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Evaluation of a New Reagent for Anti-Cytomegalovirus and Anti-Epstein-Barr Virus Immunoglobulin G

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The Enzygnost alpha method was tested against the complement fixation test and anti-VCA immunofluorescence to determine the respective titers of anti-cytomegalovirus and anti-Epstein-Barr virus immunoglobulin G antibodies. For cytomegalovirus, the Enzygnost results showed 97.99% agreement with the readings obtained by the alternative method, with 100% sensitivity and 93.7% specificity. For Epstein-Barr virus, Enzygnost showed 97.71% agreement, 100% sensitivity, and 91.11% specificity.

A herpesvirus can be detected by the presence of antibodies that reflect the condition of the patient as well as the response of his immune system. Immunosuppressed subjects are more susceptible to herpesvirus infection and do not respond well (3, 7, 10, 12, 15). Because the introduction of new serological methods should be accompanied by antibody evaluation, especially in the case of immunosuppressed and immunocompromised populations, we assessed the advantages of a new enzyme-linked immunosorbent assay (ELISA) method that determines the titers of anti-cytomegalovirus (CMV) and anti-Epstein-Barr virus (EBV) immunoglobulin G (IgG) antibodies.

A total of 1,048 serum samples were divided into four donor groups. Panel 1 consisted of 264 samples from 200 gestating females and 64 healthy male adults, panel 2 contained 140 samples from human immunodeficiency virus (HIV) carriers, panel 3 contained 588 samples from children 2 to 5 years of age, and panel 4 consisted of 56 serum samples from hemodialyzed patients awaiting a transplant.

The anti-CMV and anti-EBV IgG antibodies were analyzed with an indirect ELISA test (Enzygnost alpha method; Behring Institute) (method 1), and the results were then compared with those obtained by the complement fixation test for CMV (Virgo; Roche) and the anti-VCA immunofluorescence assay (IFA) for EBV (Epstein-Barr virus VCA; Organon) (method 2). The sera giving discrepant results were retested with a third method, which was CMVScan latex (Becton Dickinson) for CMV and the EBV-VCA-IgG IFA (Gull) for EBV. The results were classified according to the criteria representing the majority of responses.

Briefly, for method 1, the antibody titers were determined with the formula $\log_{10}$ titer of alpha = absorbance^{beta}. The alpha and beta values were batch-dependent constants given in the kit. In accordance with the manufacturers' recommendations, the anti-CMV antibodies were studied with an initial 1/8 dilution of the sample for method 2 and with nondiluted serum for method 3. The anti-EBV antibodies were studied with the recommended 1/32 dilution of the sample for methods 2 and 3.

Agreement, sensitivity, and specificity were defined as previously described (11).

The values obtained for the mean titers and the cumulative percentages of the CMV and EBV antibodies determined with Enzygnost (method 1) are given in Table 1. The mean anti-CMV titers were considerably higher for the HIV carriers of panel 2. For both CMV and EBV, the children of panel 4 showed the lowest antibody titers. Table 2 indicates the percentages of sensitivity and specificity of the results provided by Enzygnost, as well as their agreement with the results obtained with the alternative methods. These percentages were very similar for CMV and EBV. In the CMV assays, 21 possibly false readings were confirmed positive with the CMVScan test (method 3). The 24 false-negative readings obtained with method 2 for EBV were confirmed positive when method 3 was applied.

Generally speaking, when ELISA methods are used to determine antibody titers, progressive dilutions of the sample are needed or a new standard curve must be elaborated for each assay. Even so, it is difficult to precisely establish the antibody titer of a serum.

Recently, however, the laboratories of the Behring Institute introduced a new means of interpreting absorbance after reading the plate. The modification is based on the formula $\log_{10}$ titer of alpha = absorbance^{beta}. Reagents with a specific quantitative composition are used, and the absorbance of the problem serum is adjusted to a curve elaborated with a positive control. In this way, the titer can be associated with an optical density. The principal limitation of the method is its lack of sensitivity when antibodies are present in small quantities. It has not yet been tested in cephalorachidian liquid.

The serum samples from three of our four panels of study gave high percentages (ranging from 69.6% to 96.42%) of anti-CMV and anti-EBV antibodies when methods 1 and 2 were applied (in contrast with 60.03 to 63.9% among the children of panel 3). In comparison with other studies carried out in Spain or abroad (1, 2), the percentages of CMV seropositive readings that we observed are high. Blanco et al. (4) found CMV antibodies in 29% of their subjects over 10 years of age; Artieda et al. (2) concluded that, at age 16, 50% of the population is seropositive for CMV antibodies; and Cour et al. (6) and Tan and Stern (17) obtained respective immunization percentages of 67.6 and 71.6% in their studies. Likewise, studies in the United States have suggested seropositive values of around 45%, whereas in Africa and the Far East, values close to 100% have been obtained (13).

The lower percentage of prevalence among the children of our study can be attributed to the constitutional immunosuppression that characterizes this age group. Krech and Tobin (13) detected regional variations that ranged from 3% in

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TABLE 1. Anti-CMV and anti-EBV antibodies found in panels 1 to 4

Panel	CMV						EBV					
	% Positive by:		Titer (method 1)				% Positive by:		Titer (method 1)			
	Method 1	Method 2	25%[a]	75%[a]	Maximum	Mean ± SD	Method 1	Method 2	25%[a]	75%[a]	Maximum	Mean ± SD
1	81.06	75	1,300	6,400	61,000	5,829 ± 8,887	90.9	90.15	760	3,000	34,000	2,687 ± 4,737
2	90.7	87.8	700	7,000	48,000	6,834 ± 10,992	95.7	96.42	460	1,800	43,000	2,545 ± 6,549
3	60.03	60.03	460	1,800	30,000	1,497 ± 3,731	63.9	60.03	0	920	24,000	1,306 ± 3,251
4	71.4	69.6	160	4,000	28,000	4,576 ± 7,231	92.8	92.8	536	2,300	23,970	2,503 ± 4,556

[a] Positive cumulative percentage.

healthy children from Oxford, United Kingdom, to 95% among healthy children in Entebbe, Uganda.

The hemodialyzed patients of panel 4 had higher seropositivity and mean titer values than did the children of panel 3, yet they showed lower percentages than the healthy adults and the HIV carriers of panels 1 and 2. This may be related to the immunosuppression of these patients, who often suffer from reactivations or reinfection, depending on the evolution of their illness. The HIV carriers of panel 2, in turn, showed higher seropositivity in response to our tests than the healthy adults studied. Relatively high mean titers appear to accompany the high-risk behavior of this group, as other authors have suggested (5). Despite the significance attributed to CMV when AIDS first appeared (16), no direct relationship between HIV and CMV has been established to date.

The results we obtained for EBV were similar to those for CMV, with a higher prevalence of antibodies in panels 1, 2, and 4. These findings are consistent with those of other studies (9, 12, 14, 15).

The Enzygnost ELISA method yielded high percentages for both sensitivity and specificity in the detection of anti-CMV and anti-EBV IgG antibodies all across our study population. We consider these results superior to those yielded by complement fixation for CMV or the commercial IFA for EBV. Moreover, the Enzygnost technique is simpler. Because the complement fixation test detects antibodies to antigens that may appear in the acute phase of illness (8), it may prove less appropriate in prevalence studies or in cases of organ donation (7). These limitations, however, do not justify its replacement in laboratories having experience in its optimal usage or in cases of antibody detection in acute-phase serum. Finally, we found that the IFA kit marketed by Gull showed higher sensitivity in the study of anti-EBV IgG antibodies, although this may depend on the fluorochrome used.

In summary, the Enzygnost alpha method test for anti-CMV and anti-EBV IgG is a method that is easy to use and allows the exact titer of the antibodies to be determined with no need for a series of dilutions or the elaboration of a standard curve. It provides results that generally agree with those obtained by the alternative methods and the international studies that we used as points of reference.

TABLE 2. Relationship between the determination of anti-CMV and anti-EBV IgG by methods 1 and 2

Method 1	No. with given result by method 2[a]			
	CMV		EBV	
	Positive	Negative	Positive	Negative
Positive	713	21	778	24
Negative	0	314	0	246
Total	713	335	778	270

[a] Agreement, CMV, 97.99% (1,027 of 1,048); EBV, 97.71% (1,024 of 1,048); sensitivity, CMV, 100% (713 of 713); EBV, 100% (783 of 783); specificity, CMV, 93.73% (314 of 335); EBV, 91.11% (246 of 270).

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