

Letter to the Editor

In Vitro Lymphocyte Proliferative Response to HIV-1 p24 Is Associated with a Lack of CD4⁺ Cell Decline

THE T HELPER LYMPHOCYTE COMPARTMENT is the major target of human immunodeficiency virus (HIV) infection. Functional defects, evidenced by the *in vitro* lymphocyte response to recall antigens, have been demonstrated in this cell population before a significant CD4⁺ lymphocyte depletion occurs.^{1,2} Furthermore, an early depletion or functional impairment of HIV-specific CD4⁺ T helper lymphocytes can be envisioned on the basis of the assumption that these cells are more likely to encounter HIV-infected antigen-presenting cells. Lymphocyte proliferative responses to HIV antigens have been reported to be weak and sporadic in HIV-infected patients, and p24 was the antigen recognized more often by HIV-infected patients.³

We are currently investigating the proliferative response to several recombinant antigens of HIV-1 in asymptomatic HIV-infected patients. The first noticeable observation is that proliferation in response to p24 is found only in patients with stable or increasing CD4⁺ cell numbers, at least in the 2 years preceding the assay for T cell responses, and is never associated with a significant decline of CD4⁺ cell counts. Because no laboratory marker studied so far indicates clinical latency of the disease, it seems important to stress the possibility that the demonstration of HIV-specific helper activity could be suggestive of a more efficient control of the virus. At present, we have studied 31 patients with asymptomatic HIV-1 infection (CDC groups II and III) and 13 healthy HIV-seronegative subjects for the *in vitro* lymphocyte proliferative response to recombinant HIV-1 p24 (American Bio-Technologies, Cambridge, MA). Mononuclear cells (MNCs) from heparin-anticoagulated venous blood were cultured in triplicate in flat-bottomed microtiter wells at a concentration of 2×10^5 in 200 μ l of RPMI-1640 supplemented with L-glutamine, antibiotics, and 10% pooled anti-HIV-negative human AB serum. p24 was added at a concentration of 2 μ g/ml, according to preliminary titrations. Proliferation was assessed by addition after 6 days of culture of tritiated thymidine (0.5 μ Ci/well; specific activity, 25 Ci/mmol) for a further 20 hr and expressed as the ratio of the radioactivity counted in the antigen-containing cultures to that of unstimulated cultures (stimulation index, SI). The patients with HIV-1 infection were selected on the basis of having CD4⁺ lymphocyte numbers at the time of proliferation studies $> 350/\text{mm}^3$ (mean $\pm$ SD = 592 ± 142 , range, 351–904) and at least four CD4⁺ lympho-

cyte determinations over the 2 years preceding the proliferation assay. To quantify the variation of CD4⁺ lymphocyte counts over time, the regression line relative to the 2 years preceding the proliferation study was calculated by the least-squares method as described by Phillips *et al.*⁴ A negative slope of the regression line was considered indicative of CD4⁺ cell number decrease; conversely, positive slopes revealed a relative steadiness or increase of the CD4⁺ cell number, at least during the 2-year span considered.

No lymphocyte proliferation in response to p24 was found in HIV-seronegative healthy controls (SI = 0.99 ± 0.32 ; range, 0.47 ± 1.64); 7 of the 31 HIV-1-infected asymptomatic patients studied showed a p24-specific SI higher than 2, which is considered a conventional cutoff for significant proliferative responses. Raw counts of the p24-stimulated cultures of those seven subjects ranged from 1887 to 13259 cpm (mean $\pm$ SD, 6316 ± 4748) and the respective unstimulated cultures ranged from 211 to 3761 cpm (mean $\pm$ SD, 1743 ± 1358). Similarly, unstimulated cultures of the remaining subjects showing a p24-specific SI < 2 ranged from 593 to 4441 cpm (mean $\pm$ SD, 1758 ± 1018), whereas comparable counts were found in the p24-stimulated cultures of the same subjects (range, 446–4965 cpm; mean $\pm$ SD, 1736 ± 1018). The p24-specific stimulation indices of the whole group (mean, 1.77; range, 0.51–8.94; Student's *t* test vs. controls, $0.05 < p < 0.1$) were not statistically different from those of controls. No significant correlation was found between the magnitude of the proliferative response and the number or the percentage of circulating CD4⁺ lymphocytes at the time of the study.

A significant association between the slope of the CD4⁺ cell count regression line and the response to p24 was found: none of the 15 patients showing a decline in CD4⁺ cell number (negative slope) in the previous 2 years had a significant lymphocyte proliferative response to p24, whereas of the 16 remaining patients showing a steady or increasing CD4⁺ cell number (positive slope), 7 had a p24-specific SI higher than 2 (chi-square test, $p < 0.01$) (Fig. 1). According to the preliminary data reported here, we propose that the lymphocyte proliferative response to p24 could represent a useful marker of nonprogression of the disease, and should therefore be included in larger studies to be evaluated more precisely.

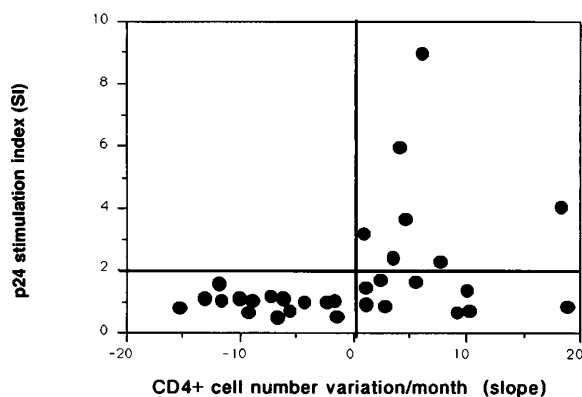


FIG. 1. Correlation between the presence of lymphocyte proliferative response to HIV-1 p24 and CD4⁺ cell number modifications. The tendency of circulating CD4⁺ cells to decline or to remain stable (or to increase) has been expressed by plotting the number of CD4⁺ cells against time (in months) and fitting the linear regression line by the least-squares method. The slope of the regression line calculated using at least four determinations in the 24 months preceding the lymphocyte proliferation assay represents the theoretical number of CD4⁺ cells decreasing (negative values) or increasing (positive values) per month. The variation in CD4⁺ cell number/month is reported on the *x* axis and the proliferative response to p24 is reported on the *y* axis. The presence of a significant proliferative response to p24 (stimulation index [SI] > 2) is never associated with an active decline of CD4⁺ cells (negative slopes).

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