Epstein-Barr Virus-Specific Serum Immunoglobulin A as an Acute-Phase Antibody in Infectious Mononucleosis

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Immunoglobulin A (IgA) antibodies to Epstein-Barr virus viral capsid antigen were assayed serially in 19 patients with infectious mononucleosis and in 38 controls. Seventy-four percent of infectious mononucleosis patients demonstrated IgA antibody, whereas this was found in 13% of controls. This antibody appeared early in infectious mononucleosis and was virtually gone 10 weeks after onset. Comparison of IgA antibody kinetics was made with IgG and IgM antibodies to viral capsid antigen, heterophile antibody, and antibody to Epstein-Barr virus early antigen and nuclear antigen. Failure to demonstrate IgA antibody was associated with severe illness, prolonged illness, delay in IgG and anti-Epstein-Barr virus nuclear antigen antibody, and low or absent heterophile and anti-early antigen antibody. Assay of IgA antibody to viral capsid antigen is a potentially useful adjunct in the serodiagnosis of infectious mononucleosis or recent Epstein-Barr virus infection, as are the other antibodies tested, but in this study IgM viral capsid antigen antibody was the only acute-phase antibody present in all patients.

Epstein-Barr virus (EBV), which numerous serological studies suggest is the most common infectious agent in humans (the prevalence of seropositive adults is or approaches 100%), is the etiological agent of infectious mononucleosis (IM) and is possibly etiological in Burkitt's lymphoma and nasopharyngeal carcinoma (see reference 11). EBV antibody responses of different classes and directed towards different EBV antigens have been reported in IM. The present study describes the time course of the immunoglobulin A (IgA) antibody response to viral capsid antigen (VCA) in IM and correlates this with other antibodies which appear in IM and with clinical events. Information about IgA antibody in EBV-related diseases is limited, although an association of nearly 100% prevalence (at high titers) in nasopharyngeal carcinoma has been noted (5, 8). We report here that IgA anti-VCA is an acute-phase antibody in IM and therefore a potentially useful adjunct in diagnosis of recent EBV infection. We also noted an unexpected absence of this antibody together with a low response of other EBV antibodies in severe and protracted illness, which suggests that IgA antibody absence is therefore a potential marker of this clinical subgroup.

MATERIALS AND METHODS

Subjects. Nineteen students with acute IM and 38 healthy students were studied. The diagnosis of IM was made from the classical clinical presentation, heterophile antibody test, and EBV serological determinations. Clinical examinations and repeated serum sampling were performed up to 37 weeks after onset of illness. The data were grouped for purposes of comparison into four time periods as previously described (12; J. Nikoskelainen, E. U. Neel, R. A. Isenberg, R. G. Miller, J. W. Halpern, A. P. Gelpi, and D. A. Stevens, In G. de The and W. Henle (ed.), Oncogenesis and Herpesviruses III, in press): <1, 1.5 to 3, 3.5 to 9, and >9.5 weeks after the onset of illness. The examining clinician was unaware of the serological results.

Sero logical methods. Assays for heterophile antibodies, IgG and IgM antibodies to VCA, antibodies to early antigen (EA) detectable by indirect immunofluorescence, and antibodies to EBV nuclear antigen (EBNA) detectable by immunofluorescence testing with the addition of complement and anti-complement conjugate were performed by previously described methods (12). EA is an antigen formed early in the viral replication cycle, and EBNA is a marker of latent EBV genome in infected cells not requiring viral synthesis. Classes of antibody to EA and EBNA antigens were not determined.

The assay for IgA antibodies to EBV VCA was similar to the IgG antibody test and used P3HR-1 target cells (Burkitt lymphoma-derived, EBV-producing lymphoblastoid cell line), but the conjugate used
was goat anti-human IgA (Hyland, Inc.) at 1:20 dilution. This conjugate did not give nonspecific staining of P3HR-1 cells or cross-reactions with IgG or IgM antibodies by immunofluorescence.

Statistical methods. The comparison indicated by the Student’s t test.

RESULTS

IgA antibody response to EBV VCA in IM. Development of IgA antibodies to VCA during IM was documented in 14 patients (74%). These antibodies developed very rapidly, and the titers declined soon after reaching their peak levels (Fig. 1). There was a significant \( P < 0.001 \) fall in the geometric mean titer comparing the convalescent-phase titers (3.5 to 9 weeks) with the preceding acute-phase titers. Only one patient had IgA antibodies after 10 weeks, and after 24 weeks all were negative.

Time course of other antibodies in IM: IgG and IgM antibodies to VCA, anti-EA, anti-EBNA, and heterophile antibodies. As shown in Fig. 2, IgG antibodies to VCA appeared soon after the onset of illness, and frequently the titer was maximal in the first serum sample. There was a slight rise in geometric titer at 1.5 to 3 weeks, but the titers then remained stable during the entire observation period. IgM antibodies to VCA likewise developed promptly, and both IgG and IgM VCA antibodies occurred in all patients studied, but the latter rapidly declined, as did the IgA antibodies. After 10 weeks only two patients had IgM VCA antibodies, and after 20 weeks all were IgM antibody negative.

Seventeen patients (89%) developed antibodies to EA, and these remained elevated, unlike the IgM and IgA VCA antibodies. In all patients who developed EA antibodies, the antibody persisted to the end of the study period.

All patients except one developed heterophile antibodies, which appeared early, peaked in the 1.5- to 3-week interval, and then rapidly declined, similar to the IgA and IgM VCA antibodies. Of these three antibodies, which correlated closely in their kinetics, the IgM antibody tended to persist slightly longer after the peak response. No patients had heterophile antibody after 10 weeks of illness.

IM patients without IgA antibody to VCA. The IM patients without IgA antibody to VCA included five patients in whom no IgA antibodies to VCA were detected during IM. In this group of patients, there were some serological and clinical peculiarities. The IgM antibody response to VCA was normal in these patients, but the IgG antibodies to VCA seemed to develop slightly slower than in the other patients. One of these patients had a seroconversion in VCA IgG antibodies during his illness, which is rare in IM (most patients are seropositive when first seen). Another had a significant rise in VCA IgG titer from 20 to 160, whereas no such rises in VCA IgG titers were seen in the IgA antibody-positive patients. The clinical course of these two patients was also exceptional. They both had extremely swollen lymph nodes, their overall illness was very severe initially, and they were given a short course of steroids between 15 and 20 and between 18 and 22 days after onset, respectively. The first serum sample with the absent or low IgG antibody titers was taken from both patients before the steroids were started.

Both of these patients had a prolonged course of illness with symptoms of fever, adenopathy, and pharyngitis up to week 9 after onset, which is unusual in this illness (12), and they also felt extreme fatigue for several weeks after recovery. The three other patients had a normal clinical course of IM. However, two of these were EA antibody negative, the only such patients in this population. The third had heterophile-negative IM, also the only such case in this population. Even the two heterophile-positive, EA antibody-negative, IgA antibody-negative patients had a very low and short heterophile antibody response, with a maximum titer of 1:56. Antibodies

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\text{\textbf{FIG. 1.} Indirect immunofluorescence assays for IgA antibody to EBV capsid in infectious mononucleosis patients as a function of time after onset of the illness. Titer expressed as reciprocal of last positive serum dilution.}
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IgA antibody serum to titers assay. 3 capsid; IgM, heterophile; antibody. (titers <1:10 healthy EBV already were patients IgA antibody-negative patients. to EBNA also seemed to develop slower in the IgA antibody-negative patients. Three of these patients were EBNA antibody negative 6.5, 9, and 31 weeks postonset, when most patients were already anti-EBNA positive.

The two patients with severe illness and a slow EBV antibody response had, however, a normal cellular response to phytogemagglutinin and EBV antigens (12). One of these had a delayed response, however, considered likely to be due to the steroids (18).

Occurrence of IgA antibodies to VCA in healthy EBV seropositive individuals. Serum samples from 38 healthy students were tested for EBV VCA IgG and IgA antibodies. VCA IgG antibodies were detected in 33 individuals (87%). Five students (13%) had IgA antibodies in their serum. Three had a low titer (≤1:20), one was positive at 1:40, and one was positive at ≥1:80. The VCA IgG antibody titers of the five individuals varied from 1:80 to 1:320, and all were negative for VCA IgM antibodies. On further questioning, none had had infectious mononucleosis during the preceding 6 months.

**DISCUSSION**

We have studied the course of appearance of IgA antibodies to EBV VCA and their relation to heterophile antibodies, IgG and IgM antibodies to VCA, and antibodies to EA and EBNA in IM, as well as their correlation with clinical events.

The temporal appearance of the antibodies studied can be helpful in diagnosis. Heterophile antibodies were detected in the acute phase in all but 1 of the 19 patients with IM. With appropriate absorptions of test sera, this antibody has been a classic hallmark of this disease. However, false-positive tests have been noted, and intercurrent illnesses can cause recrudescences in titer after convalescence, which can be confusing (9).

IgM antibodies to VCA were detected in all patients, and these disappeared after the acute phase. Therefore, the IgM antibodies to EBV are very helpful in serological diagnosis even in heterophile antibody-negative EBV infections (3, 4, 13, 14, 15, 17). Antibodies to the EA complex were detected in 89% of patients, and they persisted in all patients throughout the observation period. Thus, although EA antibodies of the D specificity are considered to be an indication of a recent and ongoing infection with EBV (7), they cannot be used to determine the approximate time of onset of illness as can the IgM antibodies to EBV. Both IgM VCA and anti-EA antibodies occur in a minority of healthy subjects in approximately the same prevalence as noted here for IgA VCA antibodies.

Assay for antibodies to EBNA can be useful in the timing of an EBV infection. All patients were EBNA antibody negative in the acute phase of illness. The first positive EBNA antibody reactions were detected 4 weeks after onset of illness. This finding is in agreement with a previous report (6).

IgA antibodies to VCA developed in 74% of the patients with IM. These antibodies were detected in the acute phase of illness, and the titers fell below detectable levels in all patients by 20 weeks after the onset of illness. Henle and Henle (5) noted the unique situation in nasopharyngeal carcinoma patients of IgA antibody to EBV VCA in high titers. Ninety-three percent of nasopharyngeal carcinoma patients had such antibody before treatment. In that study, 38% of IM patients were noted to have such antibody.
The higher percentage detected in the present study is probably related to our serial observations, for single specimens taken early or late in the disease would be less likely to have detectable IgA antibody. On the other hand, 15% of healthy EBV-seropositive students had IgA antibody to VCA. Henle and Henle (5) estimated that only 1 to 2% of healthy donors would have IgA VCA antibody. Our higher incidence may reflect the fact that students were our healthy donors, a group more likely (from epidemiological studies) to have had IM in the past several years (though none of our donors was convalescent from this disease) or a recent subclinical EBV infection. This percent of positivity for IgA antibody was somewhat surprising, since our convalescent IM patients were all seronegative by 24 weeks after onset. One might speculate that endogenously reactivated virus, or exogenous re-exposure (more likely to occur during the student years), could also transiently restimulate IgA production. From these comparative data between students with and without IM, IgA VCA antibody may be a marker of recent EBV infection (clinical IM or subclinical).

There are differing reports of the significance of virus-specific IgA antibodies as an indication of a recent infection in other viral infections. Brown and O'Leary (2) reported that 75% of patients with clinical influenza developed IgA serum antibodies which disappeared completely by 10 weeks. Conversely, IgA antibodies to rubella virus have been reported to persist in some cases for several months up to 3 years after the onset of illness (1, 10). Schmitz and Haas (17) stated that IgA antibodies to cytomegalovirus developed simultaneously with the IgM antibodies in a recent infection. Both classes of antibodies were detectable in the acute phase. The IgA antibodies to cytomegalovirus seemed to persist somewhat longer than the IgM-class antibodies. Thus, there appear to be differences in the persistence of IgA antibodies in different viral infections.

The IgA antibody-negative IM patients had some exceptional clinical and serological features. Two of these patients had delayed heterophile, IgG, and IgM antibody responses, and one of these also showed a delay in production of antibody to EA. These two patients also had an unusually severe and prolonged course of illness. It is unlikely that the steroids were responsible for the aberrant antibody kinetics, since the slow IgG response was documented before the steroids were given, and the steroids were given after the peak IgA responses were seen in the remaining patients. The three other IgA antibody-negative patients had a usual course of illness, but they had absent or low heterophile and anti-EA antibody responses and a delay in appearance of EBNA antibody. The patients with deficient IgA VCA responses were not studied with respect to IgA immunoglobulin deficiency.

It will be of interest in future studies to see whether all heterophile-negative and EA antibody-negative cases of IM (serologically proven by other tests) are also negative for IgA antibody to VCA. A lack of an IgA response in IM could be a marker for a relative deficiency in the EBV antibody response. This might in some cases be associated with a more severe illness. Since the target cell for the EBV infection is a B lymphocyte, which is essential in humoral antibody production, this association may signal a subgroup with a more severe infection. Absence of this antibody could be of prognostic value with regard to the duration of illness or of use in selecting patients for the study of drugs (e.g., steroids) for severe forms of the disease. Information on the status of IgA secretory antibody to EBV would also be of interest in IM.

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LITERATURE CITED

IgA ANTIBODY IN INFECTIOUS MONONUCLEOSIS


