Assessment of immune reconstitution to *Pneumocystis carinii* in HIV-1 patients under different highly active antiretroviral therapy regimens

Chiara Atzori\(^1\)*, Mario Clerici\(^2\), Daria Trabattoni\(^2\), Giovanna Fantoni\(^1\), Antonella Valerio\(^1\), Elisa Tronconi\(^1\) and Antonietta Cargnel\(^1\)

\(^1\)II Department of Infectious Diseases, L. Sacco Hospital, Via G.B. Grassi 74, 20157, Milan; \(^2\)Preclinic Department of Immunology, L. Sacco Hospital, Milan University, Milan, Italy

Received 11 February 2003; returned 12 March 2003; revised 29 April 2003; accepted 29 April 2003

The introduction of protease inhibitors (PIs) gave a dramatic drop in AIDS-related opportunistic events, mainly due to induced immune reconstitution. Discontinuation of prophylaxis against *Pneumocystis carinii* is considered safe when CD4 > 200 cells/mm\(^3\). Ideally, we should have specific functional tests for HIV-1-related decisions. We examined viro-immunological profiles, clinical outcome and lymphocyte proliferation (LP) to *P. carinii* and other antigens in 108 subjects: 28 AIDS presenters with *P. carinii* pneumonia (PCP) (CD4 < 200 cells/mm\(^3\)), 22 untreated asymptomatic HIV-1-infected patients (CD4 > 200 cells/mm\(^3\)), 44 HIV-1-infected patients immune-reconstituted on antiretroviral regimes and 14 HIV-1-uninfected healthy controls.

As regards viral load, there was no significant difference in therapy duration, nadir, or actual CD4, CD8, natural killer or B cell counts in immune-reconstituted patients receiving protease inhibitor (PI)-based versus those receiving PI-sparing antiretroviral regimes. Among subjects showing abnormally low *P. carinii*-specific LP, three patients receiving a non-nucleoside reverse transcriptase inhibitor (nNRTI) developed PCP despite having CD4 > 250 cells/mm\(^3\). *P. carinii*-specific LP could be considered for doubtful situations, i.e. for a safer clinical decision of discontinuing or restarting prophylaxis in patients with a low CD4 nadir or experiencing a sudden CD4 decrease under highly active antiretroviral therapy (HAART).

Keywords: immune reconstitution, lymphocyte proliferation, *Pneumocystis carinii*, highly active antiretroviral therapy, protease inhibitors

Introduction

For patients with HIV-1 infection, the beneficial effect of highly active antiretroviral therapy (HAART) in reducing opportunistic infections is currently attributed to viral suppression and T cell reconstitution.\(^1\)\(^-\)\(^3\) Before HAART introduction, 10% of *Pneumocystis carinii* pneumonia (PCP) occurred at CD4 > 200 cells/mm\(^3\).\(^4\) Interestingly, aspecific effects of HIV-1 protease inhibitors (PIs) have been observed in *vitro* against opportunistic microorganisms like *Candida*, *Pneumocystis* and *Toxoplasma*, and in *vivo* against Kaposi’s sarcoma, a potential additional benefit in those patients who have PIs included in their HAART if data could be confirmed.\(^5\)\(^-\)\(^10\)

Observational studies suggested that *P. carinii* primary and secondary prophylaxis can be safely discontinued when CD4 > 200 cells/mm\(^3\). However, sporadic PCP has been reported above that threshold.\(^11\)\(^-\)\(^15\)

In our ward, we observed three cases of PCP at CD4 > 250 cells/mm\(^3\) in patients receiving non-nucleoside reverse transcriptase inhibitor (nNRTI)-based antiretroviral therapy. We studied the response to non-specific and *P. carinii*-specific antigens (PcAg) in index cases and control groups by lymphocyte proliferation (LP), since it could be clinically useful to have functional tests for specific immunity to opportunistic agents for HIV-1-related clinical decisions.\(^16\) HIV-1 infection induces changes in CD4 T cell phenotype and repertoire depletion which are not immediately restored by antiretroviral or immuno-based therapies.\(^17\)\(^-\)\(^18\) It has been demonstrated that HIV-1 infection of human macrophages modulates cytokine responses to *P. carinii* so that a virological dissociation (high viral load with relatively high CD4 cell count under therapy) could provide clinically relevant information not provided by a CD4 count.\(^19\)

*Corresponding author. Tel: +39-02-3904-2953; Fax: +39-02-3820-0909; E-mail: c.atzori@hsacco.it

© The British Society for Antimicrobial Chemotherapy 2003; all rights reserved.
Immune reconstitution to P. carinii in HIV-1 patients

Materials and methods

After informed consent, demographic characteristics (age, sex, HIV-1 risk factors), antiretroviral therapy data (CD4 nadir, drug regimen, duration) were obtained from each patient. Blood was collected for plasma HIV-1-RNA assay, flow cytometry analysis of T cells and LP carried out for 108 enrolled subjects, divided into the following groups:

A) 28 HIV-1-infected patients naive to HAART with documented PCP and CD4 count < 200 cells/mm³.
B) 22 asymptomatic HIV-1 positive patients naive to HAART with three subsequent determinations of CD4 > 200 cells/mm³.
C) 44 HIV-1-infected patients immune-reconstituted by HAART (starting antiretroviral therapy with CD4 < 200 cells/mm³ but with CD4 > 200 cells/mm³ for at least three subsequent determinations when tested).
D) 14 healthy HIV-1-uninfected controls.

HAART was defined as a combination of two NRTIs and one protease inhibitor (PI) or nNRTI.

LP assay

After blood collection in EDTA Vacutainer tubes, peripheral blood mononuclear cell (PBMC) separation (FICOLL-Hypaque) was carried out within 4 h of withdrawal. For the LP assay, we used previously published protocols with rat-derived P. carinii antigens and human samples.20–23 LP was carried out by measuring [3H]thymidine incorporation after 7 days of culture at 37°C in a 5% CO2 incubator: 10⁵ PBMCs/well were incubated in medium alone (MED) or together with PcAg, FLU, ENV, PHA as antigens in four replicates for each antigen. PcAg was added in a 10:1 ratio of trophozoite/PBMC. On day 6, the wells were pulsed with radioactive thymidine and harvested 18 h later, and the incorporated activity measured as cpm.

P. carinii antigen preparation

Trophozoites were obtained from P. carinii-free Sprague–Dawley rats infected with P. carinii by transtracheal inoculation and expanded onto human embryonic lung (HEL 299 — ATCC CCL137) cells sheeted on microcarrier beads in spinner flasks as previously reported.24,25 Pelleted microorganisms were washed twice in PBS, counted on Giemsa-stained calibrated drops and used fresh or thawed after cryopreservation, at a 10:1 ratio of trophozoites/PBMC. To monitor the purity of P. carinii antigens, aliquots were microscopically checked for morphology, and cultured for bacteria and fungi; lipopolysaccharide (LPS) contamination was excluded by Limulus assay (E-toxate; Sigma, Italy). European guidelines for animal experimentation were followed in the animal study.

Other antigens

MED: culture medium, no stimulator, negative control, background values; PHA: phytohaemagglutinin, positive control for mitogenic activity (2.5 µg/mL); ENV: pool of five antigenic peptides from envelope of HIV-1 (25 nM);26 FLU: influenza virus vaccine prepared with a mixture of A/Taiwan, A/Shanghai and B/Victoria (24 mg/mL). LP assay was carried out twice on each patient, once with fresh PBMC and then, from the same blood sample, with frozen PBMC.

Assessment of clinical and laboratory parameters

The following data were analysed for each HIV-1-infected patient: demographic features, sex, age, risk factor, viral load, absolute counts of CD4, CD8, natural killer (NK) and B cells, and occurrence and type of antiretroviral therapy. For patients with PCP, microscopically diagnosed by bronchoalveolar lavage (BAL) after standard bronchoscopy, data were collected during the overt disease. CD4 count nadir and duration of HAART were also recorded in immune-reconstituted patients.

Informed consent was obtained from all patients and healthy controls involved in the study, according to European guidelines for good clinical practice.

Evaluation of results and statistical analysis

After verification of Gaussian distribution, statistics (two-tailed t-test with \( P < 0.05 \)) were carried out both on LP data (crude cpm to each antigen minus noise background) and stimulation index (SI), defined as the ratio of median counts per minute of quadruplicate culture with antigen to the median counts per minute in culture medium alone without antigen for PHA, ENV, FLU and PcAg in groups A, B, C and D. A two-tailed t-test with \( P < 0.05 \) was also carried out after stratification of immune-reconstituted patients (group C) into two groups (receiving PI-based or nNRTI-based HAART) in order to assess differences in age, CD4 nadir count, therapy duration, actual flow cytometry subsets, viral load, occurrence of opportunistic events, and results of LP to different antigens.

Results

The main clinical features of subjects enrolled in the study with flow cytometry subset data are reported in Table 1. HIV-1-infected patients were more often male than healthy controls (80.9% versus 35.7%, respectively); group A was entirely composed of AIDS presenters with PCP as defining illness at the first positive HIV-1 test; group B had a mean CD4 count number of 459.4 cells/mm³ with a median HIV-1 RNA of 4.3 log10 copies/mL whereas group C was mainly characterized by HIV-1 RNA < 50 copies/mL in most patients (75%). Viral load was significantly greater in group A than in groups B and C, with statistical differences between patients receiving different HAART regimens (\( P = 0.003 \)): virological suppression was achieved in 54.5% of patients under nNRTI and in 78.1% of patients receiving PLs.

Mean baseline CD4 count before starting HAART (nadir) in immune-reconstituted patients was 73.8 (s.d. 64.7) with an increase in CD4 count of 389.7 (s.d. 151.1) cells after 23.9 months of therapy, without statistically relevant differences between PI-based versus PI-sparing antiretroviral regimens. Table 2 shows the characteristics and lymphocyte proliferation responses of 43 immune-reconstituted patients treated with PI-based or nNRTI-based antiretroviral therapy. One immune-reconstituted patient receiving both nevirapine and indinavir was not included in the statistical analysis. After stratification of immune-reconstituted patients according to therapy (PI versus nNRTI regimens) no differences were seen for CD4 nadir, HAART duration, or actual CD4, CD8, NK or B cell counts.

Table 3 summarizes the results of statistical analysis of LP with different antigens in groups A, B, C and D carried out on cpm minus background noise. Statistical differences were also confirmed when data were analysed by SIs (data not shown). LP to PHA was significantly different in group A versus groups B, C and D (\( P = 0.03, P = 0.02 \) and \( P < 0.001 \), respectively). Similar results were assessed in LP to influenza antigens whereas for ENV (pool of soluble HIV-1 peptides antigens), the only statistically significant LP differences were seen between HIV-1-infected versus uninfected controls (A versus D and C versus D, \( P = 0.001 \) and 0.04, respectively).

Spinner flasks provided 3–5 × 10⁸ P. carinii trophozoites almost completely free (<1%) from feeder host cells and LPS contamination, as assessed by Limulus assay (LPS < 1.25 ng/10⁸ trophozoites).28 Rat-derived microorganisms were suitable as P. carinii antigen (PcAg) in LP. Statistically relevant differences were confirmed both in raw data (cpm), after subtraction of background noise (PcAg cpm-MED cpm) and SI calculation (PcAg cpm/MED cpm).
LP to PcAg in group D was different from all HIV-1-infected patients (P < 0.001 in D versus A, B and C). Group A had a very low LP, statistically different to group B (P < 0.001) and group C (P = 0.00012). Group C showed an LP to PcAg similar to that of group B (P = 0.28). We established as arbitrary cut-off of ‘safeness for PCP’ as the 25th percentile of Pc-specific LP values detected in naïve asymptomatic HIV-1-infected patients, since all PCP patients had an LP value below this threshold. Conversely, all healthy controls and 70% of group C had a value above the same value (Figure 1).

In total, 30% of immune-reconstituted patients (group C) showed a lymphoproliferative response to PcAg similar to group A (P = 0.16), that is below the arbitrary cut-off chosen as ‘PCP protective’, without differences between PI-treated and PI-sparing regimens. Three subjects developed PCP despite having CD4 > 250 cells/mm³.

### Table 1. Clinical features and viro-immunological data (medians and ranges) of 94 HIV-1-infected patients (groups A, B and C) and 14 healthy controls (group D)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A: AIDS presenters with PCP</th>
<th>Group B: asymptomatic HIV-1 positive</th>
<th>Group C: HIV-1 positive immune reconstituted</th>
<th>Group D: HIV-1 negative healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>28</td>
<td>22</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>23/5</td>
<td>17/5</td>
<td>36/8</td>
<td>5/9</td>
</tr>
<tr>
<td>Age, years</td>
<td>37.5 (27–72)</td>
<td>34 (23–63)</td>
<td>38.5 (25–58)</td>
<td>29 (21–39)</td>
</tr>
<tr>
<td>Risk factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDU</td>
<td>11</td>
<td>3</td>
<td>17</td>
<td>NA</td>
</tr>
<tr>
<td>MSM</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>heterosexual</td>
<td>10</td>
<td>10</td>
<td>16</td>
<td>NA</td>
</tr>
<tr>
<td>Therapy</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>PI/nNRTI</td>
<td>NA</td>
<td>NA</td>
<td>32/11</td>
<td>NA</td>
</tr>
<tr>
<td>HIV-1 RNA log₁₀</td>
<td>5.3 (2.6–7.1)</td>
<td>4.3 (2.2–5.8)</td>
<td>1.8 (1.7–5.5)</td>
<td>NA</td>
</tr>
<tr>
<td>CD⁴⁺ cells/mm³</td>
<td>32 (3–138)</td>
<td>389.5 (311–762)</td>
<td>338.5 (220–802)</td>
<td>NA</td>
</tr>
<tr>
<td>CD⁸⁺ cells/mm³</td>
<td>309 (103–1092)</td>
<td>1170.5 (405–2435)</td>
<td>963 (187–3445)</td>
<td>NA</td>
</tr>
<tr>
<td>NK cells/mm³</td>
<td>60 (7–227)</td>
<td>89 (9–392)</td>
<td>147.5 (14–795)</td>
<td>NA</td>
</tr>
<tr>
<td>B cells/mm³</td>
<td>108 (5–494)</td>
<td>143 (49–75)</td>
<td>189.5 (40–1100)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*IDU, injection drug user; MSM, men who have sex with men; NA, not applicable.

### Table 2. Immunological, virological and lymphoproliferative characteristics of 43 HIV-1 positive patients immune-reconstituted under PI-based or nNRTI-based antiretroviral regimens (group C).

<table>
<thead>
<tr>
<th></th>
<th>PI (32 patients)</th>
<th>nNRTI (11 patients)</th>
<th>Two-tailed t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>11 NFV, 8 IDV, 6 SQV, 5 LPV, 2 RTV</td>
<td>6 NVP, 5 EFV</td>
<td>NA</td>
</tr>
<tr>
<td>CD⁴⁺ nadir cells/mm³</td>
<td>64.7</td>
<td>89.0</td>
<td>0.2</td>
</tr>
<tr>
<td>HAART duration, months</td>
<td>26.7</td>
<td>26.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>26/6</td>
<td>10/1</td>
<td>NA</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.8</td>
<td>36.1</td>
<td>0.6</td>
</tr>
<tr>
<td>HIV-1 RNA (log₁₀ copies/mL)</td>
<td>2.1</td>
<td>3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>HIV-1 RNA &lt; 50 copies/mL</td>
<td>78.1%</td>
<td>54.5%</td>
<td>NA</td>
</tr>
<tr>
<td>CD⁴⁺ cells/mm³</td>
<td>385.8</td>
<td>364.4</td>
<td>0.6</td>
</tr>
<tr>
<td>CD⁸⁺ cells/mm³</td>
<td>1380</td>
<td>1202.3</td>
<td>0.6</td>
</tr>
<tr>
<td>NK cells/mm³</td>
<td>196</td>
<td>163</td>
<td>0.5</td>
</tr>
<tr>
<td>B cells/mm³</td>
<td>235</td>
<td>256.7</td>
<td>0.7</td>
</tr>
<tr>
<td>LP⁺ to PHA</td>
<td>11853</td>
<td>8256</td>
<td>0.2</td>
</tr>
<tr>
<td>LP⁺ to FLU</td>
<td>5760</td>
<td>616</td>
<td>0.8</td>
</tr>
<tr>
<td>LP⁺ to ENV</td>
<td>3131</td>
<td>5517</td>
<td>0.2</td>
</tr>
<tr>
<td>LP⁺ to PcAg</td>
<td>9514</td>
<td>8352</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Numbers are median values. NFV, nelfinavir; IDV, indinavir; SQV, saquinavir; LPV, lopinavir; RTV, ritonavir; NVP, nevirapine; EFV, efavirenz; NA, not applicable.

*cpm to specific antigen minus background noise.
they were receiving nNRTI-based HAART (two nevirapine, one efavirenz) for a mean duration of 18 months. Two-thirds of patients had a viral load >5000 copies/mL, whereas one patient had HIV-1 RNA <50 copies/mL. After stratification of immune-reconstituted patients according to antiretroviral drug regimens, no statistically significant difference was seen in LP with ENV, PHA and FLU antigens between PI-treated and nNRTI-treated patients. There were no statistically significant differences in LP responses to each antigen or in therapy duration in suppressed versus non-suppressed patients.

### Discussion

**In vitro** studies have documented reacquisition of lymphocyte proliferative response to specific mitogens under HAART.27-30 We confirmed that statistically significant differences exist in capability of responding to *Pneumocystis*, PHA and influenza antigens among HIV-1 patients, and that this phenomenon increases with higher CD4 counts. Our study showed that specific immune reconstitution against *P. carinii* occurred in 70% of patients with CD4 counts >200 cells/mm³, after HAART of 23.9 months duration. Three patients among 30% immune-reconstituted subjects who exhibited abnormally low LP to PcAg developed overt PCP although CD4 > 250 cells/mm³ and they were all receiving PI-sparing HAART. Sporadic PCP occurs in patients with CD4 counts above the threshold used to consider both instituting and discontinuing prophylaxis, but this is the first demonstration of a direct relationship between an *in vitro* specific abnormal LP to *P. carinii* and a clinically related opportunistic event.13-15,31 *Pneumocystis* has host-related species-specificity, however, rat-derived trophozoites are suitable as PcAg to be used for *in vitro* LP with human PBMC.32 The *P. carinii* antigenic moieties involved are unknown, but immunofluorescence and western blot studies have shown that organisms derived from different hosts possess both shared and species-specific antigenic determinants.33-35 Whole, washed, LPS-free trophozoites are particularly suitable for LP since it has been demonstrated that the effect of *P. carinii* on cytokine release required cell–organism contact: this may explain why human-derived recombinant antigens were only able to partially mimic the effect of intact organisms.19 Correlation between the extent of PcAg LP and stratification of patients according to CD4 counts confirmed that lymphocytes play a pivotal role in the orchestration of resistance to *P. carinii*.6,37 Despite having CD4 > 250 cells/mm³, the expected immune reconstitution was not achieved in three subjects under HAART who developed PCP. A protective host response against *P. carinii* probably involves the participation of functional CD4 lymphocytes, alveolar macrophages, and cytokines such as interleukin 1β (IL1β) and tumour necrosis factor α (TNFα) secretion.16-41 The modulation of cytokine production may be abnormal in the AIDS setting: it has been demonstrated that HIV-1 infection of human macrophages decreases the level of IL1β and TNFα when exposed to *P. carinii*.19 It has recently been demonstrated that a high level of HIV-1 replication directly impairs the proliferative response of HIV-reactive cells, whereas proliferation responses to other antigens are comparable in treated and untreated patients when controlled for circulating CD4 cell counts.18

Two-thirds of cases of PCP with relatively high CD4 number under nNRTI-based HAART may have reflected this modulation as suggested by high viral load, but in one case the HIV-1 RNA was <50 copies/mL. We previously detected antipneumocystic activity of various PIs *in vitro* whereas other authors did not confirm this observation in an animal model using a different scoring system.42,43 However, a partial, aspecific but clinically protective effect against *P. carinii* could be hypothesized for immune-reconstituted patients receiving PIs in their HAART regimens, since no patient with abnormally low Pc-specific LP receiving PIs developed PCP, although prophylaxis was stopped. In addition to the possible effect on opportunistic microorganisms, interesting studies demonstrated a non-virological possible modulation of host cell proteasome activity by ritonavir. Recently published studies suggest that subtle differences in immune restoration may exist after PI-based versus PI-sparing HAART, despite comparable degrees of viral suppression.44,45 Clinical studies have already analysed patterns of T cell repopulation, virus load reduction and restoration of T cell function in HIV-1-infected persons during therapy with different antiretroviral agents.46-48 In our study, three cases of PCP in patients with CD4 > 250 cells/mm³ occurred in nNRTI-containing HAART regimens. Among 44 immune-reconstituted patients, after stratification accord-

### Table 3: Statistical analysis (two-tailed t-test) of results of LP to *P. carinii* antigens (PcAg), phytohaemagglutinin (PHA), FLU (pool of soluble influenza virus peptides) and ENV (pool of soluble HIV-1 peptides) obtained in HIV-1-infected patients (groups A, B and C) and healthy controls (group D)

<table>
<thead>
<tr>
<th></th>
<th>PHA</th>
<th>FLU</th>
<th>ENV</th>
<th>PcAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td>0.03</td>
<td>0.008</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A versus C</td>
<td>0.02</td>
<td>0.004</td>
<td>0.06</td>
<td>0.00012</td>
</tr>
<tr>
<td>A versus D</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B versus C</td>
<td>0.3</td>
<td>0.4</td>
<td>0.9</td>
<td>0.28</td>
</tr>
<tr>
<td>C versus D</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Figure 1.** Results of lymphocyte proliferation with *P. carinii* antigens in 108 patients divided into the following groups: (A) 28 HIV-1 positive patients naive to HAART with PCP and CD4 < 200 cells/mm³; (B) 22 HIV-1 positive asymptomatic patients naive to HAART with CD4 > 200 cells/mm³; (C) 44 HIV-1 positive immune-reconstituted patients (starting HAART with CD4 < 200 cells/mm³ but with CD4 > 200 cells/mm³ when tested); (C) 3 three immune-reconstituted patients under HAART who developed PCP despite having CD4 > 250 cells/mm³ receiving nNRTI; (D) 14 healthy HIV-1-uninfected controls. The 25th percentile of the median LP value in group B was chosen as an arbitrary cut-off in our assay since all PCP patients with CD4 < 200 cells/mm³ had an LP response below this value and all healthy controls and 70% of group C had a value above the same value.
ing to therapy, we found a similar LP without statistically relevant differences in PI-containing versus nNRTI-containing associations.

A specific PcAg LP could be considered for doubtful situations (i.e. patients starting HAART with low CD4 nadir), for a safer clinical decision on discontinuing prophylaxis. In our study, the 25th percentile of the median value of LP to P. carinii of asymptomatic HIV-1 patients naive to HAART is proposed as a ‘cut-off’ value to discriminate among immune-reconstituted patients those who, showing lower values, could be at risk of developing PCP although having a CD4 count above the threshold, since all PCP patients with CD4 < 200 cells/mm³ had an LP value below this value and all healthy controls, and 70% of group C had an LP value above the same value. This value is also consistent with the observation that 10% of PCP are seen in HIV-1 patients with CD4 > 200 cells/mm³ naíve to antiretroviral therapy.

Our in vitro data confirm the clinical observation that certain HIV-1-infected patients retain a relative immunodeficiency that is not reflected in the CD4 cell counts, and are at risk of developing opportunistic events.27,45 This observation reinforces the idea that CD4 count and T helper (TH) cell function are independent variables. Thus, TH dysfunction may be observed in HIV-1-seropositive asymptomatic individuals even before a critical decline in CD4 count.46 These data also confirm that restoration of the ability to produce IL-2 upon antigen stimulation can be observed independently of dramatic changes in CD4 counts in HIV-1-seropositive individuals undergoing therapy.20,47

Such a refined measure of specific immunity, as LP to P. carinii antigen, is not hereby proposed as a routine test since it requires further prospective validation on a wider cohort. However, if validated, it could be clinically useful when focused on patients starting HAART with a very low CD4 nadir or experiencing a sudden decrease in CD4 count, for a safer decision on discontinuing or restarting therapy.50 A further application would be the discrimination of differences in immune reconstitution among patients receiving different HAART, i.e. PI- versus nNRTI-containing regimens, in addition to clinical follow up for opportunistic events.

Acknowledgements

We are grateful to Giovanna Orlando for helpful discussions and for plotting graphs, and to Henry Masur and Stefano Rusconi for critically reading the manuscript. Data partially presented at the 7th International Workshop on Opportunistic Protests, June 13–16, Cincinnati, USA, 2001 (oral presentation, platform 12) and the 1st IAS Conference on HIV Pathogenesis and Treatment, July 8–11, Buenos Aires, Argentina, 2001 (poster 647). This study was partially supported by Istituto Superiore Sanità, grant 50C.2, National AIDS Program.

References


immunodeficiency virus-infected patients to the major surface glycoprotein of *Pneumocystis carinii*. *Journal of Infectious Diseases* **177**, 238–41.


