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Progress and Problems in Understanding and Managing Primary Epstein-Barr Virus Infections

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INTRODUCTION

Epstein-Barr virus (EBV) was discovered in 1964 by electron microscopy of suspension cultures of African Burkitt lymphoma cells (51). Four years later, EBV was linked conclusively to infectious mononucleosis, which is its most common clinical manifestation (78). The unifying and perplexing characteristic of human herpesviruses, including EBV, is that acquisition results in lifelong infection after the initial viral replication has been contained (172). This review describes advances in the clinical, virologic, and immunologic aspects of primary EBV infection, which have been the focus of our research for the past decade. We discuss the spectrum of clinical illness due to primary EBV infection, risk factors for acquisition and severity of infectious mononucleosis, treatment options for EBV infections, and prospects for a vaccine. Understanding the pathogenesis of EBV infection and applying that knowledge to patient care are of great interest to basic and translational scientists and also to clinicians, especially those in family practice, pediatrics, and internal medicine.

BIOLOGY OF EBV

The biology of EBV, including virus structure, genome, strain variability, replication, and latency, has been reviewed comprehensively elsewhere (27, 57, 149, 196, 204). Thus, we focus here on the areas that are crucial for understanding pathogenesis, diagnosis, treatment, and prevention of primary EBV infections.

Virus Structure, Genome, and Strain Variability

EBV, formally designated human herpesvirus 4 (HHV-4), is one of the eight known human herpesviruses. Like those of other herpesviruses, EBV virions have a double-stranded, linear DNA genome surrounded by a protein capsid. A protein tegument lies between the capsid and the envelope, which is embedded with glycoproteins that are important for cell tropism, host range, and receptor recognition (113). Mature virions are approximately 120 to 180 nm in diameter (51, 111). The EBV genome of approximately 100 genes has been described in detail (57). There are two subtypes of EBV, which differ from each other at the EBV nuclear antigen (EBNA) loci for EBNA2, -3A, -3B, and -3C (175). Type 1 is dominant in the Western hemisphere and Southeast Asia, whereas types 1 and 2 are equally prevalent in Africa (169, 224). These isolates are distinguished by their restriction endonuclease digestion patterns and exhibit different transforming capabilities (1, 170, 199) and the ability to spontaneously enter the lytic cycle (31).

Primary Infection and Lytic Replication

Initial infection is thought to occur in the oral (tonsillar) compartment (Fig. 1). The host cells of EBV are mainly lymphocytes and epithelial cells (113). EBV attaches to B cells via binding of the viral gp350 protein to CD21 on B cells (188). EBV gp42 then interacts with B-cell HLA class II molecules and triggers fusion with the host membrane. In epithelial cells, which lack CD21, the EBV BMRF-2 protein interacts with β1 integrins (205, 219, 220), and the EBV gH/gL envelope protein triggers fusion via interaction with αvβ6/8 integrins (35). Endocytosis of the virus into vesicles and fusion of the virus with the vesicle membrane release the nucleocapsid into the cytoplasm. Once the viral nucleocapsid is dissolved, the genome is transported to the nucleus, where it is replicated by DNA polymerases. Viral DNA polymerase accomplishes linear viral replication, which occurs during the lytic phase of the viral life cycle. Tsurumi et al. (204) have published a complete review of lytic and latent replication. Briefly, there are three temporal classes of viral lytic gene products (immediate-early [IE], early [E], and late [L]). BZLF1 and BRLF1 are some of the IE products that further act as transactivators of the viral lytic program (204). Activation of lytic replication or reactivation from latency is key to transmission. The early products (e.g., BNLF2a) have a wide array of functions, including replication, metabolism, and blockade of antigen processing, while late products tend to code for structural proteins such as the viral capsid antigens (VCA) and gene products used for immune evasion (e.g., BCRF1). An important consequence of EBV infection in B cells is that they are induced to activate their growth program and trigger differentiation into memory B cells via the germinal center reaction. Infected memory B cells are released into the peripheral circulation (Fig. 1), resulting in detectable levels of virus in the blood, as discussed below. The number of infected B cells decreases over time after the onset of symptoms of primary infection (72), but these cells are never eliminated entirely.

Latency

Latency is the state of persistent viral infection without active viral production. EBV persists mostly in the memory B-cell compartment and possibly also in epithelial cells (201) (Fig. 1). Currently, it is thought that one in a million B cells carry the EBV genome in an individual after recovery from acute infection (27). It is generally thought that EBV genomes in latently infected B cells exist as episomes (3), although it is possible that the genomes exist as integrated DNA (111, 112).

In contrast to lytic replication, episomal replication during the latent phase occurs via host DNA polymerase. There is limited expression of EBNA and latent membrane protein (LMP) gene products during latency (4). These include EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA leader protein (EBNA-LP), LMP1, and LMP2. Characterization of gene expression patterns in different cell lines (i.e., Burkitt's tumors and EBV-immortalized lymphoblastoid cell lines [LCLs]) has determined that there are at least three different latency programs (201). By using different transcription programs, latent EBV genomes can multiply in dividing memory cells (type I), induce B-cell differentiation (type II),

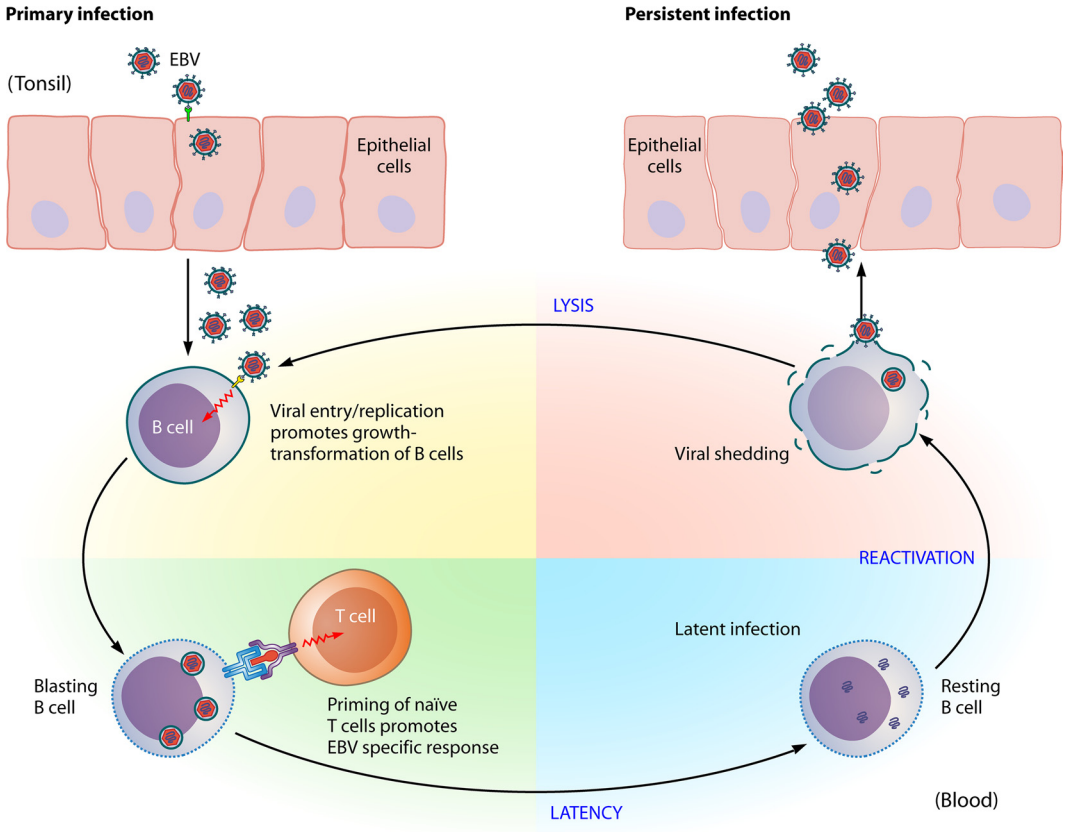

FIG. 1. EBV infection in healthy carriers. Primary EBV infection begins in the oral cavity. EBV uses different glycoproteins to infect epithelial cells and naïve B cells. Viral entry results in transport of the EBV genome into the B-cell nucleus, where replication by cellular and viral DNA polymerases begins. EBV gene products activate the B-cell growth program, resulting in the proliferation of blasting B cells. Priming of naïve T cells by antigen-presenting cells occurs in parallel. Normally, these blasting B cells are destroyed by cytotoxic T lymphocytes. Once in the circulation, previously activated memory B cells may continue to undergo lytic replication or, if EBV shuts down most of its protein-encoding genes, latency occurs. At a later time, as cells recirculate between the oral and peripheral compartments, resting B cells may be activated, resulting in viral reactivation and shedding.

activate naïve B cells (type III), or completely restrict all gene expression in a context-specific manner (189, 201). Only EBNA1 is expressed in the type I latency program, which is seen in Burkitt's lymphoma. CD8 T cells specific for many EBV antigens arise during the immune response to natural infection, but not for EBNA1, which contributes to evasion during latency (26). EBNA1 and LMP1/2A are expressed in the type II latency program, which is observed in nasopharyngeal carcinoma and Hodgkin's lymphoma. LMP1 and LMP2 are responsible for B-cell activation and induction of a growth (proliferation) program (27). The type III latency program, in which all of the latency gene products are expressed, is often detected during acute infectious mononucleosis or in certain immunocompromised individuals. Multiple factors that regulate gene expression in latency have been documented (189).

Reactivation

Latently infected B cells can occasionally be stimulated to reactivate EBV. This produces virus that can reinfect new B cells and epithelial cells, becoming a source of viral transmission (Fig. 1). Although much is known about the molecular pathways involved in viral reactivation (99), what triggers reactivation *in vivo* is not known precisely. The presumption is that it occurs when latently infected B cells respond to unrelated infections, because B-cell receptor stimulation triggers reactivation in B-cell lines. It is also not known what fraction of EBV-infected cells are in the lytic or latent phase at any time, although a technique using sera from EBV-infected individuals may prove useful in the future (23). Understanding how each gene product, whether lytic or latent, contributes to the pathogenesis of EBV-related diseases should lead to more rational and effective prevention and treatment strategies.

EPIDEMIOLOGY

Young children most likely acquire primary EBV infection from close contact that involves exchange of oral secretions via shared items such as toys, bottles, and utensils. Before the age of 10, primary infection is usually asymptomatic or produces an acute illness that is often not recognized as being due to EBV (193). In adolescents and young adults, however, primary EBV infection frequently presents as infectious mononucleosis, a clinical syndrome named by Sprunt and Evans in 1920 and discussed further below (190). Hoagland argued convincingly that infectious mononucleosis was acquired "chiefly by direct

intimate oral contact which allows for salivary exchange" (86). His hypothesis was strengthened by the lack of transmission among roommates (55, 73, 177) and the failure to infect experimental volunteers (53). He was also able to estimate its incubation period thanks to a patient who described a one-time, intense kissing experience 47 days before the onset of infectious mononucleosis. Hoagland questioned 73 subsequent patients, and 71 (97%) of them reported oral contact 32 to 49 days before developing infectious mononucleosis, which corresponds to an incubation period of approximately 6 weeks. In addition, an incubation period of 38 days has been reported for a well-documented case, which is consistent with Hoagland's observations (195).

Aside from oral transmission, EBV has been acquired from blood (62), indicating that virus present in the peripheral circulation, most likely in memory B cells (72), is or may become infectious. EBV can also be acquired from transplanted hematopoietic cells (2, 183) or solid organs (74), and such infections can be life-threatening, especially among recipients who were EBV naïve before transplantation (155). Several reports of intrauterine transmission of EBV have been published, but none has been substantiated by appropriate viral studies (64, 95). Scottish investigators believe that EBV may be transmitted via genital secretions during penetrative sexual intercourse (40, 81). However, their data are retrospective and based on only 2 questionnaires completed 3 years apart. Furthermore, because kissing and sexual intercourse are virtually inseparable, oral transmission certainly cannot be ruled out.

The seroprevalence of EBV varies widely by geographic location (34, 42, 43, 73). Data indicate that primary EBV infection occurs at a younger age among persons from lower versus higher socioeconomic backgrounds (58, 122), which has been attributed to crowded living conditions (193). Acquisition of EBV by young children indicates that it can be transmitted without deep kissing. However, this does not rule out saliva as the source of EBV, as young children commonly share objects that they put in their mouths.

Healthy people continue to shed EBV for many months after their acute infection and are potentially capable of transmitting it (16, 56). Because such virus "donors" are asymptomatic and hence not considered to be the source of infection, they often go unrecognized. For the most part, shedding becomes intermittent rather than continuous several months after the primary infection. Hadinoto et al. recently reported that EBV is shed continuously in the saliva at relatively stable levels over short periods (hours or days), but quantities varied as much as 4 to 5 $\log_{10}$ copies over months or years (72). This suggests that a person's likelihood of transmitting EBV fluctuates over time.

No clear-cut seasonal pattern for infectious mononucleosis has been recognized (30, 77). However, more cases have been documented among U.S. college and university students when school is in session than during semester breaks (30), and Leard observed over a 12-year period that there was an October peak in admissions for infectious mononucleosis to the Boston University infirmary (120). A peak incidence during June to August was reported among patients in the Israeli Defense Force (67), which the authors ascribed to increased socializing of young people during the summer.

TABLE 1. Prevalence of signs, symptoms, and laboratory abnormalities in infectious mononucleosis[a,b]

Finding	Prevalence (%)	Comment
Signs		
Pharyngitis	100	Occasionally seen without sore throat
Cervical lymphadenopathy	95	Especially posterior cervical and postauricular
Fever	50	Often masked by antipyretics
Hepatomegaly	25	
Splenomegaly	33	
Eyelid edema	10	Unusual in other acute illnesses
Rash	5	Virtually all patients given penicillin derivatives develop a rash
Symptoms		
Sore throat	95	Many patients describe this as the "worst" they have ever had
Fatigue	90	Usually the last symptom to resolve
Headache	75	Common but underappreciated
Fever	70	
Body aches	50	Patients describe this as "like the flu"
Decreased appetite	50	
Abdominal discomfort	40	Due to mesenteric adenitis or hepatosplenomegaly
Laboratory abnormalities		
Alanine aminotransferase elevation	80	Five to 10% of patients are jaundiced
Leukocystosis	40	Usually due to increase in CD8 cytotoxic lymphocytes
Thrombocytopenia	25	Thought to be autoimmune
Anemia	10	Thought to be autoimmune

[a] Based on a compilation of published series (54, 67, 84, 130, 167) and 116 subjects followed in natural history and treatment trials at the University of Minnesota (14–16).
[b] The median duration of illness is 16 days, and the mean duration is 19 days.

CLINICAL MANIFESTATIONS OF PRIMARY EBV INFECTION WITH CONTAINMENT

Infectious Mononucleosis

Infectious mononucleosis was the name chosen by Sprunt and Evans to describe a syndrome that resembled an acute infectious disease accompanied by atypical large peripheral blood lymphocytes (190). We now understand that these atypical lymphocytes, also called Downey cells, are activated CD8 T lymphocytes, most of which are probably responding to EBV-infected B cells.

Typical clinical syndrome. Infectious mononucleosis most often begins insidiously, with vague malaise, followed several days later by fever, sore throat, swollen posterior cervical lymph nodes, and fatigue. Some patients experience an abrupt influenza-like onset, with fever, chills, body aches, and sore throat. Table 1 displays the relative frequencies of signs and symptoms compiled by combining data from other investiga-

tors (54, 67, 84, 130, 167) and our own experience (14–16). It should be emphasized that the diagnosis of infectious mononucleosis cannot be made on clinical grounds alone.

Hepatitis, documented by abnormal liver function tests, is seen in 80% of cases and thus should be considered part of the acute disease rather than a complication. Liver involvement is subclinical in 90 to 95% of patients, but the remainder develop jaundice, and a few of them complain of tenderness in the right upper quadrant of the abdomen that is likely due to hepatic swelling with pressure on the liver capsule.

Eyelid edema, which gives the patient a slit-eyed appearance and may be accompanied by facial puffiness, is a useful clinical clue if present because it is unique to primary EBV infection (85).

The median duration of infectious mononucleosis is 16 days, which is much longer than the duration of most acute viral illnesses (Table 1). Recovery is gradual, and it may take months for the patient to feel entirely well (167). Fatigue interferes with productivity and quality of life and is usually the last symptom to resolve.

Recrudescence of symptoms before the acute illness ends occurs occasionally (130). However, recurrences or "second cases" of infectious mononucleosis documented by laboratory evidence of active EBV infection after recovery from the acute illness are very uncommon. Hoagland reported no recurrences in his series of 200 patients, most of whom were hospitalized during their acute illness, according to military policy (84). We have had just one laboratory-documented recurrence among 116 subjects who acquired infectious mononucleosis between the ages of 16 and 26 years (H. H. Balfour, Jr., unpublished data).

The risk of developing infectious mononucleosis after primary EBV infection correlates with the age of the patient (77). Children younger than 10 years of age are usually asymptomatic or moderately ill, with a partial infectious mononucleosis syndrome, although classic infectious mononucleosis can occur in this age group (63). Primary EBV infection among adolescents and young adults may also be asymptomatic, but at least half of them develop full-blown infectious mononucleosis (Balfour, Jr., unpublished data). The reason for this age-specific severity of illness remains elusive. The severity of primary EBV infection in adults increases with age, and patients older than 40 years of age are especially prone to serious illness (9, 94). They have more prolonged fever and more serious hepatic involvement but less noticeable lymphadenopathy than younger patients.

Complications. Complications may be due to tissue-invasive viral disease or to immune-mediated damage. Many complications have been associated with infectious mononucleosis, but nearly all of them are uncommon or rare (102, 171, 215). Table 2 lists the complications whose frequency is estimated to be at least 1%. The following complications, listed alphabetically, have been described for fewer than 1% of patients: conjunctivitis, hemophagocytic syndrome, myocarditis, neurologic diseases other than meningoencephalitis, pancreatitis, parotitis, pericarditis, pneumonitis, psychological disorders, and splenic rupture (38, 87, 102, 171, 215). Splenic rupture is a rare but greatly feared complication that excludes athletes from contact sports for various periods (see "Limitation of activities").

TABLE 2. Complications reported in ≥1% of cases of infectious mononucleosis

Complication	Comment
Airway obstruction	Due to oropharyngeal swelling and edema
Meningoencephalitis	Other neurologic complications have been reported but are rare
Hemolytic anemia	Thought to be autoimmune
Thrombocytopenia	Thought to be autoimmune
Rash	Rash due to EBV is uncommon, but maculopapular rashes occur in the majority of patients inadvertently given penicillin derivatives

Asymptomatic or Unrecognized Primary EBV Infections

As stated above, EBV infections in children under the age of 10 are often overlooked, either because they are entirely asymptomatic or because they do not present with a typical infectious mononucleosis syndrome. A clinical dilemma for making the correct diagnosis in children is that point-of-care laboratory tests, which are essentially all heterophile antibody assays, may be falsely negative (63, 92). Hence, even suspected EBV infections in children may not be confirmed. Primary EBV infection may not be recognized in adolescents and young adults, either, but 90% of them report some symptoms, especially sore throat, if seen shortly after the onset of infection (Balfour, Jr., unpublished data) and could be diagnosed with appropriate laboratory tests.

CLINICAL MANIFESTATIONS OF PRIMARY EBV INFECTION WITH LOSS OF CONTAINMENT

The vast majority of individuals who experience primary EBV infection—whether asymptomatic or ill with infectious mononucleosis—develop no serious consequences from lifelong infection. However, in rare cases, infection is not contained and results in the development of complications. EBV has a well-established oncogenic potential, which under some circumstances can be life-threatening. Additionally, EBV infection has been implicated in the pathogenesis of various autoimmune diseases, such as multiple sclerosis (139). These situations demand a better understanding of the biology of EBV infection, the host immune response, and the development of effective treatment strategies.

CAEBV

First described in the late 1940s, chronic active EBV (CAEBV) is due to the inappropriate control of viral replication (148). Although it occurs relatively rarely, there is a high morbidity and mortality rate for patients with CAEBV infection, and thus an accurate diagnosis is important. The disease is characterized by chronic infectious mononucleosis-like symptoms (fever, lymphadenopathy, and hepatosplenomegaly), with illness lasting for more than 6 months. EBV-specific antibody titers are abnormal in CAEBV (discussed below), and viral loads are elevated (148). Other complications can include pancytopenia, hypergammaglobulinemia, and B- or T-cell malignant lymphoma or lymphoproliferation (116, 149). Patients

tend to have virus in tissues and peripheral blood and high levels of antibodies to viral capsid antigen and early antigen (128). For a more thorough review of the diagnosis of CAEBV, we recommend the work of Okano et al. (147). The pathogenesis of chronic active EBV remains ill defined (115). Expansions of T cells and natural killer (NK) cells are a prominent characteristic (116, 194), although they vary between polyclonal, oligoclonal, and monoclonal populations (145). Typically, such T/NK cells are infected with EBV (103, 106–108, 114). Presumably, EBV drives the T/NK cell expansion and pathology, but why the virus escapes immune detection is unclear. Various treatment strategies for CAEBV have been attempted, including antiviral drugs, chemotherapeutic agents, immunomodulating agents, cell therapy using EBV-specific cytotoxic T lymphocytes (CTLs), and hematopoietic stem cell transplantation (65, 115). While hematopoietic stem cell transplantation has seen some success, there remains no clear consensus as to the optimal treatment regimen (37).

Lymphoproliferative Disorders

The oncogenic properties of EBV have been appreciated for a long time (221). The primary neoplasms associated with EBV are B-cell lymphomas and nasopharyngeal carcinoma, reflecting the primary cellular targets of viral infection *in vivo*: B cells and tonsillar epithelium, respectively. The virus utilizes multiple mechanisms to promote neoplasm, including activation of the B-cell growth program, immune evasion, and inactivation of tumor suppressors (221). Many lines of evidence suggest that ongoing immune control of EBV reactivation is critical to prevent transformation *in vivo* (140). This is exemplified dramatically in the case of pediatric allogeneic transplantation, where immunosuppression is used to control graft rejection. In transplant patients, lymphoproliferation may be due to viral reactivation in a seropositive individual or may result from primary exposure to infected donor tissue in a previously seronegative individual. Regardless of the source, immunosuppression can cause the immune system to lose control of EBV replication (70, 176, 210), which may result in posttransplant lymphoproliferative disorder (PTLD)—an often fatal disorder of uncontrolled B-cell proliferation, plasmacytic hyperplasia, and lymphoma (126). The incidence of PTLD correlates with the immunosuppressive dose (104), and withdrawal of immunosuppression is key to the successful treatment of PTLD (203), although this obviously compromises the likelihood of graft tolerance. For patients with PTLD, $CD8^+$ CTLs may be expanded *in vitro* and then reinfused to treat or prevent PTLD (192). An alternative strategy could include prevaccination of high-risk EBV-seronegative transplant patients with either a gp350 or peptide-based vaccine. Vaccine trials are planned or in progress but have yet to show protection against PTLD (168). Thus, the role of immunization or antiviral drugs in management of PTLD is still unclear.

Another situation of EBV-associated lymphoproliferation is X-linked lymphoproliferative disease (XLP) (136). This is a rare inherited disorder associated with mutations in the gene encoding the signaling lymphocyte activation molecule (SLAM)-associated protein (more commonly known as SAP) (36, 142, 178). The SAP protein is a signaling molecule downstream of SLAM receptors, which are important in B-cell activation of T cells and NK cells (29, 82, 152, 164). Since EBV-infected B cells are held in check by T and NK cells, individuals with XLP are at high risk for fatal infectious mononucleosis and EBV lymphomas (143, 162). Treatment of EBV-associated disease in XLP patients is through immunosuppressive cocktails and may benefit from inclusion of anti-CD20 (134). Allogeneic transplantation is the only curative therapy and is typically performed as early in life as possible (119).

PRIMARY RESPONSE TO EBV INFECTION

A potent innate and adaptive immune response occurs during primary EBV infection. This response, although it controls infection, does not eliminate it, and the virus persists for the lifetime of the infected individual. Thus, a careful balance exists between the virus and the immune system. This section summarizes what we have learned from virological and immunological studies.

Virologic Events

Infectious virus acquired by exposure to oral secretions or blood establishes a foothold in the oral and/or blood compartment in B lymphocytes, epithelial cells, or both (88, 157). After that, it takes an estimated 5 to 7 weeks for the primary EBV infection to manifest itself as infectious mononucleosis. Computer simulations based on a scenario of multiple rounds of viral replication, with amplification at each step, can predict peak viral loads consistent with those observed in natural infection (182). Unfortunately, there are few data on immune or virologic events from samples obtained during the incubation period, as patients do not seek medical care prior to the onset of symptoms. Thus, what happens during the incubation period remains a major unanswered question about primary EBV infection.

Because the onset of infectious mononucleosis is often insidious, little is known about the virus-host interactions until about the fifth day of illness, at the earliest (15, 16). Viral loads in the oral cavity, especially in oral cells, are 1 to 2 $\log_{10}$ EBV copies/ml higher, on average, than those in whole blood. As shown in Fig. 2, viral clearance from the oral compartment is much slower than that from the blood. Viral loads in oral cells and saliva remain elevated for many months (15, 16, 56, 72). In contrast, virus is eliminated from whole blood more rapidly (16). Detectable virus in the blood is eliminated by the seventh week of illness in almost every patient, but it did recur 6 months or more after primary EBV infection in 8 (10%) of 78 subjects we have been following for several years (Balfour, Jr., unpublished data).

Viral DNA detected in the blood is thought to come primarily from infected memory B cells (11, 201). It was previously held that EBV maintains latency only in B lymphocytes, based in part on the fact that infection was eradicated by an EBV-negative bone marrow transplant (66). However, viral gene expression patterns differ when the virus emerges from epithelial cells versus B cells, in a way that suggests passage back and forth (28). Furthermore, patients depleted of B lymphocytes with anti-CD20 antibody (rituximab) still shed virus from the throat (90), suggesting that there could be a reservoir of EBV latency other than the B cell.

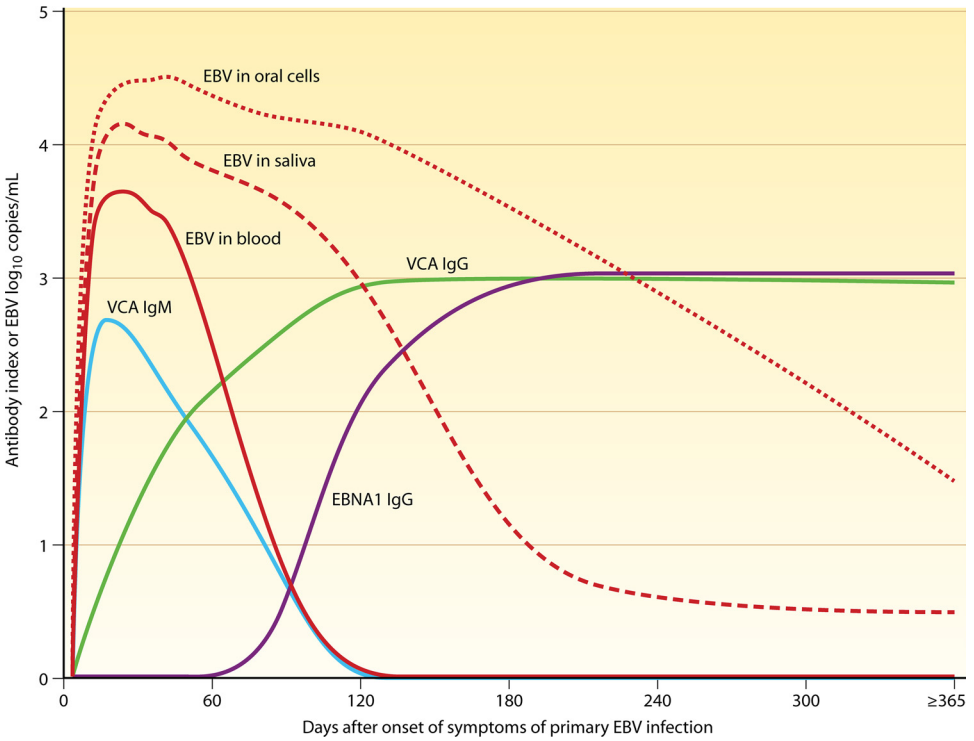

FIG. 2. Kinetics of EBV-specific antibodies and viral load in infectious mononucleosis. The graph shows the evolution of EBV replication and EBV-specific antibodies measured by EIA during primary infection. At presentation, EBV may not be detected in the blood but is usually found in large quantities in the oral cavity. Virus is cleared from the blood much more rapidly than from the oral compartment. Oral viral shedding can persist for months and recurs intermittently for years in most healthy adults. At the onset of illness, most patients have IgM antibodies to EBV VCA; these decline between 2 and 6 months after infection. VCA IgG antibodies may be detected as early as during the first 2 weeks of illness. Essentially 100% of patients have detectable VCA IgG antibodies during convalescence, and these persist for life. EBNA1 IgG antibodies do not develop until 3 to 6 months after infection but then persist for life.

One gap in our understanding of the implications of viral load on the epidemiology and pathogenesis of infectious mononucleosis is not knowing what portion of the viral load is complete and potentially infectious and what portion is in either an episomal or unencapsidated form. The latter could certainly be immunogenic but would neither be contagious nor cause tissue-invasive disease (3).

Immune Response to EBV

Innate immune response. The innate immune system is an important first line of defense against viral infections. Viruses elicit a strong type I interferon (IFN) response early after infection. This is presumed to be the case for primary EBV infection, although as alluded to above, the kinetics and quality of this response are difficult to study *in vivo* because of the long incubation period. Nonetheless, EBV potently stimulates IFN production from isolated human plasmacytoid dendritic cells *in vitro* (165). Viral DNA and protein are recognized by pattern recognition receptors such as Toll-like receptors (TLRs), which can trigger an IFN response, facilitate the activation of natural killer cells, and act in multiple ways to prime the adaptive immune response. Our laboratory recently defined the transcriptional profile of human blood during primary EBV infection, and both type I and type II interferon-regulated genes were strongly upregulated (K. A. Hogquist, unpublished data). There is evidence for the involvement of multiple TLRs in activating the innate response to EBV, including TLR2 (8), TLR3 (100), TLR7, and TLR9 (165). Interestingly, the virus may also have mechanisms for controlling TLR signaling (129).

The inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-1β are increased in tonsillar tissue from patients with infectious mononucleosis (60). Many studies have detected inflammatory cytokines in the sera of individuals with infectious mononucleosis as well (Table 3). Prominent among these is IFN-γ. IFN-γ is produced by activated T cells and NK cells. Not only is IFN-γ itself elevated, but the catabolic product neopterin, which is produced by monocytes that are stimulated with IFN-γ, is also elevated (Table 3). IFN-γ is thought to be important for control of EBV infection and reactivation, based on studies of a related gammaherpesvirus infection in mice (45, 121, 213). However, high levels of IFN-γ likely contribute to the symptoms experienced during infectious mononucleosis, as this cytokine is known to cause headache, fatigue, and fever (179). Interestingly, type I interferon (including IFN-α) is not detected consistently in the sera of infectious mononucleosis patients (125, 159, 216). This may reflect both the fact that IFN-α can be difficult to detect and the fact that it is more likely to be produced early in the response to viral infection, before the onset of infectious mononucleosis symptoms and presentation in the clinic. The

TABLE 3. Alterations in serum cytokine levels during infectious mononucleosis

Cytokine	Status during infectious mononucleosis	Possible impact on clinical pathogenesis (in terms of known function)	Reference(s)
IFN-γ	Elevated	Type II interferon, produced by NK cells and Th1 and CD8 T cells; broad immunostimulatory effects; important for control of chronic infection; likely inhibits viral replication and reactivation	24, 39, 91, 125, 180, 216
Neopterin	Elevated	A pteridine compound released from macrophages/monocytes stimulated by IFN-γ	24, 125, 180
IFN-α	Not reproducibly detected	Type I interferon, produced by monocytes and plasmacytoid dendritic cells; broad antiviral and immunostimulatory effects; important for control of acute infection	91, 125, 159, 216
IL-6	Elevated	Inflammatory cytokine produced by T cells and macrophages; mediator of fever and acute-phase response; promotes B-cell maturation	91, 125, 180, 218
TNF-α	Elevated	Inflammatory cytokine produced mainly by macrophages; activates macrophages, stimulates acute-phase response, and can cause liver dysfunction and fever	24, 218
IL-12	Elevated	Cytokine produced by dendritic cells; promotes differentiation of Th1 CD4 and CD8 T cells; enhances NK and CTL cytotoxicity	39, 216
IL-2	Occasionally elevated	Produced by activated T cells; growth factor for regulatory T cells	24, 39, 91, 216, 218
IL-10	Elevated	Immunosuppressive cytokine produced by monocytes and T cells; in combination with viral IL-10, it may suppress T-cell production of other cytokines (IFN-γ, TNF-α) and enable systemic spread of virus	197, 217, 218
TGF-β	Elevated	Immunosuppressive cytokine with pleiotropic effects	217

inflammatory cytokines TNF-α and IL-6 are also elevated during acute infectious mononucleosis. Finally, serum IL-2 is elevated during infectious mononucleosis, consistent with the dramatic expansion of CD8 T cells.

The immunosuppressive cytokines IL-10 and transforming growth factor beta (TGF-β) are also detected in the sera of infectious mononucleosis patients (Table 3). Interestingly, the EBV late gene BCRF1 acts as an IL-10 homologue and shares 84% of its amino acid sequence with human IL-10 (135). During acute infectious mononucleosis, both viral and host forms of IL-10 are detected in sera (197). Host IL-10 is produced by monocytes and lymphocytes, functions to suppress T-cell proliferation and cytokine production, and can inhibit IFN-γ production from T cells (137). Thus, it might be predicted that IL-10 counters the pathogenic effects of IFN-γ during infectious mononucleosis. Consistent with this, the highest levels of IL-10 were observed in patients with shorter durations of symptoms (217). Furthermore, high levels of IL-10 are observed in PTLD patients and are reduced as PTLD resolves with an effective antiviral response (reviewed in reference 141). Thus, overall, it would appear that IL-10 and IFN-γ play key roles in the balance of immune protection and symptoms during infectious mononucleosis.

NK cells are another important component of the immune response and are thought to play a key role in regulating chronic viral infections (118). In fact, human NK cell deficiencies are associated with increased susceptibility to several viral (and bacterial) infections, including EBV infection (150). NK cell numbers increase during infectious mononucleosis (202, 216, 223). Interestingly, their numbers are associated inversely with disease severity (216), suggesting that NK cells could play a role in limiting viral replication.

Adaptive immune response. The adaptive immune response to EBV has been studied extensively and is discussed in detail in a recent review (83). Both humoral and cellular immune responses are generated. The humoral or antibody response is critical in diagnosing infectious mononucleosis, and the cellular response (particularly the CD8 T-cell response) is critical for controlling viral replication but may also contribute to the severe symptoms of infectious mononucleosis.

The kinetics of specific antibody responses to primary EBV infection, as measured by enzyme immunoassay (EIA), are shown in Fig. 2. The first humoral response detected is an IgM class antibody directed against the viral capsid antigen (anti-VCA IgM). This antibody was present in all 70 of our subjects with primary EBV infection who were tested. Sixty-three (90%) of them were positive within 7 days after the onset of symptoms, six became positive during the second week of illness, and one did not become positive until 49 days after the onset of illness (Balfour, Jr., unpublished data). All patients develop anti-VCA IgG antibodies, which peak during the first 2 to 4 months and then persist for life. IgG antibodies to the latent antigen EBNA1 do not develop in most individuals for about 3 months, but once they appear, they persist for life (166). Antibodies to the early antigen diffuse (EA-D) are also elicited during acute infection in 60 to 80% of patients, but they are not diagnostic of a specific phase of EBV infection and hence are not generally useful (80). Anti-gp350 antibodies may be detected after natural exposure to EBV or in response to gp350 subunit vaccines (discussed below) (68, 138, 168, 187). Furthermore, it has been suggested by Turk et al. that gp350 antibodies enhance epithelial cell infection (206). This could imply another form of immune evasion, this time from neutralizing antibodies of the humoral arm that allow EBV to be maintained in an alternate reservoir (tonsillar epithelium) when its initial reservoir (B cells) is being depleted by an active immune response (T cells).

Both CD4 and CD8 T cells make a robust response to EBV antigens, and over 50 HLA class I and class II epitopes have been identified for this virus (83). Early in infection, CD8 T cells specific for lytic antigens tend to dominate the response, while CD4 and CD8 T cells specific for latent antigens do not show such a large burst but persist for life (160). The massive lymphocytosis in the blood that characterizes infectious mono-

nucleosis is thought to consist largely of CD8 T cells specific for EBV lytic antigens (83), although possible activation of bystander cells (non-EBV-specific T cells) has not been ruled out by rigorous means. This large adaptive immune response is thought to be responsible for the major symptoms of infectious mononucleosis, as disease severity correlated more closely with lymphocytosis than with viral load in a small study (184). Interestingly, EBV-specific CD8 T cells were found to be underrepresented in tonsils compared to blood early during infection. This resolved later, suggesting that efficient control of EBV infection requires tonsillar homing of CD8 T cells (82).

Ultimately, CD8 T cells are critical for control of EBV, as evidenced by the occurrence of EBV lymphoproliferation and lymphomagenesis in immunosuppressed patients (146) and the efficacy of EBV-specific CD8 T-cell therapy in controlling PTLD (79).

Pathogenesis of Infectious Mononucleosis

As mentioned above, the robust adaptive immune response is thought to be responsible for the major symptoms of infectious mononucleosis. But why is the infectious mononucleosis syndrome during primary EBV more common in adults than in children? It was proposed that adults acquire a higher viral dose through sexual activity than children do through salivary contact (41). This higher viral dose would initiate a larger CD8 T-cell response, which would cause the symptoms of infectious mononucleosis through production of inflammatory cytokines. However, at least one study found that symptomatic infectious mononucleosis was not associated with a higher viral load (184). Others have speculated that preexisting immunity to other viruses which cross-reacts with EBV (called "heterologous immunity") could provide a robust CD8 T-cell response to primary EBV (181). This could explain why adolescents and adults tend to experience infectious mononucleosis, while children are largely asymptomatic, as adults are likely to have broader immune experience in general. However, neither subunit nor peptide vaccine studies (discussed below) have suggested that preexisting immunity to EBV causes more severe infectious mononucleosis (in fact, the reverse was observed). Thus, it is unclear how heterologous immunity would impact the primary response to EBV. Another possibility relates to the innate immune response. As mentioned above, elevated NK cell numbers were shown to correlate with reduced disease severity in infectious mononucleosis (216). It was recently shown in a mouse model of chronic viral infection (murine cytomegalovirus [CMV]) that NK-cell-mediated lysis of infected dendritic cells limited the CD4 and CD8 T-cell response and, paradoxically, resulted in viral persistence (7). If the large CD8 T-cell response is responsible for disease severity during infectious mononucleosis, then NK cells may reduce it by limiting the adaptive immune response. It will be important to determine if NK cell increases are associated with lower CD8 T-cell responses in future studies. Finally, high levels of inflammatory cytokines, produced by either innate or adaptive immune cells, could also be responsible for the symptoms observed during acute infectious mononucleosis. Therefore, it is important that future studies examine multiple parameters to better understand the factor(s) that mediates pathogenesis.

DIAGNOSIS

Primary EBV infection can be diagnosed with certainty only by utilizing the appropriate laboratory tests. Patients who are mildly ill are unlikely to be identified because either they do not seek medical attention or EBV infection is not considered in the differential diagnosis. Patients with a typical infectious mononucleosis syndrome (described above) are still a diagnostic challenge because their signs and symptoms are not very sensitive or specific for EBV infection. For example, a recent report found that the classic triad of fever, sore throat, and lymphadenopathy had a sensitivity of 68.2% and a specificity of 41.9% for EBV infection (67).

Clinical Clues

Several signs and symptoms point to an EBV etiology (Table 1). These include a very sore throat that appears inflamed and swollen and sometimes has a membranous exudate, symmetrical posterior cervical and postauricular lymphadenopathy, and eyelid edema, often accompanied by facial puffiness. Clinical findings that militate against EBV infection are rhinorrhea, cough, and rash, unless the patient is taking β-lactam antibiotics, in which case the rash is due to transient hypersensitivity to penicillin derivatives induced by EBV (19, 153).

Nonspecific Laboratory Tests

Peripheral blood smear. In 1920, Sprunt and Evans reported 6 young adults with very similar acute infections who had "a mononuclear leucocytosis instead of the more usual increase in the polymorphonuclear leukocytes" (190). They illustrated the features of these mononuclear cells that distinguished them from leukemia. Several years later, Downey and McKinlay published a comprehensive description of the atypical lymphocytes seen in the peripheral blood of patients with infectious mononucleosis (44). As mentioned above, these atypical lymphocytes, also referred to as Downey cells, are activated CD8 T lymphocytes, most of which are thought to be responding to EBV-infected B cells. While they are invariably present in primary EBV infection, they may also be found in infectious mononucleosis-like illnesses due to other viruses, especially cytomegalovirus (198).

Heterophile antibodies. In 1932, Paul and Bunnell discovered that heterophile antibodies, specifically sheep cell agglutinins, were elevated during acute infectious mononucleosis but not during many other diseases and thus could be used for diagnosis (154). They defined heterophile antibodies as "having the capacity to react to certain antigens, which are quite different from, and phylogenetically unrelated to the one instrumental in producing the antibody response." Heterophile tests use various mammalian erythrocytes to detect IgM class antibodies, which are present during the generalized immune upregulation that characterizes acute primary EBV infection.

The laboratory diagnosis of infectious mononucleosis is now almost always made by a heterophile antibody test (22). Heterophile antibodies detected using bovine erythrocytes were present in 76 (96%) of 79 adults during the first 10 days of laboratory-confirmed EBV infectious mononucleosis (Balfour, Jr., unpublished data). Hence, heterophile tests are a sensitive

diagnostic method for acute infectious mononucleosis. However, they do have drawbacks. First, approximately 40% of children 4 years of age or younger do not develop heterophile antibodies during primary EBV infection (92). Thus, the heterophile test may be falsely negative for young children. Second, heterophile antibodies are nonspecific and may be present in non-EBV infections, malignancies, and autoimmune diseases (59, 93). Finally, heterophile antibodies may persist for a year or more and therefore do not always signify an acute EBV infection (25).

Liver function tests. On average, 80% of patients with infectious mononucleosis have abnormal liver function during the early stages of infection (Table 1). Elevated liver enzymes, especially alanine aminotransferase, strengthen the clinical impression of infectious mononucleosis.

EBV-Specific Assays

EBV-specific antibody tests. Indirect immunofluorescence assays or EIAs are the common platforms for the detection of EBV-specific antibodies. As discussed above and illustrated in Fig. 2, the profile of EIA antibodies present distinguishes acute primary, convalescent, and past infections. Acute primary EBV infection is characterized by IgM antibodies to the early antigen VCA in the absence of IgG antibodies to the latent antigen EBNA1. VCA IgG antibodies may be present in acute infection, but in smaller quantities than VCA IgM antibodies. During convalescence (from the third week to the third month after onset of illness), VCA IgM antibodies dwindle, while VCA IgG antibodies rise and persist for life. Between the third and sixth months, VCA IgM antibodies disappear, whereas EBNA1 IgG antibodies become detectable and persist for life. All 3 antibodies may be present in late primary infection or subclinical reactivation, which can be distinguished from each other by performing an IgG avidity assay (144). An evidence-based correlation of serologic patterns with stages of EBV infection was recently published (117). This analysis included heterophile antibody and EA-D IgG antibody in addition to VCA IgM and IgG and EBNA1 IgG.

Viral detection and quantitation. EBV can be identified in tissue samples by immunohistochemical approaches. *In situ* hybridization to detect EBV-encoded RNA transcripts (EBERs) is the gold standard for detecting EBV in tissue (69). PCR is the technique of choice for detecting and quantifying EBV in body fluids and can also be used to quantify the virus in tissue samples (70). While there are options in terms of platforms, volumes, probes, and targets, a multicenter comparison of different real-time PCR assays suggested that if samples are tested at one center on the same platform, real-time PCR is a precise technique for measuring viral load (76). However, substantial quantitative differences were found when samples were tested in different laboratories.

Transplant patients are at risk for serious EBV-related disease, including potentially fatal PTLD, and thus quantitative PCR is routinely ordered to monitor their EBV loads (12, 132). Because most assays employ a DNA template and the matrix tested is usually blood, EBV loads in blood are also referred to as EBV DNAemia. There is no consensus on the exact threshold level that should trigger a change in patient management. This is because gene targets and assay platforms differ widely among laboratories and, at present, there is no universal quantitative standard. Also, the threshold level may be lower for hematopoietic stem cell transplant recipients than for solid organ transplant recipients. At our institution and a number of others, viral loads of >4,000 copies/ml of whole blood (210, 211) or steadily rising levels often prompt a reduction of the patient's immunosuppression and initiation of specific treatment, as discussed below. Treatment is usually continued until serial viral loads are <1,000 copies/ml of whole blood.

The best matrix to use for monitoring EBV infections is debated. Some experts recommend plasma (212), but the majority favor whole blood (174, 191). Copy numbers in plasma are usually 10- to 100-fold lower than those in concomitant whole-blood samples. Cerebrospinal fluid can be tested by PCR to diagnose EBV infection of the central nervous system. A positive result, regardless of quantity, should initiate a thorough neurologic evaluation, including imaging.

In addition to its use for transplant patients, quantitative PCR may be useful for making the correct viral diagnosis for patients with atypical clinical features or for young children who have heterophile-negative infectious mononucleosis (158). Immunocompetent patients with symptomatic EBV infections have viral loads averaging 5,000 copies/ml of whole blood during the first 7 to 10 days of illness, compared with levels of 5,000 to ≥50,000 copies/ml of whole blood in transplant recipients; viral loads during latency are rarely >1,000 copies/ml of whole blood (Balfour, Jr., unpublished data).

Quantitative PCR is also useful for monitoring the effects of anti-EBV therapy and in the evaluation of candidate antiviral drugs (15). As mentioned above, when this method is used to determine the response to therapy, the goal is to drive the blood viral load below the level of detection, or at least to <1,000 copies/ml.

Quantitative PCR assays do not distinguish between integrated, episomal, whole-virion, and unencapsidated forms of EBV (3). Could DNAemia in some patients be merely a reflection of latent virus and therefore of no clinical significance? This is unlikely, because latent EBV is present in only a very small fraction of circulating memory B cells (27) and would normally be near the lower level of detection for most assays (76, 97, 161). Also, management of EBV infections is almost always based on increases in viral load over time rather than on a single result. It is reasonable to assume that when DNAemia increases, some active viral replication must be taking place.

TREATMENT

Symptomatic Management of Infectious Mononucleosis

Antipyretics. Most clinicians favor acetaminophen over aspirin to control fever because of concern that aspirin might increase the risk of hemorrhage into the spleen (10, 46, 127). Fever usually subsides within a week but has been reported to persist for up to 3 weeks (84).

Analgesics. Pain control is important during the early stages of infectious mononucleosis, especially for those patients whose throat is so sore that it keeps them up at night. Recommended pain management may include acetaminophen, nonsteroidal anti-inflammatory drugs, saltwater gargles, anesthetic throat lozenges, or viscous lidocaine hydrochloride (10, 46,

127). We prescribe codeine sulfate for subjects who do not respond to nonnarcotic pain control. Codeine can cause constipation, in which case stool softeners or laxatives are advisable (10).

Fluids and nutrition. Attention to fluid intake is important, especially for febrile patients. Maintaining adequate nutrition is also important but can be challenging because many patients are anorexic during the first week or two of illness and food is cloying to them.

Limitation of activities. Bed rest is unnecessary, but contact sports are contraindicated (10, 46, 127, 163). Patients generally adjust daily activities to the level of their exercise tolerance. When may athletes return to contact sports? There is no consensus. A recent evidence-based review suggested that they may return as early as 3 weeks after the onset of illness provided that they are afebrile, have no remaining clinical symptoms, and have normal energy (163).

Corticosteroids. A review of the medical records of Rochester Medical Center, Rochester, NY, found that nearly 45% of patients with infectious mononucleosis received systemic corticosteroids (200). Hence, the use of corticosteroids in infectious mononucleosis is a relatively common practice. However, an evidence-based literature review of 7 studies concluded that there is insufficient evidence to recommend steroids for control of the symptoms of infectious mononucleosis (33). Most authors—and this is our practice also—reserve corticosteroids for management of complications, such as impending airway obstruction, autoimmune anemia, and autoimmune thrombocytopenia (10, 46, 127, 163).

Antiviral Drugs

A number of antiviral drugs have *in vitro* activity against EBV (61, 123, 124, 131, 173, 222). Most of these drugs are nucleosides, but a few nonnucleosides are also in the pipeline. Unfortunately, there is no standard formula for equating *in vitro* susceptibility with clinical efficacy. The approach most often used is to strive for a maximum plasma concentration (C_{max}) of the antiviral drug that is at or above a certain multiple of the 50% inhibitory concentration (IC_{50}). However, the plasma C_{max} may not be the best drug exposure metric to use for this comparison. Other metrics, such as the area under the concentration-time curve (AUC) or the minimum postdose concentration (C_{min}), may be more clinically relevant.

For many nucleosides, including acyclovir and ganciclovir, plasma concentrations do not reflect the active antiviral drug moiety. Nucleoside analogues must first be taken up by virus-infected cells and phosphorylated to their active triphosphate derivatives, which then inhibit viral DNA synthesis (47). Although intracellular nucleoside triphosphate concentrations are difficult to measure analytically, the AUCs and half-lives of these active metabolites will most likely have the best correlation with *in vivo* antiviral efficacy.

Nucleosides are the only class of antiviral drugs that has been evaluated for treatment of EBV infections in controlled clinical trials. These nucleosides are DNA polymerase inhibitors. They are inactive as given, but when anabolized intracellularly to the active nucleoside triphosphate form, they act as faulty substrates for viral DNA polymerase, disrupting or terminating synthesis of the DNA chain (reviewed in reference 18). The monophosphorylation step is accomplished more efficiently by virus-encoded enzymes than by host cell nucleoside kinases, thus enhancing the activity of these compounds in herpesvirus-infected cells compared with uninfected cells (47). The enzyme responsible for monophosphorylation in EBV-infected cells appears to be a virus-encoded protein kinase rather than a thymidine kinase (71, 133).

Acyclovir. Intravenous as well as oral formulations of acyclovir have been studied for treatment of infectious mononucleosis (5, 6, 151, 207, 209). One trial evaluated oral acyclovir and prednisolone versus placebo (207). These trials have uniformly shown a reduction of EBV in the oral compartment, but clinical efficacy was not demonstrated.

Valacyclovir. Valacyclovir is a prodrug of acyclovir with an oral bioavailability of at least 50%, compared with 10 to 20% for the parent compound (214). Hence, it has the potential to exert more potent *in vivo* anti-EBV activity.

Simon et al. (185) performed a 3-arm, placebo-controlled, double-blind trial of valacyclovir, valacyclovir with prednisolone, and placebo in children aged 2 to 18 years old with "EBV illness." Fifteen subjects were enrolled in each arm. Clinical trial material was given for 14 days, and the groups were compared for improvement in signs and symptoms on the 20th day. Fatigue, feeling bad, and selected symptom scores statistically significantly favored the valacyclovir-corticosteroid group versus the placebo group. The authors concluded that "children with EBV illness treated with valacyclovir and prednisolone have a more rapid recovery and milder course." Criticisms of the study are that the diagnosis of EBV infection was not substantiated, the statistical methods were incompletely described, and a comparison of the valacyclovir arm versus the valacyclovir-plus-corticosteroid arm was not provided. The data did suggest, however, that the overall disease burden was lessened by valacyclovir with or without prednisolone compared with placebo.

We evaluated valacyclovir (3 g/day for 14 days) versus no antiviral drug in 20 University of Minnesota undergraduates with infectious mononucleosis due to laboratory-confirmed primary EBV infection (17). There was a significant reduction in viral load in the oral compartment but not in the blood. The number of reported symptoms and the severity of illness were reduced significantly among the 10 valacyclovir recipients compared with the 10 control subjects. Criticisms of our study are the small number of subjects enrolled and the lack of a placebo. However, the results of these two treatment trials encourage further investigation of valacyclovir because it is very safe (208) and relatively inexpensive now that generic formulations are available.

Hoshino et al. reported that a year of valacyclovir prescribed for suppression of recurrent genital herpes reduced the number of EBV-infected peripheral blood B lymphocytes (96). They speculated that EBV could be eliminated from the B-cell compartment by long-term administration of valacyclovir if the host is not reinfected by exogenous virus. The effect of valacyclovir or any other candidate anti-EBV drug on blood viral load is worthy of further investigation, because we have found that the whole-blood viral load correlates tightly with the overall severity of clinical illness (17). This has not been reported universally, perhaps due to diverse methods of clinical evaluation and different frequencies of whole-blood sampling. How-

ever, a reduction in EBV DNAemia could be the antiviral effect most closely associated with clinical improvement.

Ganciclovir and valganciclovir. Ganciclovir is active against EBV *in vitro* (20, 124, 173), and ganciclovir or its oral prodrug, valganciclovir, has been used to prevent posttransplant EBV disease, as discussed below. However, there are no controlled trials to support a clinical benefit of ganciclovir for treatment of EBV diseases.

Management of Serious or Life-Threatening EBV Disease

Potentially serious EBV disease in transplant patients is managed initially by a reduction of immunosuppression (155). If that is insufficient to bring the viral infection under control, humanized monoclonal anti-CD20 (rituximab) may be administered, sometimes in conjunction with chemotherapy (49). In refractory cases, adoptive immunotherapy with primed $CD8^+$ T cells (75, 109) has sometimes been successful.

PREVENTION

Minimizing Exposure to EBV

As discussed above, EBV infections can be serious and even life-threatening in transplant recipients. Primary EBV infection after transplantation could be prevented, at least in part, by finding EBV-naïve (seronegative) donors for EBV-naïve recipients. However, because >90% of adults worldwide are seropositive, identifying a suitably matched seronegative donor is impractical. Even if an EBV-naïve donor could be found, the virus still might be acquired by the natural route after transplantation. A more practical approach would be to immunize transplant candidates several months before transplantation, which is discussed below in "gp350 subunit vaccine."

Antiviral Prophylaxis

Antiviral drugs (acyclovir, valacyclovir, ganciclovir, and valganciclovir) are routinely given to patients for 3 to 6 months after transplantation to prevent or suppress herpesvirus infections, with the major focus being on prevention of CMV disease. Some transplant centers increase the antiviral drug to treatment dosage in asymptomatic patients whose CMV load is increasing. This is called preemptive therapy. Whereas antiherpesvirus drugs clearly reduce the incidence and severity of posttransplant CMV disease (89, 105), their role in the management of posttransplant EBV disease has not been established (98, 186).

Vaccines

Development of a prophylactic vaccine, in our opinion, is the most important future step toward controlling the consequences of primary EBV infection. A prophylactic EBV vaccine was proposed by Epstein and Achong in 1973 (50), but several problems—real or perceived—have hindered its progress (13). At long last, two very different EBV vaccines with adjuvants have been evaluated in placebo-controlled clinical trials (48, 187). One vaccine contains a gp350 subunit antigen, and the other contains a $CD8^+$ T-cell peptide epitope.

gp350 subunit vaccine. The EBV envelope glycoprotein, gp350 (formerly known as gp340 or EBV-induced cell membrane antigen), has been considered an attractive immunogen ever since it was shown to neutralize the virus (156). Despite a study showing that vaccine-induced anti-gp350 antibodies did not protect cottontop tamarins from developing tumors after a lymphomagenic challenge dose of EBV (52), work on a gp350 vaccine continued.

Gu et al. performed a phase I vaccine trial in Beijing, China, using vaccinia virus constructs expressing gp350 (68). After the vaccine was shown to be immunogenic in seropositive children, EBV-naïve children were studied. The subjects were 1 to 3 years of age. Nine received the vaccine, and 10 subjects served as controls. During 16 months of follow-up, 3 of 9 vaccinees were infected with EBV versus 10 of 10 individuals in the control group. The authors concluded that "it has been shown for the first time that protection against and/or delay of EBV infection by the natural route is possible."

Several years later, successful production of a recombinant gp350 construct in Chinese hamster ovary cells was reported (101). An EBV vaccine (with adjuvant) containing this antigen was subsequently employed in four clinical trials (138, 168, 187). The first two evaluated safety and immunogenicity only (138). The third was a placebo-controlled, double-blind study that also evaluated efficacy by following subjects for up to 19 months postimmunization for evidence of EBV infection (187). The vaccine did not prevent infection: 13 (14%) of 90 vaccine recipients became infected, versus 18 (20%) of 91 placebo subjects. However, it had a significant effect on clinical disease. Infectious mononucleosis developed in 2 (2%) of 90 vaccinees, compared with 9 (10%) of 91 placebo recipients. Failure to prevent EBV infection while preventing clinical illness might actually turn out to be an advantage for the vaccine, if the observation that latent infection by a murine counterpart of EBV (murine gammaherpesvirus 68) protected mice from subsequent bacterial infection applies to humans (21).

Finally, Rees et al. administered multiple doses of this vaccine to 16 pediatric renal transplant candidates (168). The vaccine was well tolerated, and all 13 evaluable subjects mounted an anti-gp350 antibody response. However, only four made a neutralizing antibody response. Because there was no control group, vaccine efficacy could not be assessed, but this small phase I trial did show that pretransplant immunization is feasible.

$CD8^+$ T-cell peptide epitope vaccine. Another strategy to control expansion of EBV-infected B cells and prevent infectious mononucleosis is to generate CD8 T-cell immunity to EBNAs (110). The potential role of these viral proteins in B-cell transformation precludes their use in whole-protein-based vaccines, and thus a peptide vaccine was generated and tested. This trial utilized an EBNA3A peptide epitope (FLR GRAYGL) restricted by HLA B8 (32), with tetanus toxoid (TT) formulated in a water-in-oil adjuvant as a source of T-cell help (48). EBV-seronegative individuals were immunized on a 2-month interval schedule. Of the 14 enrolled subjects, 4 received placebo, 2 were immunized with a 50-μg dose of peptide, and the remaining 8 individuals were immunized with a 5-μg dose. This strategy was effective at generating a peptide-specific CD8 response in most individuals, as measured by *ex*

vivo peptide-specific IFN-γ production. While this was a small study, no infectious mononucleosis occurred in 4 peptide-vaccinated subjects who subsequently acquired EBV infection, whereas it did occur in 1 of 2 subjects in the placebo group.

The general utility of epitope vaccines is limited by the fact that they target only specific HLA types. Nonetheless, epitope vaccines might be useful for PTLD patients, whose HLA type is typically known. This trial was also a "proof-of-principle" study which showed that EBV vaccines that generate CD8 immunity are safe and do not exacerbate EBV immune responses after primary infection.

SUMMARY OF PROGRESS AND PROBLEMS

In this review, we have discussed advances in the clinical, virologic, and immunologic features of primary EBV infection. These data paint a picture of lifelong viral infection that is continually and dynamically held in check by the adaptive immune system. Epidemiologic and biochemical studies have elucidated a fairly straightforward transmission and infection route, though questions remain as to whether or not the virus persists in epithelial cells. The interesting notion of a sequential passage of the virus back and forth between epithelial cells and B cells during latency deserves further study. The clinical consequences of primary infection after the onset of infectious mononucleosis have been well described for adults. However, little is known about the virologic and immunologic events that occur during the long incubation period prior to symptoms in adults or asymptomatic individuals, especially children. Further research is needed to determine means to predict who will be affected most severely and to develop therapeutic strategies to reduce symptoms.

The most severe consequences of EBV occur when the immune system fails to control infection and/or viral oncogenesis. The disease-causing role of EBV in XLP and PTLD patients is indisputable. Current knowledge of these diseases has clearly positioned the field to undertake steps toward treatment, but there are few candidate antiviral drugs, and much work remains to be done to make such therapies effective and practical. Increasing our understanding of how specific EBV gene products and expression programs contribute to pathogenesis holds promise for the development of more rational treatment strategies in the future.

Finally, an EBV vaccine could reduce the substantial disease burden due to primary EBV infection and possibly prevent or modify its chronic sequelae. However, development of an EBV vaccine has been agonizingly slow. More resources should be devoted to this endeavor, which has the potential to diminish the impact of a very common infectious disease and could even reduce the incidence of certain human malignancies, such as Hodgkin's disease, endemic Burkitt's lymphoma, and nasopharyngeal carcinoma.

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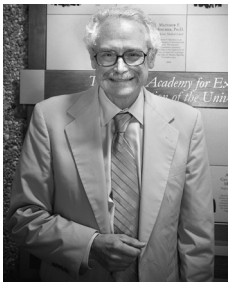

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