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Interest of Quantitative pp65 Cytomegalovirus Antigenemia Assay for Human Immunodeficiency Virus-Positive Patients

We read with interest the article of Bek et al. (1) concerning the quantitative pp65 antigenemia assay for diagnosis of cytomegalovirus (CMV) disease in AIDS patients.

Testing 174 human immunodeficiency virus (HIV)-positive patients for CMV pp65 antigenemia, the authors showed that this assay was both sensitive and rapid, high levels of antigenemia being strongly suggestive of CMV disease. However, the possibility of CMV infection cannot be excluded when antigenemia is low, especially in individuals with retinitis, 42% of whom had less than five positive cells per slide.

We made an 18-month prospective longitudinal study of 118 HIV-seropositive patients (90 male and 28 female; mean age 40 years; range, 21 to 74 years) with CD4 counts of less than 100 cells per μl to evaluate the pp65 antigenemia assay in diagnosis or prediction of CMV disease. Aliquots of 10^5 peripheral blood leukocytes from 408 blood samples (mean number of samples per patient, 3.4; range, 1 to 16) were tested by immunofluorescence using a saturation step and incubation with Clonab* anti-human CMV pp65 monoclonal antibody (Biotest, Dreieich, Germany) followed by staining with anti-mouse biotinylated $F(ab')_2$ fragments (Immunotech, Marseille, France) and streptavidin-fluorescein complex (Immunotech). The assay was considered positive when at least two cells per slide exhibited typical nuclear staining. Simultaneous appearance of clinical signs and antigenemia positivity occurred in 14 patients, with a mean of 56 positive cells per slide (range, 8 to 400), with no statistically significant difference between patients with retinitis and those with other manifestations. Six of these patients had previously shown antigenemia, and all six were positive for a mean period of 52 days before presenting clinical manifestations (range, 19 to 108 days). These results were confirmed in another study (2). Antigenemia in 12 of the patients was monitored during subsequent treatment: 11 were negative for antigenemia 3 weeks after the onset of treatment, and 1 remained positive throughout, with aggravation of symptoms until death. Five patients relapsed during maintenance therapy while remaining positive (mean of 20 cells per slide). The level of antigenemia was low (three to five cells) for patients with retinitis relapse and higher for those with visceral manifestations. Over the same period, two other patients who had a positive antigenemia result were treated without waiting for manifestations of clinical symptoms. Three months later, they are still antigenemia negative and present no symptoms.

We agree with Bek et al. that the level of antigenemia is lower during CMV disease relapse, especially in patients with retinitis. However, in our experience, the antigenemia level is high during the first episode of clinical CMV disease, even in patients with retinitis (mean of 39 positive cells per slide). The patient with the lowest result obtained during first-episode retinitis (eight positive cells) rapidly became negative during treatment, despite ophthalmological aggravation. The presence of high levels of human herpesvirus 6 DNA in the serum of this patient underlines the possibility that other pathogens may cause retinitis. Five similar cases of retinitis also occurred in patients who repeatedly tested negative for antigenemia.

The antigenemia assay seems useful for diagnosis and prediction of CMV disease. Positive results enable early treatment to be considered, even in the absence of clinical manifestations. Furthermore, this assay provides a reliable estimate of therapeutic efficiency.

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Author's Reply

Ranger-Rogez et al. raise two interesting question, i.e., whether CMV retinitis can occur with a low systemic CMV load and whether there is a significant difference in viral loads between patients with retinitis and those with other manifestations of CMV disease. In our study (1), 42% of patients with CMV retinitis had low systemic viral loads as determined by the pp65 antigenemia assay; the diagnosis of CMV retinitis was supported by response to ganciclovir treatment. This observation is in agreement with results reported by other investigators showing that CMV retinitis also occurred in a setting of low systemic viral load (5–8, 12, 13). In addition, one recent study by Rassmussen et al. and preliminary reports of two other recent studies suggest that a substantial number of patients, i.e., 11 to 48%, have no detectable virus (as determined by either PCR or the pp65 antigenemia assay) at the time of diagnosis (4, 9–11). However, as pointed out in the Discussion section of our article, other investigators did not report retinitis with low viral load, which is in agreement with the findings of Ranger-Rogez et al. Overall, the frequency of patients with low or undetectable viral loads and CMV retinitis varies between studies, possibly due to the small sample size in many of these studies, differences in assay sensitivity, and differences in the definitions of low viral load. That Ranger-Rogez et al. did not see patients with CMV retinitis and low-grade pp65 antigenemia may be due to the small sample size of their study (n = 14).

A review of the published literature on the correlation of CMV load and CMV disease suggests that CMV disease generally is associated with a higher systemic CMV load. To date, there have been no clear definitions of cutoff points for high viral load. This is, in part, due to differences in test methodology which seem to influence assay sensitivity. Available data also suggest that different breakpoints for different patient settings exist. Importantly, any manifestation of CMV disease

can occur in a setting of low viral load, both in AIDS and in transplant patients (15). However, there seem to be differences in the frequency of this phenomenon between different patient populations. For example, manifestations that are reported to occur more often in association with a low systemic viral load include CMV retinitis in AIDS patients and CMV gastrointestinal disease in marrow transplant patients (reference 2 and unpublished data). In contrast, CMV gastrointestinal disease in AIDS patients appears to be associated with a significantly higher systemic CMV load in comparison with retinitis (13).

The reason for the presence of a low systemic CMV load at the time of the diagnosis of retinitis in AIDS patients is poorly understood. One possible explanation is that CMV retinitis is a localized rather than a systemic disease, possibly on the basis of virus tropism due to strain differences (14). Technical aspects and differences in assay sensitivity may also be responsible, at least in part, for the failure to detect virus at the time of diagnosis. Finally, the diagnosis of retinitis may be incorrect or the retinitis may be due to other causes (e.g., toxoplasmosis or varicella-zoster virus). Further studies are needed to define the role of these factors in CMV retinitis in AIDS patients. These studies should include objective assessment of the diagnosis of retinitis and possibly also PCR evaluation of aqueous humor to confirm CMV as the cause of retinitis (6).

An accurate estimate of the frequency with which CMV retinitis occurs in the presence of a low systemic CMV load is important for the design of preemptive treatment strategies. Although conceptually attractive, a strategy which starts antiviral treatment at a certain level of CMV load would fail to prevent CMV retinitis in a significant number of patients, if a substantial number of patients with retinitis develop CMV disease without preceding high viral loads. The importance of this issue is illustrated by results of a recent randomized trial with marrow transplant patients. In this trial, when preemptive treatment with ganciclovir was based on systemic viral load measured by pp65 antigenemia, a significant number of patients with disease associated with low viral load were missed in comparison with the numbers when CMV prophylaxis was started at engraftment (3). Similar results might be expected with HIV patients if a significant proportion of CMV disease occurs in patients with low viral loads and anti-CMV treatment is delayed too long. In summary, several recent studies indicate that CMV retinitis can occur in patients with low systemic CMV loads. Additional studies are needed to determine the frequency and mechanism of this phenomenon.

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