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Relationship between IL-2 receptor expression and proliferative responses in lymphocytes from HIV-1 seropositive homosexual men

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SUMMARY

Previous studies have shown that exogenous IL-2 does not correct the reduction in phytohaemagglutinin (PHA)-induced proliferation of lymphocytes from HIV-1 infected (HIV^+) individuals. We investigated the mechanism of this reduction to determine if reduced expression of the complete IL-2 receptor (IL-2R) was responsible. In a series of experiments, PHA-stimulated lymphocytes from a total of 89 HIV^- and 93 HIV^+ homosexual men from the Baltimore Multicentre AIDS Cohort Study (MACS) were studied to determine the expression of messages for the α and β subunits of the IL-2R, the binding of ^{125}I-IL-2 to high affinity IL-2R, and the effect of IL-2 on cell proliferation. Compared to HIV^- donors, PHA-stimulated lymphocytes from most HIV^+ donors demonstrated (i) a reduction in high affinity IL-2R expression that correlated with the reduction in the IL-2-induced proliferative response; and (ii) a reduction in expression of both IL-2R α- and β-chain mRNA which may be responsible for decreased high affinity IL-2R expression. However, lymphocytes from some HIV^+ individuals had borderline low IL-2-induced proliferation despite normal or elevated expression of high affinity IL-2R. These results suggest that decreased expression of IL-2R may account, at least in part, for the lower proliferative response of cells from HIV^+ donors.

Keywords HIV-1 IL-2 IL-2 receptor lymphocytes phytohaemagglutinin

INTRODUCTION

Reduction of T cell proliferative responses to antigens, mitogens, and IL-2 occurs in the early stages of HIV-1 infection [1–4]. This reduction of antigen- and mitogen-induced lymphoproliferative responses in short term cultures of peripheral blood mononuclear cells (PBMC) from HIV^+ donors is not reversed by exogenous IL-2 [2,5–9]. Similarly, by limiting dilution analysis we found lower precursor frequencies of IL-2-responsive cells in PBMC from HIV^+ donors [4,10], implying that this decrease in IL-2-responsiveness reflected the functioning of single cells. The mechanism responsible for these reduced responses is not well understood, although both quantitative factors (related to the loss of CD4 lymphocytes) and qualitative factors (related to the function of the remaining CD4 lymphocytes) have been implicated. Reported to be among the latter are T cell inhibitory factors present in serum from HIV^+ individuals [11,12]; inhibitory effects of HIV proteins such as gp120, gp41, and tat [13–15]; elevated serum levels of soluble IL-2Rα [16] which can compete with cellular IL-2R for free IL-2 [17]; reduced production of IL-2 and other growth factors [5,6,17–20]; and overproduction of inhibitory factors such as transforming growth factor-beta 1 (TGF-β1) [21]. A direct viral effect seems unlikely, since the frequency of HIV-infected cells is very low (10^{-3}–10^{-6}) [22,23].

T cells stimulated by antigen or mitogen enter the G_1-S phase of the cell cycle after receiving a signal transduced by the binding of IL-2 to the high affinity IL-2R, a non-covalently associated heterodimer composed of α and β chains (Kd = 1–50 pM) [24,25]. Activated T cells express at least 20–40 times more IL-2Rα chain than required to associate with β chains [24,25]; thus, the β chain is the limiting component for the formation of the high affinity IL-2R. In addition, the β chain is essential for transduction of the IL-2 signal [26–28].

These observations suggest that a defect in expression of either the α or the β subunit of the high affinity IL-2R could explain the decrease in lymphoproliferative responses. In sup-

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port of this possibility, expression of IL-2Rα has recently been reported to be reduced on circulating lymphocytes from HIV$^+$ donors [29]. However, *in vitro* studies of the expression of the IL-2Rα subunit on mitogen-activated T cells from HIV$^+$ donors suggest that the reduction in this subunit [1,6–9] compared with HIV$^-$ donors is not sufficient to cause a reduction in the number of high affinity IL-2R in HIV-infected individuals, because the α subunit is normally expressed in such great excess. Therefore, the relationship between expression of IL-2R and lymphocyte proliferative responses in HIV-1 infection was studied. Our results indicate that there is no preferential reduction in expression of the β subunit due to HIV-1 infection and that a reduction in IL-2R accounts at least in part for the decreased lymphocyte proliferation in HIV-1 infection.

MATERIALS AND METHODS

Study participants and cell culture

Study subjects were HIV$^+$ homosexual men participating in the Baltimore centre of the Multicentre AIDS Cohort Study (MACS) and were without an AIDS-defining illness [30]. As described in detail elsewhere [31], participants in this study were followed at 6-month intervals with clinical and laboratory evaluations, including testing for the presence of antibodies to HIV-1 by ELISA with confirmatory analysis using Western blot, and measurement of T cell subsets by flow cytometry as described below. PBMC were isolated from heparinized blood by centrifugation at 400 ***g*** for 30 min over Ficoll–Hypaque [32]. PBMC were washed and suspended at 5×10^5 cells/ml in complete culture medium consisting of RPMI 1640 (Whittaker Bioproducts, Walkersville, MD), 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 100 μg/ml gentamicin. The PBMC were stimulated with 0·5 μg/ml phytohaemagglutinin (PHA) (Wellcome Research Laboratory, Janesville, NC) and, unless otherwise stated, cultivated in 75 cm^2 tissue culture flasks at 37°C in 5% CO_2 and 95% humidity. Because of limited availability of PBMC, different participants were studied in different groups of experiments; the specific characteristics of participants in each group of experiments are described in Results.

Flow cytometric analyses

Peripheral blood lymphocytes were analysed using a whole blood staining technique as previously described [33,34]. Two combinations of antibodies directly labelled with FITC or PE were used, as follows: CD3-FITC/IgG1-PE and CD8-FITC/CD4-PE (NIH Natural History of AIDS Kit, Becton Dickinson, San Jose, CA). For analysis of cultured lymphocytes, 10^6 cells were washed three times and stained with the same antibodies. In addition, cells were stained with FITC-labelled anti-IL-2Rα and a 1:1000 dilution of anti-IL-2Rβ antibody (TU27, graciously provided by Dr S. Taki, Ajinomoto Co. Inc., Central Research Laboratories, Kawasaki, Japan [35]) followed by FITC-labelled goat anti-mouse IgG antibody; normal mouse IgG was used as a control for TU27. Stained cells were fixed in 0·5% formaldehyde (Polysciences, Warrington, PA) and stored in the dark at 4°C until analysis on an EPICS C flow cytometer (Coulter Electronics, Hialeah, FL).

Cell proliferation

Lymphocyte proliferation was measured by culturing 5×10^4 PBMC per triplicate well of flat-bottomed 96-well microtitre plates in 0·2 ml with PHA or complete medium alone for 72 h, with a pulse of 1 μCi per well of ^{3}H-TdR (sp. act. 6·7 Ci/mmol, NEN) for the last 16 h. To study the effect of IL-2, cells were stimulated with PHA for 55 h, washed, and recultured overnight in the absence of lymphokines. An aliquot of these cells was cultured at 5×10^4 cells/well with or without 1 nM rIL-2 for 48 h and pulsed with 1 μCi/well of ^{3}H-TdR for the last 4 h. The incorporation of ^{3}H-TdR was quantified by standard liquid scintillation counting.

^{125}I-IL-2 binding

After stimulation with PHA for 55 h, PBMC were washed and reincubated in complete medium at 37°C overnight. The cells were isolated, washed, and suspended in ice cold complete medium at 5×10^6 viable cells/ml. ^{125}I-IL-2 binding to the cells was assayed as described previously [36] using 100 μl serial dilutions of 1·5–200 pM of ice cold ^{125}I-IL-2 (sp. act. 33–46 μCi/μg, New England Nuclear, Boston, MA). After incubation, 1 ml of ice-cold media was added to each tube, and the tubes were then centrifuged at 9000 ***g*** for 2 min. The supernatants were saved and the cell pellets suspended in 100 μl of RPMI 1640 were then layered over dibutylphthalate/dioctylphthalate (1·1:1) in chilled microcentrifuge tubes. The samples were centrifuged to pellet the cells through the oil barrier. The tips of the tubes containing the cell pellets were clipped, and radioactivity was counted in a γ counter. Non-specific binding of ^{125}I-IL-2 was determined by adding ≈100-fold excess cold rIL-2 (graciously provided by Cetus Inc, Emeryville, CA). The B max of IL-2 binding and the equilibrium dissociation constant (kd) were determined using the ENZYFIT program [37].

Cross linking and analysis of IL-2Rα and β mRNA

RNA was isolated from 20×10^6 unstimulated or PHA-stimulated PBMC by the RNAzol method (Cinna/Biotecx Laboratories, Friendswood, TX). Isolation of RNA was carried out 24 h after PHA stimulation, when IL-2Rβ mRNA expression is maximal [27] and IL-2Rα mRNA is nearly maximal ([38], Chopra, unpublished observations). For unstimulated cells 12 μg and for stimulated cells 15 μg of RNA per lane were electrophoresed, blotted, and hybridized as described previously [39] with cDNA probes specific for human IL-2Rα, β (plasmids graciously provided by Dr W. J. Leonard, NICHD, Bethesda, MD) and human actin (plasmid graciously provided by Dr L. H. Kedes, Stanford University School of Medicine, Palo Alto, CA) labelled to high specific activity with ^{32}P CTP (NEN) using the random primer method [40]. Blots were first hybridized with probes for IL-2Rα, after which the IL-2Rα probe was stripped [39] and the blots probed with IL-2Rβ and actin probes together. Autoradiograms were scanned with a densitometer (Hoefer Scientific Instruments, San Fransisco, CA) and the concentrations of mRNA bands were normalized to the concentration of actin mRNA bands (taken as 2000 arbitrary densitometric units).

Statistical analysis

The two-tailed *t*-test was used for comparisons of ^{3}H-TdR incorporation measurements and densitometry data. ^{3}H-TdR incorporation was approximately log normally distributed; these data were log transformed before use of the *t*-test and are given as geometric means and the antilog of the mean ± 1 s.d. of the logarithmic distribution of the data. IL-2 receptor data were

bimodally distributed; the Kruskal–Wallis rank order test was used to compare IL-2R expression on cells from HIV$^-$ and HIV$^+$ donors.

RESULTS

In this study, data were obtained on 89 HIV$^-$ and 93 HIV$^+$ subjects. None of the latter had AIDS at the time of study. Different individuals were studied in different sets of experiments due to limited availability of cells. Overall, CD4 measurements were available on 74 HIV$^-$ and 81 HIV$^+$ subjects. The latter had lower CD4 percentage (mean $\pm$ s.d. $= 30{\cdot}59 \pm 1{\cdot}57$ *versus* $48{\cdot}2 \pm 1{\cdot}1$ for the HIV$^-$ group) and number of CD4 cells/μl (640 ± 41 *versus* 1323 ± 54).

PHA-induced incorporation of ^{3}H-TdR was studied in PBMC from 15 HIV$^+$ and 15 HIV$^-$ donors, and as expected was significantly lower in the HIV$^+$ group, which had a geometric mean of 6442 ct/min (± 1 s.d. $= 5623$, 7379) as compared with 11 940 (10 186, 13 996) for the HIV$^-$ group ($P < 0{\cdot}006$). The HIV$^+$ donors for these experiments had CD4 lymphocyte counts ranging from 49 to 1654 cells/μl (median $= 447$). Although the CD4 count and the responsiveness to PHA were significantly correlated ($r = 0{\cdot}61$; $P = 0{\cdot}001$), the relationship between these measurements showed considerable variation (Fig. 1). Of note, HIV$^+$ donors with high numbers of CD4 cells still tended to have low PHA-induced proliferation. Therefore, further studies were carried out to investigate the decreased responsiveness of the HIV$^+$ PBMC.

Decreased response of lymphocytes from HIV$^+$ donors to PHA and IL-2 is not due to decreased in vitro *survival*

One possibility was that decreased survival of lymphocytes from HIV$^+$ donors could account at least in part for the reduced proliferative response of these cells to PHA. To test this possibility, lymphocytes were stimulated with PHA for 55 h, then washed and recultured overnight in complete medium to remove endogenously produced lymphokines which allowed the optimal expression of IL-2R (Chopra, unpublished observations). Viable cells were then counted and re-cultured at 5×10^4/well for an additional 48 h in triplicate wells with or without 1 nM rIL-2. This concentration saturates high affinity IL-2R and in preliminary experiments was found to stimulate maximal proliferation of PBMC from both HIV$^-$ and HIV$^+$ donors.

For these experiments, 22 HIV$^+$ and 22 HIV$^-$ donors were studied. For the HIV$^-$ donors, the mean CD4 count ($\pm$ s.d.) was 1286 ± 353 cells/μl ($50{\cdot}3 \pm 8{\cdot}9$% of lymphocytes), and for HIV$^+$ donors (all AIDS-free) the corresponding figures were 801 ± 429 cells/μl ($34{\cdot}9 \pm 18{\cdot}2$% of lymphocytes). As shown in Fig. 2, the proliferation of viable PBMC from HIV$^+$ donors during the 48-h reculture period in the absence of IL-2 was slightly less than that of HIV$^-$; however, this difference was not statistically significant. In contrast, although the proliferative response of PBMC from both HIV$^-$ and HIV$^+$ donors was augmented by IL-2, the mean IL-2-induced increase was significantly smaller in the HIV$^+$ group than in the HIV$^-$ group ($28\,008 \pm 21\,847$ *versus* $49\,556 \pm 22\,436$ ct/min; $P < 0{\cdot}001$). Thus, the reduced response of PHA-stimulated PBMC from the HIV$^+$ donors persisted even after adjustment for differing numbers of viable lymphocytes.

Relationship between IL-2R expression and IL-2-induced proliferation

The above results suggested that cells from HIV$^+$ donors have, on average, decreased expression of high affinity IL-2R. To evaluate this directly, the binding of ^{125}I-IL-2 to PHA-stimulated PBMC was studied under high-affinity IL-2-binding

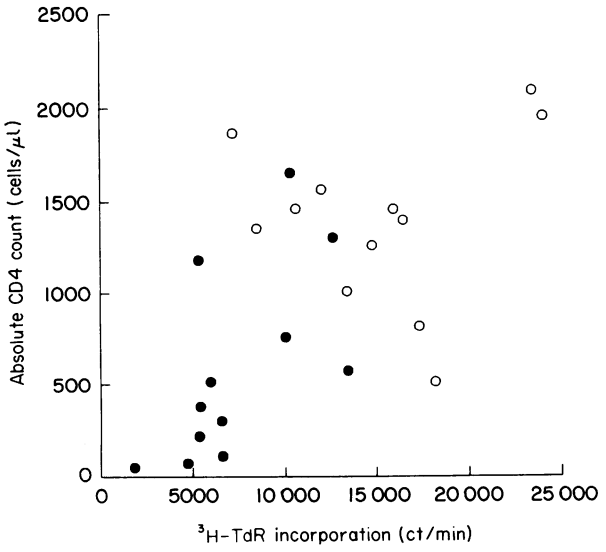

Fig. 1. Relationship between ^{3}H-TdR incorporation and CD4 counts in HIV$^-$ (○) and HIV$^+$ (●) donors ($n = 12$ in each group).

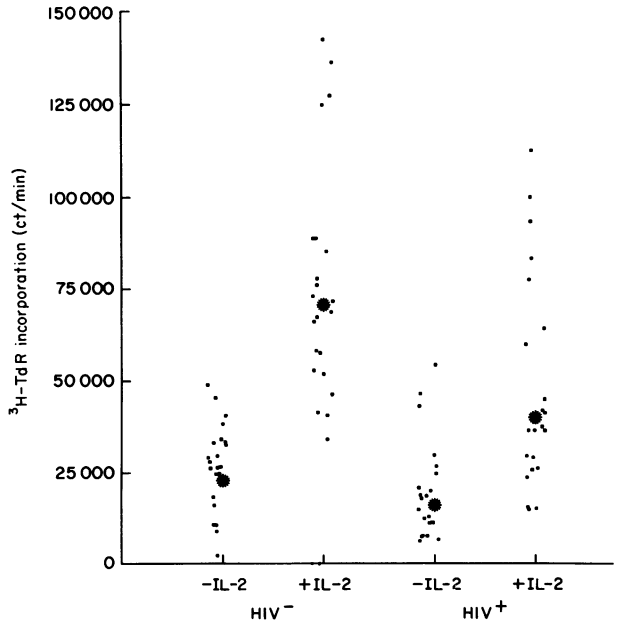

Fig. 2. Scattergram of ^{3}H-TdR incorporation in phytohaemagglutinin (PHA)-stimulated lymphoblasts from HIV$^-$ and HIV$^+$ donors ($n = 22$ in each group). PBMC were stimulated with PHA for 55 h, washed and recultivated for 16 h in complete medium. After washing, the cells were counted and recultivated at 5×10^4 cells/well in triplicate with or without 1 nM rIL-2 for 48 h. Individual data points and geometric group means (*) are shown. In the absence of rIL-2, the geometric mean ^{3}H-TdR incorporation ($\pm$ s.e.m.) was 22 803 (19 634, 26 485) and 16 181 (14 125, 18 535) for HIV$^-$ and HIV$^+$ donors, respectively. Addition of rIL-2 yielded ^{3}H-TdR incorporation of 70 795 (65 013, 77 090) and 40 179 (35 318, 45 709) for HIV$^-$ and HIV$^+$ donors, respectively.

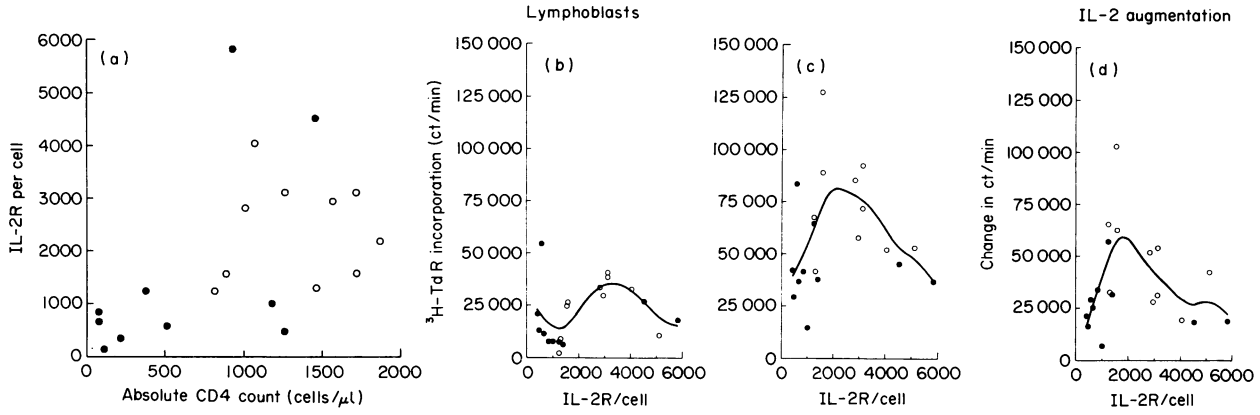

Fig. 3. (a) Relationship between high affinity IL-2R expression (average number of receptors per cell) and CD4 counts in 10 HIV$^-$ (○) and 10 HIV$^+$ (●) donors. The ^{3}H-TdR incorporation and IL-2R data are common to b–d. (b–d) Relationship between ^{3}H-TdR incorporation and IL-2R expression in HIV$^-$ (○) and HIV$^+$ (●) donors. Fifty-five hours after stimulation with phytohaemagglutinin (PHA), cells were washed and recultivated in complete medium (b) or complete medium plus 1 nM rIL-2 (c). ^{125}I-IL-2 binding was measured under high affinity IL-2 binding conditions and IL-2-induced proliferation was measured after 48 h in culture. IL-2 augmentation (d) was calculated as the difference between (c) and (b). Lines represent distance weighted least squares curve smooths of the data from both groups. The Kd of ^{125}I-IL-2 binding to receptors in HIV$^-$ donors was 26·7 ± 9·83 pM (mean ± s.e.m., $n = 10$) and 30·3 ± 17·8 pM ($n = 9$) in HIV$^+$ donors.

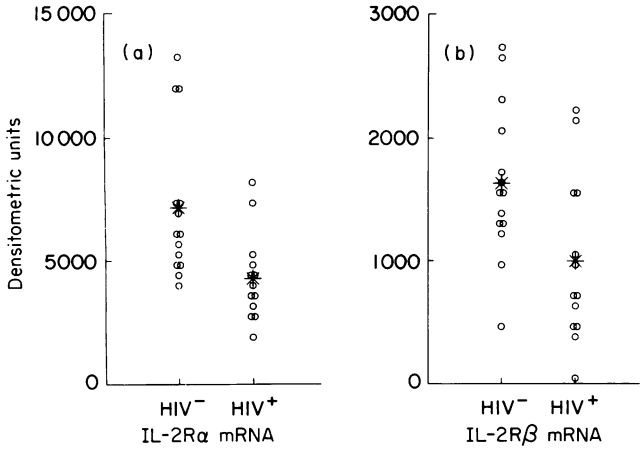

Fig. 4. (a, b) Scattered representation of densitometric analysis of IL-2Rα (a) and IL-2Rβ mRNA (b) bands for 14 HIV$^-$ and 13 HIV$^+$ individuals. After developing autoradiograms, the mRNAs bands were quantified by densitometry. The concentrations of IL-2Rα and β mRNA were normalized to 2000 densitometric units for actin in each individual. The mean IL-2Rα mRNA in HIV$^-$ donors was 7168 ± 778 (mean ± s.e.m.) and 4286 ± 480 in HIV$^+$ donors. The mean IL-2Rβ mRNA was 1634 ± 159 in HIV$^-$ donors and 997 ± 179 in HIV$^+$ donors. Individual data points and geometric means (*) are shown.

conditions (i.e. in the range of 1·5–200 pM IL-2). For these experiments, PBMC from 10 HIV$^-$ and 10 HIV$^+$ donors were studied, with mean CD4 counts of 1366 ± 360 and 619 ± 509 cells/μl, respectively. As in the preceding experiments, expression of high affinity IL-2R was studied at 72 h (55 h after stimulation with PHA, followed by washing and incubation overnight), and the responsiveness of the cells to readdition of IL-2 was also measured at this time.

High affinity IL-2R were significantly reduced on PBMC from most of the HIV$^+$ donors as compared with the HIV$^-$ donors, as shown in Fig. 3a. PBMC from most, but not all, HIV$^+$ donors had lower numbers of high affinity IL-2R than the HIV$^-$ donors, and the median number of IL-2R per cell was 2885 in the HIV$^-$ donors and 924 in the HIV$^+$ donors ($P < 0·031$). Among the HIV$^+$ donors, the correlation coefficient between the number of IL-2R and the number of CD4 lymphocytes was 0·54 ($P = 0·11$). Of note, both of the individuals with elevated expression of high affinity IL-2R had high numbers of CD4 lymphocytes at the time of study (i.e. 1452 and 928 cells/μl). In addition, both individuals had stable high CD4 lymphocyte levels, as well as antibodies to HIV-1 repeatedly, since first tested in 1984 (data not shown); neither had any HIV-1-related symptoms. On the other hand, two other individuals with high (> 900 cells/μl) CD4 counts had low expression of high affinity IL-2R (Fig. 3a). One had been HIV$^+$ since 1984 with stable high CD4 lymphocyte counts, and the other, a more recent seroconverter, had been seropositive since 1985.

To assess the relationship between IL-2R expression and IL-2-induced proliferation, we measured ^{3}H-TdR incorporation both without (Fig. 3b) and with (Fig. 3c) the addition of IL-2, and calculated the amount of proliferation induced by IL-2 (Fig. 3d, which shows the difference between Figs 3c and 3b for each donor). As shown in Fig. 3d, there was approximately a linear relationship between the number of IL-2R expressed and the induction of proliferation by IL-2 at low levels of IL-2R expression (i.e. less than 1800 IL-2R per cell, the lowest level observed in the HIV$^-$ donors). However, the two HIV$^+$ donors who had elevated levels of high affinity IL-2R also had proliferative responses to IL-2 that were slightly lower than the lowest response seen in the HIV$^-$ donors. These data show that reduction of high affinity IL-2R contributes to the lower proliferative response of HIV$^+$ PBMC, but suggest that HIV$^+$ donors with normal or elevated IL-2R may also have low proliferative responses.

IL-2Rα and β expression in PBMC from HIV$^-$ and HIV$^+$ donors

Decreased responsiveness of HIV$^+$ PBMC to IL-2 might reflect decreased expression of the β subunit of the IL-2R. We attempted to quantify expression of IL-2Rβ on PHA-stimulated lymphocytes by flow cytometry. Although no reproducible

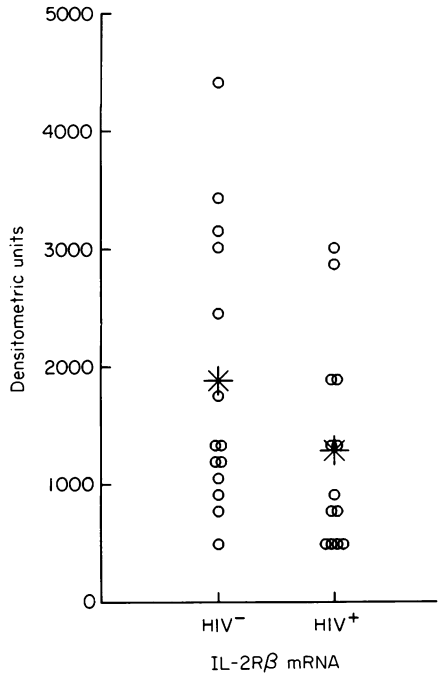

Fig. 5. Scattered representation of densitometric analysis of IL-2Rβ mRNA bands in unstimulated peripheral blood mononuclear cells (PBMC) from HIV⁻ and HIV⁺ donors. The concentrations of IL-2Rβ mRNA were normalized to 2000 densitometric units for actin in each individual. The mean IL-2Rβ mRNA in HIV⁻ donors was 1884 ± 308 (mean ± s.e.m., $n = 14$) and 1290 ± 241 ($n = 13$) in HIV⁺ donors. Individual data points and geometric means (*) are shown.

difference between cells from 12 HIV⁻ and 14 HIV⁺ donors was found in five separate experiments (data not shown), these studies were limited in that the weak fluorescence obtained with the anti-IL-2Rβ antibody did not permit accurate measurement of positively stained cells. Similar results have been reported in healthy donors [35,41–43].

As an alternative approach, therefore, we measured the relative expression of the IL-2Rα and β subunits in the high affinity IL-2R on stimulated lymphocytes from 16 HIV⁻ and 17 HIV⁺ donors (mean CD4 counts of 1154 ± 419 and 566 ± 292, respectively). ^{125}I-IL-2 at a concentration of 1 nM was covalently cross-linked to activated PBMC, and subunit expression was measured in cell lysates by SDS–PAGE/autoradiography. Both IL-2Rα and β chains were present in cells from all HIV⁻ and HIV⁺ individuals studied, and the ratio of α to β subunit expression, as measured by densitometry, was virtually the same in HIV⁻ and HIV⁺ donors (1·44 ± 0·31 *versus* 1·58 ± 0·41, respectively). In particular, no individuals with very high α/β ratios, as would be expected if there was a selective reduction in the expression of the β subunit, were found (data not shown).

Finally, we examined expression of IL-2R mRNA in both stimulated and unstimulated PBMC to determine whether HIV⁺ donors had alterations in expression of IL-2Rβ mRNA as compared to IL-2Rα mRNA. For the experiments with PHA-stimulated PBMC, cells from 14 HIV⁻ (mean CD4 count = 1459 ± 367 cells/μl) and 14 HIV⁺ donors (mean CD4 count = 671 ± 282 cells/μl) were studied. mRNA for both IL-2Rα ($P < 0·006$, Fig. 4a) and β ($P < 0·02$, Fig. 4b) were significantly lower in the HIV⁺ compared with the HIV⁻ donors, although there was considerable overlap across groups in both cases. For the experiments with unstimulated PBMC, cells from 14 HIV⁻ (mean CD4 count = 1274 ± 317 cells/μl) and 14 HIV⁺ donors (mean CD4 count = 617 ± 374 cells/μl) were studied. IL-2Rα mRNA was not detected in any of the individuals tested, but the IL-2Rβ mRNA was detected in cells from all individuals and was not significantly different for cells from HIV⁻ and HIV⁺ donors (Fig. 5).

DISCUSSION

The results of this study show that expression of high affinity IL-2R is reduced on PHA-stimulated PBMC from AIDS-free HIV⁺ donors. This reduction was shown both at the IL-2-binding level and the mRNA level, and persisted after numbers of viable cells in the tested cultures were standardized. These data confirm previous reports [1,4–8,29] and extend them by demonstrating reduced binding of ^{125}I-IL-2 under high affinity binding conditions, as well as reduced expression of the β subunit of the IL-2R, on a per viable cell basis. In addition, our data indicate that in many, if not most, HIV⁺ individuals the reduced proliferative response to IL-2 *in vitro* is closely related to the reduction in high affinity IL-2R. In contrast, it is not clearly tied to the CD4 lymphocyte count of the donor.

At the same time, our studies yielded no selective evidence to support previous suggestions of reduction in the β or α subunits of the IL-2R in HIV⁺ individuals [44]. Specifically, neither the ratio of α/β expression determined by cross-linking studies, nor the flow cytometric expression of the β subunit by single lymphocytes differed between HIV⁻ and HIV⁺ donors. The results of the cross-linking experiments and the measurements of IL-2R subunit mRNA, coupled with the reduced expression of high affinity IL-2R exhibited by most of the HIV⁺ donor cells studied, support the interpretation that a tandem reduction in the expression of both mRNA and protein for IL-2Rα and β subunits contributes to the deficiency of IL-2R chains and high affinity IL-2R. However, because cross-linking analysis may detect only about 10% of cells, and flow cytometry using antibody to the β subunit did not yield interpretable quantitative data, further studies are needed to address the question of whether individual T cells have selective changes in relative expression of the α and β subunits.

That other factors besides decreased expression of high affinity IL-2R contribute to reduced IL-2-induced proliferation by PBMC from HIV⁺ donors is suggested by the finding of borderline low IL-2 response in two HIV⁺ individuals who had normal or even increased numbers of high affinity IL-2R. Further studies will be needed to define whether this finding represents part of the normal spectrum of proliferation and IL-2R expression by T cells, or a second abnormality in T cells from HIV⁺ individuals. If the latter is true, one possible explanation would be the co-existence of populations of lymphocytes with elevated or reduced numbers of receptors. Defects in IL-2R signalling, T cell activation independent of the IL-2R, or T cell proliferation are other possibilities. Studies using limiting dilution analyses and clonal analysis [4,10,45] have demonstrated that HIV⁺ donors have increased proportions of lymphocytes that fail to respond normally to PHA and/or IL-2 and that the low or non-responsiveness of T cells to mitogens can not be attributed to the defects in accessory cell function [10]. Of note, we have previously demonstrated the accumu-

lation of non-responsive T cells in elderly humans, who are also characterized by an immune deficiency [46].

In view of the likelihood that multiple mechanisms are involved in the reduction of lymphocyte proliferation in HIV-1 infection, it will be important to define the heterogeneous types of T cells that are present in infected individuals at different stages of the infection. Such studies will clarify whether the alterations of IL-2R expression found in the present study are primary or secondary to the pathogenesis of immune deficiency caused by HIV-1.

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