positivity with this assay among patients who clearly had CMV-protective immunity. Similarly, those patients who clearly lacked CMV-protective immunity (i.e., had active CMV retinitis) did not consistently have negative results with this assay. Had we used a longer period of antigen stimulation, as done by Hsieh et al., it is still unlikely that our results would have suggested potential clinical utility, because longer incubation might increase the CD4+ T lymphocyte CD69 response to CMV stimulation, thus increasing the false positivity of the assay among patients with active retinitis.

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References


Reply

To the Editor—In response to the statements made by Jacobson et al. [1, 2], our study was not designed to study the utility of an immunological marker (such as antigen-specific CD69 expression) in monitoring the risk of development of cytomegalovirus (CMV) retinitis in human immunodeficiency virus (HIV)-infected patients during highly active antiretroviral therapy (HAART). The study design and primary goal of our investigation was to examine the T cell function profiles in patients with AIDS with CMV retinitis before and after initiating HAART [3]. After we compared these parameters of CMV-specific T cell function, we found that the development of CMV retinitis was associated with poor reactivity of CMV-specific CD4 T cells, and that no evidence of immune rebound in patients with CMV retinitis that developed after initiation of HAART. Furthermore, in our data, no patients with baseline (i.e., before any antiretroviral treatment) frequencies CMV-specific of CD69 expression on CD4 T cells (CD69 cell percentages to CMV on CD4 T cells) >0.5% developed CMV retinitis after starting HAART. However, we did not know if these patients did not receive HAART after baseline assessment or whether the immune parameter could be applied during HAART. Immunity to CMV is complex, and we did not intend to suggest in our article that the percentage of CD4+ T cells expressing CMV-specific CD69 can represent the protective immunity to CMV infection. Actually, the associated poor reactivity of CMV-specific CD4+ T cells may be a result of immune dysregulation rather than deficiency in protective immunity [4, 5].

Two critical points should be discussed with regard to CD69 expression on T cells. First, 24-h period of antigen incubation was applied in our study according to the time needed for the most steady and plateau expression of CD69 on CD4+ T cells in our preliminary experiments (figure 1). There might be an unsteady phase of CD69 expression in the initial 12 h of incubation of T cells with CMV antigen. Craston et al. [6] also showed that CD69 might be rapidly expressed on T cells in response to mitogen stimulation within 4 h; however, CD69 expression on T cells in response to antigen would not reach a steady plateau until 24–48 h of incubation. Thus, an adequate incubation time is important to avoid obtaining inconsistent data.

Second, an adequate control is essential to elucidate CMV-specific reactivity. Jacobson and Bredt [2] subtracted the background CD69 expression of control samples incubated without CMV antigen. However, the reactivity of control samples that should have been subtracted from that of experimental samples, as in our study, was the reactivity to CMV control antigen, rather than the reactivity of unstimulated samples. The CMV antigen used in our study was extracted from the CMV-infected fibroblasts, so the fibroblasts themselves might cause a non-specific immune reaction, in addition to the CMV-specific reaction. Thus, CMV control antigen, that derived from the same fibroblasts without infection by CMV (both CMV antigen and control antigen were purchased from BioWhittaker) should be used, to avoid the difficulty in data interpretation.

We would like to note again that the determination of percentages of CD4+ T cells expressing CMV-specific CD69 in CMV-seropositive patients with advanced HIV infection before
these patients receiving HAART may help identify patients who are at high risk for CMV retinitis. To know whether CD69 expression could be used as an immunological marker to monitor the risk of development of CMV retinitis in HIV-infected patients during HAART, further prospective studies with regular and standardized immunological and clinical monitoring are needed.

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