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Superantigen activation of $CD4^+$ and $CD8^+$ T cells from HIV-infected subjects: role of costimulatory molecules and antigen-presenting cells (APC)

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SUMMARY

T cell receptor (TCR) triggering via superantigens induces decreased proliferative responses and increased apoptosis in T cells from HIV-infected patients compared with controls. Our aim was to delineate the role of intrinsic T cell defects, of APC dysfunction and of cytokines and costimulatory signal dysregulation in the deficient responses of $CD4^+$ and $CD8^+$ T cells from HIV^+ subjects to the superantigen Staphylococcus enterotoxin A (SEA). Proliferation and IL-2Rα up-regulation on SEA-stimulated $CD4^+$ and $CD8^+$ T cells in whole blood were reduced in HIV^+ subjects with CD4 counts < 500, compared with controls. Neither addition of IL-2, IL-12 or phorbol myristate acetate (PMA) nor neutralization of endogenous IL-10, tumour necrosis factor-alpha (TNF-α), TNF-β or transforming growth factor-beta (TGF-β) could restore the decreased activation by SEA. Possible intrinsic T cell defects were studied by presenting SEA on HLA-DR-transfected Chinese hamster ovary (CHO) cells, co-expressing LFA3 and/or CD80, to purified T cells. In this system $CD8^+$ T cells from most HIV^+ patients were hyporesponsive with regard to IL-2 production, IL-2Rα up-regulation and proliferation, whereas clearly reduced responses were only shown in $CD4^+$ T cells from AIDS patients. Similarly, apoptosis was increased in $CD8^+$ T cells from all patients, but only in $CD4^+$ T cells from AIDS patients. During HIV infection, the responses to TCR triggering through SEA are deficient in both T cell subsets. The intrinsic defect appears earlier during disease progression in purified $CD8^+$ T than in $CD4^+$ T cells, it occurs in conjunction with both CD2 and CD28 costimulation, and it is correlated with increased levels of apoptosis.

Keywords HIV infection T cells superantigen costimulation apoptosis

INTRODUCTION

Complete activation of T cells requires ligation of the T cell receptor (TCR) by antigen–MHC complexes together with a costimulatory signal delivered by the APC [1]. B7-1 (CD80) and B7-2 (CD86) provide important costimulatory signals through interaction with CD28 on T cells [2,3], but other interactions, including those between adhesion ligand–receptor pairs, such as intercellular adhesion molecule-1 (ICAM-1)/LFA-1 or LFA-3/CD2, are also involved [4,5]. In addition, various regulatory cytokines, including IL-2, IL-12, IL-10, tumour necrosis factor (TNF) and transforming growth factor (TGF) are important in the modulation of T cell activation [6].

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Bacterial superantigens, such as Staphylococcal enterotoxins A and B (SEA, SEB), mimic nominal antigens in that they are presented on MHC class II molecules to the TCR. Superantigens do not need intracellular processing and activate T cells bearing particular TCR Vβ regions regardless of their antigen specificity [7–11]. Both purified $CD4^+$ T and $CD8^+$ T cells can be fully activated by SEA presented on Chinese hamster ovary (CHO) cell lines, transfected with HLA-DR in combination with B7-1 and/or LFA-3 [12–16].

A major characteristic of infection with HIV is the progressive depletion of $CD4^+$ T cells *in vivo* and the development of opportunistic infections [17]. Decreased proliferative responses and increased apoptosis of the T cells from HIV-infected subjects during their *in vitro* culture with mitogens, recall antigens and superantigens have been observed [18,19]. Several non-exclusive mechanisms have been proposed to explain the T cell dysfunction. T cells from HIV^+ subjects could be intrinsically refractory and

prone to apoptosis, as a consequence of *in vivo* over-activation and replicative senescence [20–25]. HIV infection could disturb the proper functioning of APC [26]. Immunosuppressive and promiscuously cytolytic activities of $CD8^+$ T cells might be involved in premature elimination of regularly activated T cells. In addition, the cytokine network seems to be skewed towards suppression because of reduced production of stimulatory factors, e.g. IL-2 and IL-12, and/or over-production of negative regulators IL-10, TGF-β and TNF [6].

Using anti-CD3 as a T cell stimulus, we have observed impaired responses of purified $CD8^+$ T cells from HIV^+ subjects, while responses of their purified $CD4^+$ T cells remained comparable to those in controls [27]. The expression and function of the B7 receptor CD28 could partly explain these observations, since CD28 expression is dramatically lowered on $CD8^+$ T cells and only marginally decreased on $CD4^+$ T cells from HIV^+ subjects [27–29]. This model, however, cannot explain the progressive loss of $CD4^+$ T cell responses to recall antigen, observed during HIV infection. Therefore, antigen triggering through TCR might reveal deficiencies in the patient's $CD4^+$ T cells, which are not evident upon anti-CD3 stimulation.

Since superantigen stimulation closely resembles recall antigen stimulation, it is an attractive model with which to explore further the mechanisms of T cell dysfunction during HIV infection [30,31]. Therefore, we studied $CD4^+$ and $CD8^+$ T cell activation after TCR stimulation with SEA in the absence or presence of various costimulatory signals. The importance of intrinsic and immunoregulatory mechanisms in the lowered responses of T cell subsets from the patients was evaluated.

PATIENTS AND METHODS

Study population

HIV^+ subjects, all out-patients at the Institute of Tropical Medicine, were screened with two recombinant HIV1/HIV2 ELISA tests (Vironostika Uniform II (+ O) from Organon Teknika N.V., Turnhout, Belgium, and Enzygnost Anti-$HIV1/2^+$ from Behringwerke, Marburg, Germany) and confirmed by Western Blot (Diagnostic Biotechnology, Singapore). The patients were classified according to their absolute CD4 counts (based on the CDC 1993 revised classification system) [32]. HIV^- controls were recruited from laboratory personnel or from the local Blood Transfusion Centre.

Reagents and flow cytometry

Recombinant IL-2 and IL-12 were purchased from R&D Systems Europe (Abingdon, UK). IL-7 was obtained from Biosource International (Immunosource, Zoerdel, Halle, Belgium). Phorbol myristic acid (PMA) was obtained from Sigma (Sigma-Aldrich, Bornem, Belgium). SEA was purified as described elsewhere [33]. The following cytokine-neutralizing MoAbs were used: anti-TNF-α, anti-TNF-β and anti-TGF-β obtained from R&D Systems and anti-IL-10 obtained from Biosource International; IL-2Rα-specific (Tac, CD25) and IL-2Rβ-specific (clone TU27, CD122) MoAbs, kindly provided by Dr T. Waldmann (NIH, Bethesda, MD) and Dr K. Sugamura (Tohoku University, Sendai, Japan), respectively. CD3, CD4, CD8, CD25 and CD28-specific MoAbs and the appropriate isotype controls, conjugated to either FITC, PE or PerCP, were obtained from Becton Dickinson (Erembodegem, Belgium).

Sample acquisition was done on a FACScan flow cytometer (Becton Dickinson). Analysis was performed, using LYSIS I software.

Cell lines

CHO cells were transfected with cDNA encoding the HLA-DR4, B7 (CD80) or LFA-3 (CD58) molecule as described in detail elsewhere [12]. Four transfectant cell lines were used in this study: CHO-DR4, CHO-DR4/B7-1, CHO-DR4/LFA-3 and CHO-DR4/B7-1/LFA-3. Surface expression of HLA-DR, B7-1 and LFA-3 was checked by FACScan analysis regularly.

Whole blood cultures

EDTA-anticoagulated venous blood from patients and controls was washed three times and resuspended in complete medium consisting of RPMI 1640 supplemented with L-glutamine 2 mM, penicillin 100 U/ml, streptomycin 100 μg/ml and 10% heat-inactivated bovine calf serum (BCS; Hyclone, Logan, UT). Blood was diluted 1:5 in complete medium and transferred into cultures tubes. SEA was added in a final concentration of 10 and 100 ng/ml.

Lymphocyte separation and culture

Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-anticoagulated venous blood by density gradient centrifugation on Ficoll–Paque. After washing, PBMC were resuspended in complete medium and kept overnight in tissue culture dishes at 37°C. PBMC were depleted of monocytes with M450 CD14 Dynabeads (Dynal, Oslo, Norway) according to the manufacturer's instructions. $CD8^+$ and $CD4^+$ T cells were then positively selected with CD8 and CD4 Dynabeads as described elsewhere [34]. $CD8^+$ and $CD4^+$ T cells were cultured in round-bottomed 96-well plates at 1×10^6/ml and $0{\cdot}5\times10^6$/ml, respectively. The CHO cells were used at a 1:10 ratio (10^5 and $0{\cdot}5\times10^5$/ml for $CD8^+$ and $CD4^+$ T cells, respectively). SEA was added at a final concentration of 0·1 ng/ml.

Parameters of activation and apoptosis

For each subject, two serum activation markers were determined by commercial ELISA techniques (Eurogenetics, Tessenderlo, Belgium): β_2-microglobulin (β_2-M) and soluble IL-2Rα.

Whole blood, PBMC or T cells were cultured during 3–4 days. The proliferation of stimulated cells was determined by addition of 0·4 μCi ^{3}H-thymidine (specific activity 25 Ci/mmol) per well, during the final 8 h of culture. Thymidine uptake was measured by liquid scintillation counting.

Expression of the activation marker IL-2Rα (CD25) on cultured cells was determined by flow cytometry. Cultured cells were labelled with anti-CD3–PerCP, anti-CD4 or anti-CD8–PE and anti-CD25–FITC. The $CD4^+$ and $CD8^+$ T cells were gated and the percentage of CD25 brightly positive cells within each subset was calculated. Bright CD25 positivity was defined as expressing more than 100 arbitrary fluorescence units, since a proportion of resting T cells (mainly $CD4^+$) expresses CD25 at low level (between 10 and 100 arbitrary units).

The percentage of apoptotic $CD4^+$ and $CD8^+$ T cells was determined by two flow cytometric methods. As a reference method, a modification of the TUNEL technique (Boehringer, Mannheim, Germany) was used [35]. In the second method, the percentage of apoptotic $CD4^+$ or $CD8^+$ T cells was determined based on their altered light scatter properties (decreased forward scatter and increased side scatter). Using a combination of fluorescence gates on $CD3^+CD4^+$ or $CD3^+CD4^-$ cells and the

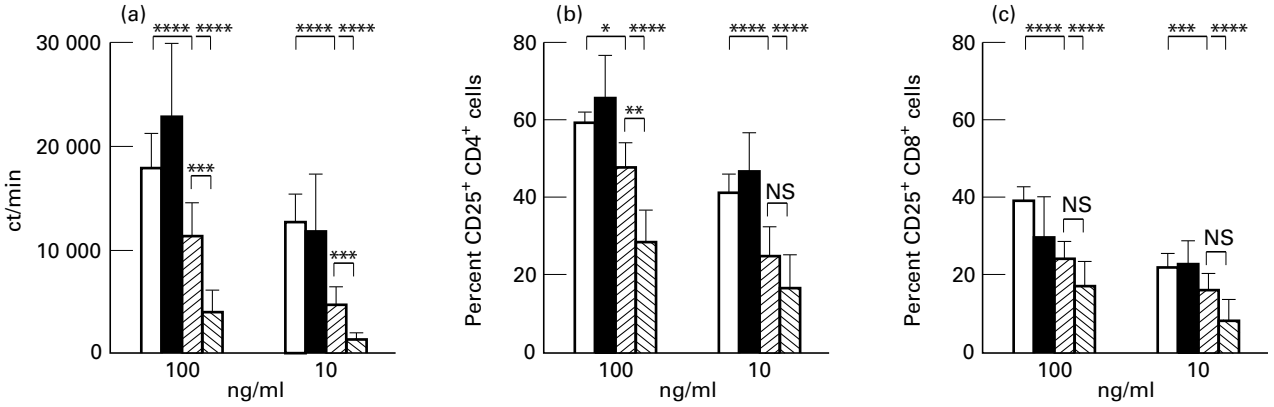

Fig. 1. Proliferative responses (a) and percentage of $CD25^+CD4^+$ (b) or $CD8^+$ T cells (c) after stimulation of whole blood with Staphylococcal enterotoxin A (SEA) (100 and 10 ng/ml). Flow cytometric analysis of CD25 expression was performed on day 4. HIV^- controls (□, $n = 46$) were compared with HIV^+ subjects grouped according to their CD4 counts: group 1, CD4 counts > 500 (■, $n = 11$); group 2, CD4 counts between 200 and 500 (▨, $n = 20$) and group 3, CD4 counts < 200 (▧, $n = 17$). Median ± confidence interval (CI) are given. Significant differences between patients and controls are indicated: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}002$; $****P < 0{\cdot}0005$. NS, Not significant.

light scatter gates, the percentage of apoptotic T cells was calculated.

IL-2 production was determined with the CTLL-2 bio-assay. The sensitivity of the IL-2 measurement was increased by adding IL-2Rα and β-chain-specific MoAbs (clones TAC and TU27, respectively) during the 72-h culture period. Blocking of cytokine–receptor interactions thus prevented IL-2 consumption [36]. The cut-off level of the assay was 50 pg/ml.

Statistical analysis

Non-parametric tests were used throughout the study. Data are presented as median ± 95% confidence interval (CI), unless stated otherwise. The Mann–Whitney *U*-test was employed to compare two different subject groups. Paired data sets were analysed using the Wilcoxon matched-pairs signed-ranks test. Correlation analysis was performed using the Spearman's rank correlation test.

RESULTS

Both $CD4^+$ and $CD8^+$ T cell subsets from HIV^+ subjects are deficient to SEA stimulation in whole blood

The responses of T cells in whole blood from 48 HIV^+ subjects and 46 controls upon stimulation with SEA were determined (Fig. 1). Proliferative responses (Fig. 1a) and expression of CD25 on $CD4^+$ (Fig. 1b) or $CD8^+$ T cells (Fig. 1c) in whole blood cultures from HIV^+ subjects with CD4 counts < 500 were significantly impaired compared with controls ($P < 0{\cdot}0005$) (Fig. 1). All responses were lower in patients with CD4 counts < 200 compared with patients with CD4 counts between 200 and 500. Since only the bright $CD25^+$ cells were considered (see Patients and Methods), CD25 expression on cells stimulated in medium was always < 1%.

Since the impaired response *in vitro* could be related to pre-existing activation *in vivo*, we determined the serum activation markers (β_2-M and sIL-2Rα) and correlated them with the *in vitro* responses for each patient. The two serum activation markers were increased in HIV-infected individuals, irrespective of their CDC classification, and both markers strongly correlated with each other ($r = 0{\cdot}70$; $P < 0{\cdot}001$). All *in vitro* responses to SEA, proliferation and up-regulation of membrane CD25 correlated with each other (r between 0·59 and 0·82, $P < 0{\cdot}001$) and inversely correlated with the stage of the disease (as defined by CDC classification; r between – 0·45 and – 0·74; $P < 0{\cdot}001$). However, no significant correlation was found between the serum activation markers and the *in vitro* responses to SEA.

Modulation of the hyporesponse in $CD4^+$ and $CD8^+$ T cells from HIV^+ persons

We determined whether a lack of costimulatory cytokines (e.g. IL-2, IL-12) or the presence of inhibitory cytokines (e.g. IL-10, TGF-β) contributed to the decreased responses of $CD4^+$ and $CD8^+$ T cell subsets from HIV^+ subjects. Whole blood from patients and controls was stimulated with SEA in the absence or presence of different cytokines or cytokine-neutralizing antibodies.

Addition of IL-2 (up to 10 ng/ml) or IL-12 (up to 50 ng/ml) had no enhancing effect on the expression of CD25 on $CD4^+$ or $CD8^+$ T cells from patients (data not shown). Furthermore, none of the neutralizing cytokine MoAbs specific for IL-10, TNF-α, TNF-β or TGF-β could restore the deficient responses to SEA (data not shown). Direct triggering of protein kinase C (PKC) with PMA induced a significant increase in IL-2Rα expression on $CD4^+$ and

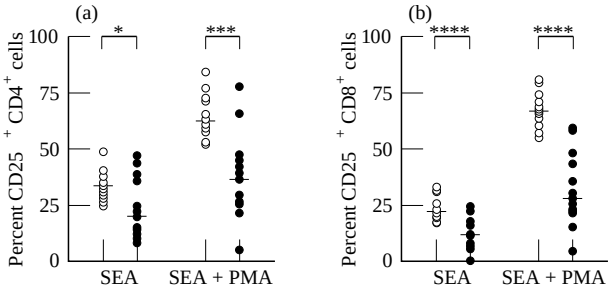

Fig. 2. Expression of CD25 on $CD4^+$ (a) or $CD8^+$ T cells (b) from HIV^- controls (○) and HIV^+ subjects (●). Whole blood was cultured with Staphylococcal enterotoxin A (SEA) alone (10 ng/ml) or with SEA (10 ng/ml) plus phorbol myristate acetate (PMA; 1 ng/ml). Paired sample analysis revealed significant differences between SEA and SEA plus PMA in both T cell subsets from both patients and controls ($P < 0{\cdot}0001$). Significant differences between patients and controls are indicated: $*P < 0{\cdot}05$; $***P < 0{\cdot}002$; $****P < 0{\cdot}0005$.

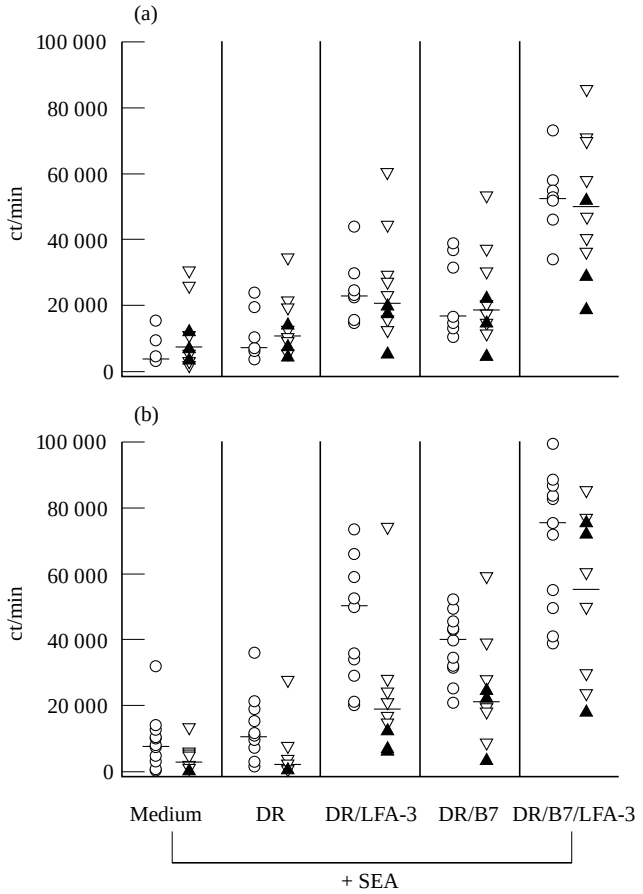

Fig. 3. Proliferative responses of purified $CD4^+$ (a) or $CD8^+$ (b) T cells after a 4-day stimulation with Staphylococcal enterotoxin A (SEA; 0·1 ng/ml) in the absence or presence of various Chinese hamster ovary (CHO) cell lines. Individual data are presented for seven (a) or 11 (b) HIV^- subjects (○), seven HIV^+ subjects with CD4 counts > 200 (▽) and three HIV^+ subjects with CD4 counts < 200 (▲). The median proliferative response for patients or controls is indicated with a horizontal line.

$CD8^+$ T cells from patients and controls. However, the differences between patient and control T cells were actually larger in cultures stimulated with SEA + PMA compared with stimulation with SEA alone (Fig. 2). PMA alone induced less than 5% $CD25^+$, $CD4^+$ or $CD8^+$ T cells.

Thus, the deficient responses of $CD4^+$ and $CD8^+$ T cells from HIV^+ subjects in this model of SEA activation are probably not due to the lack of stimulatory cytokines, an excess of inhibitory cytokines or a selectively deficient triggering of PKC.

Lowered responses in purified T cell subsets from HIV^+ subjects

Next, we determined the proliferative responses of highly purified $CD4^+$ and $CD8^+$ T cells from HIV^+ subjects and controls upon stimulation with SEA. Efficient SEA presentation was achieved with CHO cells transfected with HLA-DR and B7 and/or LFA-3. The proliferation of $CD8^+$ T cells was significantly impaired in HIV^+ persons compared with controls, even when B7-1 and LFA-3 were simultaneously present on the CHO cells (Fig. 3b) (patients *versus* controls: $P < 0{\cdot}01$ for CHO-DR/LFA-3 and $P < 0{\cdot}05$ for all other stimuli). In contrast, there was no significant difference between the responses of $CD4^+$ T cells from patients compared with controls, although the proliferation of the $CD4^+$ T cells from the AIDS patients (< 200 CD4/mm^3) was at the lower end of the spectrum (Fig. 3a).

IL-2 production upon stimulation with SEA plus the various CHO transfectants was significantly lower in purified $CD8^+$ T cells from patients compared with controls (Table 1). Moreover, the deficient proliferative responses of purified $CD8^+$ T cells could not be restored upon addition of IL-2 (up to 10 ng/ml) (data not shown).

SEA-induced apoptosis in $CD4^+$ and $CD8^+$ T cells from HIV^+ individuals

In the 3-day cultures of PBMC, spontaneous apoptosis was very low in $CD4^+$ and $CD8^+$ T cells from controls, but elevated in the T cells from patients. Stimulation with SEA selectively induced a two-fold increase of apoptosis in $CD4^+$ T cells compared with

Table 1. Production of IL-2 by purified $CD8^+$ T cells from patients and controls

	SEA + CHO-DR/LFA-3	SEA + CHO-DR/B7	SEA + CHO-DR/B7/LFA-3
HIV^- 1	1100	8050	42 800
2	700	6200	34 800
3	800	3500	4950
4	200	2600	4300
5	200	1600	2150
Mean ± s.e.m.	600 ± 320	4390 ± 2188	17 800 ± 16 800
HIV^+ 1	150	1600	1000
2	100	150	150
3	150	200	750
4	200	300	750
Mean ± s.e.m.	150 ± 25*	563 ± 519*	663 ± 256*

Production of IL-2 by $CD8^+$ T cells from five HIV^- controls and four HIV^+ subjects. Cells were stimulated with Staphylococcal enterotoxin A (SEA; 0·1 ng/ml) in the presence of various Chinese hamster ovary (CHO) transfectants. IL-2 was measured with the CTLL-2 bioassay and expressed in pg/ml (cut-off 50 pg/ml). Mean ± s.e.m. is given for each stimulus (*$P < 0{\cdot}05$). CD4 counts were 968, 156, 49 and 39 for HIV^+ donors 1, 2, 3 and 4, respectively.

medium alone, whereas the levels of apoptosis in the unstimulated and SEA-activated $CD8^+$ T cells were similar (data not shown).

We then wanted to investigate the possible effects of B7 costimulation on apoptosis and the relation between SEA-induced activation and apoptosis in purified or non-purified T cells. The degree of apoptosis was determined in $CD4^+$ and $CD8^+$ T cells upon stimulation with SEA in the absence or presence of the transfected CHO cells. In parallel, we determined the up-regulation of IL-2Rα (CD25) on the cell membrane. $CD4^+$ and $CD8^+$ T cells, either purified or within PBMC, were analysed simultaneously for each patient and control.

When cultured in PBMC, $CD4^+$ T cells from AIDS patients only (CD4 counts < 200) showed decreased up-regulation of CD25 and increased levels of apoptosis, whereas $CD4^+$ T cells from non-

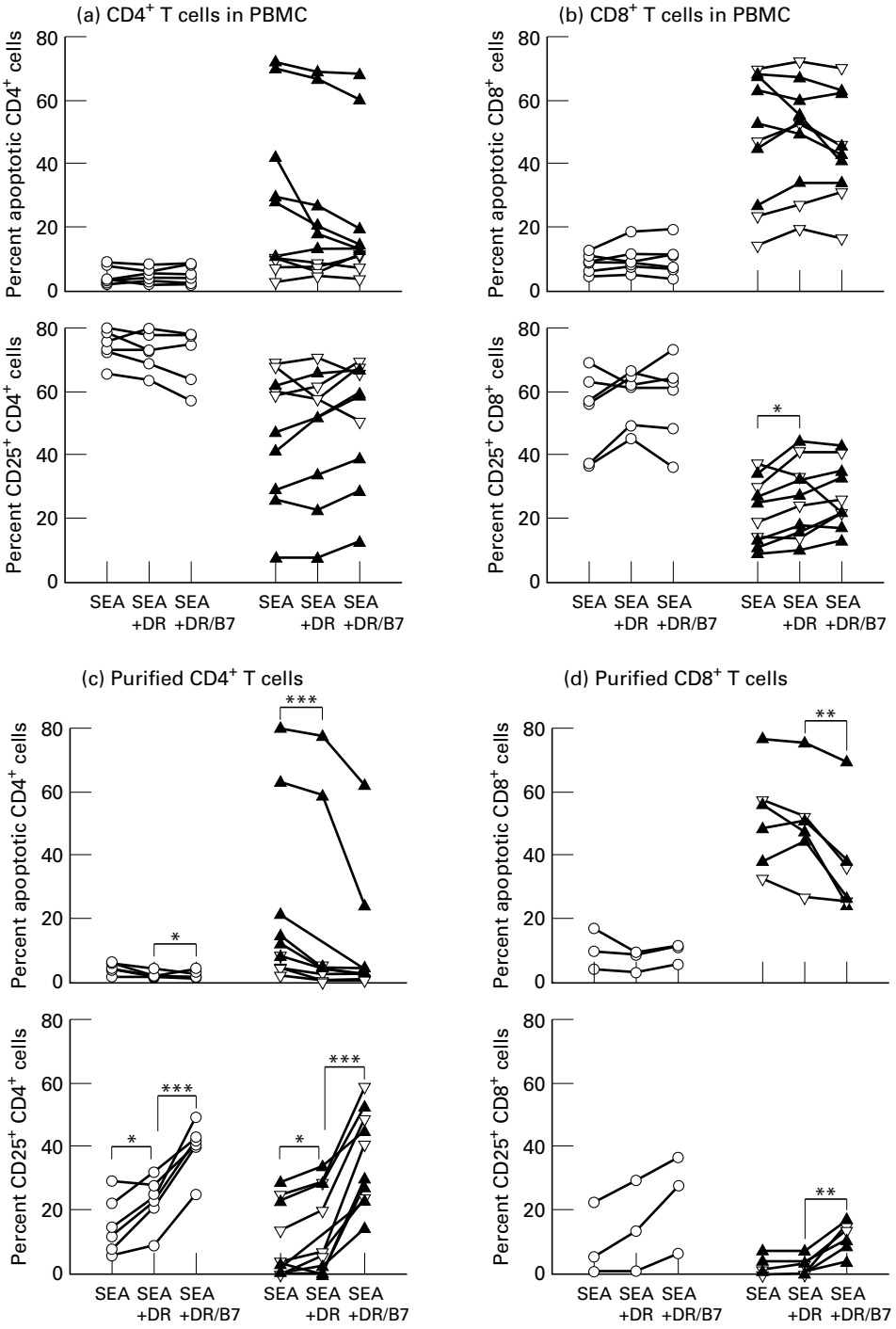

Fig. 4. Apoptosis and CD25 expression in $CD4^+$ (a,c) and $CD8^+$ T cells (b,d) from six HIV^- controls (○) and 10 HIV^+ subjects (▲). (a,b) Data obtained in peripheral blood mononuclear cell (PBMC) cultures. (c,d) Data from purified T cell cultures. The patients were divided into two groups: CD4 counts > 200 (▽) and CD4 counts < 200 (▲). The percentage of apoptotic T cells was defined by FCS-SSC analysis. Significant differences between the different stimuli are indicated: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}002$.

AIDS patients responded similarly compared with controls (Fig. 4a). In contrast, $CD8^+$ T cells, studied in the same PBMC cultures, displayed decreased CD25 up-regulation and increased apoptosis, irrespective of the disease stage (Fig. 4b).

Analysis of purified $CD8^+$ T cells showed that CD25 up-regulation was deficient in patients compared with controls. This finding parallels the experiments in PBMC from the same patients. Remarkably, $CD4^+$ T cells from the patients up-regulated CD25 almost to the same extent as controls (Fig. 4c,d). Also, purified $CD4^+$ T cells, from AIDS patients only, displayed higher levels of apoptosis, whereas purified $CD8^+$ T cells from both AIDS and non-AIDS patients showed increased cell death (Fig. 4c,d).

Addition of the CHO transfectants to PBMC cultures did not significantly alter the degree of apoptosis or the induction of CD25 on $CD4^+$ or $CD8^+$ T cells (Fig. 4a,b). In purified $CD4^+$ T cells from the patients, a significant reduction of apoptosis was seen in the presence of the CHO-DR and CHO-DR/B7 transfectant, while only the latter CHO transfectant reduced apoptosis in their $CD8^+$ T cells (Fig. 4c,d). The expression of CD25 was up-regulated by the transfectants in purified T cells from both patients and controls. It remained below normal levels in purified $CD8^+$ T cells from the patients, whereas purified $CD4^+$ T cells expressed similar levels of CD25 in both patients and controls.

DISCUSSION

The present study shows that TCR triggering with the superantigen SEA results in lowered CD4 and CD8 T cell responses (proliferation and IL-2Rα expression) in whole blood from HIV^+ subjects with CD4 counts < 500, compared with controls. Addition of costimulatory molecules or cytokines, neutralization of potentially suppressive cytokines or even direct triggering of the PKC did not restore the defect. The decreased *in vitro* responses in patients were accompanied by but not correlated with elevated levels of activation markers in the serum. Upon stimulation of purified T cells with SEA and HLA-DR expressing CHO transfectants as artificial APC, $CD8^+$ T cells of most patients showed reduced IL-2Rα expression, lower IL-2 production, deficient proliferation and increased apoptosis, whereas only $CD4^+$ T cells from AIDS patients showed increased apoptosis and a low proliferation. The strong tendency to apoptosis in purified T cells from HIV^+ subjects was reduced by superantigen stimulation in the context of the HLA-DR-expressing APC and even more so by the simultaneous triggering of CD28 on the T cells through B7-expressing APC.

Deficient responses to SEA stimulation on the level of PBMC have been described earlier [30,31]. Brinchmann *et al*. [30] already suggested that $CD8^+$, but not $CD4^+$ T cells, from HIV^+ subjects were hyporesponsive to SEA. However, these authors did not analyse the role of APC-dependent or intrinsic defects, nor consider immunoregulatory cytokines. The impaired responses in $CD8^+$ T cells, shown in the present paper, could be largely explained by an intrinsic deficiency, since the defects were present to the same extent in purified $CD8^+$ T cells as in the context of PBMC. This finding confirms our previous observations on hyporesponsiveness of $CD8^+$ T cells from HIV^+ subjects to anti-CD3 and allogenic stimulation [27,34]. The present data also imply that the $CD8^+$ T cell defect is not only the consequence of reduced CD28 expression, since the hyporesponsiveness was equally pronounced in B7- and LFA-3-dependent activation. B7 costimulation slightly reduced the elevated apoptosis. It remains to be determined whether a possibly altered expression of CTLA4, another B7 receptor, on $CD8^+$ T cells from HIV^+ patients has an impact on cell function and apoptosis. The $CD8^+$ T cell defect in HIV^+ subjects appears to be generalized and almost irreversible, since addition of various cytokines or anti-cytokine antibodies, direct activation of PKC or even costimulation via B7 could not fully reverse the lowered responses.

Recent studies in HIV-infected T cell clones have shown that Tat could induce increased IL-2 secretion upon triggering of CD3 plus CD28 [44]. This hyperactivation induced by Tat could contribute to the chronic $CD8^+$ T cell activation and predispose for the high number of apoptotic $CD8^+$ T cells in HIV^+ subjects. Recently, it was also shown that a reduced level of Bcl-2 in $CD8^+$ T cells from HIV^+ subjects was associated with a higher level of apoptosis upon *in vitro* culture [45]. Thus the profound intrinsic CD8 T cell deficiency might be due to the excessive *in vivo* activation. This activation-induced refractoriness of $CD8^+$ T cells could constitute an important factor in the failing defence against opportunistic viral and tumoural diseases during HIV infection.

A more puzzling observation in the present study, at least in patients with CD4 counts < 500, was the reduced CD25 up-regulation on $CD4^+$ T cells in whole blood, whereas a normal CD25 up-regulation was seen on purified $CD4^+$ T cells. Only purified $CD4^+$ T cells from AIDS patients (and not from those with CD4 counts between 200 and 500) showed increased apoptosis and reduced proliferation. We wondered whether insufficient costimulation or excessive suppression, exerted by other mononuclear cell types, could explain this discrepancy between purified and non-purified $CD4^+$ T cells. Neither the addition of IL-2, IL-12 or PMA nor the neutralization of various suppressive cytokines could restore the lowered expression of CD25 on the patient's $CD4^+$ T cells in whole blood. Moreover, removal of $CD8^+$ T cells, natural killer (NK) cells or monocytes from whole blood also did not result in an up-regulation of CD25 on the patient's $CD4^+$ T cells (Vingerhoets *et al*., unpublished observations).

The question whether defective APC function somehow contributed to the $CD4^+$ T cell dysfunction in the context of PBMC was further indirectly addressed by adding the CHO transfectants to SEA-stimulated PBMC, but neither increase of CD25 nor reduction of elevated apoptosis could be obtained. Nevertheless, the purified $CD4^+$ T cells from the same patients showed CD25 up-regulation in the normal range and their elevated apoptosis was reduced significantly when stimulated with SEA presented on the same CHO transfectants. Taking these data together, we conclude that the hyporesponsiveness of $CD4^+$ T cells is at least partly due to an intrinsic defect in AIDS patients, whereas the defective response of $CD4^+$ T cells from HIV^+ subjects with CD4 counts 200–500 are secondary to extrinsic factors. The presented experiments, however, did not identify the source or the nature of the suppressive mechanism.

The intrinsic defect to SEA stimulation, which we observed in $CD4^+$ T cells from AIDS patients and in $CD8^+$ T cells from many HIV^+ subjects, might result from a generalized T cell dysfunction or from a Vβ-specific deletion or anergy, induced by the hypothetical HIV-related superantigen [37–42]. For SEB, Vβ-specific anergy was recently shown in a subset of HIV-infected subjects [43]. Since the Vβ specificities of SEA and SEB are different, additional studies are needed to know whether a selective anergy might partially contribute to the presently observed intrinsic component in the T cell hyporesponse to SEA. However, the hyporesponsiveness among $CD8^+$ T cells was seen after both SEA and anti-CD3 stimulation [27,34], implying that the abnormal

response is related to an intrinsic defect rather than changes in Vβ repertoire.

In conclusion, both $CD4^+$ and $CD8^+$ T cells from HIV-infected subjects with CD4 counts < 500 are hyporesponsive to SEA stimulation. The defect in $CD8^+$ T cells seems largely due to a generalized intrinsic refractory state in this subset. The deficiency in CD4 T cells is more complex and might result from an intrinsic anergy, present in some AIDS patients, and from an earlier occurring extrinsic down-regulatory mechanism. Further studies are needed to identify the nature of immunosuppression in this system and to characterize the molecular regulation of apoptosis in $CD8^+$ T cells.

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