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An evaluation of a polyantigenic ELISA to detect Epstein-Barr virus reactivation

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Key words: Epstein-Barr virus, enzyme-linked immunosorbent assay, antibodies

Abstract

The diagnostic reliability of the Enzygnost EBV test (DadeBehring, Germany) for the detection of IgG and IgA antibodies in the diagnosis of Epstein-Barr virus (EBV) recurrent disease was investigated. Of 81 serum samples examined there were fourteen asymptomatic patients without EBV infection, 46 with past EBV infection, and 21 patients with EBV reactivation. The Enzygnost EBV test was based on an enzyme-linked immunosorbent assay with a pool of viral antigens. The reliability of IgG at >650 IU/ml, and IgA for the diagnosis of reactivation or chronic persistent EBV infection gave 100% sensitivity, 83.3% and 98.3% specificity, respectively. The data indicated that the appearance of EBV IgA was associated with EBV reactivation together with clinical manifestations.

Introduction

Epstein-Barr virus (EBV) infection is usually diagnosed by serological methods (Färber *et al.*, 1993). IgG to VCA, EBNA or EA were increased in reactivations and may be associated with increases in the IgA or IgM, although the latter was the most infrequent. In addition, the EBNA-1/EBNA-2 antibody ratio was <1, and anti-zebra antibodies appeared (Henle *et al.*, 1974a; Lennette, 1992). The EBV can be reactivated or transported in tumoral cells of lymphoid or epithelial neoplasms. In such cases the serological profile was similar to that described above, although differences existed in the anti-EA-R, anti-EA-D or anti-zebra antibody levels (Henle *et al.*, 1974b; Khanna *et al.*, 1995).

In recent times, most laboratories have investigated antibody titres by ELISA rather than IFA, because there is greater objectivity, standardization and automation (Gutiérrez *et al.*, 1994). The use of a pool of antigens in IFA (Virgo, Switzerland), ELISA (DadeBehring, Germany) and Western blot (DPC, Germany), has been proposed to reduce the number of tests needed to diagnose the infection. These methods reduce time and costs and simplify the interpretation of the results. However, their reliability for the diagnosis of recurrent EBV has not yet been demonstrated.

In this investigation we evaluated the Enzygnost EBV test (DadeBehring, Germany) for diagnosis of acute EBV infection (Gutiérrez *et al.*, 1997; Gutiérrez *et al.*, 1999), but we did not examine

patients with active chronic infection. The diagnostic reliability of the Enzygnost EBV test, which uses the ELISA with a pool of antigens for the detection of antibodies in patients with EBV reactivated infection, was assessed and is discussed.

Materials and methods

Terminology

The following acronyms and abbreviations have been used in this work: immunoglobulin G (IgG); immunoglobulin A (IgA); enzyme-linked-immunosorbent assay (ELISA); Epstein-Barr virus (EBV); viral capsid antigen (VCA); Epstein-Barr virus-asociated antigen (EBNA); early antigen (EA); early antigen, restricted (EA-R); early antigen, diffuse (EA-D); immunofluorescence assay (IFA); hepatitis B surface antigen (HBsAg); hepatitis B early antigen (HBeAg); hepatitis B core antigen (HBcAg); hepatitis B core antibody (HBcAb); hepatitis B surface antibody (HBsAb); hepatitis B early antibody (HBeAb); acquired immune deficiency syndrome (AIDS); and human immunodeficiency virus (HIV).

Methods

Serum samples (81 *in toto*) were collected from patients to investigate IgG and IgA to EBV with Enzygnost EBV. The sera were divided into three groups according to the EBV infection and criteria according to Lennette (1992). Group 1 comprised fourteen asymptomatic patients without EBV infection as defined by the absence of anti-VCA IgG, anti-VCA IgM and anti-EBNA IgG. Group 2 consisted of 46 subjects, having a mean age of 25 ± 5 years with past EBV infection defined by anti-VCA IgG and anti-EBNA IgG but not anti-VCA IgM. The group included eleven patients (group 2a) with another viral infection, and 35 healthy subjects (group 2b).

Group 2a comprised several patients. These included one acute hepatitis A virus infection (defined by positive IgM, and ELISA, Abbott, U.S.A.); one chronic hepatitis B virus infection (positive HBsAg, HBcAb and HBeAb for >6 months, and negative HBsAb, HBcAb-IgM and HBeAg; ELISA, Abbott); one HIV infection (defined by the presence of specific antibodies); and one acute HBV virus infection (positive HBsAg, HBcAb, HBeAg and HBcAb-IgM; negative HBsAb and HBeAb). In addition, there were two acute cytomegalo-virus infections defined by the presence of the p65 antigen from blood in >40 leucocytes infected by 2 x 10^5 white cells detected by the immunoperoxidase test (Insctar, U.S.A.), specific IgM with anti-IgG and IgG detected by indirect ELISA (DadeBehring) at 15 to 25 days after the onset of clinical symptoms, and the absence of IgM to the

other human herpes viruses, except for human herpes virus-7 and -8, which were not tested. The patients had fever, infectious mononucleosis or retinitis. Group 2a also included two *Rickettsia conorii* acute infections (defined by typical signs, positive IgG + IgM, IFA; BioMérieux, France) and three acute varicella-zoster virus infections (defined by typical signs, positive IgM, indirect ELISA with anti-IgG; DadeBehring).

Group 3 consisted of 21 patients (mean age of 25 ± 2 years) with chronic active infections or reactivation of virus characterized by the previous presence of specific anti-VCA IgG, fever and adenopathy, and anti-EBNA IgG above the limit of detection by the assay. Minimal requirements were enhanced typically four times in two samples by IgG titre against VCA and anti-EBNA. Samples were derived from patients with lymphoma or chronic lymphatic leukaemia (ten), epithelial carcinoma of the cavum (three), Guillain-Barré syndrome or neuritis (three), HIV-positive patients with AIDS (four) and chronic asthenia (one). Subjects who had any underlying disease known to be associated with immunological diseases, other than HIV infection, were excluded from the study.

Enzygnost EBV

Tests were carried out with an automated system (DadeBehring; ELISA Processor III) which used an indirect ELISA with peroxidase-conjugated caprine antiserum. The antigens and processes used in this test were published previously (Dopatka and Schuy, 1996; Gutiérrez *et*

Table 1 Relationship between the groups of samples and results with the Enzygnost EBV system for detecting IgG and IgA to EBV

Group	n	Enzygnost positive (%): IgG	IgA
1	14	0	0
2a	11	100	9.1
2b	35	100	0
3	21	100	100

Group 1, asymptomatic subjects without EBV infection; group 2a, patients with past EBV infection and other viral infection; group 2b, subjects with past EBV infection and no other viral infection; and group 3, patients with symptomatic reactivations.

al., 1994; Gutiérrez *et al.*, 1999). We followed the criteria of Dopatka and Schuy (1996) for IgA (cut-off optical density of 0.6) and IgG (>650 IU/ml) in the diagnosis of reactivated illness.

The sensitivity and specificity were determined for the Enzygnost IgG and IgA to compare the groups of EBV reactivation and groups 1 and 2. We used the value of an IgG level higher than 650 IU/ml, and an optical density of 0.6 for IgA to detect reactivations or persistent chronic infections (Dopatka and Schuy, 1996).

Results

The results in Table 1 show correlation with the data obtained from Enzygnost and EBV status in patients. Figure 1 shows the results for IgG obtained in groups 1 to 3. We found ten patients with high levels of IgG and one with IgA in group 2a. The reliability of IgG with more than 650 IU/ml, and IgA for the diagnosis of reactivation or chronic persistent EBV infection, was 100% sensitivity, and 83.3 and 98.3%, specificity, respectively.

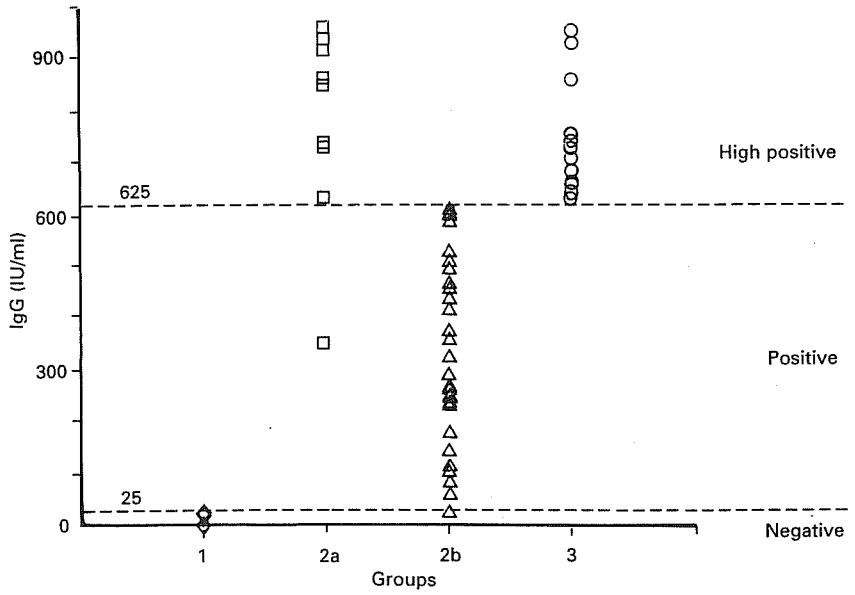

Figure 1 Rate of IgG in patients from group 1 (◆), 2a (□), 2b (△), and 3 (○). The IgG 25 and 625 IU/ml are shown by broken lines.

Discussion

EBV can be reactivated and may be responsible for infectious chronic illness in immunosuppressed patients (severe chronic syndrome) (Buchwald and Komaroff, 1991), patients with HIV infections (Rahman *et al.*, 1991), or patients with organ transplants (Ho *et al.*, 1988). In other cases, genome replication of infected neoplastic lymphoid cells also involved replication of the virion it carried. This occurred in lymphoma, nasopharyngeal carcinoma, leukaemia and gastric carcinoma. An easy method for the detection of these states has clinical significance for a specific treatment or its exclusion.

The values of anti-VCA IgG and IgA in the diagnosis and follow-up of the response to treatment in patients with lymphoma (Alexander *et al.*, 1995; Merk *et al.*, 1995), nasopharyngeal carcinoma (Sigel *et al.*, 1994), leukaemia (Schlehofer *et al.*, 1996), gastric carcinoma (Levine *et al.*, 1995) and chronic infections (Chalupa *et al.*, 1992), have also been studied. Like other authors (Zeng *et al.*, 1983) we noticed (Gutiérrez *et al.*, 1999) a lack of specificity in the detection of IgA when a cut-off point of 0.2 was used, due to the presence of low IgA titres in healthy individuals. Dopatka and Schuy (1996) demonstrated the existence of high levels of anti-EBV IgA (optical density >0.6) and IgG (>650 IU/ml) simultaneously in patients having chronic infection with a hypothetically high concentration of the virus, because of the multiplication of the virus either inside or outside the neoplastic cell.

We investigated the diagnostic reliability of these parameters in a population which was difficult to diagnose (group 3), compared with other groups in which the patients had no viral reactivation symptoms. Ten patients were found with high levels of IgG and one with IgA in group 2a. A very good degree of reliability was evident when we used the IgA test. However, when we used the first cut-off point for IgG titres, of >650 IU/ml this probably enhanced the specificity of the IgA test in samples with values near 650 IU/ml, in which IgA could be positive or negative due to stimulation of monomeric IgA synthesis, as occurred in HIV infection (Kozlowski and Jackson, 1992). Further work is needed to demonstrate the actual validity of this test and to determine which antigens against the antibodies were being detected. Such investigations are continuing in our laboratory.

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