

Epstein–Barr Virus

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Author Disclosure
Dr. Junker did not disclose any financial relationships relevant to this article.

Objectives After completing this article, readers should be able to:

1. Outline the epidemiology of primary Epstein–Barr virus (EBV) infection.
2. Describe and explain the physiologic basis of the clinical features of infectious mononucleosis.
3. Describe and explain the physiologic basis of the common complications of infectious mononucleosis.
4. List the diagnostic tests that can help distinguish acute primary from distant past EBV infection.
5. Review the EBV-associated lymphoproliferative disorders and malignancies.

Introduction

EBV was identified initially in 1964 in tumor tissue from a patient who had African Burkitt lymphoma (BL), a rapidly growing, usually fatal malignancy of the B lymphocyte. When a laboratory technician working with this new virus became ill with mononucleosis and seroconverted to EBV, the link between EBV and mononucleosis became apparent. Soon thereafter, seroepidemiologic studies revealed that EBV infects virtually all of the world's population, usually as a subclinical or trivial illness in childhood. A decade later, reports of a familial X-linked disorder of fatal infectious mononucleosis highlighted the importance of immunogenetic factors in the management of EBV infection. Now, nearly 40 years after its discovery, EBV continues to captivate clinicians, virologists, oncologists, geneticists, and immunologists attempting to understand this highly successful human pathogen.

Epidemiology

EBV is a ubiquitous, worldwide pathogen that is harbored persistently by virtually all adults, regardless of geographic location. After primary infection, the virus latently infects circulating B cells and is shed silently into saliva and genital secretions. The colloquial term “kissing disease” acknowledges the oral route by which most people are infected. Spread is associated with close personal contact, so it is not surprising that the age at first infection varies according to living conditions. Infection occurs at an earlier age in the presence of poor hygiene and crowded living conditions; infection rates in young children are higher in developing countries and underprivileged societies. Depending on the population studied, the prevalence of EBV infection during childhood ranges from 20% to 80% by age 2 to 3 years. In industrialized countries that have high standards of living, it is more common for primary EBV infection to occur in adolescence. Infection at this time causes infectious mononucleosis (IM) in 30% to 50% of cases. IM does not show

Abbreviations

BL:	Burkitt lymphoma
BLPD:	B-cell lymphoproliferative disease
CAEBV:	chronic active EBV infection
CMV:	cytomegalovirus
EBNA:	EB nuclear antigen
EBV:	Epstein-Barr virus
EBV-HLH:	EBV-associated hemophagocytic lymphohistiocytosis
HD:	Hodgkin disease
HHV:	human herpesvirus
HSV:	herpes simplex virus
Ig:	immunoglobulin
IL:	interleukin
IM:	infectious mononucleosis
NK:	natural killer
NPC:	nasopharyngeal carcinoma
PCR:	polymerase chain reaction
VCA:	virus capsid antigen
VZV:	varicella-zoster virus
XLP:	X-linked lymphoproliferative disease

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consistent seasonal or yearly variation in occurrence and rarely is associated with temporal clustering of cases. EBV infection of siblings in families can occur, and adult household members can show serologic evidence of recent (re)exposure. EBV also can be transmitted by blood product transfusion and with bone marrow and solid organ transplantation. Sexual transmission is suggested by the demonstration of virus in cervical and male genital secretions and in the significantly greater EBV seroprevalence among sexually active students. Congenital infection after primary disease of mothers during pregnancy is rare, probably because of the very low proportion of EBV-naïve women.

The two major strains of EBV (EBV type-1 and -2) differ biologically and in their geographic and ethnic prevalences, although they show no clear differences in associated clinical diseases. Minor genetic variations give rise to distinct EBV isolates or substrains. Healthy individuals can be coinfecting with more than one distinct EBV isolate. Infection with a new strain may provide the basis for some cases of recurrent IM because initial infection with one EBV strain does not preclude subsequent superinfection.

The Biology of EBV Infection

EBV is a gamma-type herpesvirus and shares the special characteristics of lymphotropism and neoplastic association with other members in this group, which includes the most recently identified Kaposi sarcoma-associated virus, human herpesvirus 8 (HHV8). The herpesviruses are large, complex DNA viruses that code approximately 100 proteins. These pathogens have coevolved with their hosts over millions of years, and both parties have developed highly sophisticated strategies for survival.

Probably no other virus group has such an impact on the pediatric population. Members include herpes simplex (HSV) 1 and 2; varicella-zoster virus (VZV), the cause of chickenpox and “shingles”; cytomegalovirus (CMV), currently the most common cause of congenital infection; and herpesviruses 6 and 7, causes of exanthema subitum (roseola infantum). The infectiousness of EBV and its propensity to infect children at a young age with mild disease are features in common with these other pathogens. CMV can cause a mononucleosis syndrome, but most children suffer only trivial respiratory infections. Primary HSV infection is common in childhood and subclinical in 85% of cases. HHV6 is a well-recognized cause of nonspecific febrile illness in toddlers.

After primary infection, it is usual for all of these viruses to establish a permanent, lifelong infection of their human host. Each virus has a preferred cell type and

site of persistence and can reactivate, intermittently, with or in the absence of clinical disease. HSV infects the motor root ganglia, and reactivation can be silent or result in “cold sores.” VZV infects dorsal root ganglia, and reactivation presents as “shingles.” EBV and CMV regularly reactivate, but without signs or symptoms of clinical disease.

Unlike the other herpesviruses, it has not been possible to identify EBV in clinical specimens by using traditional virus diagnostic laboratory techniques. EBV was identified initially by electron microscopy in BL cells, but only after these tumor cells had been “shocked” by culture as a cell suspension for some time. EBV does not replicate well in tissue culture systems, does not tend to carry out a full lytic cycle, and, thus, is not cytopathic. This feature means that clinical specimens from patients, even those heavily infected with EBV, are “culture-negative.” EBV has the ability to infect B lymphocytes. Rather than damaging the cells, EBV activates B cells and induces rapid expansion of the infected cell population by increasing cell numbers and extending cell survival. In years past, before the advent of molecular diagnostic tools, the presence of EBV was implied when filtered clinical specimens, applied to cultures of (EBV-naïve) cord blood lymphocytes, led to transformation of these cells into permanently growing, “immortalized” cell lines. EBV has been a valuable tool for immunology studies of B lymphocyte function in vitro because the virus is a potent stimulator of antibody production. Permanently growing B-lymphocyte cell lines, developed by in vitro EBV infection of patient B cells, have provided a sustainable source of material for metabolic and genetic studies.

The Virus Life Cycle

The current belief is that the B lymphocyte provides all the conditions necessary for EBV to maintain its life cycle. A balanced, generally benign, virus-host relationship has evolved, making it beneficial for both virus and host for EBV to adopt a strictly latent state in blood. If active and replicating, EBV can spread and infect tissues, but it also is vulnerable to rapid detection and elimination by cytotoxic T cells and natural killer (NK) cells. Residing in resting peripheral blood B cells, EBV remains virtually invisible to the immune system by expressing very few virus proteins. For years after acute EBV infection, latently infected B cells in peripheral blood are present at a frequency that ranges from 1 to 60/10⁶ B cells (~10 mL of blood). Each cell carries approximately two to five copies of an intact circular (latent, episomal) viral genome. Healthy individuals infected with EBV

shed infectious EBV silently, sometimes continuously, into saliva and genital secretions. The source of this virus is believed to be B cells that have become activated and circulated to the mucosal epithelium. Mucosal shedding facilitates viral spread to susceptible individuals and possibly plays a role in bolstering local defenses against infection with new EBV strains.

The Host Immune Response

The patterns of antibody responses to EBV antigens were delineated soon after their discovery. Antibodies develop during acute infection to lytic cycle proteins, including membrane antigen, virus capsid antigen (VCA), and early antigens, and appear during convalescence to the latency-associated virus nuclear antigens (EBNA). Antibodies have little role in control of established EBV infection. Much work has been done to define the specific features of immunity that protect against infection, which is important for vaccine development. The lack of a permissive virus culture system and difficulty in handling T lymphocytes in vitro have delayed analysis of EBV-directed cellular immune responses, despite their obvious importance in managing EBV infection. Individuals who have congenital or acquired cellular immunodeficiency disorders suffer more complications, even death, with primary EBV infection and are less able to maintain the virus in a latent state. In the absence of effective cellular immunity, EBV promotes activation and proliferation of its host B cell, leading to B-lymphoproliferative disorders and risk for malignant transformation.

Pathogenesis and Pathophysiology

Based on epidemiologic studies and because it is usual for patients who develop IM to have peak VCA-immunoglobulin (Ig)G levels at presentation, it is believed that EBV infection occurs at least 30 days prior to the appearance of IM signs and symptoms. There is a paucity of information about the events of early infection and an increasing suspicion that IM represents an eventually contained, but not normal, lymphoproliferative disorder. Host reactions account for the clinical manifestations of IM. The course of IM closely parallels the lymphoproliferative phase, in which there is a massive increase in the number of activated cytotoxic CD8⁺ T cells directed primarily against EBV lytic cycle proteins. This T-cellular response accounts for the traditional hematologic criteria of IM (absolute and atypical lymphocytosis) and the characteristic signs of lymphadenopathy, hepatomegaly, and splenomegaly. Fever and fatigue result from a massive T cell-induced production of cyto-

kines, which include lymphotoxin, tumor necrosis factor-alpha, interleukin (IL)-1beta, and IL-6. Disease features resolve as EBV DNA levels in serum fall and T-cell numbers normalize. The reason for this exaggerated T-cell response that focuses on a few immunodominant epitopes of the virus lytic cycle is not clear, but this response distinguishes IM from asymptomatic primary EBV infection in which homeostatic T-cell numbers and diversity are maintained. In both circumstances, systemic EBV DNA levels are high, but there is an intriguing speculation that EBV establishes a different form of infection and pattern of gene expression in these two conditions. EBV DNA detected in IM could derive from virus replication and cell lysis; the DNA in asymptomatic primary infection could derive from B cells driven to proliferate by the virus.

Complications of EBV Infection

Complications occur in about 20% of patients recently infected with EBV; essentially no body system is free from potential harm. The respiratory, neurologic, and hematologic systems are involved most frequently. Airway obstruction resulting from the virus-induced B-cell proliferation and reactive T-cell expansion can be life-threatening and is the most common reason for hospital admission.

Neurologic complications occur in about 5% of patients. EBV has been implicated in virtually every form of infection-related neurologic disorder. Quantitative polymerase chain reaction (PCR) and detection of EBV lytic cycle messenger RNA (mRNA) in cerebrospinal fluid indicates that direct EBV infection of the central nervous system is one mechanism of neuropathology.

EBV is renowned for its intriguing ability to activate B lymphocytes to produce antibodies. During acute IM, about 1/5,000 (0.02%) peripheral blood B cells are infected with EBV, which is associated with the transient appearance of unusual antibodies, including heterophile (which forms the basis of the rapid “mono” tests), Wasserman, rheumatoid factor-like, cold agglutinins of anti-i specificity, and antinuclear antibodies. Autoreactive antibodies cause some of the more frequent hematologic complications, including immune hemolytic anemia or immune thrombocytopenia. About 10% of preschool-age children develop neutropenia that is characterized by fewer than 0.5×10^9 cells. It has been proposed that the heterophile antibody, which nonspecifically agglutinates red blood cells of other species, is an autoantibody that recognizes an antigen expressed on the human fetal erythrocyte. This abnormal antibody, a sign of B-cell activation, can be present up to 1 year after acute infec-

tion; a longer duration of detectable antibody correlates with the greater geometric mean titer found in acute sera. Generally, increased levels of Ig increase the propensity for immune complexes to be formed, which may account for other disease-associated features such as rash, myalgia, and arthralgia.

Clinical Aspects

Primary Infection

Symptomatic acute EBV infection is recognized clinically as IM, characterized by fever, tonsillopharyngitis, hepatosplenomegaly, lymphadenopathy, and increased numbers of activated CD8+ T cells. The fever, present for 1 to 3 weeks, can range from a temperature of 99.5° to 104.9° F (37.5° to 40.5°C) (rectally). Lymph nodes are moderately tender and firm, but discrete. Cervical nodes, particularly those in the posterior chain, are involved principally, although generalized adenopathy can occur. The pharyngitis can vary from a mild erythema to a very painful throat that has a thick gray-white exudate. In young children, EBV seroconversion can be associated with nonbacterial tonsillitis or other poorly differentiated respiratory tract illness and enteric syndromes. Very young children may be more susceptible to failure to thrive (preceding manifestations of IM), pneumonia, and otitis media. However, classic EBV-IM is common in children and can occur in infants. The clinical picture and course of disease in young children is similar to that of adults.

Fatigue

A distinct fatigue syndrome is associated with IM, lasting about 8 weeks; resolution generally coincides with normalization of T-cell responses. No objective measures characterize self-reported continuance of fatigue after IM.

Ampicillin Rash

Antibiotic administration, particularly of ampicillin, has been linked to the subsequent development of rash in older patients who have IM. Rash occurs with IM in about 4% of older patients *not* receiving antibiotics; involves the trunk and face; and can be maculopapular, scarlatiniform, papulovesicular, urticarial, or hemorrhagic. Rash occurs with ampicillin treatment in about 10% of patients who do not have EBV infection. However, rash can develop in up to 100% of older IM patients receiving ampicillin. In young children, there may be no relationship between antibiotic administration and the occurrence of rashes. Patients who develop rashes while receiving antibiotics should not be deemed permanently

hypersensitive to the drug without subsequent documentation by appropriate skin test and challenge studies. Consistent with EBV-induced B-cell activation, patients who have IM can show a transient rise in benzylpenicilloyl-specific IgM antibodies that purportedly cause the reaction.

X-linked Lymphoproliferative Disease (XLP) – SH2D1A Deficiency

Patients who have XLP can suffer extreme complications of EBV infection. More than 50% of boys who have XLP present with overwhelming IM at approximately age 5 years, and only 4% survive. Considerable, and as yet unexplained, variation occurs in the phenotypic expression of XLP among individuals within families and between pedigrees who have the same mutation and molecular effect. Other XLP-associated conditions include: 1) no disease, even with documented EBV infection, 2) dysgammaglobulinemia or common variable immunodeficiency, 3) aplastic anemia, 4) lymphoma, and 5) lymphoid granulomatosis with vasculitis. An XLP gene defect should be considered, even in the absence of a family history, in boys who present with variable immunodeficiency or an unusual lymphoproliferative disorder with or without evidence of EBV infection.

EBV-associated Hemophagocytic Lymphohistiocytosis (EBV-HLH) and Chronic Active EBV Infection (CAEBV)

Unusual EBV infiltration into immune cells other than B cells characterizes these often fatal disorders, which present with intense, prolonged (>6 mo) signs of EBV-related illness, including fever, marked lymphadenopathy, severe hepatosplenomegaly, hepatitis, interstitial pneumonitis, and pancytopenia. Histiocytic erythrophagocytosis in bone marrow and secondary lymphoid organs distinguish EBV-HLH. In the acute phase of EBV-HLH, activated CD8+ T cells are the principal targets of EBV infection. Hypersensitivity to mosquito bites, characterized by a bulla developing into necrosis, is an unusual associated feature of CAEBV. CD4+, CD8+, and NK cells are infected in CAEBV. It is not known if EBV infection of cells other than B cells causes these disorders or if the patients have a yet undefined primary susceptibility that makes them more inclined to this pattern of infection.

B-cell Lymphoproliferative Disease (BLPD)

EBV-associated BLPD occurs in about 10% of transplant recipients, and the risk is highest for those who experience primary EBV infection while receiving high doses of

immunosuppressive agents. Thus, the incidence is increased in children. The donor organ is a common vehicle of EBV infection, and because immunosuppression is highest in the immediate posttransplant period, BLPD tends to occur early after transplant.

Burkitt Lymphoma (BL)

EBV is present in about 96% of African BL, the most frequent tumor in children ages 5 to 9 years in equatorial Africa. Severe EBV infection during the first months after birth increases the risk of developing BL. Malaria is a critically important cofactor. The incidence of BL has decreased in areas where the incidence of malaria has been reduced by mosquito eradication programs. Protection from BL is conferred by the sickle cell trait, which impairs malaria infection. All BL tumors, EBV-positive or not, have a chromosome translocation (8:14, 8:22, or 8:2) that brings the *c-myc* oncogene on chromosome 8 into one of the three immunoglobulin gene regions, resulting in its constitutive expression, which drives continuous cell proliferation and inhibits differentiation. Episodes of malaria, associated with T-cell suppression and intense antigenic stimulation, lead to increased activation of EBV-infected B cells and the potential for malignant transformation.

Nasopharyngeal Carcinoma (NPC)

All undifferentiated NPC is EBV-associated, regardless of geographic location. In southern China, NPC is the most common malignancy in men and second most common in women. NPC cells express latent EBV genes, but the events leading to tumor outgrowth have not been defined. Given that virtually everyone worldwide eventually is infected with this virus, the relative incidence of EBV-related malignancy is low. Epidemiologic studies of NPC and other EBV-associated cancers indicate that many factors are involved in the development of EBV-related malignancy in otherwise healthy individuals. These factors include particular exposure opportunities such as cultural practices, environmental pathogens, and genetic susceptibility, and temporal events such as age at first infection and exposure to exogenous factors such as carcinogens and virus-activating agents.

Hodgkin Disease (HD)

EBV is associated with about 50% of HD in Western societies, a higher incidence in developing countries, and virtually 100% of AIDS-associated HD. EBV genomes have been localized to Reed-Sternberg cells that now are recognized as B cells.

T-cell Lymphoma

EBV DNA has been identified in an increasing number of T-cell lymphomas, including nasal and extranasal angiocentric T-NK lymphoma, angioimmunoblastic lymphadenopathy, and peripheral T-cell lymphoma. It still is unclear how malignancy develops in these cells.

Gastric Carcinoma

The association of EBV with gastric carcinoma varies, depending on geographic location and histologic type. More than 80% of the rarer lymphoepithelioma type of lesions are EBV-positive. Of note, coinfection with *Helicobacter pylori* is associated with a reduced risk for gastric carcinoma.

Differential Diagnosis and Diagnostic Tests

Acute primary EBV infection can be implicated with the appearance of characteristic clinical signs and symptoms of IM and the hematologic criteria of absolute (>50%) and atypical (>10%) lymphocytosis. Documenting the presence of heterophile antibody virtually confirms acute EBV infection, and there usually is no reason for further diagnostic tests. Although very young children do not produce heterophile antibodies, there is a progressive increase with age in children who have IM syndrome and produce detectable levels. With sensitive assays, heterophile antibody is detected in more than 80% of children who have IM by age 4 years. Heterophile-negative IM patients and those who have no classic IM features in whom EBV infection is considered warrant further serologic tests. IgM-VCA is present in more than 80% of acute sera and is the most valuable serologic test. Detection of IgM-VCA depends on the serologic assay used; enzyme linked immunosorbent assays generally are more sensitive than are traditional immunofluorescence assays. Shorter persistence of IgM-VCA in the very young child relates to lower titers achieved, and it is possible to miss detection. One limitation is the potential for crossreactivity, such that EBV-directed IgM antibodies react with antigens from other herpesviruses such as CMV. IgG-VCA can be detected in virtually 100% of acute IM sera and is of no diagnostic value in IM. However, VCA and EBNA seroconversion can implicate EBV infection.

Detection of EBV DNA by PCR is becoming a useful diagnostic adjunct. Detection of cell-free virus in serum or plasma from nonimmunosuppressed patients is sensitive and specific for primary infection. Measurement of EB viral load by quantitative, competitive, or real-time PCR is useful in detecting and monitoring EBV-associated lymphoproliferative disorders and malignancies.

As with many circumstances in medicine, it is not always possible to be assured completely that a particular patient's problems are due to acute primary EBV infection. Other pathogens are capable of inducing an intense lymphoproliferative response and, in doing so, cause a mononucleosis syndrome. Other infections and inflammatory conditions can be associated with B-lymphocyte activation, resulting in heterophile antibody and other abnormal antibody production. The detection of EBV in secretions or tissues through sensitive molecular techniques is not restricted to acute infection.

Treatment

Resources to manage acute EBV infection and its complications remain limited. "Take it easy" is a usual recommendation, but little evidence supports the concept that such action shortens the disease course or prevents complications. Contact sports and activities (eg, bike riding) should be avoided until the spleen no longer is palpably enlarged. Although steroids often are recommended for management of impending airway obstruction or severe thrombocytopenia, only anecdotal evidence supports their benefit. Little appears to be gained by treating moderately ill patients with steroids. Steroid-treated ambulatory IM patients show no differences compared with controls in rapidity of resolution or im-

provement of symptoms, physical signs, or scores on the Beck Depression Inventory. The antiviral agent acyclovir [9-(2-hydroxyethoxymethyl) guanine] inhibits the replication of linear EBV DNA through inhibition of the virus DNA polymerase. Acyclovir treatment abrogates oral EBV shedding, but it does not affect levels of (circular, latent) EBV-infected B cells. Because the symptoms and signs of IM relate to the intense T-cell response, it is not surprising that there is no evidence for orally administered acyclovir affecting the course of IM.

Suggested Reading

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PIR Quiz

Quiz also available online at www.pedsinreview.org.

1. A 4-year-old boy is brought to your office because of fever, runny nose, and irritability for the last week. Physical examination reveals an axillary temperature of 101.3°F (38.5°C), respiratory rate of 28 breaths/min, and heart rate of 110 beats/min. The child is alert and has no neck stiffness. There is pharyngeal erythema and enlarged posterior cervical lymph nodes that are moderately tender. The spleen is palpable 2 cm below the costal margin. A complete blood count shows a hemoglobin of 11 g/dL (110 g/L) and a white blood cell count of $14 \times 10^3/\text{mCL}$ ($14 \times 10^9/\text{L}$), with 38% polymorphonuclear leukocytes, 60% lymphocytes, and 2% eosinophils. A peripheral blood smear shows several atypical lymphocytes. Of the following, the *most* accurate statement regarding this patient's heterophile antibody response is that:
 - A. A positive heterophile antibody test obviates the need for further testing for Epstein-Barr virus (EBV) infection.
 - B. Children younger than 4 years of age are more likely to produce heterophile antibodies than are those who are older.
 - C. Heterophile antibody is species-specific.
 - D. Heterophile antibody offers protection against reinfection with EBV.
 - E. Heterophile antibody response is more likely to occur in patients who have EBV-associated hemophagocytic lymphohistiocytosis than an infectious mononucleosis presentation.

(continued)

2. During a routine health supervision visit, the mother of a 14-year-old girl tells you that one of her daughter's soccer teammates has been diagnosed as having infectious mononucleosis. She is concerned about her daughter catching the illness. Of the following, the *most* appropriate advice is that:
 - A. Her daughter should avoid playing soccer for 2 weeks to be sure no one else on the team develops manifestations of infectious mononucleosis.
 - B. Her daughter should receive an intramuscular gamma globulin injection.
 - C. Infectious mononucleosis, also called a "kissing disease," is transmitted most often between individuals of opposite sexes.
 - D. She should not worry about her daughter catching the illness.
 - E. The teammate who has infectious mononucleosis should be isolated for 2 weeks.
3. A 6-year-old girl presents with fever, sore throat, and malaise of 1 week's duration. Physical examination reveals bilateral posterior cervical lymph nodes that are firm, discrete, and tender. The pharynx is erythematous, with thick gray-white exudates. The complete blood count shows a hemoglobin of 12 g/dL (120 g/L) and white blood cell count of $12.4 \times 10^3/\text{mcL}$ ($12.4 \times 10^9/\text{L}$), with 28% polymorphonuclear lymphocytes, 64% lymphocytes, and 8% monocytes. Multiple atypical lymphocytes are seen on the peripheral smear. The heterophile antibody test is positive. Of the following, a *true* statement regarding EBV infection is that:
 - A. A positive throat culture for EBV is highly sensitive for establishing the diagnosis.
 - B. Ampicillin rash in a child who has infectious mononucleosis is a reflection of lifelong hypersensitivity and contraindicates the use of ampicillin in future.
 - C. Atypical lymphocytosis represents activation of B cells responsible for antibody response.
 - D. The EB nuclear antigen antibody titer peaks at presentation of infectious mononucleosis.
 - E. The primary host defense mechanism is through cytotoxic T cells and natural killer cells.
4. An 8-year-old boy presents with fever and sore throat, weakness, and loss of appetite for 6 days. Physical examination reveals four to six cervical lymph nodes bilaterally that are approximately 2 to 3 cm in diameter, firm, and somewhat tender. The pharynx is erythematous and has grayish exudates. A grade 2/6 systolic murmur is audible over the left sternal border. The spleen is palpable 4 cm below the left costal margin. A complete blood count shows a hemoglobin of 10 g/dL (100 g/L) and white blood cell count of $14 \times 10^3/\text{mcL}$ ($14 \times 10^9/\text{L}$), with 24% polymorphonuclear lymphocytes, 66% lymphocytes, and 10% monocytes. The platelet count is $110 \times 10^3/\text{mcL}$ ($110 \times 10^9/\text{L}$). Multiple atypical lymphocytes are seen on peripheral smear. A rapid heterophile antibody test is positive. Over the next week, the boy improves and feels much better. His mother wants to know when he can participate in soccer. Of the following, your *best* answer is that he can start participating when:
 - A. He feels well.
 - B. His hemoglobin rises above 13 g/dL (130 g/L).
 - C. His murmur no longer is audible.
 - D. His platelet count increases above $150 \times 10^3/\text{mcL}$ ($150 \times 10^9/\text{L}$).
 - E. His spleen no longer is palpable.