determined by Spearman's rank order correlation. To account for the effect of other independent variables, partial correlation coefficients were used as an initial exploratory step; findings were tested further by multiple linear regression as needed.

Analyses were done with SPSS for Windows, version 11.0 (SPSS, Chicago, Illinois, USA) and Intercooled Stata, version 6.0 (Stata Corp., College Station, Texas, USA). Curve fitting was done with SigmaPlot, version 5.0 (Systat Software, Richmond, California, USA). All significance tests were two-tailed.

Results

Interferon-α protects T cells from spontaneous apoptosis

To examine the effect of IFN-α on T cell viability, PBMC from HIV-infected persons or from healthy donors were incubated with or without IFN-α in overnight cultures. The percentages of T cells that had undergone apoptosis were determined by annexin V staining. Initially,

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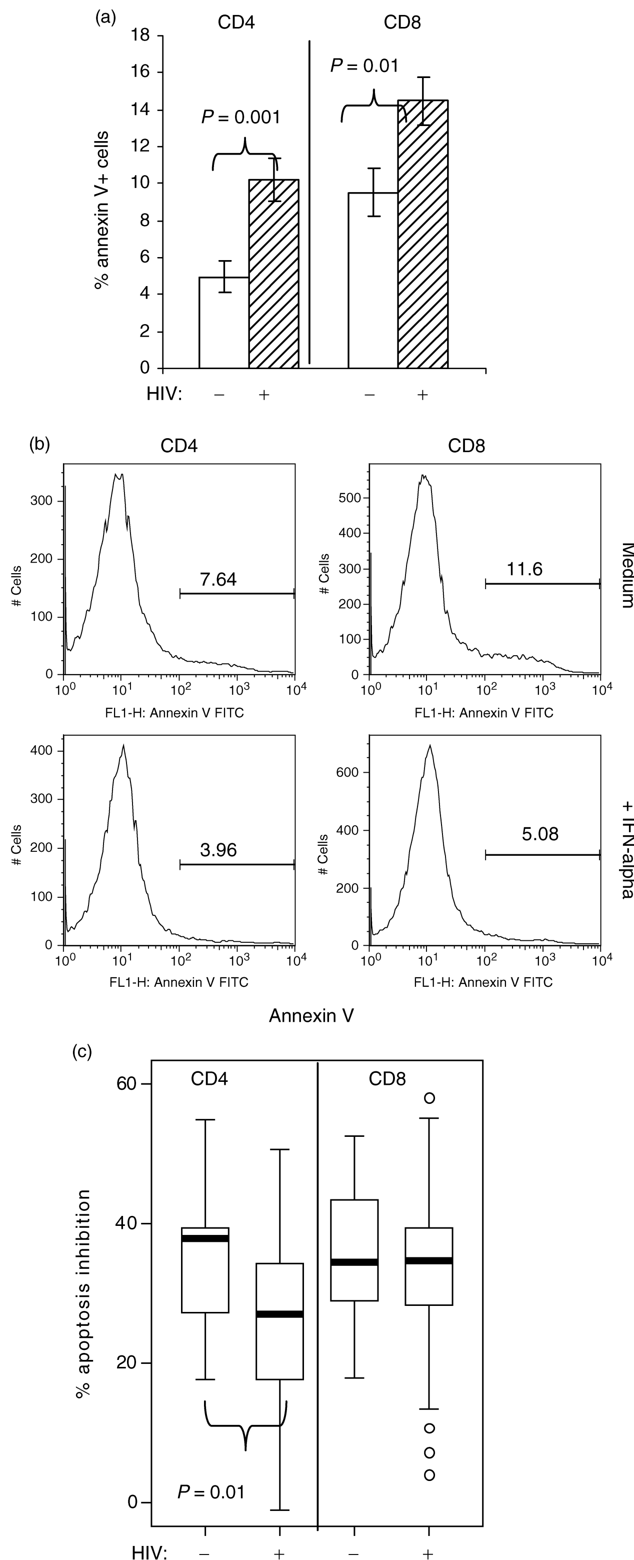

Fig. 1. Interferon-α (IFN-α) protects T cells from spontaneous apoptosis. Peripheral blood mononuclear cells were incubated overnight in medium alone or medium supplemented with 1000 U/ml IFN-α. Cells were examined the following day for expression of CD4, CD8 and phosphatidylserine. (a) Mean percentages of CD4 and CD8 T cells that were positive for annexin V (annexin V+) after overnight culture in medium alone (from 49 HIV-infected persons and 13 healthy donors). (b) Representative histograms showing the percentage of CD4 and CD8 T cells that were annexin V-positive in cell cultures from an HIV-infected person. (c) Boxplots depicting the percentage apoptosis inhibition that was mediated by IFN-α in overnight cultures. Comparison between patient and control CD4 or CD8 T cells used a two-tailed Student's *t*-test.

the analyses were restricted to lymphocytes with size (forward scatter) and granularity (side scatter) consistent with live cells. As expected, the percentages of CD4 and CD8 T cells that expressed phosphatidylserine (annexin V positive) were increased in PBMC from HIV-infected individuals (Fig. 1a). The addition of rIFN-α (1000 U/ml) reduced the levels of apoptosis in both T cell populations (Fig. 1b,c), although the degree of protection was diminished significantly among CD4 T cells derived from the HIV-infected individuals compared with that in healthy donors (Fig. 1c). Experiments with rIFN-α at one-tenth the dose (100 U/ml) also provided protection from apoptosis in cells from eight HIV-positive donors (mean percentage apoptosis inhibition with 1000 and 100 U/ml rIFN-α was 35.1 and 27.3%, respectively, for CD8 cells and 30.0 and 26.5%, respectively, for CD4 cells). Experiments with 10 U/ml ($n = 2$) or with 5 U/ml ($n = 3$) rIFN-α suggested that detectable protection from apoptosis was generally lost at concentrations < 10 U/ml (not shown).

To confirm that these results were not a consequence of excluding late apoptotic cells from the analyses, the samples were re-gated to include cells with reduced forward- and side-scatter morphology (late apoptotic cells). The conclusions were unchanged by these additional analyses (data not shown).

The specificity of the anti-apoptotic activity of IFN-α was confirmed in PBMC from two donor samples by adding anti-IFN-α/β receptor antibody (20 μg/ml) or an isotype control antibody to cells treated with IFN-α. IFN-α inhibited apoptosis by 32% and 53% for CD4 cells and by 43% and 50% for CD8 cells. This protection from apoptosis was reduced to 17% and 1% for CD4 cells and 28% and 0% for CD8 cells, respectively, when anti-IFN-α/β receptor antibody was added to the two donor cultures. Isotype control antibody had little or no effect (not shown).

Apoptosis of CD4 T cells is related to circulating CD4 cell counts independently of plasma HIV RNA levels

The relationships of apoptosis and apoptosis inhibition by IFN-α to clinical indices of age, CD4 cell counts and plasma HIV RNA levels was evaluated. For CD8 T cells,

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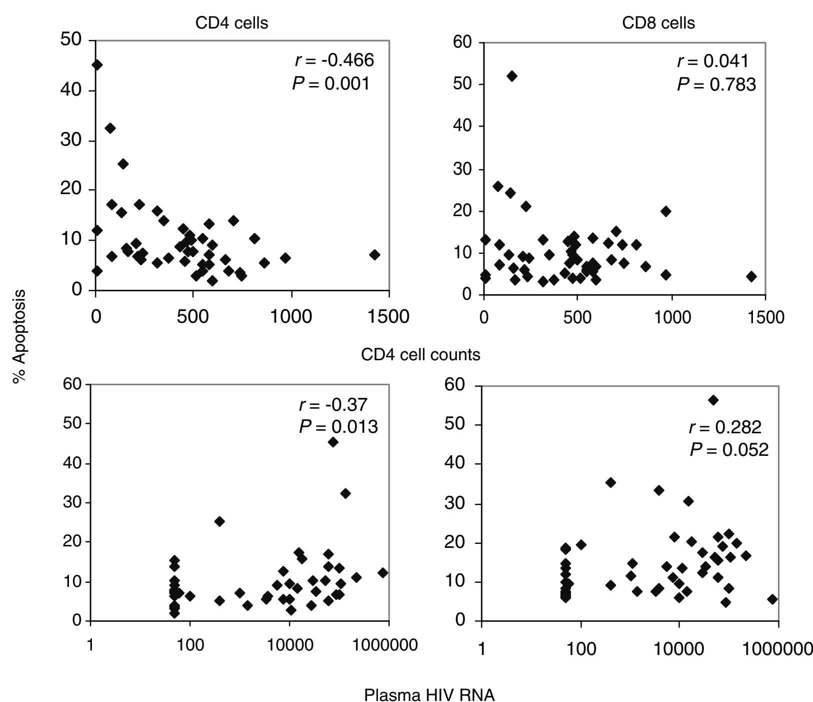

Fig. 2. Relationship between T cell apoptosis (percentages of annexin V-positive CD4 or CD8 cells) and CD4 T cell counts or plasma HIV RNA levels. Peripheral blood mononuclear cells were analyzed after overnight incubation. Spearman's correlation coefficients (*r*) are indicated along with *P* values for significance.

spontaneous apoptosis exhibited a trend towards a direct relationship with plasma HIV RNA levels while the percentages of apoptotic CD4 T cells were inversely correlated with circulating CD4 T cell counts and directly related to plasma HIV RNA levels (Fig. 2). The relationships between clinical indices and spontaneous CD4 T cell apoptosis were scrutinized further by multivariable analyses (partial correlations and linear regression models). Both methods indicated that CD4 cell counts, but not plasma HIV RNA levels, independently predicted the magnitude of spontaneous CD4 T cell apoptosis ($P = 0.007$), and the observation held whether CD4 cell count was considered as a continuous or as a categorical (by strata) variable. In contrast, the magnitude of annexin V binding on CD8 cells was only marginally and non-significantly associated with CD4 cell count or plasma HIV RNA in these multivariate analyses. These results suggest that different mechanisms may underlie the enhanced tendency for CD4 and CD8 T cells to undergo apoptosis in HIV infection and there could potentially be a connection between disease stage and the susceptibility of CD4 T cells to this mechanism of cell death.

There was no relationship between age, plasma HIV RNA levels or CD4 cell counts and the magnitude of apoptosis inhibition mediated by IFN-α among CD4 T cells ($P = 0.58$, 0.13 and 0.17, respectively). In contrast, the ability of IFN-α to inhibit spontaneous apoptosis of CD8 T cells was directly related to circulating CD4 cell counts ($r = 0.376$; $P = 0.008$). Furthermore, multivariable analyses indicated that both CD4 cell count and plasma HIV RNA levels were independently associated with the ability of IFN-α to inhibit apoptosis of CD8 T cells, although the association was marginal for HIV RNA ($P = 0.017$ and 0.08, respectively). Thus, IFN-α-mediated inhibition of CD8 T cell apoptosis may also be impaired in HIV-infected persons with more severe disease.

Spontaneous apoptosis is increased among activated T cell subsets

It was possible that subsets of activated T cells might be affected differently by exposure to IFN-α and, therefore, annexin V binding in T cell subsets differentiated by expression of an activation marker, CD38, was compared. Levels of spontaneous apoptosis were increased among CD8+CD38+ but not CD4+CD38+ cells compared with their CD38-negative counterparts in samples from HIV-infected persons and healthy donors (Fig. 3a).

While among memory CD4 T cells, CD38 may be a marker of activation, it may be expressed constitutively in the naive population [39,40]. Therefore, spontaneous apoptosis of CD4 T cells obtained from nine additional HIV-positive donors [plasma HIV RNA 50–750 000 copies/ml (median, 3625); CD4 cell count 217–753 cells/μl (median, 406)] was examined, including assessment of the T cell memory marker CD45RO and measurement of CD38 expression. During overnight incubation, apoptosis occurred predominantly within

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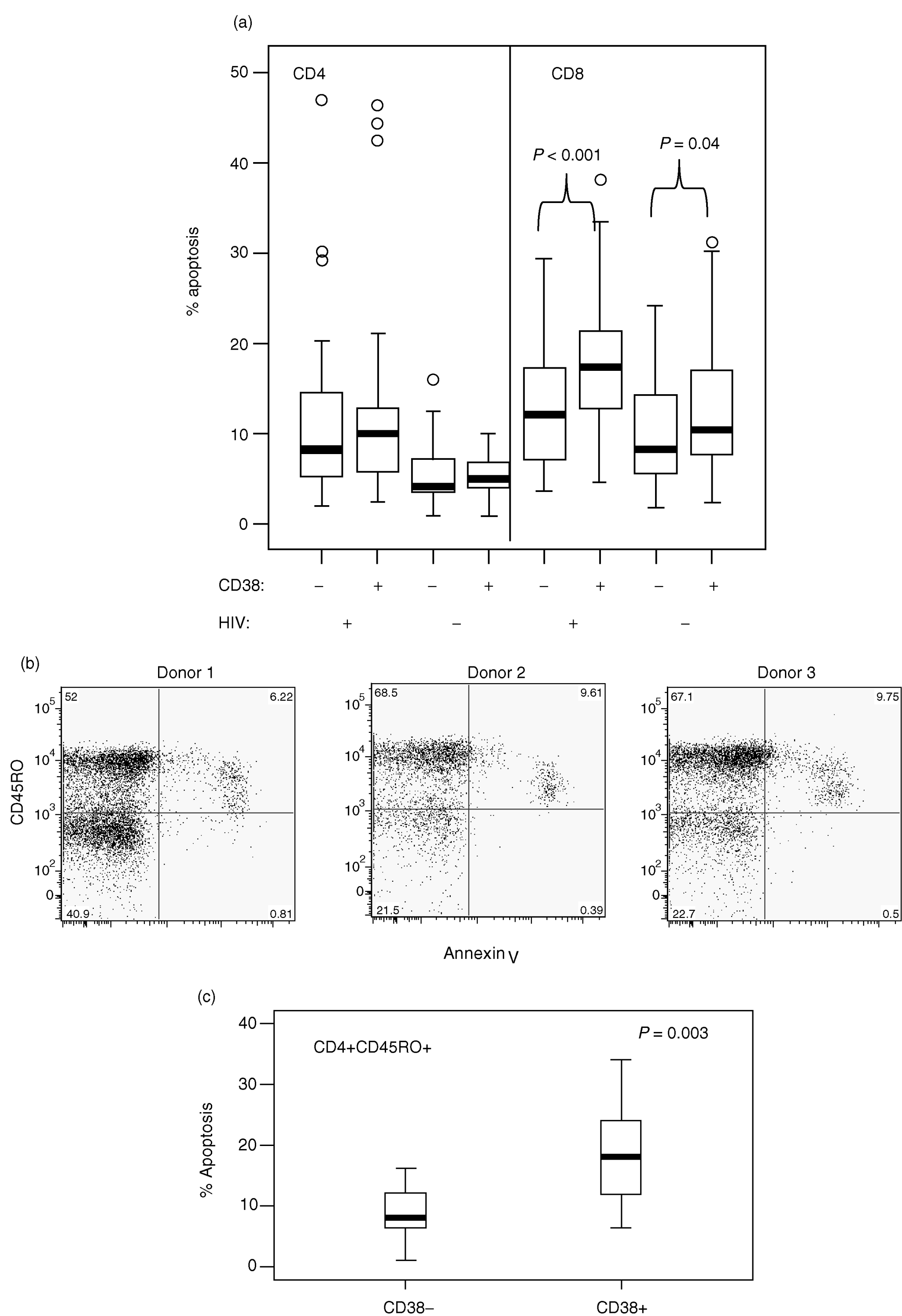

Fig. 3. Increased spontaneous apoptosis among T cell subsets. (a) Peripheral blood mononuclear cells (PBMC) were incubated overnight in medium alone and stained the following day for expression of CD4, CD8, CD38 and surface phosphatidylserine. Percentages of annexin V-positive T cells are shown for HIV-positive and HIV-negative donors. Comparisons between percentages of annexin V-positive cells in CD38-positive and CD38-negative subsets were made using paired Student's *t*-test. (b) PBMC from three HIV-infected donors were incubated overnight and examined the next day for coexpression of CD4, CD45RO, CD38 and surface phosphatidylserine. Dotplots indicate the coexpression of CD45RO and phosphatidylserine (annexin staining) on cells gated for lymphocyte scatter characteristics and CD4 expression. Similar observations were made in an additional six subjects. (c) Percentages of apoptotic cells were measured by annexin V staining in CD38-positive and CD38-negative cells that were gated for lymphocyte scatter characteristics and coexpression of CD4 and CD45RO. P value was derived by paired t-test ($n = 9$).

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CD4 T cells expressing CD45RO (Fig. 3b) and CD4+CD45RO+CD38+ cells were more likely to die after overnight incubation than were CD4+CD45RO+CD38− cells (Fig. 3c). Therefore, activated memory CD4 T cells from HIV-positive donors were especially susceptible to spontaneous cell death *in vitro*.

Presence of CD38 or CD45RO does not predict the ability of IFN-α to rescue CD4 memory T cells from apoptosis

IFN-α was more effective in inhibiting apoptosis of CD4+CD38− cells than of CD4+CD38+ cells (mean percentage inhibition, 31.1 and 18.6%, respectively; $P < 0.001$) in overnight incubations. The increased potential for IFN-α to rescue CD4+CD38− T cells compared with CD4+CD38+ T cells was also observed in samples obtained from healthy donors (not shown). These observations raised the possibility that diminished inhibition of apoptosis by IFN-α in HIV disease could be fully explained by a proportional increase in the percentage of CD4+CD38+ cells in HIV infection. If so, the percentage of CD4+CD38+ T cells should be inversely correlated with the ability of IFN-α to inhibit spontaneous apoptosis among CD4 T cells. No such relationship was observed ($r = -0.041$; $P = 0.75$), suggesting that the differential susceptibility of CD38-positive and CD38-negative cells to IFN-mediated protection from apoptosis does not entirely explain the diminished effectiveness of IFN-α to block apoptosis in HIV infection.

These relationships were further tested in cells from the nine HIV-infected persons where CD38 expression was measured among the CD4+CD45RO+ cells in freshly isolated preparations. The percentages of CD38 cells among the freshly isolated CD4+CD45RO+ T cell subset were inversely related to CD4 cell counts and directly related to apoptosis in the cultured CD4+CD45RO+ subset (Table 1). Nevertheless, the expression of CD38 among the CD4+CD45RO+ T cell subset was not significantly related to the ability of IFN-α to inhibit T cell death in these cultures, suggesting that enrichment of activated memory CD4 T cells alone is not likely to account for the diminished responsiveness of CD4 T cells in HIV-positive patients to IFN-α.

It was also possible that diminished IFN-α/β receptor expression might account for poor responsiveness of T cells from HIV-positive patients to the anti-apoptotic activity of IFN-α. Analyses of IFN-α/β receptor expression among freshly isolated CD4+CD45RO+ T cells from the nine HIV-infected persons and the four healthy control donors suggested that there was no deficiency in receptor expression in HIV disease (95% and 84% INF-α/β receptor-positive cells in HIV-infected and healthy control donors, respectively). Furthermore, there was no relationship between the percentages of IFN-α/β receptor-positive cells and the degree of apoptosis inhibition mediated by IFN-α in cells from these subjects (Table 1).

In general, higher plasma HIV RNA levels and lower CD4 cell counts were associated with increased spontaneous apoptosis of CD4+CD45RO+ cells, but neither index predicted the ability of IFN-α to protect memory CD4 T cells from apoptosis (Table 1). These observations demonstrate that simple models of immune activation, disease stage or levels of IFN-α/β receptor expression cannot readily account for the complexity of IFN-α responsiveness in T cells from HIV-infected persons.

IFN-α mediates induction of CD38 expression, particularly among CD8 T cells

Interpreting the effects of IFN-α on apoptosis of CD8+CD38+ and CD8+CD38− subsets after IFN-α treatment was compromised by a surprising ability of IFN-α to induce CD38 expression, especially among CD8 T cells from HIV-infected persons (Fig. 4a). A 10-fold lower concentration of IFN-α (100 U/ml) also mediated detectable induction of CD38 (mean induction of CD38 was14.6% for 1000 U/ml and 10.1% for

Table 1. Spearman's correlations restricted to CD4+CD45RO+ T cells from nine HIV-positive donors.

	Apoptosis (%)	Apoptosis CD38 (%)	Apoptosis CD38 negative (%)	Apoptosis inhibition (%)	Apoptosis inhibition CD38 (%)	Apoptosis inhibition CD38 negative (%)
CD38 (%)						
r	0.800	0.700	0.817	−0.100	−0.483	−0.083
P	0.010*	0.036*	0.007*	0.798	0.187	0.831
Interferon-α/β receptor (%)						
r	−0.477	0.653	−0.544	−0.100	0.326	−0.444
P	0.194	0.057	0.130	0.797	0.391	0.232
Plasma HIV RNA						
r	0.635	0.731	0.731	−0.228	−0.563	−0.048
P	0.091	0.040*	0.040*	0.558	0.146	0.910
CD4 cell count						
r	−0.900	−0.583	−0.883	0.083	0.517	0.133
P	0.001*	0.099	0.002*	0.831	0.154	0.732

*Significant at $P < 0.05$.

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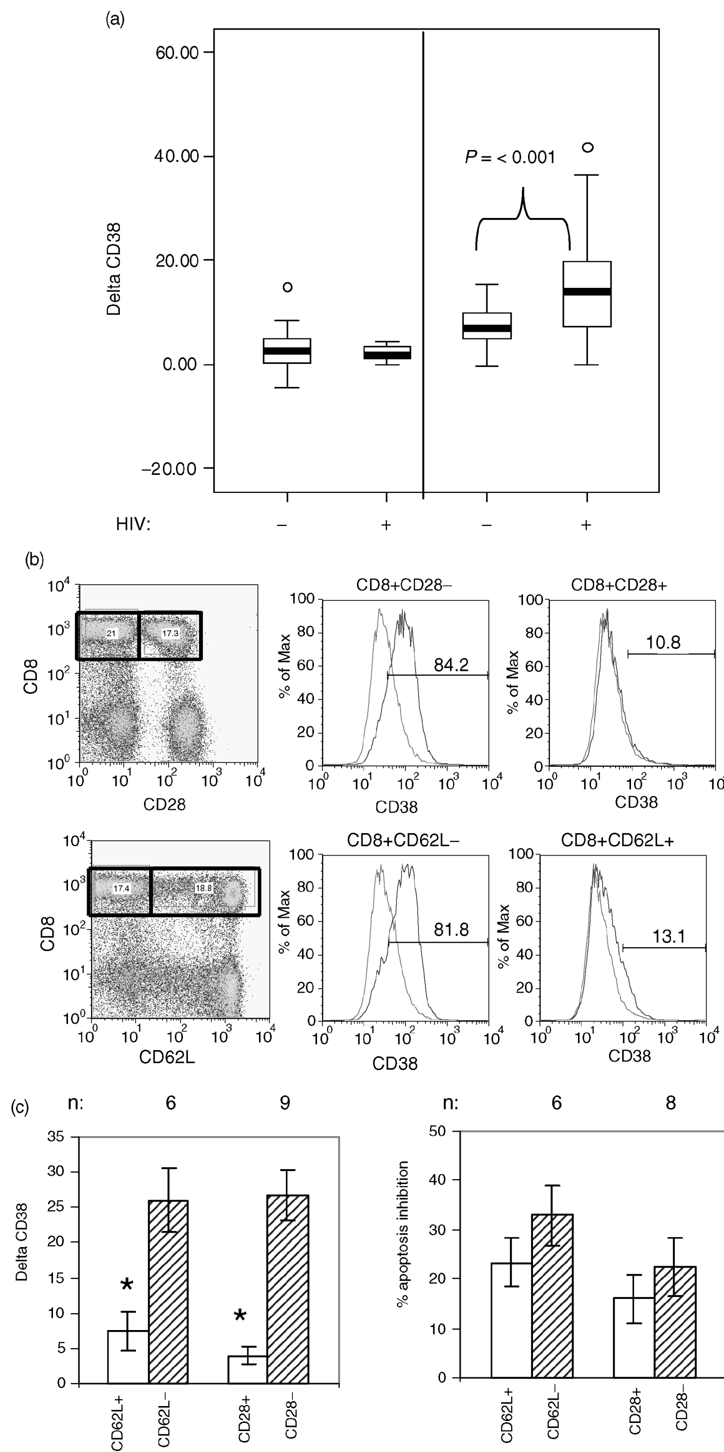

Fig. 4. Interferon-α (IFN-α)-induced CD38 expression among CD8 T cells from HIV-positive donors. (a) Peripheral blood mononuclear cells (PBMC) were incubated with IFN-α overnight and examined the next day for CD4, CD8 and CD38 expression. Cumulative data from 48 patients and 13 controls showing the change in CD38 expression (percentage CD38 cells in cultures treated with IFN-α minus the percentage CD38 cells in cultures in medium alone). A Student's two-tailed *t*-test was used for comparisons of mean values. (b) PBMC were incubated with IFN-α overnight and expression of CD28 or CD62L was examined along with expression of CD38 or phosphatidylserine in CD8 T cells. Representative histograms showing the gating strategies of CD8/CD28 and CD8/CD62L costains and the frequency distribution histograms indicating CD38 expression among the various CD8 T cell subsets for two different HIV-positive donors. (c) Cumulative data showing the means and standard error of the means for the change in CD38 expression on the CD8 subsets induced by IFN-α or the percentage apoptosis inhibition mediated by IFN-α. Statistical differences were determined by paired Student's *t*-tests. $^{*}P < 0.01$.

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100 U/ml; $n = 8$) in cells from HIV-infected donors. The specificity of this activity was confirmed in experiments with cells from two HIV-infected individuals wherein the induction of CD38 expression was diminished by addition of anti-IFN-α/β receptor antibody (20 μg/ml) from 5% to 1.3% and from 5.9% to 1.2%, but was not changed by addition of isotype control antibody at the same concentration. In addition, depletion of CD38 T cells prior to treatment with IFN-α verified that IFN-α induced CD38 expression on CD8 T cells (data not shown).

The induction of CD38 expression among CD8 T cell subsets differentiated by expression of CD28 and/or CD62L was also examined. These markers are absent on differentiated CD8 effector/memory T cells. The induction of CD38 by IFN-α occurred almost exclusively among CD28-negative and CD8+CD62L−/low T cells (Fig. 4b). In contrast, protection from apoptosis occurred in all CD8 T cell subsets examined (Fig. 4c). Incubation of PBMC with IFN-α did not change the expression of CD28 or CD62L on CD8 cells from that seen in incubation of PBMC in medium alone. In addition, comparison of freshly isolated cells with cells cultured overnight ($n = 3$) indicated that cultured CD8 cells did not spontaneously lose expression of either CD28 or CD62L (not shown). Therefore, the induction of CD38 on CD8+CD28−CD62L±− cells is not the result of CD38 cells losing expression of CD28 or CD62L during incubation with IFN-α.

Since previous studies indicate that CD8+CD62L− and CD8+CD28− cells increase proportionally as HIV infection progressed [21,22,41], it was hypothesized that IFN-α induction of CD38 on CD8 T cells would increase as CD4 T cell numbers decline with disease progression. Surprisingly, instead of an inverse relationship, induction of CD38 on CD8 T cells was directly related to CD4 cell counts ($r = 0.383$; $P = 0.007$) and marginally related to age ($r = 0.239$; $P = 0.059$).

Discussion

The expression of IFN-α in HIV infection may have important consequences for T cell function and survival. IFN-α mediates anti-apoptotic effects in both CD4 and CD8 T cells cultured *in vitro* without additional stimuli. Importantly, the ability of IFN-α to inhibit CD4 T cell death is impaired in HIV disease whereas the ability of IFN-α to inhibit CD8 T cell death is only diminished among patients with more severe disease (lower CD4 cell counts and higher plasma HIV RNA levels). Nonetheless, there is heterogeneity in both susceptibility to spontaneous apoptosis *in vitro* and the ability of IFN-α to protect cells from apoptosis among T cell subsets. Spontaneous apoptosis is increased among CD8+CD38+ cells in both HIV infection and in health, suggesting that activated CD8 cells are more susceptible to apoptosis than quiescent CD8 cells. In contrast, CD38 did not distinguish cells that were more readily induced to undergo apoptosis among CD4 T cells; however, this was probably a consequence of the constitutive expression of CD38 among naive CD4+CD45RO− cells. When the analyses were restricted to CD4+CD45RO+ cells, which were the predominant cell type undergoing spontaneous apoptosis within the CD4 T cell subsets, the expression of CD38 strongly predicted spontaneous apoptosis. Moreover, the CD4+CD45RO+CD38+ cells were more prone to spontaneous apoptosis than the CD4+CD45RO+CD38− subset. Therefore, activated CD4 memory cells were more likely to undergo apoptosis *in vitro*. The percentage of CD38-expressing cells within either the whole CD4 T cell subset or the CD4+CD45RO+ T cell subset, however, did not predict the ability of IFN-α to rescue CD4 T cells (or CD4+CD45RO+) from apoptosis. This suggests that simple enrichment of activated T cells, as occurs in HIV infection, is not likely to explain the diminished ability of IFN-α to rescue CD4 T cells from spontaneous apoptosis.

Previous studies have suggested that there are reduced levels of IFN-α/β receptor expression in PBMC from HIV-infected persons [42], perhaps underlying reported impairments of IFN-α responsiveness among certain cell types in HIV infection [43]. Nevertheless, our initial analyses of type I IFN-α/β receptor expression among CD4+CD45RO+ cells from HIV-infected persons demonstrated that the majority of these cells did express type I IFN-α/β receptor and that there was no discernable relationship between the expression of this receptor and responsiveness to the anti-apoptotic activity of type I IFN. These observations suggest that any defects in type I IFN-responsiveness is probably not a consequence of diminished receptor expression in HIV infection.

We also attempted to explore the relationship between IFN-α levels in cryopreserved plasma samples from our study subjects and T cell responses to IFN-α *in vitro*. Though we were unable to find a correlation between IFN-α levels in cryopreserved plasma and T cell responsiveness to this cytokine, only 17 of 44 plasma samples had detectable IFN-α. To reduce the possibility of a type II error, a second analysis was restricted to only those samples with a positive IFN-α result in an enzyme-linked immunosorbent assay (ELISA). Even with this approach, we failed to find a correlation between IFN-α detected in plasma and IFN-α responses (measured by apoptosis inhibition or CD38 induction). The percentage of CD8 cells that expressed CD38 in untreated PBMC cultures was related to plasma IFN-levels ($r = 0.557$; $P = 0.031$), suggesting that *in vivo* levels of IFN-α may contribute at least in part to the increased frequencies of CD38+CD8+ T cells observed in HIV disease. We are not, however, fully confident that cryopreserved plasma samples are ideal for IFN-α measurement or that these

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levels reflect reliably the *in vivo* exposure. Therefore, these data suggest but do not assure that *in vivo* exposure to IFN-α may contribute to a heightened 'activation' state in HIV infection.

The concentration of IFN-α levels in plasma of late-stage HIV-infected persons is reported to average 15–84 U/ml, with levels as high as 500–600 U/ml in some patients [1,44]. These concentrations are within the range of biological activity that we report here. Obviously, our analyses of IFN-α in plasma could not confirm these high levels of cytokine in HIV disease but this may stem from our sample source (cryopreserved plasma) or our method of detection (ELISA rather than biological assays). Alternatively, lower levels of type I IFN production might be anticipated in HIV infection since the primary cell type responsible for production of this cytokine is depleted (at least from peripheral blood) and functionally impaired in HIV infection [45–51]. Further assessment of type I IFN expression *in vivo* may help to clarify these issues in HIV disease.

In addition to questions surrounding the production of type I IFN in HIV infection, the role of this cytokine in HIV pathogenesis remains uncertain. While some investigators propose administration of IFN-α to inhibit HIV replication [52–55], others propose that interferon activity should be blocked to attenuate its immunopathological effects [56–58]. Our results suggest that the increased expression of IFN-α in HIV infection is likely to have complex consequences for T cell viability and activation. Moreover, the effects of IFN-α in HIV infection may not be predictable, since we have demonstrated here and elsewhere [43] that cellular responsiveness to IFN-α is altered in HIV infection. Our work underscores the complex activities of this cytokine in HIV infection and in health and suggests that strategies utilizing or inhibiting this cytokine may have profound and heterogeneous effects on lymphocyte activation and survival.

Acknowledgements

We thank the patients who participated in this study and Dr. Robert Asaad for obtaining patient samples.

Sponsorship: This work was supported by National Institutes of Health grants AI-55793 and AI-36219 CFAR (Center for AIDS Research, Case Western Reserve University).

References

1. Piasecki E, Knysz B, Gasiorowski J, Gladysz A. **Decrease of enhanced interferon alpha levels in sera of HIV-infected and AIDS patients receiving combined antiretroviral therapy.** *Arch Immunol Ther Exp (Warsz)* 1999; **47**:37–44.
2. Stylianou E, Aukrust P, Bendtzen K, Muller F, Froland SS. **Interferons and interferon (IFN)-inducible protein 10 during highly active anti-retroviral therapy (HAART)-possible immunosuppressive role of IFN-alpha in HIV infection.** *Clin Exp Immunol* 2000; **119**:479–485.
3. Swindells S, Baldwin T, Kelly C, Baca-Regen L, Loomis L, Post D, *et al*. **Regulation and characterization of the interferon-alpha present in patients with advanced human immunodeficiency virus type 1 disease.** *J Interferon Cytokine Res* 1996; **16**:127–137.
4. von Sydow M, Sonnerborg A, Gaines H, Strannegard O. **Interferon-alpha and tumor necrosis factor-alpha in serum of patients in various stages of HIV-1 infection.** *AIDS Res Hum Retroviruses* 1991; **7**:375–380.
5. Yamamoto JK, Kruzel ML, Louie H, Georgiades JA. **Inhibition of human immunodeficiency virus type 1 replication by human interferons alpha, beta and gamma.** *Arch Immunol Ther Exp (Warsz)* 1993; **41**:185–191.
6. Meylan PR, Guatelli JC, Munis JR, Richman DD, Kornbluth RS. **Mechanisms for the inhibition of HIV replication by interferons-alpha, -beta, and -gamma in primary human macrophages.** *Virology* 1993; **193**:138–148.
7. Mace K, Gazzolo L. **Interferon-regulated viral replication in chronically HIV1-infected promonocytic U937 cells.** *Res Virol* 1991; **142**:213–220.
8. Lapenta C, Santini SM, Proietti E, Rizza P, Logozzi M, Spada M, *et al*. **Type I interferon is a powerful inhibitor of in vivo HIV-1 infection and preserves human CD4(+) T cells from virus-induced depletion in SCID mice transplanted with human cells.** *Virology* 1999; **263**:78–88.
9. Hartshorn KL, Neumeyer D, Vogt MW, Schooley RT, Hirsch MS. **Activity of interferons alpha, beta, and gamma against human immunodeficiency virus replication in vitro.** *AIDS Res Hum Retroviruses* 1987; **3**:125–133.
10. Luft T, Pang KC, Thomas E, Hertzog P, Hart DN, Trapani J, *et al*. **Type I IFNs enhance the terminal differentiation of dendritic cells.** *J Immunol* 1998; **161**:1947–1953.
11. Montoya M, Schiavoni G, Mattei F, Gresser I, Belardelli F, Borrow P, *et al*. **Type I interferons produced by dendritic cells promote their phenotypic and functional activation.** *Blood* 2002; **99**:3263–3271.
12. Paquette RL, Hsu NC, Kiertscher SM, Park AN, Tran L, Roth MD, *et al*. **Interferon-alpha and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells.** *J Leukoc Biol* 1998; **64**: 358–367.
13. Tanabe Y, Nishibori T, Su L, Arduini RM, Baker DP, David M. **Cutting edge: role of STAT1, STAT3, and STAT5 in IFN-alpha beta responses in T lymphocytes.** *J Immunol* 2005; **174**:609–613.
14. Schandene L, Roufosse F, de Lavareille A, Stordeur P, Efira A, Kennès B, *et al*. **Interferon alpha prevents spontaneous apoptosis of clonal Th2 cells associated with chronic hypereosinophilia.** *Blood* 2000; **96**:4285–4292.
15. Scheel-Toellner D, Pilling D, Akbar AN, Hardie D, Lombardi G, Salmon M, *et al*. **Inhibition of T cell apoptosis by IFN-beta rapidly reverses nuclear translocation of protein kinase C-delta.** *Eur J Immunol* 1999; **29**:2603–2612.
16. Pilling D, Akbar AN, Girdlestone J, Orteu CH, Borthwick NJ, Amft N, *et al*. **Interferon-beta mediates stromal cell rescue of T cells from apoptosis.** *Eur J Immunol* 1999; **29**:1041–1050.
17. Lombardi G, Dunne PJ, Scheel-Toellner D, Sanyal T, Pilling D, Taams LS, *et al*. **Type 1 IFN maintains the survival of anergic CD4+ T cells.** *J Immunol* 2000; **165**:3782–3789.
18. Dondi E, Roue G, Yuste VJ, Susin SA, Pellegrini S. **A dual role of IFN-alpha in the balance between proliferation and death of human CD4+ T lymphocytes during primary response.** *J Immunol* 2004; **173**:3740–3747.
19. Marrack P, Kappler J, Mitchell T. **Type I interferons keep activated T cells alive.** *J Exp Med* 1999; **189**:521–530.
20. Stylianou E, Yndestad A, Sikkeland LI, Bjerkeli V, Damas JK, Haug T, *et al*. **Effects of interferon-alpha on gene expression of chemokines and members of the tumour necrosis factor superfamily in HIV-infected patients.** *Clin Exp Immunol* 2002; **130**:279–285.
21. Fagnoni FF, Vescovini R, Passeri G, Vescovini R, Sansoni P, Passeri M, *et al*. **Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging.** *Blood* 2000; **95**:2860–2868.

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22. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA. **CD8 naive T cell counts decrease progressively in HIV-infected adults.** *J Clin Invest* 1995; **95**:2061–2066.
23. Rabin RL, Roederer M, Maldonado Y, Petru A, Herzenberg LA. **Altered representation of naive and memory CD8 T cell subsets in HIV-infected children.** *J Clin Invest* 1995; **95**:2054–2060.
24. Sherman GG, Scott LE, Galpin JS, Kuhn L, Tiemessen CT, Simmank K, *et al*. **CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients.** *Pediatr Res* 2002; **51**:740–745.
25. Kestens L, Vanham G, Vereecken C, Vandenbruaene M, Vercauteren G, Coleblunders RL, *et al*. **Selective increase of activation antigens HLA-DR and CD38 on CD4+CD45RO+ T lymphocytes during HIV-1 infection.** *Clin Exp Immunol* 1994; **95**:436–441.
26. Liu Z, Hultin LE, Cumberland WG, Hultin P, Schmid I, Matud JL, *et al*. **Elevated relative fluorescence intensity of CD38 antigen expression on CD8+ T cells is a marker of poor prognosis in HIV infection: results of 6 years of follow-up.** *Cytometry* 1996; **26**:1–7.
27. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. **Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4 cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **16**:83–92.
28. Mahalingam M, Peakman M, Davies ET, Pozniak A, McManus TJ, Vergani D. **T cell activation and disease severity in HIV infection.** *Clin Exp Immunol* 1993; **93**:337–343.
29. Fleury S, Rizzardi GP, Chapuis A, Tambussi G, Knabenhans C, Simeoni E, *et al*. **Long-term kinetics of T cell production in HIV-infected subjects treated with highly active antiretroviral therapy.** *Proc Natl Acad Sci USA* 2000; **97**:5393–5398.
30. Hazenberg MD, Cohen Stuart JW, Otto SA, Borleffs JC, Boucher CA, de Boer RJ, *et al*. **T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART).** *Blood* 2000; **95**: 249–255.
31. Kovacs JA, Lempicki RA, Sidorov IA, Adelsberger JW, Herpin B, Metcalf JA, *et al*. **Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV.** *J Exp Med* 2001; **194**:1731–1741.
32. Lempicki RA, Kovacs JA, Baseler MW, Adelsberger JW, Dewar RL, Natarajan V, *et al*. **Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4+ and CD8+ T cell turnover in HIV-infected patients.** *Proc Natl Acad Sci USA* 2000; **97**:13778–13783.
33. Sachsenberg N, Perelson AS, Yerly S, Schockmel GA, Leduc D, Hirschel B, *et al*. **Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen.** *J Exp Med* 1998; **187**:1295–1303.
34. Sieg SF, Rodriguez B, Asaad R, Jiang W, Bazdar DA, Lederman MM. **Peripheral S-phase T cells in HIV disease have a central memory phenotype and rarely have evidence of recent T cell receptor engagement.** *J Infect Dis* 2005; **192**:62–70.
35. Naora H, Gougeon ML. **Interleukin-15 is a potent survival factor in the prevention of spontaneous but not CD95-induced apoptosis in CD4 and CD8 T lymphocytes of HIV-infected individuals. Correlation with its ability to increase BCL-2 expression.** *Cell Death Differ* 1999; **6**:1002–1011.
36. Adachi Y, Oyaizu N, Than S, McCloskey TW, Pahwa S. **IL-2 rescues in vitro lymphocyte apoptosis in patients with HIV infection: correlation with its ability to block culture-induced down-modulation of Bcl-2.** *J Immunol* 1996; **157**:4184–4193.
37. Boudet F, Lecoeur H, Gougeon ML. **Apoptosis associated with ex vivo down-regulation of Bcl-2 and up-regulation of Fas in potential cytotoxic CD8+ T lymphocytes during HIV infection.** *J Immunol* 1996; **156**:2282–2293.
38. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, *et al*. **Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load.** *Blood* 2004; **104**:942–947.
39. Benito JM, Lopez M, Lozano S, Ballesteros C, Martinez P, Gonzalez-Lahoz J, *et al*. **Differential upregulation of CD38 on different T-cell subsets may influence the ability to reconstitute CD4+ T cells under successful highly active antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2005; **38**:373–381.
40. Dianzani U, Funaro A, DiFranco D, Garbarino G, Bragardo M, Redoglia V, *et al*. **Interaction between endothelium and CD4+CD45RA+ lymphocytes. Role of the human CD38 molecule.** *J Immunol* 1994; **153**:952–959.
41. Choremi-Papadopoulou H, Viglis V, Gargalianos P, Kordossis T, Iniotaki-Theodoraki A, Kosmidis J. **Downregulation of CD28 surface antigen on CD4+ and CD8+ T lymphocytes during HIV-1 infection.** *J Acquir Immune Defic Syndr* 1994; **7**:245–253.
42. Lau AS, Read SE, Williams BR. **Downregulation of interferon alpha but not gamma receptor expression in vivo in the acquired immunodeficiency syndrome.** *J Clin Invest* 1988; **82**:1415–1421.
43. Jiang W, Lederman MM, Salkowitz JR, Rodriguez B, Harding CV, Sieg SF. **Impaired monocyte maturation in response to CpG oligodeoxynucleotide is related to viral RNA levels in human immunodeficiency virus disease and is at least partially mediated by deficiencies in alpha/beta interferon responsiveness and production.** *J Virol* 2005; **79**:4109–4119.
44. Brockmeyer NH, Barthel B, Mertins L, Goos M. **Effect of zidovudine therapy in patients with HIV infection on endogenous interferon plasma levels and the hepatic cytochrome P450 enzyme system.** *Chemotherapy* 1998; **44**:174–180.
45. Barron MA, Blyveis N, Palmer BE, MaWhinney S, Wilson CC. **Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals.** *J Infect Dis* 2003; **187**:26–37.
46. Chehimi J, Campbell DE, Azzoni L, Bacheller D, Papasavvas E, Jerandi G, *et al*. **Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals.** *J Immunol* 2002; **168**:4796–4801.
47. Donaghy H, Gazzard B, Gotch F, Patterson S. **Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1.** *Blood* 2003; **101**:4505–4511.
48. Donaghy H, Pozniak A, Gazzard B, Qazi N, Gilmour J, Gotch F, *et al*. **Loss of blood CD11c(+) myeloid and CD11c(−) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load.** *Blood* 2001; **98**:2574–2576.
49. Feldman S, Stein D, Amrute S, Denny T, Garcia Z, Kloser P, *et al*. **Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors.** *Clin Immunol* 2001; **101**:201–210.
50. Pacanowski J, Kahi S, Baillet M, Lebon P, Deveau C, Goujard C, *et al*. **Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection.** *Blood* 2001; **98**:3016–3021.
51. Soumelis V, Scott I, Gheyas F, Bouhour D, Cozon G, Cotte L, *et al*. **Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients.** *Blood* 2001; **98**: 906–912.
52. Hatzakis A, Gargalianos P, Kiosses V, Lazanas M, Sypsa V, Anastassopoulou C, *et al*. **Low-dose IFN-alpha monotherapy in treatment-naive individuals with HIV-1 infection: evidence of potent suppression of viral replication.** *J Interferon Cytokine Res* 2001; **21**:861–869.
53. Emilie D, Burgard M, Lascoux-Combe C, Laughlin M, Krzysiek R, Pignon C, *et al*. **Early control of HIV replication in primary HIV-1 infection treated with antiretroviral drugs and pegylated IFN alpha: results from the Primoferon A (ANRS 086) Study.** *AIDS* 2001; **15**:1435–1437.
54. Skillman DR, Malone JL, Decker CF, Wagner KF, Mapou RL, Liao MJ, *et al*. **Phase I trial of interferon alfa-n3 in early-stage human immunodeficiency virus type 1 disease: evidence for drug safety, tolerance, and antiviral activity.** *J Infect Dis* 1996; **173**:1107–1114.
55. Haas DW, Lavelle J, Nadler JP, Greenberg SB, Frame P, Mustafa N, *et al*. **A randomized trial of interferon alpha therapy for HIV type 1 infection.** *AIDS Res Hum Retroviruses* 2000; **16**:183–190.
56. Zagury D, Lecoq H, Gervi I, Le Buanec H, Zagury JF, Bizzini B, *et al*. **Anti-IFN alpha immunization raises the IFN alpha-neutralizing capacity of serum–an adjuvant to antiretroviral tritherapy.** *Biomed Pharmacother* 1999; **53**:90–92.

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57. Gringeri A, Santagostino E, Cusini M, Muça-Perja M, Marinoni A, Mannucci PM, *et al.* **Absence of clinical, virological, and immunological signs of progression in HIV-1-infected patients receiving active anti-interferon-alpha immunization: a 30-month follow-up report.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1996; **13**:55–67.
58. Gringeri A, Musicco M, Hermans P, Bentwich Z, Cusini M, Bergamasco A, *et al.* **Active anti-interferon-alpha immunization: a European–Israeli, randomized, double-blind, placebo-controlled clinical trial in 242 HIV-1-infected patients (the EURIS study).** *J Acquir Immune Defic Syndr Hum Retrovirol* 1999; **20**:358–370.

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