Comparative Analysis of Human Cytomegalovirus-Specific CD4+ T-Cell Frequency and Lymphoproliferative Response in Human Immunodeficiency Virus-Positive Patients

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Evaluation of human cytomegalovirus (HCMV)-specific T-helper immunity could contribute in optimizing anti-HCMV therapy in human immunodeficiency virus (HIV)-infected patients. Testin the lymphoproliferative response (LPR) is the standard technique used to evaluate T-helper response, but its use in the routine diagnostic laboratory setting can be problematic. The most promising new alternative technique is the determination of HCMV-specific CD4+ T-cell frequency by flow cytometry detection of intracellular cytokine production after short-term antigen-specific activation of peripheral blood mononuclear cells. HCMV-specific LPR and CD4+ T-cell frequency were compared in a group of 78 blood samples from 65 HIV-infected patients. The results showed concordance in 80.7% of samples. In addition, comparative analysis of sequential blood samples from 13 HIV-infected patients showed that while in about half of patients the T-helper HCMV-specific immune response remained stable during highly active antiretroviral therapy (HAART), in the other half declining levels of the HCMV-specific CD4+-mediated immune response were determined by either one or both assays. In conclusion, our data suggest that the determination of HCMV-specific CD4+ T-cell frequency can be considered a valuable alternative to the LPR test for the detection of HCMV-specific T-helper response in HIV-infected patients. It could facilitate wider screening of anti-HCMV T-helper activity in HIV-infected patients, with potential benefits for clinicians in deciding strategies of discontinuation or maintenance of anti-HCMV therapy.

In the last few years, highly active antiretroviral therapy (HAART) has greatly reduced the incidence of human cytomegalovirus (HCMV) disease in human immunodeficiency virus (HIV)-infected patients in Western countries (5, 19, 20). However, at least two reasons suggest that careful monitoring of HCMV disease in HIV-infected patients is still important: first, some cases of HCMV disease in patients with relatively high CD4+ T-cell counts have been reported (7, 10, 11, 15); second, the guidelines for the discontinuation of maintenance anti-HCMV therapy are mainly based on the CD4+ T-cell count (12, 13, 16, 18, 24, 25, 27), regardless of any possible information on the reconstitution of the HCMV-specific immune response. Evaluation of specific anti-HCMV immunity could influence the clinical decision to discontinue or maintain anti-HCMV therapy in patients with previous HCMV disease and intermediate CD4+ T-cell counts. In this respect, evaluation of the HCMV-specific T-helper response is of particular interest (23). For several years, T-helper immune response has been evaluated by testing the antigen-specific lymphoproliferative response (LPR). However, the impact of the LPR assay in guiding clinical decisions is still limited, since the assay is time-consuming and poorly standardized and, being based on the use of tritiated thymidine, requires specific containment measures and facilities.

The most promising new alternative technique for the determination of HCMV-specific T-helper response is the evaluation of HCMV-specific CD4+ T-cell frequency by flow cytometry detection of intracellular cytokines after short-term antigen-specific activation of peripheral blood mononuclear cells (PBMC), as recently reported (1, 9, 14, 21, 26). This technique provides results in a few hours, does not require the use of radioactive compounds, is easy to standardize, and is applicable to frozen PBMC samples. Previous reports have shown that HCMV-specific CD4+ T cells are almost totally polarized toward Th1 phenotype and therefore that the frequency of CD4+ T cells producing tumor necrosis factor alpha (TNF-α) or gamma interferon (IFN-γ) after exposure to HCMV antigens can be considered the overall frequency of HCMV specific CD4+ T cells (21, 26). Some authors have described the analysis of HCMV-specific CD4+ T-cell frequency in different stages of HIV disease (14, 21), but LPR remains the most commonly used test to evaluate the HCMV-specific T-helper response. At present, a single report has been published comparing the HCMV-specific CD4+ T-cell frequency and LPR in two groups of HIV-seropositive patients (9).

In this study, we compared the two techniques by testing a series of samples from HIV-infected patients. Samples from patients showing a wide range of CD4+ T-cell counts, either with or without HCMV-specific T-helper response, were examined. Our data suggest that HCMV-specific CD4+ T-cell frequency correlates with LPR and is a reliable alternative to the HCMV-specific LPR test.
**MATERIALS AND METHODS**

Patients. A total of 78 blood samples from 65 adult patients (20 females, 45 males) that were both HIV and HCMV seropositive were analyzed. The number of CD4+T cells per microliter of blood was assessed by the Ortho ImmunoCount Flow Cytometer System (Raritan, N.J.), while the HIV type 1 (HIV-1) viral load was determined by the bDNA technique (Bayer/Chiron Corp., Emeryville, Calif.). At first evaluation, of the 65 patients, 6 (3 with and 3 without HCMV disease) were HAART naïve with a median HIV load of 272,551 RNA copies/ml (range, <50 to 1,000,000 copies/ml) and 21 CD4+T cells/μl (range, 1 to 221 cells/μl), and 6 (3 with and 3 without HCMV disease) had been treated with short-term HAART for 12 months (range, 8 to 20 months), reaching the level of (<50 HIV RNA copies/ml and 271 CD4+ T cells/μl (range, 65 to 410 cells/μl). In addition, of the remaining 53 patients treated with long-term HAART for 46 months (range, 29 to 57 months), 11 with HCMV disease were tested either during anti-HCMV treatment (n = 6) or after its discontinuation (n = 5), while 42 were examined in the absence of HCMV disease. At initial observations, the patient group showed a median HIV load of 59 RNA copies/ml (range, <50 to 150,070 copies/ml) and a CD4+T-cell count of 421/μl (range, 50 to 1,256 cells/μl).

PBMC preparation and storage. PBMC were isolated by standard Ficoll gradient centrifugation of heparinized blood samples and frozen by resuspending 5 × 106 cell aliquots in freezing medium (RPMI 1640 supplemented with 10% dimethyl sulfoxide and 5% human albumin). When necessary, cells were thawed rapidly at 37°C and washed twice with RPMI 1640 containing 5% pooled human serum (AB type; Euroclone, Wetherby, West York, United Kingdom). Viability was evaluated by trypan blue exclusion staining. The rare samples with a viability of <95% were discarded.

HCMV-specific lymphoproliferative assay. Cells resuspended in RPMI 1640 containing 5% pooled human serum (AB type) were plated in triplicate at a 105 cell density by scattering parameters. For each tube, FACSCalibur (BD Biosciences) was used to analyze surface marks (anti-CD4 fluorescein isothiocyanate, anti-TNF-α, and anti-CD28 phycoerythrin (PE), or anti-IFN-γ-PE plus anti-CD28-PerCP). Cells were suspended with a FACScalibur flow cytometer (Becton Dickinson) equipped with CellQuest software. Viable lymphocytes were identified by scattering parameters. For each sample, 50,000 viable CD4+ T lymphocytes were evaluated. CD4+ T cells expressing TNF-α or IFN-γ and CD69 were considered activated cells. The HCMV-specific CD4+ T cell frequency was calculated by subtracting the value of the control sample incubated with control antigen (consistently ±0.05%). The value of HCMV-specific CD4+ T cells per ml of blood was calculated by multiplying the

| Subject | HCMV-specific CD4+ T-cell frequency (net cpm) |
|---------|---------------------------------------------|
|         | Fresh | Frozen | Fresh | Frozen |
| 1       | 0.48  | 0.96   | 6     | 18,000 | 14,800 |
| 2       | 1.70  | 0.82   | 7     | 10,400 | 1,800  |
| 3       | 1.24  | 0.79   | 8     | 60,400 | 800    |
| 4       | 0.53  | 0.32   | 9     | 22,600 | 12,000 |
| 5       | 0.37  | 0.12   | 10    | 28,800 | 8,000  |

* All subjects except subject 4 were HIV positive.
CD4⁺ T cells by flow cytometry analysis: the simultaneous presence of an HCMV-specific CD4⁺ T-cell frequency of ≥0.1% and ≥400/ml of blood. In parallel, the LPR response was evaluated in 23 samples from 10 immunocompetent HCMV-seropositive subjects and in 8 samples from 4 immunocompetent HCMV-seronegative subjects (Table 3). Patients were considered responders (R) for LPR to HCMV when the SI was ≥3 and the net cpm was ≥3,000 and defined as nonresponders (NR) when either one or both of these conditions were not satisfied. All of the nine HCMV-seropositive healthy subjects tested by both assays were found to be positive for both LPR to HCMV and a measurable frequency of HCMV-specific CD4⁺ T cells.

Comparison of HCMV-specific CD4⁺ T-cell frequency and LPR in HIV-infected patients. Seventy-eight PBMC samples from 65 HCMV-seropositive HIV-infected patients (13 patients were evaluated twice) were tested by both methods. In summary, according to the criteria reported above, 43 samples (55.1%) were found to be positive and 20 samples (25.6%) were found to be negative by both assays. In addition, seven samples (9.0%) were found to be positive for HCMV-specific CD4⁺ T-cell presence but negative for LPR, whereas eight samples (10.4%) were negative for HCMV-specific CD4⁺ T cells but positive for LPR (Table 4). Therefore, 80.7% of assayed samples gave concordant results by both techniques. As for discordant results, the evaluation of HCMV-specific CD4⁺ T-cell frequency was repeated for most samples, confirming previous results.

In addition, further aliquots of the same samples positive for LPR and negative for HCMV-specific CD4⁺ T-cell presence were tested. At first, impairment of cytokine production (possibly due to the freezing procedure) was excluded by evaluating the intracellular TNF-α production in seven samples by stimulating PBMC with SEB. The percentage of SEB-responsive CD4⁺ T cells was 6.56 ± 1.6 (mean ± standard deviation), i.e., only slightly lower than that found in samples from healthy subjects, thus indicating that even in discordant samples the vast majority of CD4⁺ T cells could be stimulated in vitro. Furthermore, an abnormal cytokine pattern production was excluded in three samples by evaluating the intracellular production of IFN-γ instead of the production of TNF-α. The analysis of the expression of the two cytokines gave similar results. Finally, to exclude a varying reactivity to antigens of different sources, the HCMV-specific frequency of three samples was determined by using the same HCMV antigen preparation used for LPR that confirmed results obtained previously with the commercial antigen preparation. In addition, the commercial HCMV antigen was found to induce LPR to a level comparable to that seen with the antigen prepared in the laboratory.

However, when we examined discordant samples in detail, the few samples with positive LPR but negative for HCMV-specific CD4⁺ T-cell frequency appeared to show a low-level LPR (net cpm < 10,000; see Fig. 1). In contrast, at least three samples had an HCMV-specific CD4⁺ T-cell frequency of ≥1% but a totally impaired HCMV-specific LPR (Fig. 1).

As for the different patient groups, concordant results were found in 6 of 6 treatment-naive patients (4 NR patients with CD4⁺ T cells at <50/μl and 2 R patients), 4 of 6 patients (3 NR, 1 R) treated with short-term HAART, 8 of 11 (7 R, 1 NR) patients with HCMV disease treated with long-term HAART, and 34 of 42 (28 R, 6 NR) patients treated with long-term HAART in the absence of HCMV disease.

Correlation of HCMV-specific CD4⁺ T-cell count and LPR. The correlation of HCMV-specific CD4⁺ T-cell count and LPR was significant (R = 0.327, P = 0.003) (Fig. 1), whereas no significant correlation was found between CD4⁺ T-cell count and either the net cpm of LPR (Fig. 2A) or the HCMV-specific CD4⁺ T-cell frequency (Fig. 2B). It was evident that

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**TABLE 2. HCMV-specific CD4⁺ T-cell frequency in healthy HCMV-seropositive and -seronegative control subjects**

| HCMV serostatus group (n) | % HCMV-specific CD4⁺ T cells | No. of HCMV-specific CD4⁺ T cells/ml of blood |
|---------------------------|-----------------------------|---------------------------------------------|
|                           | Median Range                | Median Range                                |
| Positive subjects (9)     | 1.69 0.24–4.73              | 12,248 1,796–37,604                          |
| Negative subjects (6)     | 0.01 0.00–0.04               | 39 0–218                                    |

**TABLE 3. LPR to HCMV in 10 HCMV-seropositive and -seronegative immunocompetent subjects**

| HCMV serostatus group (n) | SI | Net cpm |
|---------------------------|----|---------|
|                           | Median Range | Median Range |
| Positive samples (23)     | 24.6 3.9–395.2 | 28,293 9,629–67,422 |
| Negative samples (8)      | <1.0 <1.0–4.5 | 185 0–2,699 |

**TABLE 4. Comparison of HCMV-specific CD4⁺ T-cell frequency and LPR in 78 samples from 65 HIV-infected patients**

| HCMV-specific CD4⁺ T-cell presence | HCMV-specific LPR | No. of samples (%) | Median CD4⁺ T cells/μl (range) |
|------------------------------------|-------------------|--------------------|--------------------------------|
| Positive                            | Positive          | 43 (55.1)          | 424 (56–1,256)                 |
| Negative                            | Negative          | 20 (25.6)          | 210 (1–1,108)                  |
| Positive                            | Negative          | 7 (9.0)            | 406 (50–977)                   |
| Negative                            | Positive          | 8 (10.4)           | 378 (113–655)                  |

**FIG. 1. Correlation of HCMV-specific CD4⁺ T-cell frequency and LPR as net cpm in 78 PBMC samples from 65 HIV-infected patients. Dotted lines indicate the cutoffs for the HCMV-specific CD4⁺ T-cell presence (0.1% of total CD4⁺ T cells) and for HCMV-specific LPR (3,000 net cpm).**
several patients with CD4+ T-cell count of <150/μl showed a high anti-HCMV T-helper activity, as documented by both assays. On the other hand, 5 patients (patients 1, 8, 10, 11, and 12) showed declining levels of HCMV-specific T-helper response by both or either assay, and only one patient (patient 9) displayed a positive response by flow cytometry at the time of the second evaluation only (Fig. 3B).

Clinically, patients 4 to 13 all had CD4+ T-cell counts of <50/μl prior to HAART, and all had been treated with long-term HAART at the time of the first examination. Of these, patients 4 to 8 had had HCMV disease in the past and had been treated with anti-HCMV therapy, which was discontinued for patients 7 and 8 prior to testing because of high CD4+ T-cell counts, whereas patients 9 to 13 had no HCMV disease. All of these patients had a negative LPR to HCMV prior to HAART.

DISCUSSION

HAART has been found to be effective in restoring CD4+ T-cell counts and reconstituting the immune system function in the majority of HIV patients (2, 5, 17, 19, 20, 22). However, some cases of HCMV retinitis in HIV patients with high CD4+ T-cell counts have been reported (7, 10, 15), suggesting that the HCMV-specific immune response could not be restored in
those patients, possibly due to failure of reconstitution of the complete T-cell-receptor repertoire (4, 6, 8). In addition, current guidelines suggest maintenance or interruption of anti-HCMV therapy only based upon CD4⁺ T-cell count (12, 13, 16, 18, 24, 25, 27). Furthermore, as shown in this study, some patients with low CD4⁺ T-cell counts possess an apparently adequate anti-HCMV immune response and so would not require anti-HCMV therapy.

As a matter of fact, one of the major aims of research in this field is to develop an immunoassay that can evaluate the HCMV-specific immune response (in particular, the T-helper response) and predict which patients will relapse or reactivate HCMV disease. Lack of this information is partially due to the poor standardization and the large intrinsic variability of the most common method, the LPR assay. In this respect, one of the most promising alternative techniques is the evaluation of HCMV-specific CD4⁺ T-cell frequency by cytokine flow cytometry. Despite the fact that this technique has been already used in several clinical studies (1, 14, 21, 26), its correlation with the well-known LPR assay has, to our knowledge, only preliminarily been reported (9).

The most interesting finding of the present study is that, in a population of HIV-seropositive patients with a wide range of CD4⁺ T-cell counts, analysis of presence of HCMV-specific T-helper response by LPR and HCMV-specific CD4⁺ T-cell frequency gave concordant results in more than 80% of cases. Agreement between the two assays is only slightly higher than that reported in a recent study, in which the HCMV-specific CD4⁺ T-cell frequency was determined by HCMV-specific stimulation of whole blood instead of PBMC (9). The lack of an even higher concordance between the two techniques could be explained by considering the two groups of discordant results separately. In the group of patients positive for LPR but negative for HCMV-specific CD4⁺ T-cell frequency, the positivity for LPR was consistently weak. As a matter of fact, it is possible that in a few samples very rare HCMV-specific CD4⁺ T cells are present with a high proliferative potential that can be detected by LPR but not by flow cytometry analysis. On the other hand, in a few samples HCMV-specific CD4⁺ T-cell presence was detected (in two cases, at high frequency), but the same PBMC samples were not able to proliferate in vitro in response to HCMV antigen. This discordance could be explained by considering that LPR measures the expansion of several antigen-specific T-cell clones, displaying different effector functions, whereas the HCMV-specific CD4⁺ T-cell frequency was calculated by taking into account only TNF-α-and/or IFN-γ-producing cells. It is conceivable that HIV-positive individuals showing a negative LPR, in the presence of a measurable frequency of HCMV-specific CD4⁺ T-cell frequency, lack some other antigen-specific T-cell subsets, such as IL-2-producing cells, that are necessary for optimal T-cell expansion. Alternative explanations could include (i) a suboptimal antigen-presenting-cell function, which might be inadequate for sustaining T-cell expansion but sufficient to activate TNF-α- and/or IFN-γ-producing cells, and (ii) the presence of a high proportion of HCMV-specific T cells with suppressor function that can inhibit lymphocyte proliferation (3).

The evidence that the HCMV-specific LPR assay and the evaluation of HCMV-specific CD4⁺ T-cell frequency in a proportion of HIV-positive individuals give rise to discrepant results suggests that the evaluation of HCMV-specific immune recovery after HAART therapy should include both assays at best. Nevertheless, the results of the present study suggest that, whenever the availability of PBMC is not sufficient to perform both assays, the evaluation of HCMV-specific CD4⁺ T-cell frequency is as reliable as LPR.

The concordance of results obtained by the two assays on the great majority of samples tested was confirmed by the similarity of results found in the short-term follow-up of the 13 HIV-infected patients tested some months apart. While a fair proportion of patients showed stable results, another substantial aliquot displayed results showing declining levels of the HCMV-specific CD4⁺ T-cell-mediated immune response by either one or both assays after 3 to 4 years of HAART. These findings are in agreement with results recently acquired in our laboratory showing a decrease or loss of the rescued LPR to HCMV in AIDS patients after 3 to 4 years of HAART (unpublished data).

Present data do not provide an explanation for this phenomenon. However, it is conceivable that immune recovery achieved after HAART therapy might bring about a decrease in the frequency of HCMV reactivation and, as a consequence, a reduction in the number of circulating HCMV-specific T cells, which are no longer stimulated by chronic antigen exposure.

The significant correlation of CD4⁺ T-cell frequency and LPR supports the concept that the two assays may be interchangeable. On the other hand, the lack of correlation between CD4⁺ T-cell count and either the CD4⁺ T-cell frequency or LPR documents that the absolute CD4⁺ T-cell count and the other two assays do not express a comparable immunological condition, thus indicating that the CD4⁺ T-cell count alone is insufficient for deciding upon strategies of discontinuation of anti-HCMV therapy in AIDS patients with HCMV retinitis.

In conclusion, our data suggest that the determination of HCMV-specific CD4⁺ T-cell frequency can be considered a valid alternative to the LPR test for the detection of HCMV-specific T-helper response in HIV-infected patients. This technique offers evident advantages over LPR. It does not require the use of radioactive compounds, and it is faster and easier to standardize. Furthermore, it could facilitate wider screening of anti-HCMV T-helper activity in HIV-infected patients, with potential benefits for clinicians in deciding strategies of discontinuation or maintenance of anti-HCMV therapy.

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REFERENCES

1. Asanuma, H., M. Sharp, H. T. Meacker, V. C. Maino, and A. M. Arvin, 2000. Frequencies of memory T cells specific for varicella-zoster virus, herpes simplex virus, and cytomegalovirus by intracellular detection of cytokine expression. J. Infect. Dis. 181:859–866.
2. Autran, B., G. Carcelaint, T. S. Li, G. Gorochov, C. Blanc, M. Renaud, M. Durali, D. Mathez, V. Calvez, J. Leibowitch, C. Katlama, and P. Debre, 1999. Restoration of the immune system with anti-retroviral therapy. Immunol. Lett. 66:207–211.
Boussiotis, V. A., E. Y. Tsai, E. J. Yunis, S. Thim, J. C. Delgado, C. C. Dascher, A. Bereczkyvaka, D. Roussel, J. M. Reynolds, and A. E. Goldfield. 2000. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. J. Clin. Invest. 105:1317–1325.

Connors, M., J. A. Kovacs, S. Krevat, J. C. Gea-Banacloche, M. C. Sneller, M. Flanigan, J. A. Metcalf, R. E. Walker, J. Fallono, M. Baseler, R. Stevens, I. Feuerstein, H. Masur, and H. C. Lane. 1997. HIV infection induces changes in CD4 T-cell phenotype and depletions within the CD4 T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. Nat. Med. 3:533–540.

Deayton, J. R., P. Wilson, C. C. Sabin, C. C. Davey, M. A. Johnson, V. C. Emery, and P. D. Griffiths. 2000. Changes in the natural history of cytomegalovirus retinitis following the introduction of highly active antiretroviral therapy. AIDS 14:1163–1170.

Gea-Banacloche, J. C., L. Martino, J. M. Mican, C. W. Hallahan, M. Baseler, R. Stevens, L. Lambert, M. Polis, H. C. Lane, and M. Connors. 2000. Longitudinal changes in CD4 T-cell antigen receptor diversity and naive/memory cell phenotype during 9 to 26 months of antiretroviral therapy of HIV-infected patients. AIDS Res. Hum. Retrovir. 16:1877–1886.

Gillquin, J., C. Piketty, V. Thomas, G. Gonzales-Canali, L. Belic, and M. D. Kazatchkine. 1997. Acute cytomegalovirus infection in AIDS patients with CD4 counts above 100 × 10^6 cells/l following combination antiretroviral therapy including protease inhibitors. AIDS 11:1659–1660.

Goroschov, G., A. U. Neumann, A. Kereveur, C. Parizot, T. Li, C. Katlama, M. Karmochnache, G. Raguin, B. Autran, and P. Debire. 1998. Perturbation of CD4+ and CD8+ T-cell repertoire during progression to AIDS and regulation of the CD4+ repertoire during antiretroviral therapy. Nat. Med. 4:215–221.

Jacobson, M. A., R. Schrier, J. M. McCune, F. J. Torriani, G. N. Holland, J. J. O'Donnell, W. R. Freeman, and B. M. Bredt. 2001. Cytomegalovirus (CMV)-specific CD4+ T lymphocyte immune function in long-term survivors of AIDS-related CMV end-organ disease who are receiving potent antiretroviral therapy. J. Infect. Dis. 183:1399–1404.

Jacobson, M. A., M. Zegans, P. R. Pavan, J. J. O'Donnell, F. Sattler, N. Rao, S. Owens, and R. Pollard. 1997. Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 349:1443–1445.

Johnson, S. C., C. A. Benson, D. W. Johnson, and A. Weinberg. 2001. Recurrences of cytomegalovirus retinitis in a human immunodeficiency virus-infected patient, despite potent antiretroviral therapy and apparent immune reconstitution. Clin. Infect. Dis. 32:815–819.

Jouan, M., M. Saves, R. Tubiana, G. Carcelain, N. Cassoux, C. Aubron-Olivier, A. M. Fillet, M. Nciri, B. Senechal, G. Chene, C. Tural, S. Lasry, B. Autran, and C. Katlama, and the RESTIMOP Study Team. 2001. Discontinuation of maintenance therapy for cytomegalovirus retinitis in HIV-infected patients receiving highly active antiretroviral therapy. AIDS 15:23–31.

Kirk, O., J. D. Lundgren, C. Pedersen, H. Nielsen, and J. Gerstoft. 1999. Can chemophrophylaxis against opportunistic infections be discontinued after an increase in CD4 cells induced by highly active antiretroviral therapy? AIDS 13:1647–1651.

Komanduri, K. V., M. N. Viswanathan, E. D. Wieder, D. K. Schmidt, B. M. Bredt, M. A. Jacobson, and J. M. McCune. 1998. Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. Nat. Med. 4:953–956.

Komanduri, K. V., J. Feinberg, R. K. Hutchins, R. D. Frame, D. K. Schmidt, M. N. Viswanathan, J. P. Lalezari, and J. M. McCune. 2001. Loss of cytomegalovirus-specific CD4+ T cell responses in human immunodeficiency virus type 1-infected patients with high CD4+ T cell counts and recurrent retinitis. J. Infect. Dis. 183:1285–1290.

Kovacs, J. A., and H. Masur. 2000. Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. N. Engl. J. Med. 342:1416–1429.

Li, T. S., R. Tubiana, C. Katlama, V. Calvez, H. Ait Mohand, and B. Autran. 1998. Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. Lancet 351:1682–1686.

Macdonald, J. C., F. J. Torriani, L. S. Morse, M. P. Karavellas, J. B. Reed, and W. R. Freeman. 1998. Lack of reactivation of cytomegalovirus (CMV) retinitis after stopping CMV maintenance therapy in AIDS patients with sustained elevations in CD4 T cells in response to highly active antiretroviral therapy. J. Infect. Dis. 177:1182–1187.

Mocroft, A., C. Katlama, A. M. Johnson, C. Pradier, F. Antunes, F. Mulcahy, A. Chiesi, A. N. Phillips, O. Kirk, and J. D. Lundgren. 2000. AIDS across Europe, 1994–98: the EuroSIDA study. Lancet 356:291–296.

Palella, F. J., Jr., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. F. Fuhrer, G. A. Satten, D. J. Aschman, and S. D. Holmberg. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N. Engl. J. Med. 338:853–860.

Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Kouop, V. C. Maino, and L. J. Picker. 1999. HIV-1 specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. 5:518–525.

Pontesilli, O., S. Kerkhof-Garde, N. G. Pakker, D. W. Notermans, M. T. Roos, M. R. Klein, S. A. Danner, and F. Miedema. 1999. Antigen-specific T-lymphocyte proliferative responses during highly active antiretroviral therapy (HAART) of HIV-1 infection. Immunol. Lett. 66:213–217.

Schrier, R. D., W. R. Freeman, C. A. Wiley, and J. A. McCutchan. 1995. Immune predispositions for cytomegalovirus retinitis in AIDS. The HNRC Group. J. Clin. Invest. 95:1741–1746.

Tural, C., J. Romeu, G. Sirera, D. Andreu, M. Conejero, S. Ruiz, A. Jov, A. Bonjoch, L. Ruiz, A. Arno, and B. Clotet. 1998. Long-lasting remission of cytomegalovirus retinitis without maintenance therapy in human immunodeficiency virus-infected patients. J. Infect. Dis. 177:1080–1083.

Vabrec, T. R., V. F. Baldassano, and S. M. Whitcup. 1998. Discontinuation of maintenance therapy in patients with quiescent cytomegalovirus retinitis and elevated CD4+ counts. Ophthalmology 105:1259–1264.

Waldrop, S. L., C. J. Pitcher, D. M. Peterson, V. C. Maino, and L. J. Picker. 1997. Determination of antigen-specific memory effector CD4+ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency. J. Clin. Invest. 99:1739–1750.

Whitcup, S. M., E. Fortin, A. S. Lindblad, P. Griffiths, J. A. Metcalf, M. R. Robinson, J. Manischewitz, R. Baird, C. Perry, I. M. Kidd, T. Vabrec, R. T. Davey, Jr., J. Fallono, R. E. Walker, J. A. Kovacs, H. C. Lane, R. B. Nussenblatt, J. Smith, H. Masur, and M. A. Polis. 1999. Discontinuation of anticytomegalovirus therapy in patients with HIV infection and cytomegalovirus retinitis. JAMA 282:1633–1637.