Analysis of the effect of highly active antiretroviral therapy during acute HIV-1 infection on HIV-specific CD4 T cell functions

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Background: It has been reported that antiretroviral therapy (HAART) during acute HIV-1 infection may rescue HIV-1-specific CD4 T cell responses.

Objective: To determine the duration of this preserved response by investigating the long-term effects of HAART during acute infection on HIV-specific CD4 T cell function related to possible immune control during subsequent therapy interruption.

Methods: A longitudinal analysis followed HIV-specific CD4 T cell reactivity in 17 individuals with well-documented acute HIV-1 infection where five out of 11 HAART-treated patients stopped therapy and six were untreated. Peripheral blood mononuclear cells were stimulated with overlapping peptide pools derived from Gag and Nef. Production of interferon-γ (IFN-γ) and interleukin-2 (IL-2) by CD4 T cells was analysed together with proliferative responses.

Results: Absolute numbers, but not percentages, of Gag-specific IFN-γ-, IL-2- or IFN-γ/IL-2-producing CD4 T cells were increased in treated compared with untreated individuals up to 2 years after seroconversion. HAART during acute HIV-1 infection was associated with lower viral load but did not result in increased proliferation of HIV-specific CD4 T cells. One out of five individuals who discontinued therapy showed evidence for immune control. However, patients who failed to control viraemia also had measurable proliferative HIV-specific CD4 T cell responses and preserved numbers of cytokine-producing CD4 T cells.

Conclusions: Early HAART during acute HIV-1 infection resulted in higher numbers of HIV-specific IFN-γ- and IL-2-producing CD4 T cells, but this preservation in four out of five patients was not associated with control of viraemia upon treatment interruption.

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Introduction

During infection with HIV, the presence of HIV-specific CD8 cytotoxic T lymphocytes (CTL) has been associated with suppression of viral replication and delayed disease progression [1–4]. Eventually, control of HIV by these cells fails, leading to progression to AIDS [5,6]. It has been shown that loss of CD8 cytotoxic T cell control is not a

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result of physical depletion but is a consequence of impaired functionality of these HIV-specific CD8 cells [7]. This may occur because of loss of CD4 T cell help, which is important for the induction and maintenance of CD8 cytotoxic T cell function during chronic infection in both mice and humans [8–11].

Proliferative HIV-specific CD4 T cell responses have been demonstrated in individuals who are able to control viral replication, such as individuals with non-progressing HIV-infection and individuals who receive highly active antiretroviral therapy (HAART) during acute (primary) infection [12–14]. This p24-specific proliferation was shown to correlate inversely with plasma HIV-1 RNA [13]. Interestingly, analysis of cytokine production showed that interferon-γ (IFN-γ)-producing HIV-specific CD4 T cells could be found in both viraemic and non-viraemic infection [15] and did not correlate with either the magnitude of the specific CD8 T cell response or disease markers [15–17]. Further studies demonstrated that the fraction of Gag-specific CD4 T cells expressing interleukin-2 (IL-2), CD28 *and* IFN-γ correlated inversely with plasma HIV-1 RNA and may be associated with slower progression of HIV-1 infection, but not the fraction of IFN-γ-producing cells [18]. This was confirmed in a recent study by Harari *et al.* [19] in which skewing towards IFN-γ-secreting CD4 T cells together with an absence of IL-2-producing or IFN-γ/IL-2-producing CD4 T cells was observed in subjects with progressive disease.

HAART treatment of chronically infected individuals has been shown to lead to a decrease in HIV-specific cellular immune responses [15], and a decrease in plasma HIV-1 RNA. Low levels of viraemia are, however, associated with a sustained increase in CD4 T cell counts [20] and a preferential increase in antigen-specific CD4 T cell proliferation [21,22].

The effect of treatment during primary infection on HIV-specific CD4 T cell function has previously been investigated by IFN-γ Elispot analysis [14,23–25], [^{3}H]-thymidine incorporation after stimulation with recombinant HIV proteins [13,25,26] or both [25,27]. The main observation was preservation of HIV-1-specific CD4 T cell proliferative responses, which was shown to be related to the low viraemic state induced by HAART [28]. However, studies reported so far have been limited with respect to detailed longitudinal data on CD4 T cell responses because of the small sizes of study groups or limited follow-up [23–26]. In a previous study, five out of eight patients who received HAART treatment during acute infection showed some evidence of immune control after treatment interruption, as reflected by HIV-specific CD4 T cell proliferative responses [25].

Here, we report results of longitudinal analyses of the effects of early HAART on HIV-specific CD4 T cell responses in untreated and treated individuals, of whom five discontinued therapy. All individuals were treated with an intensive, triple-class five-drug regimen. To our knowledge, this is the first study to determine HIV-specific CD4 T cell function during primary infection comparing treated and untreated individuals, using a combination of cytokine production (IFN-γ and IL-2) and proliferative capacity with a follow-up of 2 years after seroconversion.

Materials and methods

Patients

In this prospective study, 17 individuals who experienced acute HIV-1 infection (based on clinical symptoms of acute HIV-1 infection [29] or an incomplete Western blot) were selected. Eleven individuals starting antiretroviral therapy within weeks following signs of infection with an intensive, triple-class five-drug regimen were compared with six controls who remained untreated. Seroconversion dates of both treated and untreated individuals are known. Five of the treated individuals stopped therapy. The virological effects in two patients who stopped therapy (ERA 024 and 026) have been reported elsewhere [30] as well as clinical features of untreated individuals [31–34]. Sampling started within days of presentation with acute HIV-1 infection for all individuals, and blood was collected every 2/3 months. Peripheral blood mononuclear cells (PBMC) were cryopreserved according to a standard protocol in a computerized freezing device. As a control, 10 HIV-negative healthy bloodbank donors were included.

Intracellular cytokine staining after antigenic stimulation

Cryopreserved PBMC were thawed using RPMI-1640 medium supplemented with penicillin, streptomycin and 20% inactivated fetal calf serum (FCS) and subsequently washed in 10% FCS medium. Cells were sampled at 2×10^6 cells/ml in round bottom tubes (polystyrene; Falcon, Beckton Dickinson, San José, California, USA). Cells were stimulated with either Gag or Nef peptide pools (15-mers with 11 overlap; HXB2; NIH AIDS Research and Reagent Program, Bethesda, Maryland, USA) in the presence of co-stimulation (2 μg/ml αCD28; Sanquin Reagents, Amsterdam, the Netherlands) and 2 μg/ml αCD49d (Pharmingen, San José, California, USA). The concentration of the individual peptides within each pool was 2 μg/ml. Phorbol 12-myristate 13-acetate/ionomycin was used as a positive control. Stimulations were performed for 6 h at 37°C under 5% carbon dioxide. After 1 h, brefeldin A (Beckton Dickinson, San José, California, USA) was added. Next, cells were fixed and permeabilized (permeabilization kit; Beckton Dickinson) and stained with αCD3–peridinin–chlorophyll *a*

complex protein, αCD4–allophycocyanin, αIL-2–phycoerythrin and αIFN-γ–fluoroscein isothiocyanate (Beckton Dickinson) for 20 min at 4°C. Cells were then fixed in Cellfix (Beckton Dickinson) and at least 300 000 events were acquired using a FACSCalibur flow cytometer (Beckton Dickinson). Data were analysed using the software program CELL Quest (Beckton Dickinson). Frequencies of IFN-γ- and IL-2-producing cells were reported after subtraction of the frequencies in medium controls. Cytokine production above the levels observed in HIV-negative individuals was considered positive (median 0.02% of CD4 T cells for Gag-induced cytokine production and median 0.01% for Nef-specific cytokine production; data not shown).

Antigen-specific proliferation

In vitro T cell proliferation to HIV peptide pools was measured using 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Leiden, the Netherlands) according to the manufacturer's protocol. Briefly, PBMC were thawed and cells were labelled using CFSE for 8 min; labelling was stopped using human pool serum. Cells were washed and 2×10^6 cells in 0.5 ml RPMI-1640 medium were added to round bottom tubes. Cells were then stimulated with either Gag or Nef peptide pools (concentration of individual peptides in pool 2 μg/ml) or 1/100 dilution of cytomegalovirus (CMV) lysate (Microbix Biosystems, Toronto, Ontario, Canada). As a positive control, 0.02 μg αCD3 and 2 μg αCD28 (Sanquin Reagents) was used. After 6 days of incubation at 37 °C under 5% carbon dioxide, cells were stained using αCD4 and αCD3 as described above.

Stimulation indices (SI) were calculated by dividing the percentage proliferation of CD3+CD4+ T cells after stimulation by the percentage proliferation of unstimulated cells. SI values > 1 were considered positive based on SI values observed in HIV-negative individuals (mean SI for Gag 1.0; mean SI for Nef, 0.9; data not shown).

Plasma HIV-1 RNA determination

Plasma HIV-1 RNA was measured using the NucliSens HIV-1 QT assay (bioMérieux, Boxtel, the Netherlands). When the RNA concentration decreased to < 400 copies/ml, an adaptation of the protocol was used to allow ultrasensitive detection, resulting in a lower quantification limit of 5 copies/ml [35].

Statistical analyses

Differences between the patient groups were analysed using Mann–Whitney tests. Wilcoxon tests were performed to determine differences in HIV-specific CD4 T cell percentages and numbers over time. Correlations were tested using the Spearman's non-parametric correlation test. All statistical analyses were performed using the software program SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics

Seventeen individuals with acute HIV-1 infection with a median follow-up of 145 weeks took part in the study. Six individuals remained untreated; 11 individuals received a potent drug regimen consisting of drugs from all three classes of currently available antiretroviral drugs (Table 1). Treatment was initiated directly at onset of symptoms (median start 1 week after seroconversion), so during the acute phase of the infection. Five individuals decided to stop therapy after a median of 53 weeks (range, 22–98). At baseline (before therapy was initiated in the treated individuals), no differences between the treated and untreated individuals were observed for CD4 T cell numbers, CD8 T cell numbers and viral load (Table 1). Treatment with HAART resulted in a decrease in plasma HIV-1 RNA to < 5 copies/ml in all patients except one (MAN 12) and plasma HIV-1 RNA remained < 5 copies/ml during therapy. In untreated patients, plasma HIV-1 RNA remained high (median, 2 209 500 copies/ml; data not shown).

The use of HAART during acute infection resulted in an increase in CD4 T cell numbers to a median of 965×10^6 cells/l late in infection (≥ 2 years after seroconversion) ($P = 0.02$, Wilcoxon). In untreated individuals, the number of CD4 T cells decreased over time to a median of 275×10^6 cells/l ($P = 0.03$, Wilcoxon), resulting in a significant difference in CD4 T cell numbers at 104 weeks after seroconversion ($P = 0.004$, Mann–Whitney). This effect was not observed in the CD8 T cell compartment (HAART treated, late median, 885×10^6 cells/l; untreated median, 745×10^6 cells/l; $P = 0.63$, Mann–Whitney) (data not shown).

The effect of antiretroviral therapy on cytokine-producing HIV-specific CD4 T cells

Early in infection (at the time therapy was initiated) Gag-specific CD4 T cells producing IL-2, IFN-γ or both (IFN-γ/IL-2) were detectable in all individuals (for a representative FACS plot see Fig. 1a) to a similar extent: median IFN-γ-, IL-2- and IFN-γ/IL-2-producing cells were 0.10%, 0.03% and 0.02%, respectively, in HAART-treated individuals and 0.05%, 0.04% and 0.02%, respectively, in untreated individuals (Fig. 1b–d). During follow-up, percentages of cytokine-producing CD4 T cells tended to decrease, although this was not significant. Late in infection, no significant differences in percentages of IFN-γ-, IL-2- and IFN-γ/IL-2-producing CD4 T cells were found between treated (medians, 0.02%, 0.02% and 0.01%, respectively) and untreated (medians, 0.02%, 0.01% and 0.01%, respectively) individuals ($P = 0.87$, $P = 0.46$ and $P = 0.28$, respectively, Mann–Whitney). Similar observations were made for Nef-specific CD4 T cells, although frequencies of Nef-specific CD4 T cells were slightly lower (Fig. 1h–j) than Gag-specific CD4 T cells.

Table 1. Characteristics of study participants.

Group and subject	Therapy	Follow-up (weeks)[a]	Baseline CD4 cell count ($\times 10^6$ cells/l)[b]	Baseline CD8 cell count ($\times 10^6$ cells/l)[b]	Baseline viral load (10^3 copies/ml)
HAART					
ERA 16	AZT+3TC+ABC+IDV+NVP	235	370	1440	83
ERA 18	AZT+3TC+ABC+IDV+NVP	219	340	1630	190
ERA 21	d4T+3TC+ABC+IDV+NVP	136	670	780	44
ERA 22	d4T+3TC+ABC+RTV+IDV+NVP	129	880	2570	43
ERA 23	d4T+3TC+ABC+RTV+IDV+NVP	133	730	2840	340
ERA 25	d4T+3TC+ABC+RTV+IDV+NVP	139	930	2440	110
Median		138	700	2035*	96.5
Untreated					
A02	–	132	660	1000	39 000
A03	–	159	790	2700	140
A04	–	78	680	800	nd
A05	–	108	670	1000	7.7
A06	–	118	1150	300	0.4
A07	–	248	810	1100	510
Median		125	735	1000	325
Stop therapy					
ERA 24	d4T+3TC+ABC+RTV+IDV+NVP	201	450	1200	29
ERA 26	d4T+3TC+ABC+RTV+IDV+NVP	153	660	800	45
MAN 01	ddI+3TC+ABC+RTV+IDV+NVP	166	740	1180	18
MAN 05	ddI+3TC+ABC+RTV+IDV+NVP	121	750	960	9
MAN 12	ddI+3TC+ABC+RTV+IDV+NVP	97	550	1140	3181
Median		153	660	1140	29

HAART, highly active antiretroviral therapy; 3TC, lamivudine; d4T, stavudine; ABC, abacavir; RTV, ritonavir; IDV, indinavir; NVP, nevirapine; ddI, didanosine; nd, not determined.
[a]Seropositive follow-up.
*$P < 0.05$ HAART versus stopping treatment.

Early in infection, no differences in absolute numbers of Gag-specific IFN-γ-, IL-2- and IFN-γ/IL-2-producing CD4 T cells were found between the different groups (median values with HAART: 528, 204 and 136 × 10^3 cells/l, respectively; median untreated values 372, 252 and 109 × 10^3 cells/l, respectively; Fig. 1e–g). However, 2 years after seroconversion, more IFN-γ-, IL-2- and IFN-γ/IL-2-producing CD4 T cells were found in treated individuals (median 170, 198 and 99 × 10^3 cells/l, respectively) than in untreated individuals (median 52, 29 and 22 × 10^3 cells/l, respectively) ($P = 0.02$, $P = 0.04$, $P = 0.001$, respectively, Mann–Whitney; Fig. 1e–g). Similar trends were found in Nef-specific CD4 T cells (Fig. 1k–m).

Therefore, 2 years of treatment with HAART that was initiated during acute HIV-1 infection resulted in higher absolute numbers of HIV-specific cytokine-producing CD4 T cells in comparison with untreated individuals, despite similar fractions of cytokine-producing CD4 T cells. Within all groups, no differences between Gag- and Nef-induced cytokine production were observed.

The effect of antiretroviral therapy during acute infection on HIV-specific CD4 T cell proliferative capacity

Proliferation of HIV-specific CD4 T cells was analysed using the fluorescent dye CFSE, which is diluted when cells proliferate. As is shown in Fig. 2, no differences were observed between the different groups in Gag-specific proliferative capacity. Nef-specific proliferation in HIV-positive individuals was only detectable at the latest time point in untreated individuals (mean SI, 1.8; Fig. 2b).

To determine proliferative capacity of CD4 T cells with other specificities in these individuals, proliferation of CMV-specific CD4 T cells was analysed (Fig. 2c). Proliferation of these cells was higher in individuals treated with HAART (mean SI, 14.1) than in untreated individuals (mean SI, 1.3) ($P = 0.03$, Mann–Whitney) early in infection. This difference persisted during HIV infection (HAART treatment: mean SI, 28.8; untreated: mean SI, 1.6; $P = 0.09$, Mann–Whitney). Therefore, HAART-treated individuals showed stronger CMV-specific proliferative responses than individuals who remained untreated, but treatment did not enhance HIV-specific proliferative capacity.

Therapy interruption and HIV-specific CD4 T cell function

HIV-specific CD4 T cell function was analysed in more detail in relation to HIV-1 load in five individuals who discontinued HAART (Fig. 3). This did not result in dramatic changes in total CD4 and CD8 T cell numbers. The median values for CD4 and CD8 cells were 605 and 1050 × 10^6 cells/l, respectively, at baseline and 800 and

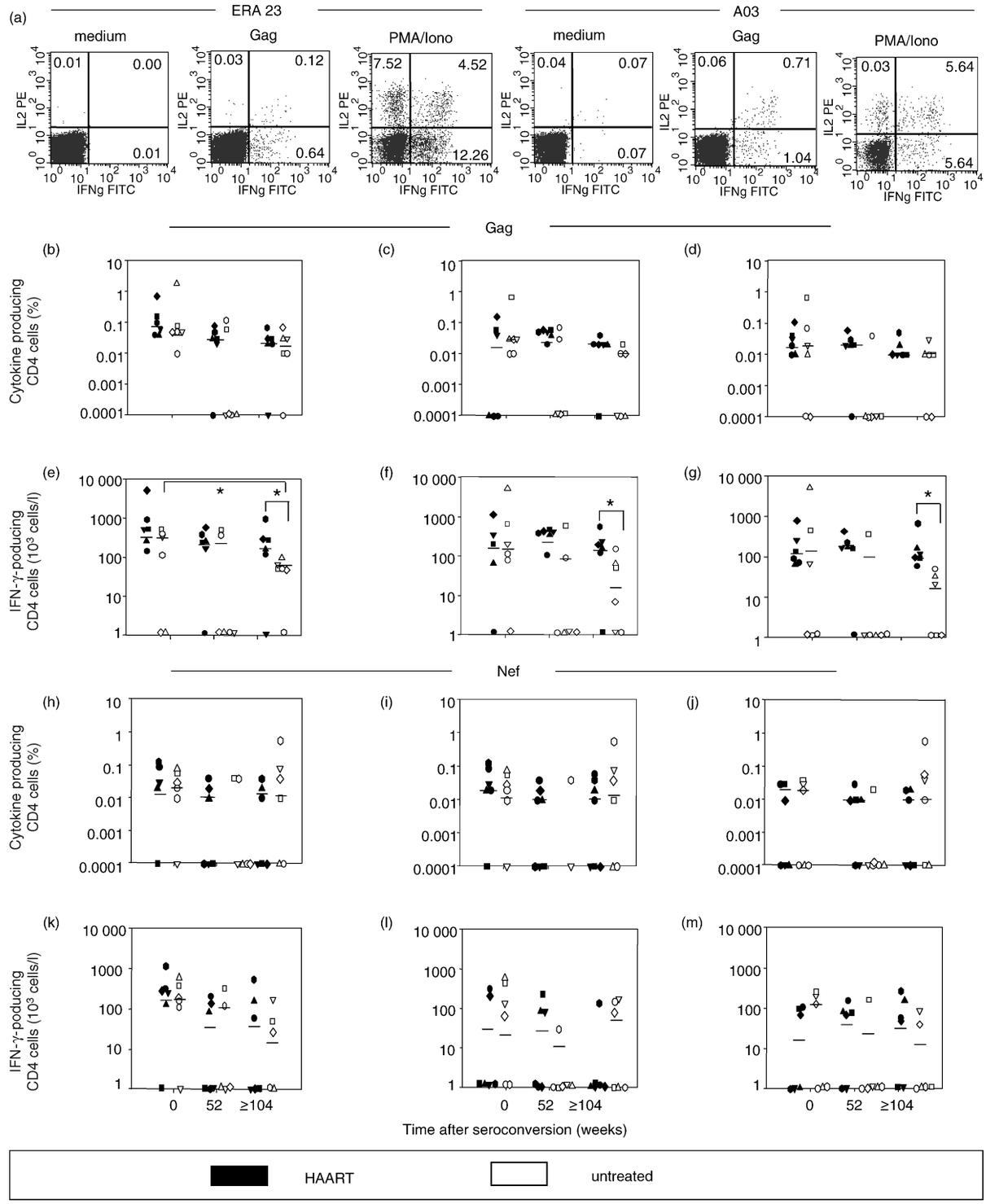

Fig. 1. Preservation of HIV-specific CD4 T cell numbers, but not frequencies, by highly active antiretroviral therapy during acute HIV-1 infection. Production of interferon-γ (IFN-γ; left), interleukin-2 (IL-2; middle) and both IFN-γ and IL-2 (right) was determined after stimulation with Gag (upper panels) and Nef (lower panels) peptide pools. (a) Representative FACS plots for a treated (ERA 23) and an untreated individual (A03) at baseline. Results are gated on the CD3+CD4+ T cells and percentages of cytokine-producing CD4 T cells are indicated for Gag- (b–d) and Nef-specific (h–j) T cells. Absolute numbers of cytokine-producing CD4 T cells were calculated and numbers of cells producing IFN-γ (e,k), IL-2 (f,l) and IFN-γ plus IL-2 (g,m) are shown. *P* values ≤ 0.05 are considered to be significant and are indicated by an asterix.

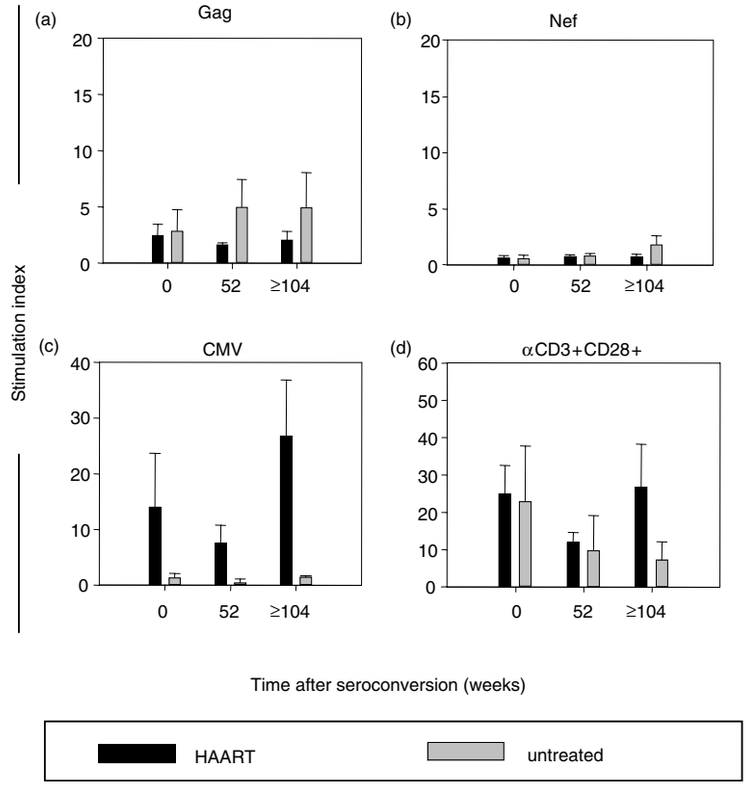

Fig. 2. Highly active antiretroviral therapy treatment during acute HIV-1 infection does not enhance HIV-specific CD4 T cell proliferation. Proliferation of CD4 T cells was analysed using 5,6-carboxyfluorescein diacetate succinimidyl ester labelling. Stimulation indices were calculated (see text). Mean stimulation indices (±SEM) are shown after stimulation with Gag peptide pool (a), Nef peptide pool (b), cytomegalovirus (CMV) lysate (c) and αCD3+αCD28 cells (d).

700×10^6 cells/l, respectively, at the time of restarting therapy (or latest time point off-HAART that was available). Viral load rebounded in four out of five individuals who stopped medication.

With regard to CD4 T helper cell dynamics, three patterns were observed. In ERA 26 and MAN 1 (Fig. 3a and 3b, respectively), interruption of therapy resulted in an increase in plasma HIV-1 RNA load. Despite this, HIV-specific CD4 T cells remained detectable and were stable during the period off HAART. Restart of HAART resulted in undetectable plasma HIV-1 RNA accompanied by a slight increase in HIV-specific CD4 T cell frequencies. The second pattern occurred in ERA 24, where interruption of HAART resulted in a fall in HIV-specific CD4 T cells, which had been quite high, while plasma HIV-1 RNA increased (Fig. 3c). When therapy was restarted, a slight increase in HIV-specific CD4 T cells was seen, together with an increase in plasma HIV-1 RNA. This individual, therefore, failed to control plasma HIV-1 RNA after the second period off therapy. Finally, the third pattern was seen in MAN 12 and MAN 5 (Fig. 3d and 3e, respectively). In these patients, stopping HAART had no immediate effect on plasma HIV-1 RNA load. However, in MAN 5 only, interruption of HAART was accompanied by a transient rise in HIV-specific CD4 T cell numbers, which resulted in long-term control of plasma HIV-1 RNA.

After therapy interruption, MAN 1, ERA 24 and MAN 12 demonstrated Gag-specific proliferative responses which were equal to responses observed in treated individuals (Fig. 3b–d, respectively); in ERA 26 responses were even higher (Fig. 3a). In MAN 5, Gag-specific proliferative responses diminished after treatment was abrogated, whereas viral load remained suppressed (Fig. 3e).

Taken together, treatment interruptions of HAART initiated during acute HIV-1 infection showed various effects on HIV-specific CD4 T cell function. In this small group of patients, HIV-specific CD4 T cell cytokine production did not correlate with control of viraemia.

Correlations of Gag- and Nef-specific cytokine production and proliferation of CD4 T cells

Correlations between cytokine production, proliferative responses and plasma HIV-1 RNA were analysed in both early and late infection. As the groups contained only five or six individuals each, correlations (Spearman's correlation) both early and late infections were calculated using the complete dataset. Early in infection, a positive

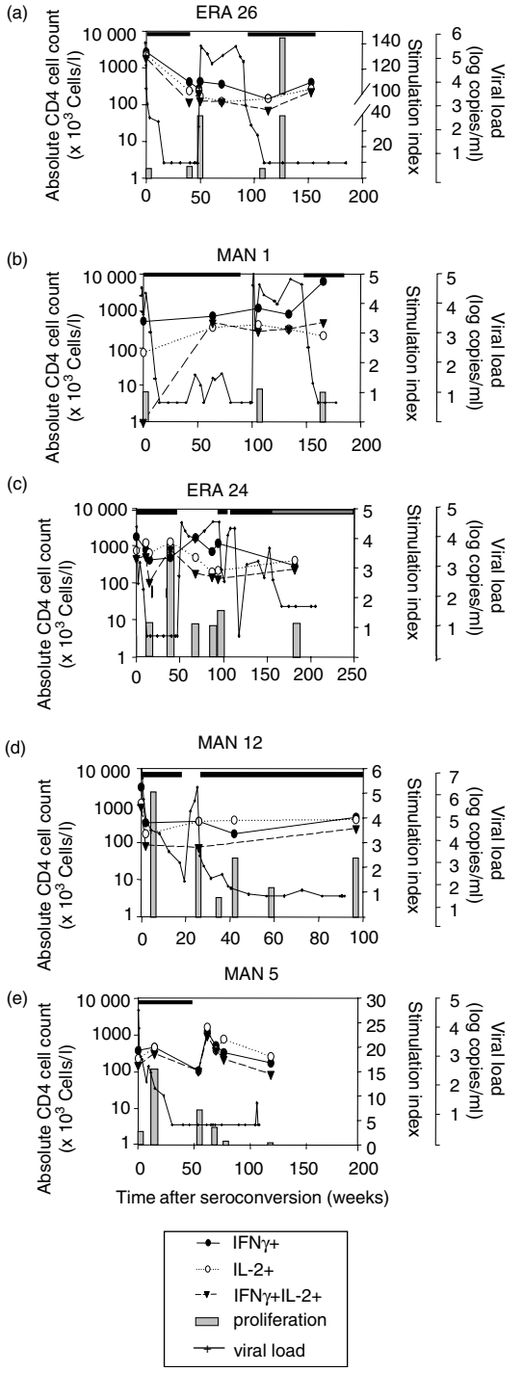

Fig. 3. Treatment interruption has no effect on preservation CD4 function by early highly active antiretroviral therapy (HAART). Absolute numbers of Gag-specific CD4 T cells producing interferon-γ (●), interleukin-2 (○) or both (▼) are shown in five individuals who underwent interruption of therapy. Horizontal black bars indicate periods on HAART and horizontal dark grey bars represent a change in drug regimen. Gag-specific proliferation is indicated by vertical grey bars. Viral load is shown as black crosses.

correlation was found between plasma HIV-1 RNA and numbers of HIV-specific IL-2- or IFN-γ/IL-2-producing CD4 T cells [$r = 0.54$ ($P = 0.02$) and $r = 0.50$ ($P = 0.03$), respectively]. Furthermore, early in infection, a positive correlation was found between Gag-specific proliferative responses and IL-2- or IFN-γ/IL-2-producing CD4 T cells [$r = 0.59$ ($P = 0.01$) and $r = 0.62$ ($P = 0.006$), respectively], which suggests that IL-2 production may be a major factor in driving proliferative responses. The production of IFN-γ correlated with IL-2 production [Gag-specific early response, $r = 0.49$ ($P = 0.03$); late response $r = 0.75$ ($P = 0.08$)] and Gag- and Nef-specific responses also correlated both early [early IL-2-producing, $r = 0.56$ ($P = 0.01$); IFN-γ/IL-2-producing, $r = 0.60$ ($P = 0.006$)] and late [IL-2-producing, $r = 0.66$ ($P = 0.12$); IFN-γ/IL-2-producing, $r = 0.76$ ($P = 0.06$)]. Surprisingly, no significant correlations were found between HIV-1 RNA load and proliferative responses.

Discussion

The immunological benefit of early treatment during acute HIV-1 infection has been investigated in this longitudinal study in 17 HIV-1-infected individuals, with or without successful antiretroviral therapy.

Production of both IFN-γ and IL-2 was determined after stimulation with Gag and Nef peptide pools. No differences between the groups were found in the percentage CD4 T cells producing IFN-γ, IL-2 or both. However, absolute numbers of Gag-specific IL-2-producing CD4 T cells were lower in untreated individuals late in infection, paralleled by a decrease in the number of Gag-specific IFN-γ- and IFN-γ/IL-2-producing CD4 T cells. Similar results were observed for Nef-specific CD4 T cells, although these differences were less distinct, possibly because of lower frequencies of these cells. Therefore, early treatment of HIV-1 infection resulted in a preservation of the number of cytokine-producing CD4 T cells, which was mainly the result of the increase in CD4 T cell numbers following treatment, but no increase in the fraction of cytokine-producing CD4 T cells.

It has been suggested that CD4 T cells producing IL-2 or both IFN-γ and IL-2 are critical in protection against HIV [18]. Skewing towards IFN-γ-secreting CD4 T cells with a lack of IL-2- or IFN-γ/IL-2-producing CD4 T cells was observed in subjects with progressive disease [19]. Another study suggested that CD4 T cells with IL-2 production and proliferative capacity are the crucial difference between controlled and uncontrolled chronic HIV-1 infection [17]. Indeed, CD4 T cells producing IL-2 (and IFN-γ) were observed only in long-term

non-progressors, acutely treated patients who became aviraemic and patients successfully treated during chronic infection [19,28,36,37]. However, in our hands, IFN-γ-, IL-2- and IFN-γ/IL-2-producing HIV-specific CD4 T cells were preserved to the same extent by HAART during acute HIV-1 infection.

Treatment with HAART during acute HIV-1 infection did not enhance HIV-specific proliferative capacity. Furthermore, we did not find significant correlations between HIV-1 RNA load and proliferative responses. Although untreated individuals had lower CMV-specific proliferative responses compared with treated individuals already at baseline, no difference in the expression of the activation marker CD38 on CD8 T cells was found between untreated [median, 58% (range 49–92)] and treated individuals [median, 63% (range 57–90)] ($P = 0.22$, Mann–Whitney; data not shown). Also, no differences in viral load and CD4 T cell numbers were observed between untreated and treated individuals. Expression of the activation marker CD38 on CD8 T cells [38,39], as well as viral load and CD4 T cell numbers [40,41], have been reported to be prognostic markers for progression to AIDS, indicating that both groups were at comparable stages of HIV infection.

Although the inverse relation between high plasma HIV-1 RNA load and proliferation is well accepted, it is still not clear what the exact causal mechanism is. It has been suggested that high HIV-specific proliferation results in low plasma HIV-1 RNA [13]. Connors *et al.* [14,15] have suggested that high viral load impairs HIV-specific proliferation but does not physically delete HIV-specific T helper cells. This could be caused by the high levels of inhibitory cytokines induced during HIV- induced immune activation [21,42]. Younes *et al.* [28] have suggested that CD4 T cells that proliferate and produce IL-2 in response to antigen are less-differentiated cells and that persistent viraemia induces these cells to differentiate, upon which they lose these functional properties but retain the capacity to release IFN-γ.

In the study of Younes *et al.* [28], all individuals received HAART during primary infection and were subsequently divided into a treated group (aviraemic individuals) and an untreated group (viraemic individuals). This is essentially different from our study, where we compared responses in treated individuals with responses in individuals who never received HAART. Furthermore, in our studies, patients were treated with a very effective five regimen antiretroviral drug combination. This results in a very rapid fall in plasma HIV-1 RNA load to undetectable levels. Lack of boosting of HIV-specific CD4 T helper cells may be explained by the extremely low viral loads that are achieved [43].

To induce and maintain CD4 helper cells, low viral loads are required, but persistent very high loads may exhaust T helper cell function [28]. Thus, there seems to be a bell-shaped relation between plasma HIV-1 RNA load and proliferative responses (Fig. 4). Chronic exposure to low-level viraemia drives proliferation and expansion of CD4 T cells until the optimal relation between plasma HIV-1 RNA and proliferation is reached. If viral load further increases, proliferative capacity gets progressively impaired [21] and this results in a negative correlation between plasma HIV-1 RNA load and proliferation. At this stage, interruption of therapy will result in an increase in load, associated with a further decrease in proliferative capacity (A′ to A). The treated individuals in our study had low viral loads and, with respect to the load–proliferation relationship, are located at the left-hand side of the curve: in the phase where proliferation is driven by plasma HIV-1 RNA load. Indeed, if therapy is interrupted in these patients, the subsequent increase in the amount of virus can still result in an increase in proliferative responses (B′ to B). Untreated individuals persistently have high amounts of virus and are located at the right-hand side of the curve; so increases in plasma HIV-1 RNA cannot lead to increases in proliferation anymore.

Recently, Lichterfeld *et al.* [44] reported strong *ex vivo* HIV-specific CD8 T cell proliferative capacity during acute HIV-1 infection. In the presence of ongoing viral replication, this proliferative capacity was lost, paralleled

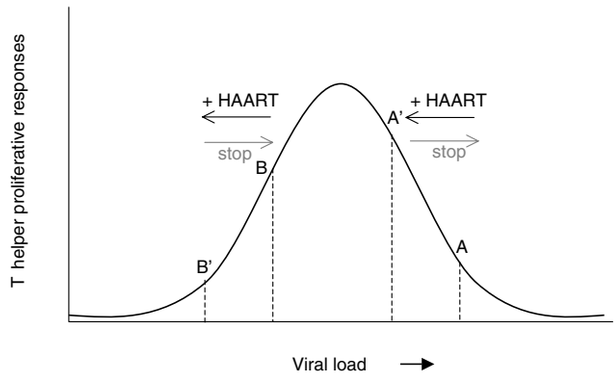

Fig. 4. Model to describe the relation between viral load and proliferative responses of HIV-specific CD4 T cell responses. Supposing that HIV-specific CD4 T cell proliferative responses are driven by viral load [22], we propose the following model. Early in HIV infection, viral load will drive proliferation of T helper cells until the optimal relation between viral load and proliferation is reached. If viral load further increases, proliferative capacity is impaired (A). Treatment with highly active antiretroviral therapy will decrease viral load, which results in an increase in proliferation (A′). Therapy interruption during this stage will result in an increase in the amount of virus, associated with a further decrease in proliferative capacity (A′ to A). Treatment early in HIV-1 infection, when viral load is low (B), will result in a low level of proliferation of HIV-specific CD4 T cells (B′). Interruption of therapy will increase the amount of virus, subsequently increasing the proliferative capacity (B′ to B).

by a loss of HIV-specific CD4 T cell proliferation. Early suppression of viral replication by antiretroviral therapy preserved these proliferative responses, also suggesting that persistent viraemia results in a progressive impairment of T cell function.

Preservation of proliferative capacity 1 year after acute infection as a result of early treatment has been shown by others [45], but in these studies individuals were treated with a standard regimen rather than the intensive regimen we used.

Interruption of therapy had variable effects on CD4 T cell function and kinetics, but overall preservation of the initial cytokine production of HIV-specific CD4 T cells was found, together with preserved proliferation of Gag-specific CD4 T cells. In only one out of five patients who stopped treatment was control of viraemia observed, despite the fact that CD4 T cell responses could be detected in all of them during HAART. Therefore, our data show that the capacity of HIV-specific CD4 T cells to produce IL-2 and IFN-γ was spared by early HAART and could be boosted by increasing viral loads. However, preserved functional CD4 T cells were not necessarily protective after treatment interruption. Although control of viraemia has been initially reported in a subset of patients [25], long-term follow-up in a small group of patients (14) who were treated with HAART during acute infection has more recently shown poor control of viraemia during treatment interruption [46].

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