Epstein-Barr Virus Infections: Biology, Pathogenesis, and Management

Moderator: Stephen E. Straus, MD; Discussants: Jeffrey I. Cohen, MD; Giovanna Tosato, MD; and Jeffery Meier, MD

Epstein-Barr virus (EBV) encodes genes that ensure its persistence in human B lymphocytes. Some of the genes encourage B-cell proliferation; others are poised to evade or defeat immune recognition. Immune restraints on the virus, however, are typically so effective that most infections are never symptomatic. In contrast, acute infectious mononucleosis, a self-limited lymphoproliferative illness, is common in adolescents and young adults. Unbridled proliferative illnesses arise when cellular immunity is grossly defective. Treatment of EBV-associated syndromes is largely supportive. Antiviral drugs have no proven role except in patients with oral hairy leukoplakia. Vaccine development is technically feasible but is not considered a high priority for developed nations.


Dr. Stephen E. Straus (Medical Virology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, Maryland): Epstein-Barr virus (EBV) is a ubiquitous pathogen that has evolved an effective strategy for infection, persistence, and spread; EBV infection occurs in more than 90% of the population, most often without evident consequences (1, 2). The clinical outcome of infection rests on the compromise struck between the virus and the host—a fragile interplay of competing forces. Epstein-Barr virus, for its part, unswervingly pursues a genetic mission to infect B lymphocytes, to endow them with endless potential to pass the virus on to their progeny, and to do so while evading immune clearance. The host response is less predictable and determines whether an EBV infection becomes symptomatic. An ineffectual response, which occurs in persons with congenital or acquired immunodeficiency, permits the unbridled proliferation of virus-infected B cells. In contrast, the curious predilection of adolescents and adults to experience symptomatic infection and the features of the mononucleosis syndrome that ensues suggests that an excessive host response to EBV can also lead to illness.

The biology of EBV, the diseases it provokes, the nature of the virus-host interaction and how it is controlled or subverted, and the limited means available to manage EBV infections are the subjects of this conference.

Epidemiology

The paradigmatic disease associated with EBV is infectious mononucleosis. The description and epidemiology of infectious mononucleosis predated by decades the recognition of EBV as its dominant cause; cytomegalovirus, human immunodeficiency virus (HIV), and Toxoplasma gondii are infrequent causes of mononucleosis-like illness (3, 4). Infectious mononucleosis occurs at all ages, but most cases occur during adolescence and early adulthood (5). It is now understood that the restricted age distribution of infectious mononucleosis is delimited at the lower end by the greater capacity of children to resolve a primary EBV infection with few or no symptoms. Although adults do not handle EBV as well as children, by the age of 25 years most are already seropositive and not susceptible to reinfection.

In developing countries, EBV infection occurs early in life. Nearly the entire population of a developing country becomes infected before adolescence. Thus, symptomatic infectious mononucleosis is uncommon in

Abbreviations and Glossary

EA early antigen: a complex of viral nonstructural proteins expressed during active infection
EBV Epstein-Barr virus
EBER Epstein-Barr virus-encoded RNA: a viral RNA that is expressed in the cell nucleus during latent infection
EBNA Epstein-Barr virus nuclear antigen: a complex of viral proteins in the cell nucleus expressed during latent infection
IL interleukin: an immunomodulatory protein encoded by the cellular genome
LMP Epstein-Barr virus latent membrane protein: a viral protein expressed on the surface of the cell during latent infection
VCA viral capsid antigen: a complex of viral structural proteins expressed during active infection

1 January 1993 • Annals of Internal Medicine • Volume 118 • Number 1 45
Table 1. The Most Common Symptoms and Signs of Infectious Mononucleosis*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sore throat</td>
<td>82</td>
</tr>
<tr>
<td>Malaise</td>
<td>57</td>
</tr>
<tr>
<td>Headache</td>
<td>51</td>
</tr>
<tr>
<td>Anorexia</td>
<td>21</td>
</tr>
<tr>
<td>Myalgias</td>
<td>20</td>
</tr>
<tr>
<td>Chills</td>
<td>16</td>
</tr>
<tr>
<td>Nausea</td>
<td>12</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>9</td>
</tr>
<tr>
<td>Cough</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Serologic Responses of Patients with Epstein-Barr Virus-associated Diseases*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Anti-VCA</th>
<th>Anti-EA</th>
<th>Anti-EBNA</th>
<th>Heterophile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgA</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Uninfected</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>+</td>
<td>++</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Convalescent</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Past infection</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chronic active infection</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Post-transplant lymphoproliferative disease</td>
<td>-</td>
<td>++</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>-</td>
<td>++</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

* Adapted from Okano and colleagues (17), with permission. The symbols denote the absence (-), variable presence (±), or usual presence in relatively low (+), moderate (++), or high (+++) titers of the indicated antibodies. Anti-VCA = antibody to the Epstein-Barr viral capsid antigen; Anti-EA = antibody to the Epstein-Barr virus early antigen; Anti-EBNA = antibody to Epstein-Barr virus nuclear antigen.

Clinical Spectrum

Infectious Mononucleosis

The classic syndrome of mononucleosis is defined as a clinical triad of fever, pharyngitis, and adenopathy (14), although many other symptoms and signs also occur (Table 1). The diagnosis of acute EBV infectious mononucleosis is based on clinical presentation and supportive laboratory findings, including an absolute lymphocytosis in which more than 10% of cells are atypical, as well as heterophile antibody titers of at least 1:56 by the traditional Paul-Bunnell-Davidsohn test or positive rapid slide assays such as the Mono-Latex test (Wampole Laboratories, Cranbury, New Jersey) (15). About 93% of patients who fulfill the above criteria have primary EBV infection; if the criteria are relaxed, the proportion decreases (16).

The fortuitous discovery in 1967 that EBV causes infectious mononucleosis led quickly to the development of virus-specific antibody tests that, despite broad

Salivary tissues are the recognized repositories of EBV, and periodic shedding from such tissue is a necessary feature of this virus's biology. Shedding is sustained for months after infection and then falls gradually; in 15% to 20% of all attempts, the virus can be recovered from saliva (7). Immune compromise in transplant recipients and patients with the acquired immunodeficiency syndrome (AIDS) leads to higher rates of shedding (average, 50% to 80%) (8).

Evidence of the virus’s presence in cervical epithelium and semen has emerged, but sexual transmission has not been proved (9). Exchange of saliva would appear sufficient to explain EBV transfer to partners and the corresponding difficulty of transmitting this virus in the home, in the hospital, or in other confined quarters where the issue has been carefully examined (5, 10). The virus can be transmitted to susceptible recipients by blood transfusion or bone marrow transplantation (11, 12), but such transmission is rare. Studies of natural or experimental transmission of infectious mononucleosis documented an incubation period of 3 to 7 weeks (13).
### Table 3. Complications of Infectious Mononucleosis*

<table>
<thead>
<tr>
<th>Category</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td>Hemolytic anemia, thrombocytopenia, granulocytopenia, aplastic anemia, hemophagocytic syndrome</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Encephalitis, transverse myelitis, the Reye syndrome, the Guillain-Barré syndrome, Bell palsy, psychosis, optic neuritis</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Myocarditis, pericarditis</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Streptococcal pharyngitis, laryngotonsillar obstruction, pneumonia, pleuritis, hilar lymphadenopathy, lymphocytic interstitial pneumonitis, nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Dermatologic</td>
<td>Ampicillin-associated rash, leukocytoclastic vascularitis, acrocyanosis, cold-mediated articular, oral hairy leukoplakia</td>
</tr>
<tr>
<td>Renal</td>
<td>Interstitial nephritis, glomerulonephritis</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Hepatitis, massive hepatic necrosis. Reye syndrome</td>
</tr>
<tr>
<td>Spleenic</td>
<td>Rupture</td>
</tr>
<tr>
<td>Immunologic</td>
<td>Anergy, hypogammaglobulinemia, hypergammaglobulinemia, X-linked and non-X-linked lymphoproliferative syndromes, Burkitt and non-Hodgkin B- and T-cell lymphomas</td>
</tr>
</tbody>
</table>

* Reproduced from Straus (19), with permission.

### Lymphoproliferative Disorders

Many EBV-associated lymphoproliferative lesions are recognized in persons with congenital or acquired cellular immune deficiency (20). X-linked lymphoproliferative disease is a rare disorder of young boys who develop fulminating mononucleosis after EBV infection. The disease is characterized by B- and T-cell infiltration of organs with displacement of bone marrow elements (21). Over two thirds of affected patients die of hemorrhage or infection within weeks, and 100% die by 40 years of age. Survivors of the acute infection experience aplastic anemia, hypo- or hypergammaglobulinemia, opportunistic infections, or lymphomas. Epstein-Barr virus-associated lymphoproliferative disease also occurs in 1% to 3% of bone marrow, kidney, or liver transplant recipients. About 5% to 10% of heart or heart-lung transplant recipients have developed EBV-associated B-cell tumors (20). The marked degree of immunosuppression in these patients may allow outgrowth of EBV-infected B cells, resulting in lymphoproliferative lesions. The reversal of some of these lesions is achieved by reduction in immunosuppressive therapy and the role of the immune system in controlling EBV infection in the normal host. In transplant recipients, as well as in patients with AIDS, EBV-associated B-cell tumors may have either polyclonal, monoclonal, or mixed phenotypes. Death from progressive expansion of tumors, immunodeficiency, and opportunistic infections is frequent.

Chronic infectious mononucleosis is a rare, heterogeneous, and poorly understood syndrome that arises in previously well men and women (22, 23). During the past 13 years, we have studied six patients, including one girl, who developed chronic mononucleosis between the ages of 7 and 23 years. Their illnesses lasted 2 to 14 years and, thus far, three of the patients have died. Prominent features include reactive adenopathy and hepatosplenomegaly, uveitis, pneumonitis, and polyneuropathy. Progressive cellular and humoral im-
mune deficiencies evolve in the course of follow-up, making it impossible to discern whether active infection causes the immune impairment or persists because of it. The features of chronic infectious mononucleosis establish it as distinct from the far more common chronic fatigue syndrome (23).

Other Syndromes

Nasopharyngeal carcinoma, Burkitt lymphoma, non-Hodgkin B-cell and central nervous system (CNS) lymphomas, rare T-cell lymphomas (24), and some thymomas have been associated with EBV infection. One particularly instructive disorder caused by EBV is oral hairy leukoplakia, which is characterized by an exophytic growth of epithelial cells of the tongue and buccal mucosa (25).

None of these chronic or lymphoproliferative sequelae can be diagnosed by serologic methods alone. The persistence of anti-EA and of higher-than-usual levels of IgG anti-VCA and the absence of anti-EBNAs are common in patients with disorders described above, but these features can also be seen in any patient with cellular immune impairment. Very high titers of anti-EA (≥ 1:640) and IgG anti-VCA (≥ 1:5120), which can sometimes be seen in immunodeficient patients, are compatible with chronic mononucleosis. As indicated earlier, isolation of the virus in seropositive persons is too common for it to be of diagnostic value (7, 8). Diagnoses are established histologically, and evidence of increased EBV levels in the tissues must be confirmed by immunohistochemical or nucleic acid hybridization techniques (25).

Molecular Biology of Epstein-Barr Virus and Its Mechanism of B-Cell Transformation

Structure and Replication

Dr. Jeffrey I. Cohen (Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, Maryland): The EBV genome is a double-stranded DNA molecular of approximately 172 000 base pairs; as in other herpesviruses, the molecule is divided into unique, internal repeat, and terminal repeat domains (26) (Figure 1). The genome encodes approximately 80 proteins. The function of many of the genes involved in viral replication has been inferred from their homology to herpes simplex virus genes; however, genes expressed during latent infection of B cells do not have recognized counterparts in other human herpesviruses.

Two strains, or types, of EBV infect humans. These strains differ in the sequences of the viral genes expressed during latent infection and in their ability to transform B lymphocytes. Although earlier studies suggested that type A (or EBV-1) virus infection was more prevalent in North America and Europe and that type B (or EBV-2) virus infection was more prevalent in Africa, more recent studies suggest that both strains are prevalent in the United States (27) and that persons can be coinfected with both strains.

Epstein-Barr virus infects epithelial cells of the oropharynx (28) and of the cervix (9) and resting B lymphocytes. The receptor for the virus on epithelial cells and B lymphocytes is the CD21 molecule, formerly called CR2, which is also the receptor for the C3d component of complement (29). Infection of epithelial...
Table 4. Diseases Associated with Persistent Epstein-Barr Virus Gene Expression*

<table>
<thead>
<tr>
<th>Disease</th>
<th>EBERs</th>
<th>EBNA-1</th>
<th>EBNA-2</th>
<th>LMP-1</th>
<th>ZEBRA</th>
<th>gp350</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt lymphoma</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Not studied</td>
<td>-</td>
</tr>
<tr>
<td>Lymphoproliferative disease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Not studied</td>
<td>-</td>
</tr>
<tr>
<td>Hodgkin disease</td>
<td>+</td>
<td>Not studied</td>
<td>-</td>
<td>+</td>
<td>Not studied</td>
<td>-</td>
</tr>
<tr>
<td>Oral hairy leukoplakia</td>
<td>-</td>
<td>Not studied</td>
<td>Not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* EBERs = Epstein-Barr-virus-encoded RNAs; EBNA-1 = Epstein-Barr virus nuclear antigen 1; EBNA-2 = Epstein-Barr virus nuclear antigen 2; LMP = latent membrane protein 1. ZEBRA = Z Epstein-Barr virus replication activator. The symbols denote the usual expression (+), occasional expression (±), or lack of expression (–) of the proteins indicated.

Cells results in replication of the virus, with release of progeny virions from the cells. In contrast, infection of B lymphocytes usually results in latent infection without replication or release of virus. Recently, the CD21 molecule was inserted into epithelial cell lines that normally are not infectable by EBV. Thus, these cells can be infected with EBV, with the resulting expression of certain latent gene products and production of infectious virus (30).

The virus can be reactivated from latently infected B lymphocytes by certain chemicals or by antibody to immunoglobulin. These stimuli lead to expression of the EBV BZLF1 gene product, or ZEBRA (Z EBV replication activator) protein, an immediate early gene product. The ZEBRA protein acts as the switch that triggers viral replication in latently infected B cells (31). This protein also transactivates (or up-regulates) expression of other immediate early genes as well as expression of ZEBRA itself. These genes, in turn, up-regulate the expression of early gene products, which include the viral DNA polymerase and thymidine kinase, important for viral DNA replication. Thymidine kinase phosphorlates acyclovir, thus permitting the drug to inhibit viral replication (32). Finally, the late gene products are made, including structural components of the virion such as the VCA and gp350, which is the major envelope glycoprotein of the virus. Another late viral protein, termed BCRF1, shares 70% amino acid identity with human interleukin-10 (33); this aspect is discussed more fully in the section on lymphocyte responses to EBV.

**Latency and Transformation**

When EBV infects B lymphocytes, its linear genome circularizes to form an episome, or extrachromosomal element, in the nucleus of the cell (34). The result is a transformation of the infected B cells, which acquire the capacity to proliferate indefinitely. In vitro, these latently infected B cells express only 10 of the approximately 80 genes encoded by the virus (see Figure 1). The state of latent infection is maintained by the EBNA-1 protein; it binds to a nucleotide sequence, termed oriP, which is part of the viral origin of DNA replication. The binding of EBNA-1 to oriP allows the viral genome to be maintained in the nucleus of the B cell.

Two of the EBV latent genes encode proteins that transactivate other viral genes. Epstein-Barr virus nuclear antigen 1 leads to transactivation of the EBNA proteins, whereas EBNA-2 transactivates the expression of two EBV latent membrane proteins, LMP-1 and LMP-2. Epstein-Barr virus latent gene products also transactivate the expression of B-cell genes. Epstein-Barr virus nuclear antigen 2 transactivates CD21, CD23, and c-fgr; EBNA-3C also transactivates CD21, whereas LMP-1 transactivates CD23 and the intercellular adhesion molecules ICAM-1, LFA-1, and LFA-3. The secreted (truncated) form of CD23 may be a B-cell growth factor, but the full-length molecule may be a receptor, thus providing autocrine stimulation of EBV-infected B cells (35). CD23, also a low-affinity receptor for IgE, may be important for antigen presentation in association with major histocompatibility complex (MHC) II antigens and was recently shown to interact with CD21 (36). A member of the src oncogene family, c-fgr encodes a protein kinase that may be important for the regulation of B-cell growth. Two latent gene products, the EBV-encoded RNAs (EBERs), are the most abundant RNAs expressed in vitro; they do not code for proteins and are dispensable for transformation in vitro (37).

One EBV latent gene product, LMP-1, acts as a direct oncogene in transformation assays (34). Expression of LMP-1 in epithelial cells transforms them morphologically (38), and in B-lymphoma cells, LMP-1 prevents programmed cell death, or apoptosis (39). Latent membrane protein 2 associates with a cellular tyrosine kinase and colocalizes in the cell with LMP-1 (42). Genetic analyses using viral mutants indicate that EBNA-2 and LMP-1 are essential for transformation (41). Thus, transformation of B cells is a complex process requiring the function of several EBV gene products that serve together to maintain the viral genome in the cell, transactivate viral and cellular gene products important for B-cell growth, interact with B-cell proteins, and prevent apoptosis.

**Lymphoproliferation and Neoplasia**

Studies have begun to clarify the molecular properties of several EBV-associated lymphoproliferative and neoplastic diseases, as well as the role of the virus in their development (Table 4). Burkitt lymphoma is a mono-
Figure 2. Lymph node specimens from patients with Epstein-Barr virus lymphoproliferative disease. Specimens were stained with monoclonal antibody to Epstein-Barr virus nuclear antigen 2 (EBNA-2) (top panel) or latent membrane protein 1 (LMP-1) (bottom panel). Note the nuclear staining with nucleolar exclusion with EBNA-2 antibody and cytoplasmic membrane staining with LMP-1 antibody. Reproduced with permission from Gilligan and colleagues (51) (top panel) and Cohen (20) (bottom panel).

The B-cell lymphoproliferative disorders arise in congenitally immunosuppressed infants and children, as well as in immunosuppressed organ or bone marrow transplant recipients (20). These disorders can evolve during acute EBV infection, but most often they result from previous infection with the virus. The lesions may exhibit B-cell hyperplasia or lymphoma; regardless of the pathologic findings, the chromosomal translocations characteristic of Burkitt lymphoma are usually not present. Tissues from immunocompromised patients with B-cell lymphoproliferation contain EBV genomes and usually express the full complement of latent gene products (46, 47) (Figure 2). The marked degree of immunosuppression may allow EBV-infected B cells to express all the latency proteins and still expand without selective pressure from cytotoxic T cells.

About one third of B-cell lymphomas in patients with AIDS contain EBV genomes (20). Unlike the tumors seen in congenitally or iatrogenically immunosuppressed patients, these tumors show a Burkitt or Burkitt-like histology and contain chromosomal translocations similar to those seen in Burkitt lymphoma in patients without AIDS. Most of these tumors in patients with AIDS also express the full repertoire of latent genes.

Patients with Hodgkin disease often have elevated levels of antibody to EBV antigens before or at the time of presentation of lymphoma; this condition was long presumed to reflect evolving impairment in cellular immunity. Recently, however, tissues from 20% to 40% of patients with Hodgkin disease were found to contain EBV genomes, usually within the Reed-Sternberg cells (48). Most cases associated with EBV are of the more aggressive, nodular sclerosing or mixed cellularity subtypes of Hodgkin disease (49). Tumors that contain EBV DNA show expression of LMP-1 but not of EBNA-2.

Unlike the EBV-associated tumors discussed above, oral hairy leukoplakia represents a nonmalignant infection of epithelial cells, with extensive replication of EBV. Lesions contain EBV DNA in the upper epithelial layers, and herpesvirus-like particles have been seen on electron microscopy (25); EBV DNA is not present in the lower layers. Thus, EBV may not be latent in the lower layers of the epithelium, and infection may be sustained by continuous infection of maturing cells in the upper layers. EBV replicative genes (such as ZEBRA, VCA, gp350) and LMP-1 are expressed, but the EBERs are not (50, 51).

Lymphocyte Responses to Epstein-Barr Virus Infection

Dr. Giovanna Tosato (Laboratory of Immunology, Center for Biologies Evaluation and Research, Food and Drug Administration, U.S. Department of Health and Human Services, Bethesda, Maryland): Most normal adults harbor EBV-infected B lymphocytes that have the potential for unlimited growth; however, lyr-
phoproliferative disorders involving EBV-infected cells only rarely occur. The successful restraint of EBV-infected B cells is primarily attributable to T-cell immunity (52, 53). Several observations form the basis for this assertion. First, if T cells are removed or functionally inactivated from the blood mononuclear cells of most normal EBV-seropositive persons, EBV-immortalized cell lines spontaneously arise in vitro (52). Second, after injection of EBV-seropositive blood intraperitoneally into mice with severe combined immunodeficiency, tumors that are composed of EBV-infected human B cells often arise (54) because of both the immunodeficiency of the host and the limited survival of the human T cells in the murine host. Finally, severe T-cell immunodeficiency in humans, such as occurs in solid organ transplant recipients and in patients with AIDS, is associated with lymphoproliferative syndromes involving B lymphocytes naturally infected with EBV (20).

B Cells Infected with Epstein-Barr Virus

Most of the studies of immunity to EBV infection used EBV-immortalized B cells as targets for monitoring immune functions. During the past several years, it has become apparent that EBV-immortalized B cells undergo several phenotypic changes that facilitate immortalization. One important change is the establishment of an autocrine loop whereby the EBV-immortalized B cells produce growth factors that stimulate their proliferation (55, 56). The existence of this process was suggested by the observation that EBV-immortalized cell lines die if cultured at critically low cell densities; supplementation of the culture medium with cell-free “conditioned” supernatants of EBV-immortalized cell lines cultured at optimal cell densities prevents this outcome, however. Recently, the growth stimulatory factors in the conditioned media of EBV-immortalized cells were shown to include lactic acid and interleukin-6. Lactic acid alone accounts for most of the stimulatory activity in serum-free supernatants of EBV-immortalized cell lines (57). Under culture conditions that include serum, stimulatory compounds other than lactic acid and interleukin-6 may also be involved (36, 58).

Little is known about the requirements for growth and survival of EBV-infected B cells in vivo. During acute EBV-induced infectious mononucleosis, at least 1 in 10^4 circulating B cells is infected with EBV (52). These cells probably become infected with the virus as they travel through lymphoid tissues in the oral cavity. In normal EBV-seropositive persons, however, the proportion of circulating B cells that are chronically EBV-infected is less than 1 in 10^5 (Figure 3) (52). There has been controversy over the derivation of this pool of residual B cells infected with the virus. Moss and colleagues (59) noted that EBV can be detected frequently in the saliva years after the primary infection, and they proposed that virus in the oropharynx serves as a reservoir to repeatedly infect B cells, which then migrate to the periphery where they are susceptible to killing by cytotoxic T cells (59). In this view, the pool of virally infected B cells would be turning over constantly.

An alternative model, now more widely accepted, is that the EBV-infected cells are long-lived; they are perhaps the very cells that were originally infected or their progeny (60). In support of this latter model, acyclovir therapy was shown to eliminate, or at least reduce, EBV shedding in the saliva, but it had no effect on the number of circulating B cells infected with EBV (52, 61). Moreover, repeated analyses of circulating EBV-infected cells during a 3-year period in a patient who had undergone ablative therapy and subsequent bone marrow transplantation showed them to be donor derived (62). During these 3 years, no B cells that carried the recipient's precise viral genotype could be found. Thus, EBV-infected cells can be long-lived, and de novo infection with virus intermittently released in the oropharynx or elsewhere may be uncommon after primary infection.

T-Cell Regulation of Epstein-Barr Virus Infection

Persons who are EBV seropositive and patients with acute EBV-induced infectious mononucleosis harbor EBV-infected B cells with the potential for unlimited growth. That all infected persons do not succumb to lymphoproliferative disorders points to the existence of potent, durable restraints on the EBV-transformed cells.

Figure 3. Frequency and geometric mean of spontaneous B-cell outgrowth in the peripheral blood of patients with acute Epstein-Barr-virus-induced infectious mononucleosis and of normal persons seropositive for Epstein-Barr virus. The geometric mean is indicated by the horizontal bar.
viral interleukin-10 may limit host responses directed at growth of EBV-infected cells without killing them can and mitogen (64) (Figure 4). Although it is not yet predicted amino acid sequences of murine interleukin-10, the T cells were cultured for 3 days in phytohemagglutinin glutinin-stimulated T cells by human and viral interleukin-10. The T cells were cultured for 3 days in phytohemagglutinin (PHA), 0.5 μg/mL, either alone or with interleukin-10, 0.1 to 10 U/mL. Proliferation was measured as counts per minute (cpm) of H-thymidine incorporation during the final 18 hours of culture. IL = interleukin.

Early in the course of acute infectious mononucleosis, activated cytotoxic T cells are found in the peripheral blood, but they disappear during convalescence (52, 53). These cytotoxic cells are neither EBV-specific nor HLA-restricted, because they kill EBV-negative and MHC-incompatible targets. Thus, they resemble activated killer cells induced in vitro by T-cell exposure to mitogens, interleukin-2, or EBV-immortalized cells.

In addition to this predominantly virus-nonspecific response, cytotoxic T-cell clones that are both specific for virus-infected cells and HLA-restricted can be derived from the blood of patients with infectious mononucleosis (53). Other T-cell clones that inhibit the growth of EBV-infected cells without killing them can also be derived from the blood of patients with infectious mononucleosis (63).

Recent studies have shown extensive homology in the predicted amino acid sequences of murine interleukin-10, human interleukin-10, and the protein product of BCRF-1 (viral interleukin-10); BCRF-1 is an open-reading frame in the EBV genome known to be expressed during replication (see Figure 1) (32). Functionally, human and viral interleukin-10 share several properties, including inhibition of gamma-interferon secretion and suppression of T-cell proliferation in response to antigen and mitogen (64) (Figure 4). Although it is not yet known whether viral interleukin-10 is produced during acute infectious mononucleosis, the generally poor response of T cells from patients with infectious mononucleosis to in-vitro stimulation with mitogens and antigens could be an effect of viral interleukin-10. It is intriguing to note that during evolution, EBV may have captured a cellular gene that aids its survival. By inhibiting T-cell growth and gamma-interferon production, viral interleukin-10 may limit host responses directed at eliminating the virus.

Although present during acute EBV-induced infectious mononucleosis, activated cytotoxic and suppressor cells are not detected among T cells from persons who have recovered from the infection; however, by culturing T cells from EBV-seropositive persons under conditions that involve restimulation with autologous EBV-infected B cells, both cytotoxic and suppressor T-cell activities directed against EBV-positive B cells can be elicited (52, 53). Early studies showed that cytotoxic T cells derived in this manner have strong reactivity against autologous EBV-infected targets but fail to kill EBV-negative targets, even those that have been activated by mitogens and that are restricted by HLA class I determinants. Recently, the virus specificity of cytotoxic T-cell clones derived from EBV-seropositive persons was mapped. These clones recognized EBNA-2, EBNA-3A, EBNA-3C, and LMP (65-67). Interestingly, no cytotoxic T-cell clones that recognize EBNA-1 have yet been isolated. The lack of detectable EBNA-1-specific T-cell clones may have important implications regarding the cellular immune containment of EBV-infected cells. Epstein-Barr virus nuclear antigen is required to maintain the EBV episomal genome in B cells, so its expression is obligatory; however, the expression of other latency genes if not required (68). The failure of EBV to express certain genes, such as LMP and EBNA-2, in vivo may allow B cells to avoid cytotoxic T-cell recognition and clearance.

**Figure 4. Inhibition of the proliferation of normal phytohemagglutinin-stimulated T cells by human and viral interleukin-10.** The T cells were cultured for 3 days in phytohemagglutinin (PHA), 0.5 μg/mL, either alone or with interleukin-10, 0.1 to 10 U/mL. Proliferation was measured as counts per minute (cpm) of H-thymidine incorporation during the final 18 hours of culture. IL = interleukin.

Epstein-Barr Virus-related Lymphoproliferative Disorders

Cellular immune restraints on EBV-infected B cells sometimes fail, and lymphoproliferative disorders involving EBV-infected B cells occur. Recent attention has focused on the potential role of growth factors as contributors to the various processes of transformation (69). Because EBV-immortalized cells respond to interleukin-6 with increased proliferation in vitro, the effects of interleukin-6 on B-cell tumorigenesis have been assessed (70, 71). Expression of the human interleukin-6 gene in EBV-transformed human B cells rendered them tumorigenic in athymic mice (70, 71). The parental cells and those clones transduced with a control plasmid (interleukin-6-nonexpressing) were generally nontumorigenic. The studies showed that the mechanism of interleukin-6 tumorigenicity was unrelated to a direct oncogenic effect of this cytokine on the EBV-immortalized cells. Rather, interleukin-6 appeared to inhibit markedly cytotoxic functions in the mouse, and it was the further reduction in immune effector activity that permitted tumor outgrowth (71).

What relevance might these observations have to the development of tumors in humans? One study found that serum interleukin-6 levels are abnormally elevated in HIV-seropositive persons, a group predisposed to EBV-positive lymphomas (72). Six of seven patients who developed EBV-associated post-transplant lymphoproliferative disorders had markedly abnormal serum interleukin-6 levels (85 to 1040 U/mL) at various times after they received the transplant; in contrast, none of six patients with normal interleukin-6 levels (5 to 15 U/mL) developed post-transplant lymphoproliferative disorders (range, 45 to 215 days) (73). Thus, interleukin-6, an autocrine and paracrine growth factor of
EBV-immortalized B cells in vitro, appears to be associated with increased tumorigenicity of EBV-immortalized cells in athymic mice and an increased rate of post-transplant lymphoproliferative disorders. Interleukin-6 may play an important role in the pathogenesis of EBV-related lymphoproliferative disorders, either by further limiting host immune responses or by directly promoting the growth of the EBV-infected B cells. A better understanding of the role of interleukin-6 in the multistep process of lymphomagenesis by EBV-infected B cells may provide a rational basis for novel approaches to therapy.

Management and Prevention of Epstein-Barr Virus Infections

Dr. Jeffery I. Meier (Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, Maryland): Epstein-Barr virus infections pose few problems for which more than supportive care is warranted. When required, the adjunctive therapeutic options often reflect strategies that either directly target the replication of EBV or, more often, the immunopathologic responses to viral infection.

Uncomplicated Acute Infectious Mononucleosis

General Considerations

Patients with uncomplicated acute infectious mononucleosis usually require only symptomatic therapy. Contact sports should be avoided for at least 1 month or until resolution of splenomegaly has been verified. Splenic enlargement is not always clinically appreciable and generally resolves within the first month of illness. The enlarged spleen is susceptible to rupture (74, 75); an ultrasonographic examination can exclude subclinical splenomegaly.

Table 5. Results of Controlled Studies of Corticosteroid or Corticotropin Therapy for Uncomplicated Acute Infectious Mononucleosis

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Patients, n</th>
<th>Corticosteroid, Initial Dose, and Taper Duration</th>
<th>Time of Assessment</th>
<th>P Value for Effects of Corticosteroids*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bennike (76)</td>
<td>Unblinded</td>
<td>Placebo Cortico-steroid Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>16</td>
<td>Corticosteroid, 60 IU intramuscularly, 5 days Premixone, 60 mg/d orally, 5 days without taper</td>
<td>Daily‡</td>
</tr>
<tr>
<td>Antila et al. (77)</td>
<td>Double-blind</td>
<td>5 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schumacher et al. (78)</td>
<td>Double-blind</td>
<td>41 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prout and Dubrymple (79)</td>
<td>Case-control</td>
<td>66 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bender (80)</td>
<td>Double-blind</td>
<td>13 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klein et al. (81)</td>
<td>Double-blind</td>
<td>21 26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value reflects shortening of the duration of symptoms or signs by corticosteroids.
† The laboratory and physical examination indices that were monitored varied; tonsillopharyngitis, lymphadenopathy, and hepatosplenic abnormalities were often evaluated.
‡ Daily assessments until substantial overall improvement was observed.
§ Trend = beneficial trend, not statistically significant.

The exudative tonsillopharyngitis that accompanies infectious mononucleosis often leads to a search for bacterial infection. The percent of random-surveillance throat cultures in patients with infectious mononucleosis that grow group A beta-hemolytic streptococci ranges widely, from 3% to 30%, and as many as 30% of these show serologic evidence of recent streptococcal infection (76). Accordingly, treatment of beta-hemolytic streptococci with penicillin or erythromycin for 10 days is warranted to prevent poststreptococcal sequelae. Ampicillin- or amoxicillin-containing regimens should not be used in persons with infectious mononucleosis, because they frequently cause a rash.

Corticosteroid Therapy

The use of glucocorticoids for the routine management of acute infectious mononucleosis is controversial. The cumulative results of several small controlled clinical studies (Table 5) suggest that corticosteroids hasten the resolution of fever and tonsillopharyngeal symptoms of uncomplicated acute infectious mononucleosis, but they do not provide significant or reproducible benefit for lymphadenopathy or hepatosplenic involvement (77-82). Despite these studies, corticosteroids are still used by some physicians with the goal of quickly returning students to school. In part because of rare reports of an association between encephalitis or myocarditis and corticosteroid use in patients with acute infectious mononucleosis (83-85), other investigators have cautioned against the use of corticosteroids. Further, corticosteroids might adversely influence the biology of the infection—the long-term immunity to EBV or the number of lymphocytes latently infected by the virus. On balance, we support recent guidelines that advise against the routine use of corticosteroids in patients with uncomplicated acute infectious mononucleosis (86).
Table 6. Results of Double-Blind, Placebo-controlled Trials of Acyclovir Therapy for Acute Infectious Mononucleosis

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Patients, n</th>
<th>Dosage</th>
<th>Day of Culture from Start of Therapy</th>
<th>Viral Shedding</th>
<th>Overall Clinical Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo Group</td>
<td>Acyclovir Group</td>
<td>Placebo Group</td>
<td>Acyclovir Group</td>
<td>Placebo Group</td>
</tr>
<tr>
<td>Pagano et al. (88)</td>
<td>10</td>
<td>10</td>
<td>1500 mg/M² body surface area per day intravenously for 5 days</td>
<td>0</td>
<td>1/5</td>
</tr>
<tr>
<td>Andresson et al. (89)</td>
<td>16</td>
<td>15</td>
<td>30 mg/kg, per day intravenously for 7 days</td>
<td>0</td>
<td>15/16</td>
</tr>
<tr>
<td>Andresson et al. (90)</td>
<td>28</td>
<td>28</td>
<td>800 mg 5x/d, p.o., 7 d</td>
<td>0</td>
<td>19/20</td>
</tr>
<tr>
<td>Pagano et al. (88)</td>
<td>10</td>
<td>10</td>
<td>1500 mg/M² body surface area per day intravenously for 5 days</td>
<td>0</td>
<td>1/5*</td>
</tr>
<tr>
<td>Andresson et al. (89)</td>
<td>16</td>
<td>15</td>
<td>30 mg/kg, per day intravenously for 7 days</td>
<td>0</td>
<td>15/16</td>
</tr>
<tr>
<td>Andresson et al. (90)</td>
<td>28</td>
<td>28</td>
<td>800 mg 5x/d, p.o., 7 d</td>
<td>0</td>
<td>19/20</td>
</tr>
<tr>
<td>van der Horst et al. (91)</td>
<td>58</td>
<td>62</td>
<td>600 mg 5 times a day orally for 10 days</td>
<td>0</td>
<td>50%</td>
</tr>
</tbody>
</table>

* The proportion (expressed as n/n or as a percent) of saliva specimens from which Epstein-Barr virus could be isolated.
† Difference was statistically significant.
§ P < 0.011.
|| Significant decrease in geometric mean virus titer, P < 0.001.

Antiviral Therapy

Several antiviral agents, such as acyclovir, ganciclovir, bromovinyldeoxyuridine, zidovudine, and foscarin, as well as human alpha-, beta-, and gamma-interferon, effectively inhibit the replication of EBV or initial B-cell transformation by the virus in vitro (87). Only EBV DNA synthesis dependent on the viral polymerase (the linear form of the genome) is susceptible to inhibition. The latent circular form of the genome, which replicates in concert with the cell cycle using the cellular DNA polymerase, is not selectively inhibited.

Because both forms of replication transpire in the course of infectious mononucleosis, it was initially uncertain whether antiviral therapy would prove to be beneficial. The efficacy of acyclovir has been assessed in controlled studies of uncomplicated acute infectious mononucleosis (Table 6) (88-91). Oropharyngeal shedding of EBV is inhibited by intravenous or high-dose oral acyclovir therapy that yields peak serum levels exceeding 1 μg/mL, the concentration needed to suppress EBV genome replication by 50% in in-vitro studies. This effect is temporary, however; viral shedding resumes shortly after therapy is discontinued. Despite this virologic response, the trials documented little or no clinical benefit in treating uncomplicated acute infectious mononucleosis with acyclovir.

Complications of Acute Infectious Mononucleosis

Complications of acute infectious mononucleosis usually necessitate additional supportive measures such as tracheostomy for airway obstruction or transfusions for critical hemolytic anemia or thrombocytopenia. Corticosteroids may be useful for some of these complications. Small case series indicate that corticosteroids quickly reduce obstructive tonsillar enlargement so as to obviate tracheostomy in some instances (78, 92). Such therapy may also ameliorate autoimmune hemolytic anemia and thrombocytopenia (93, 94). Reports that aplastic anemia responds to corticosteroids or antithymocyte globulin suggest that these therapies can be contemplated as an initial alternative to bone marrow transplantation (95-97). In addition to these uses, corticosteroids may be cautiously considered in cases of encephalitis, myocarditis, and pericarditis (86).

Acyclovir might be given with corticosteroids to restrict the potentially enhanced opportunity of the virus to replicate in the setting of steroid-induced immunosuppression. Andersson and Ernberg (85) conducted an open clinical trial in which the combination of prednisolone and acyclovir was assessed for treating immunocompetent patients with complicated acute infectious mononucleosis. They showed that acyclovir could suppress oropharyngeal shedding of EBV even when corticosteroids were given. Although the combined regimen appeared to be clinically beneficial, the individual contribution of acyclovir to this effect was not delineated.

Acute Progressive Infection

Treatment of fulminant infections that develop in the setting of immunologic deficiency has been disappointing (19). This result is typified by the general experience in patients with X-linked lymphoproliferative disease, in whom neither acyclovir nor corticosteroids seem to alter the explosive progression of primary EBV infection or its sequelae (98, 99). Gamma-interferon and the combination of alpha-interferon and immunoglobulin may provide transient benefit to such patients (100, 101).

Chronic Infectious Mononucleosis

Anecdotal reports have linked intravenous acyclovir therapy with improvement of fever, interstitial pneumonitis, and lymphocyte CD4/CD8 ratio in patients with
chronic infectious mononucleosis (22). During the past 13 years, we have found that only one of six such patients responded to acyclovir and that three of five responded well to long-term treatment with prednisone plus a cytotoxic agent. In the isolated instances where alpha- or gamma-interferon or intravenous immunoglobulin was used, no evidence of benefit was seen.

Other EBV-associated Disorders

Oral Hairy Leukoplakia

A benign lesion commonly found in HIV-infected persons, oral hairy leukoplakia is associated with minimal symptoms (or none) and may resolve spontaneously (26, 102). Interestingly, this is the only lesion that appears to arise as a direct consequence of replication of the linear genome of EBV and, therefore, is amenable to antiviral therapy. Several small series suggest that intravenous or oral acyclovir is effective in resolving hairy leukoplakia, but lesions frequently recur after therapy is discontinued (103). It has also been reported that hairy leukoplakia responds to ganciclovir.

Lymphoproliferative Disorders

The EBV-associated lymphoproliferative disorders that arise in solid organ and bone marrow transplant recipients pose another problem for which the treatment options are often unsatisfactory and untested by controlled trials. Measures should be taken to improve immune function by withdrawing or reducing immunosuppressive therapy, if possible. Otherwise, several regimens can be considered. Remissions of both polyclonal and monoclonal tumors have been associated with the combination of alpha-interferon and intravenous gammaglobulin (104). An initial report indicates that infusions of anti-B-cell antibodies hold promise for oligoclonal lymphoproliferative disorders not involving the central nervous system (105). Although earlier studies suggested that acyclovir was effective in cases of polyclonal lymphoproliferative disorders, subsequent research has cast doubt on this conclusion (20). Surgical resection of localized tumors appears to be beneficial in certain cases; debulking of extensive tumors may also be useful. Aggressive tumors may require cytotoxic or radiation therapy, or both.

Lymphomas associated with EBV that arise in persons with AIDS or congenital immunodeficiency syndromes are typically aggressive, high-grade B-cell tumors that may respond to cytotoxic agents or radiation therapy.

Prevention

Restricting intimate contact during acute mononucleosis can reduce the transmission of EBV but will needlessly hamper contact with many persons who are already seropositive and, in cases of susceptible children, may even delay virus acquisition to an age when symptomatic mononucleosis is more likely. In addition, the transmissibility of EBV during asymptomatic viral shedding remains problematic long after the acute illness. Nevertheless, avoidance of intimate contact is reasonable in the few instances when the complications of infection could be devastating. Nosocomial transmission of EBV is largely preventable by attention to universal precautions and hand-washing. Isolation of persons with acute infectious mononucleosis is generally unnecessary (106). Because recipients of EBV-containing blood products or tissue allografts may become infected, the use of irradiated blood products or EBV-negative tissues in seronegative recipients is justified in special circumstances (for children with severe combined immunodeficiency and selected transplant recipients, for example).

Vaccine Development

The major impetus for vaccine development arises from the desire to prevent infection in regions of the world where EBV-associated malignancies are highly endemic and to prevent infection in persons with immunodeficiency disorders. In addition, vaccination of all children in developed countries could be considered for the prevention of infectious mononucleosis.

Because a live EBV vaccine, however attenuated, could retain its oncogenic potential, research is being concentrated on a subunit vaccine or an inactivated virus vaccine. Subunit vaccine development has focused on use of the EBV gp350 envelope glycoprotein, which binds to the virus receptor on B cells. Serum from humans infected with EBV contains antibodies to gp350 antigens. These antibodies are active in in-vitro assays such as virus neutralization, complement fixation, and antibody-dependent cellular cytotoxicity. In addition, most persons also have salivary IgA antibodies to gp350. Moreover, gp350 elicits a cellular immune response, and CD4-positive T-cell clones from infected humans recognize epitopes on gp350.

Experimental preparations of gp350 appear to be promising. Cotton-top tamarins (Old World primates) invariably develop EBV-associated lymphoproliferation within 3 weeks after infection. After vaccination with gp350, the animals develop substantial levels of EBV-neutralizing antibody and are protected from B-cell tumors after subsequent challenge with EBV (107).

Despite the progress made in technical aspects of EBV vaccine development, several obstacles remain. There is concern that vaccine-induced immunity would wane and merely delay the age of primary infection or alter immune responses to the infection and make the disease worse. Also, it is not known whether the existing candidate vaccines would protect against virus acquired by a natural route of infection. It will be some time before a vaccine is considered suitable for routine prevention of EBV infection in the United States.

Acknowledgments: The authors thank S. E. Pike, K. Jones, K. Taga, P. Frugoni, J. Tanner, M. K. Breining, J. McKnight, and A. Rosenberg for helping with this work; B. R. Marshall, E. Caruso, and M. Stoughton for providing editorial assistance; and S. Rosen for organizing this conference and providing invaluable advice on the manuscript.

Requests for Reprints: Stephen E. Straus, MD, Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Building 10, Room 11N228, Bethesda, MD 20892.

Current Author Addresses: Drs. Straus, Cohen, and Meier: Medical Virology Section, Laboratory of Clinical Investigation, NIAID, NIH, Building 10, Room 11N228, Bethesda, MD 20892.
References


Cellular immunological responses to the virus infection.


But I am now as much afraid of drinking, as of bathing; for, after a long conversation with the Doctor, about the construction of the pump and the cistern, it is very far from being clear with me, that the patients in the Pump-room don’t swallow the scours of the bathers. I can’t help suspecting, that there is, or may be, some regurgitation from the bath into the cistern of the pump. In that case, what a delicate beverage is every day quaffed by the drinkers; medicated with sweat and dirt, and dandriff; and the abominable discharges of various kinds, from twenty different diseased bodies, parboiling in the kettle below. In order to avoid this filthy composition, I had recourse to the spring that supplies the private baths on the Abbey-green; but I at once perceived something extraordinary in the taste and smell; and, upon inquiry, I find that the Roman baths in this quarter, were found covered by an old burying ground, belonging to the Abbey; through which, in all probability, the water drains in its passage; so that as we drink the decoction of living bodies at the Pump-room, we swallow the stainings of rotten bones and carcasses at the private bath—I vow to God, the very idea turns my stomach!—

Tobias Smollett
Humphrey Clinker

Submitted by: Theodore B. Schwartz, MD
Boise, ID 83705

Submissions from readers are welcomed. If the quotation is published, the sender’s name will be acknowledged. Please include a complete citation, as done for any reference.—The Editors