Serum IgA Antibodies to Epstein-Barr Virus (EBV) Early Lytic Antigens Are Present in Primary EBV Infection

Sumita Bhaduri-McIntosh,1 Marie L. Landry,2 Sarah Nikiforow,3 Marisa Rotenberg,1 Ayman El-Guindy,4 and George Miller1,4,5

Departments of 1Pediatrics, 2Laboratory Medicine, 3Internal Medicine, 4Molecular Biophysics and Biochemistry, and 5Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut

Primary Epstein-Barr virus (EBV) infection is characterized by the presence of IgM antibodies to viral capsid antigen and the absence of antibodies to EB nuclear antigen. Here, using a flow cytometry–based assay, we investigated whether IgA antibodies are a marker for primary infection. Serum IgA antibodies in 15 individuals with primary EBV infection reacted with 15%–55.6% of HH514-16 Burkitt lymphoma cells expressing early lytic antigens (EAs), whereas IgA antibodies in serum samples from 15 healthy EBV-seropositive individuals reacted with 0.02%–2% of cells with EAs (P < .0001). IgA antibodies in primary infection were directed against the Bam Z Epstein-Barr replication activator (ZEBRA) (BZLF1) and diffuse EA (BMRF1) EAs. Thus, IgA antibodies to EBV EAs are produced during primary EBV infection and are likely to be stimulated as a result of lytic EBV replication in mucosal sites. Detection of IgA antibodies to EA may be developed into a diagnostic tool for primary EBV infection.

Control of primary Epstein-Barr virus (EBV) infection is achieved by a combination of EBV-specific antibodies, cytotoxic T lymphocyte responses against latent and lytic EBV gene products, increased NK cell activity, and secretion of cytokines [1–5]. Serologic responses to primary EBV infection can be classified into heterophile antibody responses and responses directed against specific EBV antigens. Most infected individuals develop EBV-specific antibodies directed against viral capsid antigens (VCAs), early (pre-DNA replication) antigens (EAs), and EB nuclear antigens (EBNAs). They are conventionally detected by indirect immunofluorescence assay (IFA) or ELISA. The serologic responses in primary infection, such as infectious mononucleosis (IM), are characterized by the sequential appearance of IgM antibodies to VCAs, followed by the appearance of IgG antibodies to VCAs and EAs [6]. Antibodies to EBNAs appear last, sometimes after several months.

IgA antibodies directed against VCAs have been detected in 38% and 74% of individuals with IM in 2 different studies [7, 8]. Lytic viral replication in pharyngeal squamous epithelial cells during IM would suggest that IgA antibody responses directed against proteins of the viral DNA replication machinery exist. These include the products of BZLF1, BRLF1, BALF2, BALF5, BMRF1, BBLF4, BSLF1, and BBLF2/3 and are collectively known as EAs [9]. We hypothesized that an IgA response to EAs is elicited during primary infection with EBV and might be involved in the control of the early lytic phase of infection. We recently developed a highly sensitive, specific, and quantitative flow cytometry–based system that allows detection of IgG antibodies directed against both total and early lytic EBV antigens [10]. Using a modification of this assay, we searched for IgA antibodies directed against EBV EAs. Here, we compare the occurrence of IgA serologic re-
responses to EBV total antigens and EAs during primary EBV infection and in healthy individuals persistently infected with EBV.

SUBJECTS, MATERIALS, AND METHODS

Subjects. A convenience sample of serum from 15 individuals between the ages of 5 and 45 years with acute primary EBV infection was selected based on the presence of IgG and IgM antibodies directed against VCAs and the absence of antibodies directed against EBNAs. These tests were performed in the Clinical Virology Laboratory at Yale–New Haven Hospital (Merifluor EBV IFA kits; Meridian Bioscience). Serum samples from 12 individuals were tested and found to be positive for the presence of heterophile antibodies. All 15 individuals had EBV-specific serologic tests confirming primary infection. Five individuals presented with IM and 1 presented with thrombocytopenic purpura, whereas the clinical manifestations of 9 individuals remained unknown. Serial serum samples were also obtained from 2 individuals at intervals after IM. Twenty-one healthy individuals between the ages of 13 and 40 years were determined to be either EBV seropositive (n = 15) or EBV seronegative (n = 6) on the basis of the detection of serum IgG antibodies directed against small VCA (BFRF3) and EBNA1 (BKRFL) by Western blot analyses using B95-8 and BJAB cell lines. Seroreactivity to EBV was confirmed in each individual by a newly developed flow cytometry–based assay [10].

Cell lines and chemical induction of EBV lytic cycle. Cell lines HH514-16, B95-8, and BJAB have been described elsewhere [11–13]. HKB5/B5 is an EBV-negative cell line formed by the fusion of HH514-16 cells with 293T cells [14]. Chemical induction of HH514-16 cells has been described elsewhere [10].

Expression plasmids and transfection. HKB5/B5 cells were transfected 2 days after subculture using DMRIE-C reagent (Invitrogen) in accordance with the manufacturer’s instructions. pRTS/Rta was used for expressing full-length Bam R transactivator (RTA), whereas pRTS was used as the empty vector [15]. pHD1013/Z, which expresses ZEBRA under the control of cytomegalovirus (CMV) promoter, has been described elsewhere [16]. Plasmid (pGS113) expressing the BMLF1 product was provided by S. Swaminathan (University of Florida, Gainesville). Expression vector pFLAG-CMV2 was provided by R. Sun (University of California at Los Angeles). Plasmid (pFLAG-CMV2/BMRF1) expressing diffuse EA (EA-D) was generated by inserting a polymerase chain reaction (PCR)–amplified product of BMRF1 into EcoRI and XhoI sites of pFLAG-CMV2. Similarly, pFLAG-CMV2/BLRF2 expressing BLRF2 gene product was generated by inserting a PCR-amplified product of BLRF2 into EcoRI and BamHI sites of pFLAG-CMV2. Sequences of the primers used to amplify BMRF1 and BLRF2 are available on request.
bands were detected using an extended chemiluminescence kit (Jackson ImmunoResearch Laboratories). IgA-immunoreactive samples from individuals with primary EBV infection or nitrocellulose membrane. The blots were probed with serum SDS-PAGE, and the separated proteins were transferred to a phosphonoacetic acid (butyrate+PAA) were resolved using 10% cytochrome for another hour.

cells were incubated with a 1:500 dilution of avidin-conjugated monoclonal antibody (BD Pharmingen) for 1 h. After 3 washes, a 1:200 dilution of biotinylated mouse anti–human IgA1/IgA2 protection of serum IgA antibodies, cells were first incubated with mouse anti–human IgM monoclonal antibody (BD Pharmingen). Incubation with a 1:25 dilution of R-phycoerythrin–conjugated antibody has been described elsewhere [10].

ELISA for detection of total IgA antibodies in serum samples. Ninety-six-well polystyrene microtiter plates (Corning) were coated overnight at 25°C with a 1:20 dilution of serum samples from individuals with acute or convalescent primary EBV infection or from healthy EBV-seropositive individuals. This was followed by incubation with a 1:20,000 dilution of HRP-conjugated goat anti-human serum IgA (Jackson ImmunoResearch laboratories) at 25°C for 1 h. Color development was performed using a TMB substrate kit (34021; Pierce Laboratories). The optical density at 450 nm was determined using a Universal Microplate Reader (Bio-Tek Instruments).

Statistical analyses. The unpaired t test was used to compare the mean values from 2 groups of interest.

RESULTS

IgG antibodies directed against EBV EAs present in serum samples from most healthy EBV-seropositive individuals. Our earlier study established that IgG antibodies in serum samples from healthy EBV-seropositive individuals detected the subpopulation of HH514-16 cells that were induced into the EBV lytic cycle [10]. To determine whether serum samples from healthy EBV-seropositive individuals detected early viral or late lytic cycle products, butyrate+PAA-treated HH514-16 cells were used as a source of antigen. PAA blocks viral DNA replication and synthesis of late lytic cycle proteins [17]. Figure 1A–1C and table 1 show that IgG antibodies in 11 of 13 serum samples from healthy EBV-seropositive individuals (except individuals 1 and 8) recognized 25%–50% of cells treated with butyrate+PAA. In contrast, serum samples from 5 healthy EBV-seronegative individuals bound to a mean of 1.3% (range, 0.2%–4%) of butyrate+PAA-treated cells. Serum samples from 2 patients with IM recognized a larger percentage of butyrate+PAA-treated cells (59% and 67%, respectively) than did serum samples from healthy EBV-seropositive individuals (figure 1B). IgG antibodies in serum samples from healthy EBV-seropositive individual 1, which failed to recognize EAs in the FACS-based assay (figure 1A), did not recognize those antigens on an immunoblot, whereas serum sample 2 from an individual with acute IM reacted strongly with EAs (figure 1D).

The efficacy of PAA in blocking late gene expression was demonstrated by Western blot analyses (figure 1D). Two randomly selected serum samples from healthy EBV-seropositive individuals detected a 21-kDa polypeptide representing small VCA [18], a late lytic cycle protein whose expression is dependent on lytic viral DNA replication, only in cells treated with butyrate and not in cells that were untreated or had been treated with butyrate+PAA. Serum samples from a randomly selected patient with IM detected the EA-D complex in butyrate-
rate-treated and butyrate+PAA-treated cells but not in untreated cells.

Differentiation between individuals with primary EBV infection and healthy EBV-seropositive individuals by use of the newly developed flow cytometry–based assay. Serum IgG antibodies directed against EBV EAs could not differentiate between primary and persistent EBV infection on the basis of the detection of the number of cells with EAs. However, IgG antibodies from patients with IM did react with greater intensity to cells expressing EAs (figure 1). We therefore investigated whether IgM antibodies could be detected by the flow cytometry–based assay. Figure 2A–2C shows that IgM antibodies in serum samples from individuals with primary EBV infection detected between 11.5% and 72.6% of butyrate-treated cells (mean detection, 38.9%). In contrast, IgM antibodies directed against 0.04%–2.3% (average, 0.7%) of cells were present in serum samples from healthy EBV-seropositive individuals (P < .0001). Healthy EBV-seropositive or EBV-seronegative individuals could not be distinguished based on IgM antibody reactivity to EBV lytic antigens (figure 2C).

IgA antibodies directed against EBV lytic antigens present in serum samples from individuals with acute primary EBV infection. We examined the ability of the flow cytometry–based assay to detect IgA antibodies directed against lytically induced cells. Figure 2D–2F and table 2 show that between 10% and 45% of cells were detected by IgA antibodies in serum samples from all 15 individuals with primary EBV infection. In contrast, 0.02%–1% and 0.1%–0.9% of cells were detected by serum samples from healthy EBV-seropositive and EBV-seronegative individuals, respectively (figure 2F). The average percentage of butyrate-treated cells detected by IgA antibodies in serum samples from individuals with primary EBV infection, EBV-seropositive individuals, and EBV-seronegative individuals was 28.8%, 0.3%, and 0.4%, respectively (P < .0001, for difference between primary infection and EBV seropositive or EBV seronegative). IgA antibodies present in serum samples from

Figure 2. Presence of IgM and IgA antibodies directed against butyrate-treated HH514-16 cells during primary Epstein-Barr virus (EBV) infection. Panels A, B, D, and E are dot-plot analyses of butyrate-treated HH514-16 cells incubated with serum samples from healthy EBV-seropositive individuals (seropositive serum 2 in panel A and seropositive serum 2 in panel D) or an individual with acute infectious mononucleosis (IM; IM serum 3 in panel B and IM serum 5 in panel E) followed by incubation with either phycoerythrin-conjugated anti–human IgM antibody (A and B) or biotin-conjugated anti–human IgA antibody and avidin–cytochrome (D and E). Nos. in the upper-right-hand quadrants indicate the percentage of cells recognized by IgM or IgA antibodies in serum samples. C and F, Comparison of the fraction of butyrate-treated HH514-16 cells detected by IgM (C) or IgA (F) antibodies present in serum samples from individuals with primary EBV infection, healthy EBV-seropositive individuals, and EBV-seronegative individuals. The Y-axis represents the percentage of butyrate-treated cells detected by IgM or IgA antibodies in serum samples. Horizontal bars represent mean values.
Table 2. Fraction of lytic cells recognized by IgA antibodies in serum samples from healthy Epstein-Barr virus (EBV)–seropositive individuals and individuals with primary EBV infection.

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NOTE. Data are percentages. PAA, phosphonoacetic acid.

individuals with primary EBV infection or healthy EBV-seropositive individuals did not detect untreated HH514-16 cells (data not shown).

IgA antibodies directed against EBV EAs present, during the acute phase of illness, in serum samples from individuals with primary EBV infection. Because serum samples from individuals with primary EBV infection contained IgA antibodies directed against lytically infected cells, we asked whether only late lytic EBV proteins were targeted by IgA antibodies, as has been reported elsewhere [7, 8]. HH514-16 cells that were lytically induced with butyrate while viral DNA replication was inhibited using PAA were used as antigen. As shown in figure 3 and table 2, between 15% and 55.6% of cells were detected by IgA antibodies present in serum samples from 15 individuals with primary EBV infection. In contrast, only 0.02%–2% and 0.1%–0.5% of cells were detected by serum samples from 15 healthy EBV-seropositive and 5 healthy EBV-seronegative individuals, respectively. The average percentage of cells detected by serum samples from individuals with primary EBV infection, healthy EBV-seropositive individuals, and EBV-seronegative individuals was 42.3%, 0.5%, and 0.2%, respectively (P<.0001).

After induction of the viral lytic cycle by butyrate+PAA, HH514-16 cells express proteins of the immediate early and early phases of the viral lytic cycle. We therefore asked whether IgA antibodies directed against butyrate+PAA-treated cells were directed against known EBV EAs such as ZEBRA, RTA, and EA-D. Total extracts of butyrate+PAA-treated HH514-16 cells were resolved by SDS-PAGE followed by immunodetection with serum samples from 2 individuals with IM or 2 healthy EBV-seropositive individuals. Bound IgA antibodies were detected by use of an anti-human IgA antibody. As shown in figure 4A, IgA antibodies in serum samples 3 and 5 from individuals with IM detected bands that were consistent with multiple phosphorylated forms of EA-D ranging in size from 45 to 50 kDa. In contrast, serum samples 2 and 5 from healthy EBV-seropositive individuals did not contain IgA antibodies directed against viral lytic proteins. Hybridization with an anti-β-actin antibody established that an equivalent amount of extract was present in each lane. Figure 4B demonstrates that IgA antibodies in serum sample 6 from an individual with IM but not serum sample 6 from a healthy EBV-seropositive individual detected ZEBRA and EA-D expressed in HKB5/B5 cells after transfection with vectors expressing BZLF1 and BMRF1, respectively. In contrast, HKB5/B5 cells expressing EBV lytic cycle proteins such as RTA, EBV transactivating factor 2 (BMLF1), small VCA (BFRF3), and BLRF2 gene product (BLRF2) were not recognized by IgA antibodies in serum sample 6 from an individual with IM or serum sample 6 from a healthy EBV-seropositive individual. All the recombinant polypeptides were expressed and detected by monospecific antibodies (data not shown).

Transient presence of total serum IgA and serum IgA antibodies specific for EBV lytic antigens. Figure 5A and 5B compares the percentages of HH514-16 cells detected by IgM and IgA antibodies at intervals after acute IM in patients 1 and 2. IgM antibodies in patient 1 decreased to almost nondetectable levels between 2 and 3.5 months after acute IM, whereas detectable levels of IgM antibodies persisted in patient 2 at 3 months after acute IM. In contrast, IgA antibodies directed against EBV total lytic antigens and EAs decreased to nondetectable levels by 1 month after acute IM in both patients. Figure 5C shows that, in acute phase serum samples from individuals
Figure 3. Presence of IgA antibodies directed against butyrate and phosphonoacetic acid (butyrate+PAA)–treated HH514-16 cells during primary Epstein-Barr virus (EBV) infection. A, Histogram of butyrate+PAA-treated HH514-16 cells incubated with serum samples from 2 healthy EBV-seropositive individuals (seropositive serum 2 and 5) and 2 patients with acute infectious mononucleosis (IM; IM serum 3 and 5) followed by incubation with biotin-conjugated anti–human IgA antibodies and avidin-cychrome. Panels B and C are dot-plot analyses of butyrate+PAA-treated HH514-16 cells incubated with serum samples from a healthy EBV-seropositive individual (B) or an individual with acute IM (C) followed by incubation with biotin-conjugated anti–human IgA antibody and avidin-cychrome. Nos. in the upper-right-hand quadrants indicate the percentage of cells recognized by IgA antibodies in serum samples.

D, Comparison of the fraction of butyrate+PAA-treated HH514-16 cells detected by IgA antibodies in serum samples from individuals with primary EBV infection, healthy EBV-seropositive individuals, and EBV-seronegative individuals. The Y-axis represents the percentage of butyrate+PAA-treated cells detected by IgA antibodies in serum samples. Horizontal bars represent mean values.

with IM, the levels of total IgA antibodies were >2-fold higher than those present in serum samples from healthy EBV-seropositive individuals. By 1 month after clinical presentation, the levels of total IgA antibodies in serum samples from both patients with IM decreased to levels that were comparable to those present in serum samples from healthy EBV-seropositive individuals (figure 5D). Thus, the transient increase in total serum IgA levels in 2 patients with IM paralleled those observed for IgA antibodies directed against total and early lytic EBV antigens.

DISCUSSION

Our examination of the serologic response in healthy EBV-seropositive individuals and in primary EBV infection using a recently described flow cytometry–based assay has led to 3 discoveries that broaden our understanding of persistent EBV infection and the pathogenesis of primary EBV infection. First, serum samples from individuals with primary EBV infection contained IgA antibodies directed against EBV EAs. Western blot analysis revealed that such IgA antibodies were directed against EA-D and ZEBRA. Second, serum IgA antibodies directed against EBV lytic antigens decreased rapidly and were almost undetectable by 1 month after acute illness in 2 patients with IM. In contrast, IgM antibodies continued to be produced until 3.5 months in 1 patient and beyond 3 months in another patient. Third, an IgG response directed against early lytic EBV proteins was found in serum samples from 11 of 13 healthy, persistently infected individuals.

The timing of the reduction of total serum IgA in 2 patients with IM correlated with the disappearance of IgA antibodies directed against EBV lytic antigens. The differential rates of decrease of IgM and IgA antibodies in convalescent serum samples from both patients with IM provided additional information about the biological half-life of these 2 classes of EBV-specific antibodies. Because the half-life of both IgA and IgM antibodies is ~5 days [19], the differential rates of decrease of IgA and IgM antibodies in convalescent serum samples reflect differences in production and not destruction of the 2 immunoglobulin isotypes.

Characterization of initial humoral immune response to primary EBV infection by the presence of IgA antibodies directed against EA-D and ZEBRA. To our knowledge, few
Figure 4. Detection of IgA antibodies directed against Epstein-Barr virus (EBV) early lytic antigens (EAs) by immunoblotting. Total extracts from butyrate and phosphonoacetic acid (butyrate+PAA)–treated HH514-16 cells (A) and HKBS5/5 cells transfected with constructs expressing EBV lytic gene products ZEBRA, RTA, diffuse EA (EA-D), EBV transactivating factor 2 (EB2), small viral capsid antigen (VCA), or BLRF2 gene product or empty vectors pFLAG–cytomegalovirus (CMV) 2 or pRTS (B) were resolved using SDS-PAGE. The blots were probed with serum samples from either individuals with acute infectious mononucleosis (IM; IM serum 5 or IM serum 3 in panel A; IM serum 6 in panel B) or healthy EBV-seropositive individuals (seropositive serum 2 or seropositive serum 5 in panel A; seropositive serum 6 in panel B) followed by detection of IgA antibodies with horseradish peroxidase–conjugated anti–human IgA antibody in an enhanced chemiluminescence detection system. The blots were also probed with an antiserum to β-actin followed by detection with goat anti–mouse antibody and 125I-protein A.

studies have examined serum samples from individuals with primary EBV infection for the presence of IgA antibodies directed against lytic EBV antigens. Nikoskelainen et al. found that 74% of patients with IM developed IgA antibodies directed against VCAs [8]. In contrast, a study by Henle et al. revealed the presence of IgA antibodies directed against VCAs in only 38% of patients with IM [7]. Both studies used indirect IFAs to detect IgA antibodies directed against VCAs. In our study, serum samples from all patients with primary EBV infection contained IgA antibodies directed against lytically induced HH514-16 cells, underscoring the increased sensitivity of the FACS-based assay.

EA-D, an EBV EA, is a well-described antigenic target for the humoral immune system during primary EBV infection. Between 66% and 100% of patients with IM produce IgG antibodies directed against EA-D during the acute phase of their illness [20, 21]. Using the flow cytometry–based assay, we have now detected the presence of IgA antibodies directed against EA-D in individuals with primary infection in 73% of patients with nasopharyngeal carcinoma and IM [7]. In this study, 73% of patients with nasopharyngeal carcinoma had IgA antibodies directed against EA-D, but only 1 of 37 patients with IM had evidence of very low titer serum IgA antibodies directed against EA-D by indirect IFA.

During primary EBV infection, EBV gains entry into the host via the mucosal route and actively undergoes lytic replication in the oropharyngeal epithelial cells during the acute phase of illness. EAs, such as EA-D and ZEBRA, which are essential components of the viral DNA replication machinery, are very likely to elicit an IgA response by triggering B cells in the mucosa-associated lymphoid tissue present in the salivary glands and upper-respiratory and gastrointestinal tracts. Cells secreting IgA antibodies to EBV EAs may also represent B cells that are infected with EBV after recruitment to mucosal sites for defense against primary EBV infection. Polyclonal activation of B cells with all 3 major classes of immunoglobulin receptors is a hallmark of primary EBV infection [22]. Thus, B cells that secrete IgA antibodies would be readily available for infection with EBV. Additionally, IgA-secreting lymphoblastoid cells lines
Figure 5. IgM and IgA in serum samples. A and B, Time course of detection of IgM and IgA antibodies in serum samples from individuals with infectious mononucleosis (IM) directed against lytically induced HH514-16 cells. Butyrate-treated (●, ■) or butyrate and phosphonoacetic acid–treated (▲) HH514-16 cells were subjected to flow cytometric detection by IgM antibodies (●) or IgA antibodies (■, ▲) present in serum samples obtained from 2 individuals (IM serum 1 in panel A; IM serum 2 in panel B) during acute IM infection and at intervals thereafter. The Y-axes represent the percentages of cells recognized by antibodies in serum samples. C and D, Detection of total IgA in serum samples by ELISA. Serum samples from patients with IM during the acute phase of their illness and healthy EBV seropositive individuals (IM 1, 2, and 5 and seropositive 3 and 5; C) or serum samples from patients with acute and convalescent phase IM (IM 1 and 2; D) were tested at dilutions of 1:20. IgA antibodies were detected using horseradish peroxidase–conjugated goat anti–human IgA antibody.

can be generated after in vitro infection with EBV by use of peripheral blood mononuclear cells derived from patients with IM [23, 24]. Isolation of IgA-secreting lymphoblastoid cell lines directly from individuals with primary EBV infection would provide definitive evidence for the second scenario. We have not yet studied the distribution of monomeric versus polymeric IgA antibodies in serum samples from individuals with primary EBV infection; however, earlier studies have demonstrated that initial serum IgA responses to rubella, measles, and herpes simplex virus infections are characterized by the presence of predominantly polymeric IgA antibodies [25, 26].

Ramifications of rapid decrease of serum IgA antibodies directed against lytic EBV antigens. Infectious agents differ in the kinetics of IgM and IgA responses. For example, in rubella infection IgM antibodies can be detected for about a month, whereas IgA antibodies persist from 2 months to several years after the onset of disease [27, 28]. Similarly, in human toxoplasmosis IgA antibodies persist longer than IgM antibodies [29]. The presence of IgA antibodies against EBV EAs is likely to be useful as an additional diagnostic marker for acute infection with EBV and would benefit from evaluation in a larger cohort of subjects. The rapid decrease in IgA antibodies after acute infection raises the question whether IgA antibodies may be involved in the control of viral replication in mucosal sites or elsewhere. Diagnostically, detection of serum IgA antibodies will be important in unusual situations in which an IgM anti-VCA response is absent in adults with primary infection [30] or in individuals who have persistently elevated levels of IgM anti-VCA antibodies for several months after infection [31]. IgA antibodies to EBV EAs may be a surrogate marker for high virus loads in the oropharynx and blood. Thus, such antibodies might be used to screen blood, organ, or tissue donors for individuals who are more likely to transmit EBV.

IgG response to EBV early lytic proteins detectable in most healthy individuals after recovery from primary EBV infection. The presence of IgG antibodies in persistently infected individuals directed against EA-D has been used as a marker for EBV reactivation [32]. ELISA-based studies have
revealed that serum samples from only 10% of healthy EBV-seropositive individuals contain low titers of IgG antibodies directed against EA-D [33]. Our data show that at least 85% of healthy EBV-infected individuals have IgG antibodies directed against EBV early lytic proteins. Viral reactivation is likely to be frequent and important in maintaining humoral immune responses to EBV lytic proteins. The use of whole cells expressing a mixture of all early proteins combined with a highly sensitive technique competent to evaluate several thousand cells are the most likely reasons for the frequent detection of IgG antibodies to EBV EAs by FACS, when indirect IFA, Western blot analysis, and ELISA studies have failed to do so.

The presence of IgA antibodies to EAs needs to be examined during periods of viral reactivation in healthy individuals persistently infected with EBV. Presence of IgG antibodies to EBNAs would differentiate reactivation from primary infection. Other clinical conditions in which IgA antibodies to EBV lytic antigens are present include nasopharyngeal cell carcinoma and systemic lupus erythematosus. The presence of IgA antibodies directed against EA-D serves as a sensitive and specific marker for diagnosis of nasopharyngeal cell carcinoma [32, 33], whereas seroprevalence of VCA-specific IgA antibodies is strongly associated with lupus in African American individuals [34].

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References


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