Epstein-Barr Virus Serology

Introduction

Epstein-Barr virus (EBV) is a herpesvirus with enveloped icosahedral nucleocapsid and double stranded DNA, first isolated in 1964 by Epstein, Achong and Barr from cultured Burkitt lymphoma cells. Although the virus is ubiquitous geographically, it is capable of infecting only B lymphocytes. A complement receptor found on B lymphocytes alone may serve as the site of viral attachment. The virus is capable of producing a latent, lytic or transforming infection and transforms B lymphocytes in vivo and in vitro. Infected lymphs may contain up to 50 copies of viral DNA in a repressed episomal form.

Cells with EBV infection (containing EBV DNA in the nucleus) show Epstein Barr nuclear antigen (EBNA) localized to the nucleus. When the EBV genome becomes de-repressed and viral infection enters a lytic cycle, infected cells begin to express EA (early antigen) first in the nucleus and cytoplasm diffusely (D form), then as a compact mass in cytoplasm (R form). As the virus enters the replicative phase of its life cycle, viral capsid antigens (VCA) and intact viral particles begin to appear in the infected cell. In active infection (lytic phase) both EA and VCA are expressed in large quantity.

Clinical Spectrum of EBV Infections

A number of distinct clinical entities are associated with EBV infection. Infectious mononucleosis, a disease of young adults, classically presents with fever, pharyngitis, lymphadenopathy, atypical mononucleosis, splenomegaly and liver dysfunction. A similar clinical picture may be seen with CMV, adenovirus and toxoplasmosis. Burkitt's lymphoma (African), associated with three specific chromosomal translocations at the distal region of chromosome 8 involving the c-myc oncogene, is a malignant neoplasm of B lymphocytes geographically and climatically associated with holoendemic malaria. Nasopharyngeal carcinoma (S.E. Asia) is the only human carcinoma known to be regularly associated with a virus. The viral genome resides in all NPC cells, which are EBNA positive. The mode of entry of the virus into the nasal pharyngeal epithelial cell is unknown. Polyclonal B cell lymphoma associated with EBV is found in the immunosuppressed host and associated with X-linked immunodeficiency syndrome, ataxia-telangiectasia, and post transplantation. Serologically, most patients show evidence of reactivation of EBV illness. EBV has recently been implicated in a chronic unexplained illness (greater than 1 year duration) characterized by low grade fever, weight loss, lymphadenopathy, splenomegaly, emotional disorders and fatigue.
Methodology (Serologic Testing)

EBV is maintained in the laboratory in lymphoblastoid cell lines: 1) producer cell lines synthesize intact virus particles or structural antigens, and 2) non-producer cell lines do not. Both types have the EBV genome in a repressed form and show EBNA.

**Anti-VCA.** HR-1 cells, a producer lymphoblastoid cell line in which 10% of cells are producing structural viral antigens or particles, are used as target cells. Patient's serum is added to a smear of HR-1 cells which is then washed and flooded with fluorescent anti-IgG or anti-IgM. Cells producing viral particles stain with anti-VCA.

**-Anti-EA.** EBV latent-infected Raji cells are treated with iododeoxyuridine which de-represses the viral genome. The cells begin a replicative cycle and produce EA. These cells are then used as target cells to detect anti-EA.

**-Anti-EBNA (anti-complement IF test).** EBV latent-infected Raji cells expressing the EBNA are smeared on a slide and patient serum (heat inactivated) is added. Human complement is added to smear. After complement fixation, the slide is overlayed with anti-complement antibody. Positive and negative control sera are necessary and negative control cells needed to exclude non-specific staining with anti-nuclear antibodies.

Serology of EBV Infection

Heterophile antibodies (HA) of infectious mononucleosis are present in approximately 90% of patients in the acute phase of disease. These are antibodies directed against sheep and horse RBCs which are not absorbed by guinea pig RBCs. Young patients are often heterophile antibody negative. HA lasts 3-6 months in typical EBV infectious mononucleosis. Ab's predominantly IgM and are not directed against specific EBV antigens.

EBV specific antibodies (see illustration)

**-anti-VCA** appear in infection, often have reached peak upon presentation. Anti-VCA-IgM lasts a few weeks to 3 months, and is typically elevated in reactivation infection. Anti-VCA-IgG lasts for life. Although it was previously believed that a single high titre of anti-VCA-IgG (> 1:320) was diagnostic of acute infection, more recent studies (Lamy, et al.) argue against this. Patients with BL and NPC have approximately 10X titres of previously-infected controls.

**-anti-EA** next to develop, most often positive at 1 month after presentation, typically lasts for 2-3 months, may last up to 6 months in low titres. D form greatly elevated in NPC. In one large series, all patients (17) with NPC had elevated -VCA (as did all controls) and 76% of NPC patients had elevated -EA (D form) compared to 2-10% of controls. R form is greatly elevated in BL. Antibody to EA as elevated in EB reactivation infection. Anti-EA may be found in some patients with Hodgkins disease, CLL and some malignancies.
-anti-EBNA appears as anti-VCA-IgM and anti-EA fall and remains elevated for life.

In a study of 307 individuals with heterophile antibody positive infectious mononucleosis and 323 controls, Lamy obtained the following results:

<table>
<thead>
<tr>
<th>HA+, acute phase %</th>
<th>age matched controls %</th>
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<tbody>
<tr>
<td>-EA+</td>
<td>94</td>
</tr>
<tr>
<td>-VCA+</td>
<td>100</td>
</tr>
<tr>
<td>-VCA-IGM+</td>
<td>100</td>
</tr>
<tr>
<td>-EBNA+</td>
<td>2.28</td>
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</tbody>
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In patients with infectious mononucleosis, 94% has positive VCA, positive EA, and negative EBNA serology. Of controls, less than 1% had this pattern: 57% of controls had VCA+, EA-, EBNA+ serology. Note that 23.5% of controls were EA+.

Several recent studies (Jones, 1985; Straus, 1985) suggest that EB virus serology may be helpful in diagnosing a recently proposed EB virus associated syndrome characterized by fatigue, malaise, depression, fluctuating fever, adenopathy and arthralgia/myalgia. However, the specific indications for performing the test in the diagnosis of this syndrome are not well defined. Most patients in the recent studies had extensive workups to exclude other possible etiologies of this type of chronic illness. The studies were both inadequately controlled. The lack of specificity of some EB viral antigens (especially EA) may make them unreliable indicators of ongoing active EB virus infection.

References


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<thead>
<tr>
<th>Antibody</th>
<th>Time Period</th>
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<tbody>
<tr>
<td>HA</td>
<td>reactivation</td>
</tr>
<tr>
<td>anti-VCA-IGM</td>
<td>lifelong</td>
</tr>
<tr>
<td>anti-VCA-IGG</td>
<td>may be found, low titres</td>
</tr>
<tr>
<td>anti-EA</td>
<td>lifelong</td>
</tr>
<tr>
<td>anti-EBNA</td>
<td></td>
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**Patterns of Heterophile Antibody and EBV-Specific Antibody Following Infection**