

Long-term evaluation of T-cell subsets and T-cell function after HAART in advanced stage HIV-1 disease

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Objectives: Evaluation of immunological reconstitution after 2 years of highly active antiretroviral therapy (HAART) in AIDS patients.

Design: Previous data showed the effectiveness of HAART but conflicting evidence of immune reconstitution has been found in severely immunocompromised patients. Therefore, T-cell subsets and functions were analysed during 24 months of HAART in 21 AIDS patients (mean baseline CD4 cell count, $20 \times 10^6/l$).

Methods: Subjects were tested at baseline and after 4, 12 and 24 months of therapy for clinical symptoms and the following investigations were carried out: plasma HIV RNA, T-cell subsets and lymphoproliferative responses to mitogens (phytohaemagglutinin, anti-CD3), and recall antigens (*Candida* mannoprotein, tetanus toxoid and recombinant glycoprotein 160).

Results: Increase in body weight, improvement of Karnofsky's score and reduction of opportunistic infections were observed. All patients showed an initial increase in the CD4 memory subset, whereas naïve CD4 cells consistently increased only after 1 year. The magnitude of immune recovery was stronger in patients showing a significant reduction in viral load. However seven out of 21 patients who did not reach a sustained suppression of viral load showed also an increase in T-cell subsets. The majority of patients recovered lymphoproliferative responses to mitogens, whereas only four subjects showed a functional response to *Candida* mannoprotein. No patients showed a response to HIV recombinant glycoprotein 160 or tetanus toxoid.

Conclusions: The immune recovery observed is slower and not complete in severely immunocompromised patients. Our data suggest that HAART may be continued also in the absence of a significant HIV RNA decrease if alternative drugs are not available.

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AIDS 1999, **13**:1187–1193

Keywords: HAART, immunocompromised patients, naïve CD4 T cells, memory CD4 T cells, lymphoproliferative responses, mitogen, recall antigen, HIV

Introduction

Infection with HIV-1 leads to a severe depletion of peripheral CD4 T lymphocytes. This decrease is associated with phenotypic alterations of lymphocyte

subpopulations [1] and progressive impairment of functional activity [2]. Highly active antiretroviral therapy (HAART) including one protease inhibitor plus two nucleoside reverse transcriptase inhibitors has been capable of potently suppressing viral replication below

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Sponsorship: Supported by grants no. 980.2.3, 1996 and 40A.0.02, 1997–98 from the Istituto Superiore di Sanità, Italian Department of Health.

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Received: 28 October 1998; revised: 24 March 1999; accepted: 31 March 1999.

detection limits for at least 1 year in the majority of patients who remain under treatment [3]. Decrease of HIV-1 plasma viraemia is generally accompanied by clinical improvement and disease stabilization, even in drug-experienced subjects [4]. Effective therapy causes an early and consistent increase in the number of circulating CD4 T cells even in patients starting HAART at an advanced stage of the disease [3].

Analysis of naive and memory T-cell subsets in patients responding to HAART reveals that an increase in memory cells is evident from the first months of therapy, whereas the number of naive cells is not modified [5] or increases more slowly later compared with the baseline number of circulating CD4 T cells [6]. Persistence of naive and memory T cell levels is observed also in patients with rebounds in viral load [7]. Moreover, subjects responding to HAART show reduced CD8 T-cell activation [5,7].

However, the degree of immune suppression susceptible to restoration, the extent of immune function recovery and its duration, are still controversial topics. Some authors have shown that restoration of T-lymphocyte function may only be identified regarding immune responses present before therapy [8]. In contrast, others have demonstrated that this restoration depends on the amplitude and duration of viral load reduction and the increase of memory CD4 T cells [7]. These studies were performed in small groups of patients with a follow-up of 1 year. In addition, the baseline mean value of CD4 T cells was higher than $100 \times 10^6/l$, with the majority of patients showing CD4 T-cell counts of $100\text{--}300 \times 10^6/l$ [5,7,9].

Our aim was to assess whether these modifications could also be obtained in patients with AIDS and CD4 T-cell counts below $50 \times 10^6/l$ and to verify if the immune response may be restored against other antigens at present not investigated by others.

The effects of HAART were studied in a cohort of patients who, before HAART, showed high viral load and a loss of response to mitogens and recall antigens. During 24 months of follow-up lymphoproliferative responses (LPR) to mitogens, recall antigens and HIV-1 antigens were evaluated. Furthermore, changes in key surface molecules on T cells and plasma HIV-1 viral load were assessed and the clinical outcome of patients after treatment described.

Materials and methods

Patients

Twenty-one AIDS patients from the Department of Allergy and Clinical Immunology at University of Rome 'La Sapienza' were enrolled in an open prospec-

tive study with protease inhibitor (indinavir, Merck Sharp & Dohme Ltd. Hoddesdon, UK and ritonavir, Abbott Laboratories Ltd. Queenborough, UK). Of the 21 patients, 14 were male. The median age at entry was 36 years (range, 27–48 years). According to risk factors, the patients were subdivided as follows: 11 injecting drug users, six homo/bisexuals and four heterosexuals. According to Centers for Disease Control classification [10], all patients belonged to the C3 group. The mean CD4 T-cell count on entry was $20 \times 10^6/l$ (range, $2\text{--}44 \times 10^6/l$) and mean plasma HIV-1 RNA was 5.05 $\log_{10}$ copies/ml (range 4.0–5.98 $\log_{10}$ copies/ml). All subjects were nucleoside reverse transcriptase inhibitors-experienced (mean previous therapy with nucleoside reverse transcriptase inhibitors was 3.4 years) and protease inhibitor-naïve. No concomitant active opportunistic infections were present at enrollment. Patient follow-up was 24 months. Karnofsky's score, clinical signs and symptoms, HIV-related and AIDS-defining events were monitored monthly; delayed-type hypersensitivity was evaluated on entry and every 12 months. Blood samples were taken on entry and every month for haematology, biochemistry and liver function tests, and on entry, at months 4, 12, and 24 for immunologic and virologic investigation. All subjects gave informed written consent according to the Rome University Ethical Committee procedures.

Lymphocyte phenotyping

Lymphocyte phenotype was determined in whole blood samples by immunofluorescence with monoclonal antibody (MAb) combinations CD3–fluoresceine isothiocyanate (FITC), CD28–phycoerythrin (PE), CD4–peridin-chlorophyll-protein (PerCP), CD45RA–FITC, CD45R0–PE, HLA-DR–PE (Becton-Dickinson, San Josè, California, USA), CD3–FITC, CD4–PE (Ortho Diagnostic Systems, Raritan, New Jersey, USA), CD45R0–FITC (Dako, Glostrup, Denmark), CD8–tricolor (Caltag Laboratories, San Francisco, California, USA). Lymphocyte populations were quantitated by flow cytometry according to standard techniques, using a Cytoron Absolute (Ortho Diagnostic Systems).

Mitogen-induced LPR

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by density gradient centrifugation on Lymphoprep (Nycomed AS, Oslo, Norway) at 800 g for 30 min. Separated PBMC (1×10^5 cells/ml) were cultured in 200 μ l of RPMI 1640 with 10% human AB serum negative for anti-HIV-1 antibodies (N.A.B.I., Miami, Florida, USA) in 96-well flat-bottom cell culture plates (Falcon, Lincoln Park, New York, USA). CD3 MAb (OKT3, Ortho Diagnostic Systems) and phytohemagglutinin (Murex Diagnostics, Dartford, UK) at a final concentration of 25 ng/ml and 1 μ g/ml respectively were added. Cultures were set up in triplicate.

Antigen-specific LPR

Cultures were set up essentially as described before for mitogen cultures with the following differences: 1×10^6 PBMC were seeded in each well in 100 μ l of RPMI 1640 with 10% foetal calf serum added in the presence of recombinant glycoprotein (rgp) 160 (American Bio-Technologies Inc., Cambridge, Massachusetts, USA) at a concentration of 2 μ g/ml [11]. To test proliferative response to recall antigens *Candida albicans* mannoprotein [12] and tetanus toxoid (Wyeth labs., Marietta, Pennsylvania, USA) were added to the cultures at a concentration of 50 μ g/ml and 1 Lf/ml respectively. Cultures were set up in triplicate.

Evaluation of LPR

After 3 days of culture at 37°C in a humidified 5% CO₂ atmosphere (6 days for cultures with mitogens), 0.5 μ Ci of tritiated thymidine (specific activity 25 Ci/mmol; Amersham, Beckenham, UK) were added to each well. After further 24 h (4 h for cultures with mitogens) cells were harvested onto glass fibre filters by means of a cell harvester and incorporated radioactivity was measured in a scintillation counter and expressed as counts per minute (c.p.m.). Stimulation index (SI) was calculated by dividing c.p.m. of stimulated cultures by c.p.m. of the unstimulated ones. We considered as non-defective an anti-CD3- and phytohemagglutinin- driven proliferation with a SI > 15.6 and > 25 respectively. LPR to recall antigens were considered to be positive if SI > 5. These cut-off values are 10% of the HIV-1 seronegative controls SI average. If patients were positive at baseline, a two fold increase in SI was considered at least as a significant amelioration. LPR to viral antigens were considered positive if SI > 2. This cut-off value was chosen by considering that the mean $\pm$ 2 SD of SI obtained in cultures of PBMC from HIV-1 seronegative controls for each HIV-1 antigen tested was always < 2. The same cut-off has been used by other authors in similar assays with HIV-1 antigens [13].

Plasma HIV-1 RNA determination

Plasma HIV-1 RNA load was measured by RT-PCR (Amplicor HIV-1 Monitor, Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA). HIV-1 viral RNA copy number was calculated on the basis of the manufacturer's reference standards. The threshold of detection for 30 amplification cycles is 200 HIV-1 RNA copies/ml (2.3 log₁₀).

Delayed type hypersensitivity reaction

Reactions to intradermal administration of recall antigens were obtained by Multitest IMC (Pasteur Merieux, Lyon, France), before and after 12 and 24 months of therapy.

Statistical analysis

Arithmetic mean change from baseline ($\pm$ SD) in T-cell subset numbers, log₁₀ HIV-1 RNA load and SI of LPR during the 24 months follow-up of patients were eval-

uated by Student's t test for paired and unpaired data, as appropriate. Polynomial regression analysis was used to correlate HIV-1 RNA levels and CD4 T-cell count increase from baseline after 24 months.

Results

Clinical evaluation

All patients reached the 24th month of follow-up, regardless of the initial treatment assigned. The HAART regimen consisted of one protease inhibitor (indinavir in 15 patients and ritonavir in six) plus two nucleoside reverse transcriptase inhibitors (zidovudine + didanosine, zidovudine + lamivudine, stavudine + didanosine and lamivudine + stavudine). Constitutional symptoms, when present, resolved shortly after starting HAART. Relapses of opportunistic infections occurred only in two patients in the first 2 months of therapy (cytomegalovirus retinitis and cryptococcal meningitis). No major protease inhibitor-related toxicities were observed in this group. The majority of patients experienced a gain in body weight from baseline values in the first 6 months of therapy (mean increase 2.8 ± 0.6 kg), which persisted during follow-up. Karnofsky's score increased in all subjects from a mean of 74.7 at baseline to a mean of 91.4 at month 24. Three patients with cytomegalovirus disease permanently discontinued gancyclovir maintenance therapy after 12 months of HAART, remaining free of relapses. Another four patients stopped *Pneumocystis carinii* pneumonia prophylaxis. In one patient Kaposi's sarcoma skin lesions disappeared without other treatment.

Delayed type hypersensitivity

Delayed type hypersensitivity against seven antigens (PPD, streptokinase-streptodornase, tetanus, diphtheria, *Candida*, *Proteus* and *Trichophyton*) was negative in all subjects before starting therapy. After 24 months skin test reactivity against at least one antigen was present in four patients showing a mild infiltrate, whereas in other eight subjects an erythematous reaction was observed.

Lymphocyte phenotyping and plasma HIV-RNA

Table 1 shows mean values (ranges) $\pm$ SD of T-cell subsets. A significant increase in CD4 T cells was observed from baseline to month 4 (from $20 \pm 15 \times 10^6/l$ at entry, to $162 \pm 83 \times 10^6/l$ at month 4; $P = 0.0009$). This rise continued during follow-up ($334 \pm 107 \times 10^6/l$ 24 months later; $P < 0.0001$ versus baseline); however, the number of CD4 cells remained far below normal values. CD8 T cells also showed a significant and consistent increase during the 2 years of follow-up (from $457 \pm 327 \times 10^6/l$ at entry, to $1153 \pm 589 \times 10^6/l$ at month 24; $P = 0.0002$). Analysis of HLA-DR expression on CD8 T cells did not show a significant variation during the follow-up, despite a

Table 1. Changes in T-cell subsets [mean $\pm$ SD (range)] during follow-up.

	Baseline	Month 4	Month 12	Month 24
CD4 ($\times 10^6/l$)	20 $\pm$ 15 (2–44)	162 $\pm$ 83 (52–334) * $P = 0.0009$	253 $\pm$ 82 (154–359) $P < 0.0001$	334 $\pm$ 107 (130–566) $P < 0.0001$
CD8 ($\times 10^6/l$)	457 $\pm$ 327 (114–1302)	995 $\pm$ 467 (434–1648) $P = 0.01$	970 $\pm$ 303 (501–1446) $P = 0.002$	1153 $\pm$ 589 (410–2344) $P = 0.0002$
CD8HLA-DR ($\times 10^6/l$)	171 $\pm$ 169 (1–580)	149 $\pm$ 134 (40–479) $P = NS$	139 $\pm$ 121 (78–387) $P = NS$	107 $\pm$ 106 (43–466) $P = NS$
CD8CD28 ($\times 10^6/l$)	111 $\pm$ 49 (51–185)	291 $\pm$ 87 (159–407) $P = 0.0001$	283 $\pm$ 61 (231–400) $P < 0.0001$	443 $\pm$ 205 (215–906) $P < 0.0001$

* P -values are versus baseline.

trend towards a decline after 24 months (from $171 \pm 169 \times 10^6/l$ at baseline, to $107 \pm 106 \times 10^6/l$ 24 months later; $P = 0.3$, non-significant). CD8 CD28 T cells showed a significant increase at month 4 (from $111 \pm 49 \times 10^6/l$ at entry, to $291 \pm 87 \times 10^6/l$ at month 4; $P = 0.0001$). Thereafter CD8 CD28 T cells continued to increase, reaching $443 \pm 205 \times 10^6/l$ 24 months after starting HAART ($P < 0.0001$ from baseline values).

A significant mean reduction in HIV-RNA plasma load ($> 1.5 \log_{10}$ copies/ml) was observed in the whole group from month 4 and was maintained during the follow-up period (from 5.0 ± 0.5 to 3.3 ± 1.0 at month 4, 3.1 ± 1.1 at month 12 and 3.3 ± 1.1 at month 24; $P < 0.0001$ at each time-point versus baseline). Six patients reached undetectable levels of plasma viremia (below 200 copies/ml) at month 4, nine patients reached this at months 12 and 24, whereas in another four patients HIV-RNA fell below 400 copies/ml at months 4 and 12. In 14 patients the decrease of viral load reached $2 \log_{10}$ copies/ml at month 24 compared with baseline levels. In seven cases HIV-RNA persisted unchanged or showed a small decrease ($< 0.5 \log_{10}$); these were considered to be non-responders, at least in terms of viral burden reduction. Despite the persistent high HIV-RNA levels, these patients increased CD4 T-cell counts from $28 \pm 17 \times 10^6/l$ at entry, to $291 \pm 150 \times 10^6/l$ at month 24. However, patients who consistently had plasma HIV-RNA levels below limits of detection showed an increase of circulating CD4 T cells significantly higher at month 24 (400 ± 65 versus $301 \pm 111 \times 10^6/l$; $P < 0.05$). Moreover, regression analysis of CD4 increase versus HIV-RNA levels at month 24 showed a significant inverse relationship (r , 0.52, $P = 0.015$, $n = 21$). Mean CD8 T-cell count was higher, although not significantly so, in non-responder patients at month 24 in comparison with the virological responder subjects (1415 ± 736 versus $1013 \pm 462 \times 10^6/l$). CD28 expression on CD8 T cells was the same in the two groups of patients after 24 months of HAART, whereas CD8DR T cells showed a more pronounced decrease in virological responder subjects (78 ± 63 versus $170 \pm 150 \times 10^6/l$).

Memory and naive CD4 T-cell subsets

Analysis of CD45R0 and CD45RA expression on

CD4 T cells showed that memory cells (CD45R0) mainly contributed to the initial increase of CD4 T cells. The greater rise was evident in the first 4 months of therapy (from $11 \pm 12 \times 10^6/l$ at entry to $130 \pm 77 \times 10^6/l$ at month 4; $P = 0.001$), whereas at 12 and 24 months a smaller increase of CD45R0 cells was observed ($155 \pm 56 \times 10^6/l$ and $160 \pm 65 \times 10^6/l$, respectively). The rise of naive (CD45RA) CD4 T cells was more pronounced between months 12 and 24 ($133 \pm 61 \times 10^6/l$; $P < 0.0001$ versus baseline) also if an initial small, but statistically significant, increase was observed. However, their number remained below normal range, reflecting the predominance of the memory subset in the CD4 T-lymphocyte expansion (Fig. 1). The increase in memory and naive CD4 T-cell subsets was similar in virological responder and in non-responder subjects (data not shown).

LPR to mitogens

Before starting HAART all patients showed a defective LPR to anti-CD3 MAb (mean SI, 10.70 ± 2.77 ; range, 6.58–14.84), but after 24 months of treatment in all but one patient a recovery of anti-CD3 driven lymphoproliferation was observed, up to normal values (SI, 52.14

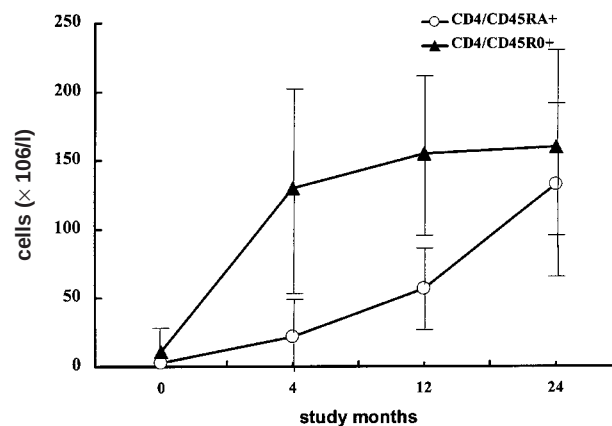


Fig. 1. Mean $\pm$ SD of memory ($\blacktriangle$ CD45R0) and naive ($\circ$ CD45RA) CD4+ T cells during HAART in the 21 patients. Staining was by triple immunofluorescence and quantification was performed by flow cytometry. CD45R0 cell number was significantly increased at each time-point ($P = 0.001$ at month 4, $P = 0.0001$ at month 12 and $P < 0.0001$ at month 24). A similar increase was observed for CD45RA cells ($P = 0.03$ at month 4, $P = 0.0003$ at month 12 and $P < 0.0001$ at month 24).

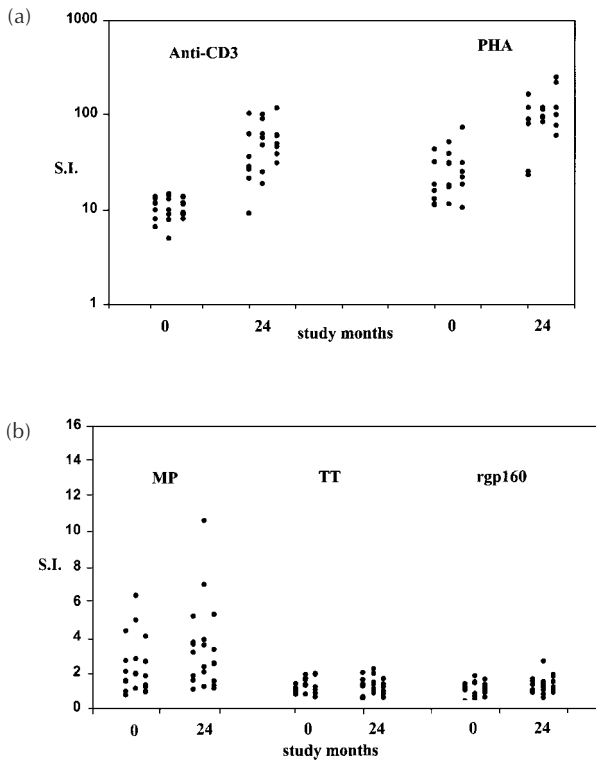


Fig. 2. Mean $\pm$ SD of SI in response to phytohaemagglutinin and anti-CD3 (a) and to mannoprotein, tetanus toxoid and rgp160 (b) at baseline and after 24 months of therapy. Isolated PBMC were cultured for 3 days (a) or 7 days (b). LPR was assessed by [^{3} H]thymidine incorporation at the end of the culture period measured by a scintillation counter. A significant increase was observed ($P < 0.0001$) for both mitogens, whereas minor or no changes were recorded for LPR to recall and HIV-1 antigens.

± 29.97 ; range, 9.04–116.30). This significant ($P < 0.0001$) increase of LPR to anti-CD3 was associated with a significant increase of phytohemagglutinin-driven LPR compared with baseline values (SI, 25.65 ± 15.54 ; range, 10.45–72.28 before HAART and SI, 105.93 ± 52.50 ; range, 22.96–216.68 after 24 months of treatment; $P < 0.0001$). The patient who did not show recovery of LPR to anti-CD3 after HAART did not restore lymphoproliferation to phytohemagglutinin either (from 12.80 to 22.96). LPR to mitogens are shown in Fig. 2a. LPR to anti-CD3 did not differ in patients with persistent high viral load in comparison with subjects with a significant decrease in plasma HIV-RNA levels after HAART.

LPR to recall and HIV-1 antigens

Before treatment only two patients showed a weak response to mannoprotein (SI, 5.01 and 6.39) and none responded to tetanus toxoid or rgp160. After 24 months of HAART two other patients showed a SI > 5 (5.21 and 5.34). The proportion of responder patients who showed positive LPR to mannoprotein increased from 9.5 to 19%. After 24 months of study

only one patient responded to rgp160 (SI, 2.62), and none responded to tetanus toxoid. LPR to recall and HIV-1 antigens are shown in Fig. 2b.

Discussion

Response to HAART can be demonstrated by several clinical parameters such as absence or disappearance of recurrent opportunistic infections, increase of body weight and Karnofsky's score increase. Immune restoration may be revealed by quantitative analysis of lymphocyte subpopulations and recovery of responses to recall or immunization antigens [5–7,14].

In this study we have provided an analysis of T-cell subset changes and immune functions in 21 drug-experienced and severely immunocompromised HIV-1 patients during 24 months of HAART. The improvement in Karnofsky's index, the body weight increase and the decrease of opportunistic infections observed in our cohort are clinical signs indirectly pointing to immunological recovery. An early increase in memory CD4 T lymphocytes was observed shortly after starting HAART, probably due to a de-trapping of cells from lymphoid tissue, as hypothesized previously [15–17]. Naive CD4 T-cells also showed an increase; however, in our patients this increase was observed later than in less immunocompromised patients studied by others [5,7,14] as it consistently occurred only after 12 months of therapy. The comparison between CD4 and CD8 T-cell increase after HAART is clearly in favour of CD4 T cells (an increase of almost 17 times the baseline level) as opposed to CD8 T cells (an increase of 2.5 times the baseline level). This seems to contrast with the hypothesis of blind T-cell homeostasis [18], and may be explained by a strong CD4 T-cell proliferation [19] and perhaps also by thymic differentiation [14,20]. This latter possibility may be supported by the regeneration of naive cells and also by the improvement in CD4 T-cell receptor repertoire observed by others [21]. Alternatively, a decrease in decay rate may be different in CD4 and CD8 T cells after HAART. In previous reports other authors described a subsequent decrease of CD8 T cells [5,14]. We did not observe this decrease perhaps due to the different characteristics of the patients. The expansion of CD8 T cells is accompanied by the decreased expression of activation markers and an increase of CD28 expression, and it is probably due to a reduction of spontaneous cell death [22]. However after 4 months this expansion wanes, perhaps because of the reduction of CD8 stimulation by infected cells due to viral load decrease [23].

The decline of HIV-RNA levels in virological responder patients, even though partial, seems to represent the most important event caused by HAART. However

these subjects were all treated previously with one or two nucleoside reverse transcriptase inhibitors and therefore the possibility that they were partially resistant to these drugs cannot be ruled out. Seven patients who did not show a HIV-RNA decrease had the same clinical improvement such as weight increase and regression of opportunistic infections, increased their naive and memory CD4 T-cell counts and also had a partial functional recovery of LPR. Similar results were recently observed by others [9]. This phenomenon is difficult to explain, but the following hypotheses can be proposed: (i) possible protease inhibitor action on non-viral targets participating in the mechanisms of CD4 T-cell depletion; (ii) presence of low cytopathic or defective viral strains; (iii) transient or partial reduction of viral load (less than $0.5 \log_{10}$) may have been sufficient to allow cell proliferation or to decrease mortality rate and thus to compensate for CD4 T-cell loss. Further studies will be needed in order to verify these hypotheses.

In the course of HIV infection, viral replication plays a pivotal role [24,25]. However, approximately 20% of patients rapidly progress to AIDS even in the presence of low virus replication and, conversely, a proportion of cases (10–25%) with high viral load do not show rapid progression [25]. These facts demonstrate that the immune system is subjected to a balance regulated by the regenerative capacity of progenitor cells, the different cellular mortality rate and the average life span of CD4 and CD8 T lymphocytes. Therefore, although viral replication is the main cause of impairment of immune responses, it represents only one aspect of the balance.

The partial immunological recovery obtained in our patients is only shown by functional tests such as LPR to phytohemagglutinin and anti-CD3 MAb. Responses to mannoprotein and tetanus toxoid were largely unmodified after 24 months of therapy. This could be explained by considering that some recall antigens without antigen stimulation (i.e. tetanus toxoid) could lack memory cell function whereas the naive cells without antigen contact are blind. Only tetanus toxoid booster administration or immunization with new antigens (i.e. haemocyanin), if ethical, could assess the extent of immune reconstitution by priming the newly produced naive T cells. However in their paper, Li *et al.* [7] observed recovery of responses to cytomegalovirus and PPD in some patients. Probably these are ubiquitous antigens in immunocompromised patients and they were able to stimulate lymphocytes. Nevertheless, we did not observe an immune response to *Candida albicans* antigen, which is also a ubiquitous microorganism. We can not exclude that in a prolonged follow-up restoration of response to recall antigens may be observed. The response to HIV-1 rgp160 was not restored as in the patients described by Pontesilli *et al.* [26]. The diminution of viral antigens

following HAART may not help CD4 and CD8 T-cells to mount an HIV-specific response. However the seven patients with increased CD4 T cells and high viral load also failed to present a specific T-cell response to gp160. Other studies are needed to clarify the mechanism of immunologic correction and the possible stimulation with specific antigens *in vivo*, possibly in conjunction with adjuvants. We [27] and others [28] have demonstrated previously that the response to specific viral antigens can be obtained only by active immunization.

In conclusion, our data demonstrate the possibility of a partial restoration in T-cell subsets and immune functions also in patients starting HAART at an advanced stage of HIV-1 disease. The clinical benefits and the decrease of opportunistic infections observed also suggest that our immunological tests are not able, at present, to indicate precisely the degree of immune recovery. In some patients the immunological and clinical recovery may be independent of the virological outcome and suggest that even a partial reduction in viral load may be beneficial, at least in this group of patients.

Combined treatments of HAART, cytokines (interleukin-2, interleukin-12, etc.) and vaccines (proteins and peptides) may be considered for a strategy to obtain full immune reconstitution in HIV-1-infected patients.

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