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$CD8^+$ T cells and not $CD4^+$ T cells are hyporesponsive to CD28- and CD40L-mediated activation in HIV-infected subjects

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SUMMARY

T cell dysfunction in HIV-infected subjects could be the consequence of altered sensitivity of $CD4^+$ or $CD8^+$ T cells to various costimulatory signals. Therefore, we studied proliferation and cytokine production in highly purified $CD8^+$ and $CD4^+$ T cells from HIV-infected and HIV^- subjects, induced by co-activation via cell-bound CD80, CD86 and CD40 or by allo-activation. Regardless of the nature of the first and the costimulatory signal, $CD8^+$ T cells from patients proliferated consistently less than controls, while responses from $CD4^+$ T cells were similar in patients and controls. This phenomenon was observed after ligation of CD28 combined with anti-CD3 or phorbol myristate acetate (PMA), but also after allogeneic stimulation and after activation by CD40 and anti-CD3. Anti-CD3 combined with CD80 or CD86 induced a mixed Th1/Th2-type cytokine profile in both $CD4^+$ and $CD8^+$ T cells from controls, whereas anti-CD3 plus CD40 induced only low levels of Th2-type cytokines and no interferon-gamma (IFN-γ) in $CD4^+$ T cells. Compared with controls, $CD4^+$ T cells from patients produced slightly lower levels of IL-10 but equal amounts of IFN-γ, IL-4 and IL-5, while $CD8^+$ T cells from patients produced less of all cytokines tested. In conclusion, responses of purified $CD4^+$ T cells from HIV^+ subjects to various costimulatory pathways are relatively intact, whereas $CD8^+$ T cells are hyporesponsive at the level of proliferation and cytokine production. A generalized intrinsic $CD8^+$ T cell failure might contribute to viral and neoplastic complications of HIV infection.

Keywords HIV CD28 CD40L cytokines $CD8^+$ T lymphocyte

INTRODUCTION

One of the major characteristics of infection with HIV is a progressive depletion of $CD4^+$ T cells associated with the development of opportunistic infections [1]. In addition, both $CD8^+$ and $CD4^+$ T cells display phenotypical and functional signs of excessive activation and anergy [2–4]. $CD8^+$ T cells have increased promiscuous cytotoxic and immunosuppressive activity, while proliferative responses of $CD4^+$ T cells to recall antigens disappear early in the course of infection [5–8]. Furthermore, HIV infection has been associated with dysregulation of the Th1/Th2-type cytokine profile: a Th1 to Th2 shift has been observed by some authors, while others described more complex changes [9–12].

Optimal proliferation and cytokine production of T cells requires ligation of the T cell receptor (TCR) by antigen–MHC complexes together with a costimulatory signal delivered by the antigen-presenting cell (APC) [13]. The most important costimulatory signals are provided by the members of the B7 family (CD80 and CD86) which interact with CD28 on resting $CD4^+$ and $CD8^+$ T cells and with both CD28 and CTLA4 on activated T cells [14]. Another important costimulatory pathway involves CD40–CD40 ligand (CD40L) interaction. CD40L expression is mainly but not exclusively induced on $CD4^+$ T cells after optimal activation [15]. CD40 cross-linking on B cells or APC by T cells expressing CD40L results in activation and differentiation of the former [16,17]. $CD4^+$ and $CD8^+$ T cells can be activated by accessory cells through cross-linking of either CD40L or CD40 [18,19]. The effect of CD80, CD86 or CD40 costimulation on cytokine production in normal T cells seems to vary according to the activation and maturation state of the T cell [18,20–22].

Apart from $CD4^+$ T cell depletion, other mechanisms might be responsible for the progressive immunodeficiency in HIV-infected individuals. Inadequate APC function, dysregulation of $CD4^+$–$CD8^+$ T cell interactions and intrinsic defects in T cell activation have all been investigated, with variable and contradictory results.

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Although in HIV-infected individuals a decreased expression of CD28 and decreased proliferation of T cells to CD28-dependent stimuli has been documented [23–26], it is still not clear whether this defect is due to impaired signalling through TCR/CD3 (signal one) or through CD28 (signal two) [27–30]. Also, no data are available on the role of other costimulatory pathways, including CD40-CD40L, in HIV$^+$ subjects.

In the present study, intrinsic T cell responses from HIV-infected and HIV$^-$ persons were compared after triggering of distinct costimulatory pathways. Highly purified CD8$^+$ and CD4$^+$ T cell subsets were assayed in parallel and both proliferative capacity and production of Th1-type and Th2-type cytokines were measured.

MATERIALS AND METHODS

Study population

HIV$^+$ subjects, all out-patients at the Institute of Tropical Medicine, were screened with a recombinant HIV1/HIV2 ELISA (Abbott Diagnostica, Wiesbaden, Germany) and confirmed by Western blot (Dupont de Nemours, Singapore). Their median age was 37 years (range 23–64 years). Patients were classified according to the CDC 1993 revised classification system and their absolute CD4$^+$ T cell count [31]. The absolute number of CD4$^+$ T cells/μl (mean $\pm$ s.d.) was 415 ± 173 (range 206–911) and 93 ± 55 (range 11–195) for HIV$^+$ subjects, with >200 CD4$^+$ T cells/μl and $\leq$200 CD4$^+$ T cells/μl, respectively. HIV$^-$ controls were recruited from laboratory personnel (median age 34 years, range 25–43 years).

Monoclonal antibodies and flow cytometry

Anti-CD3 (clone UCHT1) was purified from hybridoma supernatant. Anti-CD28 (clone 9.3) was kindly provided by Dr C. June (Naval Medical Research Institute for Immunology, Bethesda, MD). CTLA4-Ig fusion protein was donated by Dr P. Linsley (Bristol-Meyers-Squibb Pharmaceutical Research Institute, Seattle, WA). Anti-CD80 (clone B7-24) and anti-CD86 (clone IT2.2) were previously described [32,33]. MoAbs to the following CD antigens were purchased from Becton Dickinson (Erembodegem, Belgium): CD3, CD4, CD8, CD14, CD16, CD28, CD56 and HLA-DR. Samples were analysed on a FACScan (Becton Dickinson) using LYSIS I software.

Lymphocyte subsets

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 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) or 10% pooled normal human serum (pNHS). PBMC were kept overnight in tissue culture dishes at 37°C. CD8$^+$ and CD4$^+$ T cells were then positively selected with an immunomagnetic separation technique (Dynabeads M-450 CD8 or M-450 CD4 and DETACHaBEAD; Dynal, Oslo, Norway). The purity (median $\pm$ confidence interval (CI)) for CD8$^+$ T cells was $92 \pm 1\%$ and $90 \pm 2\%$ and for CD4$^+$ T cells, $93 \pm 2\%$ and $97 \pm 1\%$ in patients and controls, respectively. The main contaminating subset in the CD8$^+$ fraction consisted of natural killer (NK) cells, while the main contamination subset in the CD4$^+$ T cell fractions consisted of CD8$^+$ T cells. Monocyte contamination was always <0·5%.

Cell lines

The 3T6 fibroblast transfected with human FcγRIIa and human CD80, designated 3T6-CD32/B7 [34], was cultured as described [28]. P815 cells either not transfected (parental) or transfected with CD40, CD80 or CD86 were obtained from DNAX Research Institute of Molecular and Cellular Biology (Palo Alto, CA) [35]. P815 cells were cultured in complete medium with 10% BCS. Two allogeneic Epstein–Barr virus (EBV)-transformed B cell lines (ARC and Raji) expressing CD80, CD86 and CD40, and a CD80/CD86$^+$ T cell line (MT4) were maintained in complete medium with 10% BCS.

Cell cultures

Purified CD8$^+$ or CD4$^+$ T cells were activated using 3T6-CD32/B7 cells as described [28]. Briefly, 10^4 3T6-CD32/B7 transfectants, treated with mitomycin C (50 μg/ml for 30 min), were cocultured with 5×10^4 CD8$^+$ or CD4$^+$ T cells in the presence of anti-CD3 (clone UCHT1, 15 μg/ml). In parallel, T cells were stimulated with 3T6-CD32/B7 cells and phorbol myristate acetate (PMA; 10 ng/ml) or with PMA and anti-CD28 MoAb (clone 9.3, 100 ng/ml). Stimulation with PMA alone or with UCHT1 and 3T6 cells, transfected with CD32 alone, did not induce proliferation in CD8$^+$ or CD4$^+$ T cells. Cocultures of 2×10^4 mitomycin C-treated P815 transfectants with 10^5 CD8$^+$ or CD4$^+$ T cells in the presence of anti-CD3 (OKT3, 1 μg/ml) were performed during 4 days in 96-well flat-bottomed plates in complete medium with 10% BCS. Stimulation with OKT3 alone did not induce a proliferative response in T cells.

The MLR was performed in 96-well round-bottomed plates by coculturing $2{\cdot}5 \times 10^5$ CD4$^+$ or CD8$^+$ T cells with 10^4 mitomycin C-treated ARC, Raji or MT4 cells in complete medium with 10% pNHS for 6 days. In some cases, the interaction between CD28 and/or CTLA4 on the T cells with B7 molecules was blocked by preincubating the stimulator cells either with anti-CD80 MoAb (1 μg/ml) and/or anti-CD86 MoAb (1 μg/ml), or with CTLA4-Ig (10 μg/ml) for 15 min at 37°C before the addition of responder CD8$^+$ or CD4$^+$ T cells. In two experiments, cyclosporin A (CsA; 1 μg/ml; Sandoz, Basel, Switzerland) was added in combination with anti-CD80 and anti-CD86 MoAb.

Proliferation of stimulated cells was determined by addition of 0·4 μCi ^{3}H-thymidine (specific activity 5·0 Ci/mmol) per well, during the final 8 h of culture. Thymidine uptake was measured by liquid scintillation counting.

Cytokine determinations

Supernatants from cultures of stimulated CD8$^+$ or CD4$^+$ T cells were harvested at 72 h and stored at -30°C. Interferon-gamma (IFN-γ) was measured by ELISA (Eurogenetics, Tessenderlo, Belgium). IL-4, IL-5 and IL-10 were determined using capture MoAb and biotinylated detection MoAb purchased from Pharmingen (San Diego, CA). Streptavidin–horseradish peroxidase (HRP) was purchased from Jackson ImmunoResearch Labs (West Grove, PA).

Statistical analysis

Non-parametric analysis was used throughout the study. Data are presented as median $\pm$ 95% CI, unless stated otherwise. Levels of significance were determined using the Mann–Whitney *U*-test. Linear regression was performed to determine the correlation of the *in vitro* response in function of the absolute CD4$^+$ T cell count or the *in vivo* expression of CD28.

RESULTS

CD8$^+$ but not CD4$^+$ T cells from HIV$^+$ subjects have reduced responses to CD28 costimulation, regardless of the primary signal

To extend and clarify our previous findings on response to CD28 costimulation, we compared different methods of CD28-dependent activation in T cells from HIV$^-$ controls and HIV$^+$ patients at various stages of the disease. Purified CD8$^+$ and CD4$^+$ T cells were activated via anti-CD3 or PMA, as signal one, and 3T6-CD32/B7 cells or an agonistic anti-CD28 MoAb, as signal two. CD8$^+$ T cells from the patients showed clearly lower levels of proliferation than CD8$^+$ T cells from controls in all culture conditions (Table 1a). In contrast, CD4$^+$ T cells from HIV$^+$ and HIV$^-$ subjects showed no difference in proliferative responses to costimulation via CD28 (Table 1b).

A significant positive correlation was found between the initial CD28 expression on CD8$^+$ T cells and their subsequent response to costimulation through this receptor (Fig. 1a). There was no correlation with the absolute CD4$^+$ T cell count (Fig. 1b), nor with the clinical stage (CDC-A, B or C) (Fig. 1a,b).

Stimulation with PMA + 3T6-CD32/B7 and PMA + anti-CD28 yielded similar correlations as for UCHT1 + 3T6-CD32/B7.

CD8$^+$ but not CD4$^+$ T cells from HIV$^+$ subjects show impaired proliferative responses to allogeneic stimulation

Allo-responses are known to depend on multiple costimulatory signals. Since decreased responses of PBMC from HIV$^+$ persons to an allogeneic stimulus are a marker of immunodeficiency, we studied the responses of pure T cell subsets to different allogeneic stimulators, including two B cell lines (Raji and ARC) and one T cell line (MT4). CD8$^+$ T cells from HIV$^+$ persons proliferated less than control CD8$^+$ T cells to all cell lines tested (Table 2a). In contrast, CD4$^+$ T cells from patients and controls responded to the same extent (Table 2b). No correlation was found between expression of CD28, absolute CD4$^+$ T cell count or the clinical stage on one hand, and the proliferative responses of CD8$^+$ T cells on the other hand, for any of the allogeneic stimuli used (Fig. 1c,d).

The allo-stimulation of CD8$^+$ T cells induced by ARC or Raji was only partially inhibited by blocking B7–CD28 interactions using anti-CD80/CD86 MoAb or CTLA4-Ig, but could be completely blocked by the combination of anti-CD80/CD86 MoAb with CsA (Fig. 2). Importantly, CD8$^+$ T cells from patients remained deficient compared with control CD8$^+$ T cells, even after blocking of CD28 signalling. Taken together with the absence of correlation with CD28 expression, data indicate that the observed deficiency was not due to impaired CD28 function alone.

Triggering through CD40L induces deficient responses in CD8$^+$ but not CD4$^+$ T cells from HIV$^+$ subjects

To investigate whether the CD8$^+$ T cell deficiency was present in other costimulatory pathways, we compared CD40L-dependent and CD28-dependent costimulation of T cells using P815 cells transfected with CD40, CD80 or CD86 together with anti-CD3. First, CD8$^+$ and CD4$^+$ T cells from HIV$^+$ and HIV$^-$ subjects proliferated more strongly to CD80 and CD86 compared with CD40 costimulation (Fig. 3). Parental P815 also induced some proliferation, possibly as a consequence of the interaction between mouse and human adhesion molecules [18]. Proliferative responses of CD8$^+$ T cells from HIV$^+$ persons were always significantly lower than in controls, whereas the responses of CD4$^+$ T cells were not different between HIV$^+$ subjects and controls. In fact, CD40 costimulation more than doubled the proliferative response in control CD8$^+$ T cells (median ct/min increased from 5751 to 15 188 in P815-par and P815-CD40, respectively) but failed to enhance proliferation in CD8$^+$ T cells from patients.

Cytokine production in response to CD40L and CD28 triggering is impaired in CD8$^+$ T cells from HIV$^+$ subjects

Several reports described a shift from Th0/Th1-type to Th2-type

Table 1. Proliferative responses of CD8$^+$ T cells (a) or CD4$^+$ T cells (b) to CD28 costimulation

a. CD8$^+$ T cells

Stimulus	HIV$^-$	(*n*)	HIV$^+$	(*n*)	*P*
Medium	119 ± 66	(12)	137 ± 68	(14)	NS
UCHT1 + 3T6-CD32/B7	111 995 ± 29 263	(12)	46 601 ± 10 970	(17)	<0·001
PMA + 3T6-CD32/B7	39 133 ± 24 611	(12)	16 087 ± 3386	(16)	<0·01
PMA + anti-CD28	35 382 ± 21 958	(12)	18 762 ± 7381	(15)	0·08

b. CD4$^+$ T cells

Stimulus	HIV$^-$	(*n*)	HIV$^+$	(*n*)	*P*
Medium	214 ± 78	(8)	224 ± 78	(6)	NS
UCHT1 + 3T6-CD32/B7	137 615 ± 20 306	(8)	141 524 ± 35 416	(10)	NS
PMA + 3T6-CD32/B7	70 551 ± 18 275	(8)	57 839 ± 9247	(10)	NS
PMA + anti-CD28	43 017 ± 11 008	(8)	55 009 ± 13 615	(10)	NS

Data are expressed as median ct/min ± CI. The number of subjects tested is indicated between parentheses. Percentage of CD28 expression (median ± CI) on the different subsets were: HIV$^-$ CD8$^+$ T, 78·7 ± 8·4; HIV$^+$ CD8$^+$ T, 43·1 ± 8·4 ($P < 0·001$); HIV$^-$ CD4$^+$ T, 99·9 ± 0·1 and HIV$^+$ CD4$^+$ T, 97·2 ± 2·2 ($P < 0·01$).

PMA, Phorbol myristate acetate.

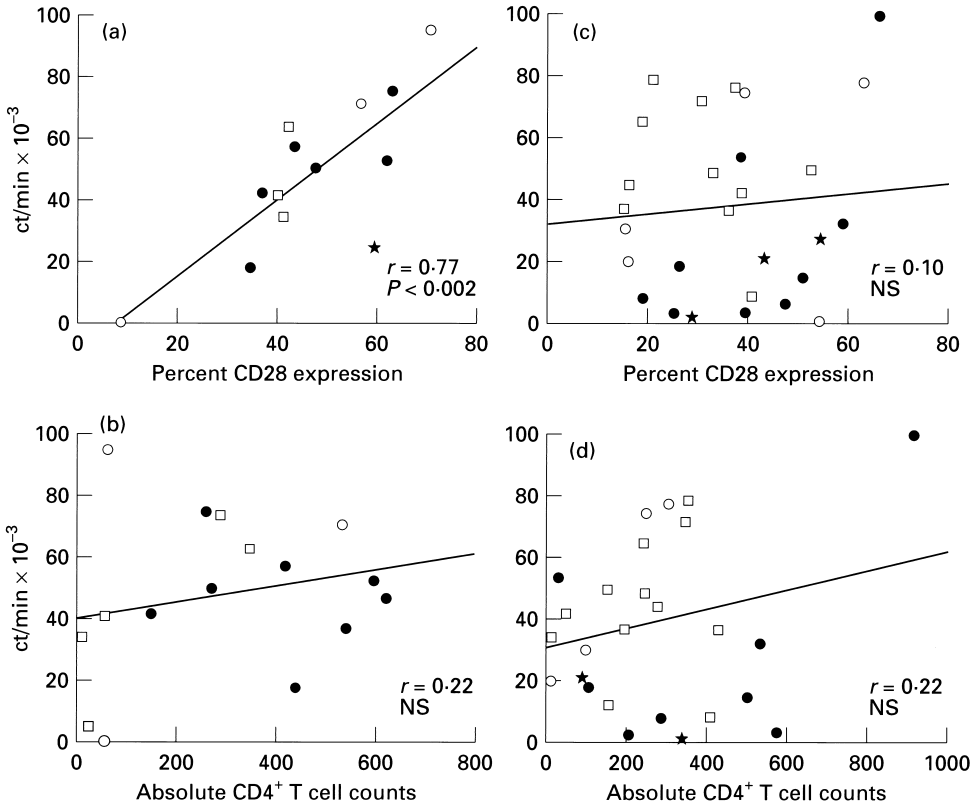

Fig. 1. Correlation of CD28 expression on CD8$^+$ T cells (a,c) or absolute CD4$^+$ T cell counts (b,d) with the proliferative response of CD8$^+$ T cells to UCHT1 + 3T6-CD32/B7 (a,b) or to allogeneic stimulation with ARC (c,d) in HIV$^+$ subjects. The different symbols represent the following clinical categories: CDC-A (●), CDC-B (□), CDC-C (○), unclassified (*). CD28 expression on CD8$^+$ T cells was determined at the onset of culture. Correlation coefficients (*r*) and *P* values are indicated on the figure. Note: absolute CD4$^+$ T cell counts were not available for a few patients.

Table 2. Proliferative responses of CD8$^+$ T cells (a) or CD4$^+$ T cells (b) to allostimulation

a. CD8$^+$ T cells

Stimulus	HIV$^-$	(*n*)	HIV$^+$	(*n*)	*P*
Medium	97 ± 17	(25)	88 ± 21	(28)	NS
Raji	25 725 ± 11 974	(19)	15 087 ± 4994	(25)	<0·05
ARC	70 937 ± 15 052	(25)	32 443 ± 11 900	(29)	<0·001
MT4	5761 ± 6536	(12)	2167 ± 2410	(14)	<0·05

b. CD4$^+$ T cells

Stimulus	HIV$^-$	(*n*)	HIV$^+$	(*n*)	*P*
Medium	222 ± 122	(14)	134 ± 75	(15)	NS
Raji	59 349 ± 11 762	(14)	55 106 ± 10 693	(16)	NS
ARC	74 079 ± 10 758	(14)	67 745 ± 14 454	(15)	NS
MT4	33 022 ± 13 806	(10)	30 615 ± 17 346	(10)	NS

Allogeneic B cell lines (ARC and Raji) or T cell lines (MT4) were cocultured with T cell subsets. Data are expressed as median ct/min ± CI. The number of subjects tested is indicated between parentheses. Percentage of CD28 expression (median ± CI)) on the different subsets were: HIV$^-$ CD8$^+$ T, 83·1 ± 3·2; HIV$^+$ CD8$^+$ T, 37·9 ± 7·3 ($P < 0·001$); HIV$^-$ CD4$^+$ T, 99·9 ± 0·4 and HIV$^+$ CD4$^+$ T, 96·5 ± 2·5 ($P < 0·001$).

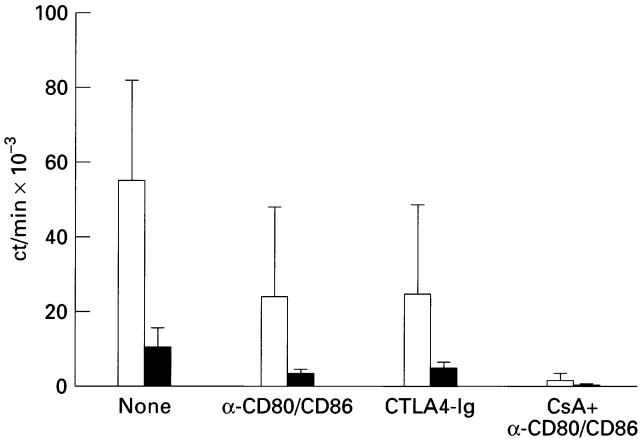

Fig. 2. Blocking of alloantigen-induced proliferation of $CD8^+$ T cells from HIV^- and HIV^+ subjects. Results of stimulation with ARC cells are expressed as median ct/min ± confidence interval (CI) of four HIV^- (□) and four HIV^+ (■) donors. Percent inhibition after addition of anti-$CD80^+$ anti-CD86 or of CTLA4-Ig varied between 40% and 60%. Percent inhibition after addition of cyclosporin A (CsA) and anti-CD80 + anti-CD86 varied between 93% and 99%. The proportional degree of inhibition, compared with medium alone (none) was not different in patients or controls.

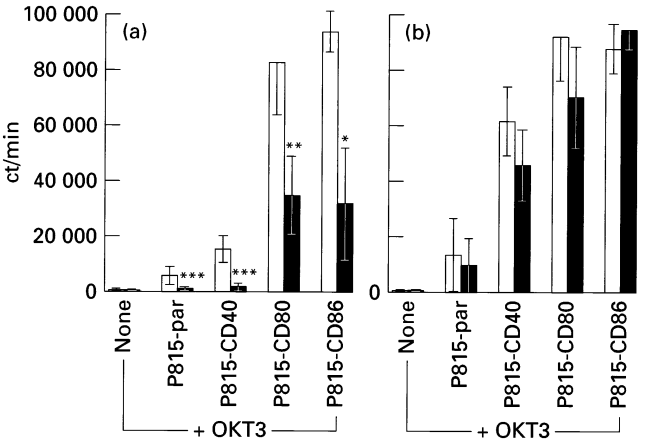

Fig. 3. Proliferative responses of $CD8^+$ (a) or $CD4^+$ (b) T cells from HIV^- (□) or HIV^+ (■) to stimulation with OKT3 alone or to stimulation with OKT3 plus P815 cells either transfected with CD40, CD80 or CD86 or untransfected (parental). Data are expressed as median ct/min ± confidence interval (CI) for *n* individuals ($n = 12$ and 16 for $CD8^+$ T cells from HIV^- and HIV^+, $n = 10$ and 12 for $CD4^+$ T cells from HIV^- and HIV^+, respectively). Levels of significance: $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}005$; $^{***}P < 0{\cdot}0005$.

cytokines in stimulated PBMC from HIV^+ compared with HIV^- subjects. We therefore wanted to know whether a similar shift was present at the level of purified T cell subsets, and measured IFN-γ, IL-10, IL-4 and IL-5 in culture supernatants of $CD4^+$ or $CD8^+$ T cells. CD28 triggering of $CD8^+$ T cells via CD80 or CD86 induced high IFN-γ and IL-5 production, whereas IL-4 and IL-10 production was close to the detection limit in both HIV^+ subjects and controls (Fig. 4). Median IFN-γ and IL-5 production by $CD8^+$ T cells from patients was lower than in controls. $CD8^+$ T cells from patients and controls did not produce measurable cytokine levels upon CD40L triggering via CD40. Activation of $CD4^+$ T cells via CD28 induced similar levels of IFN-γ, IL-4 and IL-5 in patients and controls, but IL-10 levels in HIV^+ subjects tended to be lower. CD40 costimulation induced mainly IL-4 and IL-5 but only very low levels of IL-10 and no IFN-γ in $CD4^+$ T cells, with no difference between HIV^+ subjects and controls. The parental P815 cells did not induce any cytokine production in $CD4^+$ or $CD8^+$ T cells from patients and controls (data not shown).

DISCUSSION

In the present study, we showed that CD28- and CD40L-induced costimulation, as well as allogeneic activation of $CD8^+$ T cells from HIV^+ subjects, are deficient: both the proliferation and the production of Th1-type and Th2-type cytokines by $CD8^+$ T cells from patients were clearly reduced compared with controls. In contrast, responses of $CD4^+$ T cells from HIV^+ subjects were not significantly altered in any of these conditions, with the exception of a lowered production of IL-10.

Recently, it has been shown that HLA-DR^- T cells, including both $CD4^+$ and $CD8^+$ cells, from HIV^+ subjects have an impaired capacity to respond to CD28-mediated costimulation. It was suggested that this defect resided in the TCR/CD3 activation pathway and not in the CD28 costimulatory signal [29]. The data presented here clearly indicate that the defect was restricted to $CD8^+$ T cells, and show that bypassing the TCR/CD3, by the use of PMA, does not prevent the hyporesponses. It is therefore suggested that the deficiency did not depend exclusively on the primary stimulus but also on CD28 triggering via either CD80, CD86 or anti-CD28.

Nevertheless, decreased activation through CD28 is not the only explanation for the overall deficiency of $CD8^+$ T cells in HIV^+ subjects. Earlier observations demonstrated that both $CD28^+$ and $CD28^-CD8^+$ T cells from HIV^+ subjects were hyporesponsive to TCR triggering [28,30]. In this study, we showed that the hyporesponse to allogeneic stimuli (i) did not correlate with CD28 expression, and (ii) remained present after the complete block of CD28–B7 interactions. In addition, we clearly demonstrated that $CD8^+$ T cells are also deficient in CD40L-mediated signals in the absence of CD28 ligation.

The generalized hyporesponsiveness of $CD8^+$ T cells from HIV^+ persons might relate to an excessive activation *in vivo*, as reflected in the increased expression of HLA-DR and CD38 on both the $CD28^+$ and the $CD28^-$ subset [3,28]. It has been shown that HLA-DR^+ and not HLA-DR^- $CD8^+$ T cells from HIV^+ persons have a reduced clonogenic capacity *in vitro* [36]. It is also known that the increased state of activation of $CD8^+$ T cells *in vivo* is related to a higher degree of apoptosis *in vitro* [26,37]. The refractory state of $CD8^+$ T cells, caused by a chronic *in vivo* activation, might contribute to the development of opportunistic viral infections and neoplasms, which are typically controlled by $CD8^+$ T cells.

The increased expression of CD38 and HLA-DR is much less pronounced on $CD4^+$ T cells than on $CD8^+$ T cells from HIV^+ subjects [4]. Still, the preserved function of purified $CD4^+$ T cells from HIV^+ subjects, described in this study, was surprising, since dysregulation of $CD4^+$ T cell-mediated immunity, such as the early loss of responses to recall antigens in PBMC cultures and the decrease in DTH *in vivo*, is an important feature of HIV infection. The preserved responses of $CD4^+$ T cells to costimulation via CD28 paralleled the relatively normal expression of this receptor on

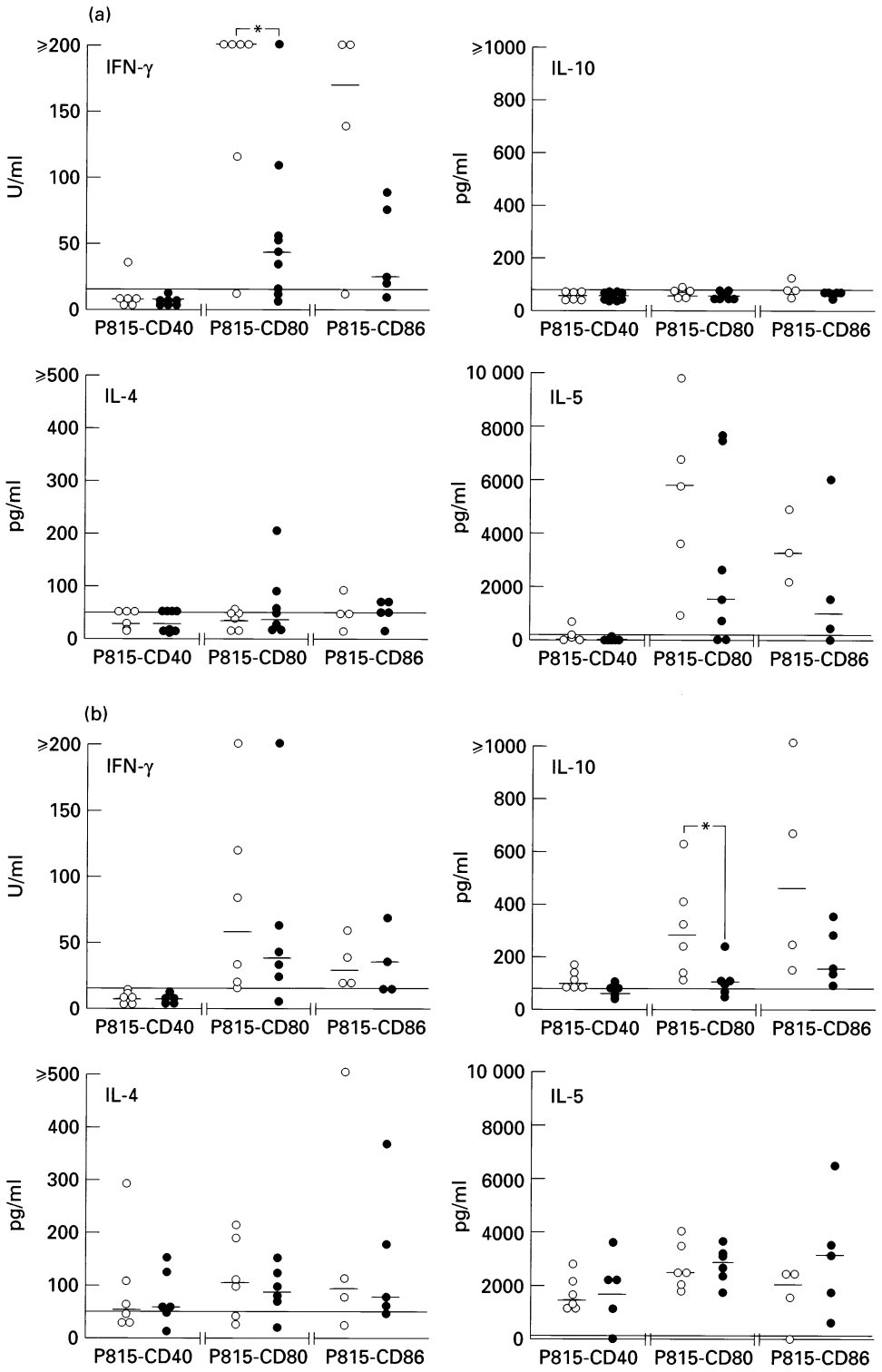

Fig. 4. Production of IFN-γ, IL-10, IL-4 and IL-5 by CD8$^+$ (a) and CD4$^+$ (b) T cells from HIV$^-$ (○) and HIV$^+$ (●) persons. Supernatants were collected after 72 h of coculture with untransfected (parental) cells (P815-par) or different P815 transfectants indicated as P815-CD40, P815-CD80 or P815-CD86. Each dot represents one individual and the median value for each group is indicated with a horizontal dash. Detection limits for the assays are indicated as horizontal lines (20 U/ml for IFN-γ, 80 pg/ml for IL-10, 50 pg/ml for IL-4 and 40 pg/ml for IL-5). *$P < 0{\cdot}05$.

ex vivo $CD4^+$ T cells from HIV-infected persons [30]. Similarly, the data in this study demonstrate that the responses of $CD4^+$ T cells from the patients to other stimuli, such as allo-activation and costimulation via CD40, were not impaired. The normal response to CD40-mediated costimulation is in agreement with the finding that after *in vitro* activation, an equal expression of CD40L on $CD4^+$ T cells was observed in HIV^+ subjects and controls [38]. Furthermore, Hickey *et al.* have demonstrated normal TCR-triggered calcium signalling in $CD4^+$ T cells from HIV-infected patients, and a similar percentage of responding cells in patients and controls [39]. The loss of allo-response described by Clerici *et al.* [7] was observed in an experimental system using a pool of irradiated PBMC from unrelated HIV^- donors as stimulator cells and patient PBMC, but not purified $CD4^+$ T cells, as the responder population. We used an MLR system that consisted of allogeneic EBV-transformed cells, as stimulators and purified $CD4^+$ T cells from patients as responders. Therefore, neither defective APC nor suppressive $CD8^+$ T cells could have influenced the response of the $CD4^+$ T cells, which, in contrast, is possible when the responders are PBMC [40,41,50]. Meyaard *et al.* [49] reported a decreased T cell precursor frequency to allo-antigen stimulation in HIV infection. In their system, purified $CD4^+$ T cells from HIV^+ subjects were stimulated with a pool of purified monocytes from unrelated HIV^- donors. Their observations are clearly in contrast with the present study, but this might be related to the different allo-stimulator cells used.

Several authors have described a shift from Th0/Th1 to Th2-type cytokine production [7,10], while others have reported no shift or even an increased production of IFN-γ both *in vivo* and *in vitro* [11,12,42–44]. The present data on a relatively unchanged cytokine profile in purified $CD4^+$ T cells are in line with other studies [12,45]. We also showed that purified $CD8^+$ T cells from HIV^+ subjects produced reduced levels of both Th1 and Th2-type cytokines after *in vitro* activation. In conclusion, our data indicate that the shift from Th1 to Th2-type cytokines observed by others in PBMC is not present at the level of purified T cell subsets. Functional alteration in accessory cells might explain this apparent paradox [46–48].

The present study has clearly demonstrated that $CD8^+$ T cells from HIV^+ individuals are intrinsically hyporesponsive in all tested systems. In our patient group, this hyporesponse was independent of absolute $CD4^+$ T cell count or clinical stage. The chronic *in vivo* activation of $CD8^+$ T cells during HIV infection leads to an anergic state which could explain the appearance of opportunistic diseases. $CD4^+$ T cells from HIV^+ subjects, however, behave normally. To understand the *in vivo* deficiency of $CD4^+$ T cell function, additional studies on $CD4^+$ T cell–APC and $CD4^+$–$CD8^+$ T cell interaction during HIV infection are in progress.

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