clinical expression of HCMV mononucleosis has been reported to include fever, malaise, and myalgias that can be persist for weeks. Pharyngitis appears to be less frequent and less severe than that seen in infectious mononucleosis caused by Epstein-Barr virus. Laboratory abnormalities can include hemolytic anemia, thrombocytopenia, reactive lymphocytosis, and elevated aminotransferases. Similar findings have been reported in children acutely infected with HCMV, although the incidence of symptomatic HCMV in normal children is unknown but likely to be exceedingly low.[236] Even in individuals with asymptomatic infections, virus can be shed from saliva, urine, and vaginal secretions for weeks to months.[237] Treatment is symptomatic, and clinical and laboratory abnormalities are self-limited.

Transfusion-Acquired Infection

In addition to mononucleosis syndromes, HCMV has been demonstrated to be a cause of clinical disease in transfusion-acquired infections in normal hosts. Transfusion-associated HCMV infections in surgical patients were well described in older literature, but have been dramatically reduced by the use of leukocyte-reduced blood products (see "Routes of Transmission and Sources of HCMV Infection"). There has been more recent interest in the contribution of HCMV infection to the morbidity and possibly mortality in severely ill patients with septic shock and patients with thermal burns.[238–246] In this patient population, HCMV reactivates from an existing latent infection, and replicating virus can be detected over a broad time interval in these patients, ranging from within the first week of hospitalization to several weeks.[238,239,242] Although most case series do not describe increased mortality secondary to replicating HCMV in patients with severe sepsis or burns, it appears that HCMV replication in these patients is associated with an increased length of stay in an intensive care unit.[238–240,242] A randomized trial using ganciclovir treatment to control viremia and virus shedding in patients with severe sepsis failed to demonstrate that treatment was associated with altered levels of the proinflammatory cytokine interleukin-6 in the sera from these patients or length of intensive care unit stay when compared to control patients.[247] Thus HCMV replication in these patients may be only an indirect marker of systemic inflammation and does not directly impact the clinical course of these patients. Alternatively, this initial trial could have selected noninformative end points for analysis, and additional trials will be required to definitively address the role of HCMV in the morbidity of patients with severe sepsis or burns.

Association With Inflammatory Bowel Disease

A consistent association between HCMV and exacerbation of symptoms of inflammatory bowel disease (IBD) has been described in numerous case reports and in several larger patient series, particularly in patients with steroid-refractory ulcerative colitis.[248–255] It is estimated that about 25% of patients with IBD who present with severe colitis will have active HCMV infections, a rate that increases to over 30% in patients with symptoms that failed to respond to steroid treatment.[29,248,256,257] Although definitive evidence that HCMV infection can lead to the development of IBD has not been reported, clinical observations have strongly argued that the presence of HCMV in the intestinal mucosa contributes to the severity of flares of disease in these patients. The diagnostic criteria for HCMV in patients with IBD and severe colitis have included the detection of HCMV DNA in blood by PCR and, more definitively, the detection of HCMV in intestinal biopsy specimens by immunohistochemistry, PCR, or both.[28,258] The severity of colitis in these patients, including intestinal inflammation, has been shown to be related to the number of HCMV inclusion-bearing cells in biopsy specimens and the quantity of HCMV DNA in biopsy specimens.[259,260] Consistent with the observed relationship between virus replication and disease severity, treatment with antiviral agents has been shown to hasten resolution of clinical symptoms and to reduce the need for surgical interventions in IBD patients with flares of colitis.[248,251,259,261–263] Finally, well-defined mechanisms that account for the role of HCMV infection in the development of severe colitis in patients with IBD remain to be defined; however, recent studies have suggested that HCMV infection may alter the phenotype of intestinal macrophages, resulting in the release of proinflammatory mediators and increased intestinal inflammation.[264,265]

HCMV Infections in the Immunocompromised Host

HCMV Infections in Solid-Organ Transplant Recipients (See Also Chapter 308)

In the mid-1960s, Rifkind and colleagues described histopathologic findings consistent with HCMV infection in tissue specimens from patients undergoing renal transplantation and isolated infectious HCMV from these patients.[10,11,266,267] These investigators also linked immunosuppression with clinical disease associated with this recently described virus. Shortly thereafter, other investigators confirmed these reports.[268] HCMV has subsequently become one of the most recognized causes of disease in allograft recipients. An illustration of the importance of HCMV infection in the posttransplantation period is illustrated by an early report of an *epidemic* of HCMV in a transplant recipient population that was thought to have originated in a hemodialysis unit utilized by allograft recipients prior to transplantation.[269] HCMV infections continue to represented one the most frequent posttransplantation infection in both SOT and HSCT recipients, occurring in approximately 50% of allograft recipients. Prior to the widespread use of antiviral prophylaxis, HCMV infection and disease ranged from about 50% in renal, liver, and heart transplant recipients to over 70% in lung allograft recipients.[270–272] Although substantial progress in treatment with antiviral drugs coupled with sensitive and rapid diagnostics have resulted in decreased in morbidity and mortality associated with HCMV infections in allograft recipients, HCMV remains a common infection in the posttransplantation period, and clinically apparent infections can develop in as many as 20% of high-risk recipients.[273–278]

With the dramatic reduction in blood product transfusion transmission of HCMV, the major sources of HCMV infection in SOT recipients are virus present in the transplanted organ (donor CMV-positive [D+]) or reactivation of persistent infection (recipient CMV-positive [R+]) following immunosuppression of allograft recipients.[279,280] Transplantation of an organ from an HCMV-seropositive donor into an HCMV-seronegative recipient (D+/R–) represents a high risk for both virus transmission and the severity of the ensuing infection, whereas transplantation of an organ from a seropositive donor into a seropositive recipient (D+/R+) or an organ from a seronegative donor into a seropositive recipient (D–/R+) represent an intermediate risk (Table 137.2). Transplantation of an organ from a seronegative donor into a seronegative recipient (D–/R–) is classified as low risk. These risk stratifications were derived from observations of patients infected during the first 6 months

TABLE 137.2 Identified Clinical Risks for Significant HCMV Infections in Allograft Recipients

ALLOGRAFT RECIPIENT	RISK CATEGORY	REFERENCES
Solid-Organ Transplant		
Donor+/Recipient–	High	279, 280
Donor+/Recipient+ or Donor–/Recipient+	Intermediate	273, 280
Donor–/Recipient–	Low	
T-cell depletion (antithymocyte globulin; anti-CD52; anti-CD25)	Can modify risk stratification	281, 286, 289, 291
Hematopoietic Stem Cell Transplant		
Donor+/Recipient+ or Donor–/Recipient+	High	387–389
Donor+/Recipient–	Intermediate	387, 389
Donor–/Recipient–	Low	
Degree of HLA mismatches between donor and recipient; umbilical cord stem cell transplant into + recipient; T-cell depleting antibodies; graft-versus-host disease requiring steroid therapy	Can modify risk stratification	390, 391, 393

HLA, Human leukocyte antigen.

following transplantation, when immunosuppression was most intense, and during a period of time when antiviral therapies were not available. It was argued that HCMV infection following D+/R− transplantation was equivalent to primary infection in a recipient with a compromised immune system and that clinical symptoms developed more frequently in seronegative recipients than seropositive recipients, secondary to residual adaptive immunity to HCMV in seropositive recipients. While this remains a paradigm in SOT recipients, an analysis of a large series of SOT recipients demonstrated that while about 20% of D+/R− transplant recipients developed symptomatic HCMV infections, 8.1% of D+/R+ and 9% of D−/R+ recipients also developed symptomatic HCMV infections.[273] Thus when the total number of patients in all risk categories were considered, the number of CMV-positive recipients with symptomatic infection was equivalent to the number of patients in the high risk D+/R− group with symptomatic infection.[273] Lastly, different T-lymphocyte–depleting and nondepleting antibodies that are commonly used during induction of immunosuppression and for treatment of allograft rejection have been reported to differentially increase the risk for HCMV infection in the posttransplantation period (see Table 137.2). Three antibody preparations, a polyclonal antithymocyte globulin and two monoclonal antibodies, anti-CD25 (basiliximab) and anti-CD52 (alemtuzumab), have been reported to confer different risks for HCMV infection and disease in the posttransplantation period.[281–290] A consensus on the relative risk for increased HCMV infections and disease conferred by each antibody is difficult to define from available literature, although a recent review has suggested that use of rabbit anti-thymocyte globulin is associated with a higher rate of HCMV infection in recipients as compared to the monoclonal anti-CD52 or anti-CD25 antibodies.[291]

The importance of the level of suppression of T-lymphocyte responses as a risk for HCMV infection and disease in SOT recipients in the posttransplantation period in contemporary studies have confirmed observations made more than 3 decades ago that demonstrated the relationship between HCMV T-lymphocyte responses and disease in the posttransplantation period.[292] Furthermore, the finding that both seronegative and seropositive SOT recipients could develop invasive HCMV infections, when viewed together with more contemporary data, has led to the paradigm that in an individual allograft recipient, the level of HCMV-specific T-cell immunity in the posttransplantation period is the major determinant in the control of HCMV infection and disease. More recently, standardized assays that can accurately quantify HCMV-specific T-lymphocyte responses have convincingly shown that the level of T-lymphocyte responses in the posttransplantation period can define the risk of HCMV infection in allograft recipients.[129,130,293–301] Thus exposure to HCMV in the posttransplantation period, whether from a persistent infection or from a transplanted organ, presents a risk for infection and disease that can be modified by the level of HCMV-specific T-lymphocyte responsiveness.

The widespread use of antiviral prophylaxis and, in some centers, rigorous monitoring for HCMV replication followed by aggressive preemptive therapy has resulted in HCMV infections that become apparent late (defined as >120 days, or after discontinuing antiviral prophylaxis) following transplantation. Although combined into a single category as late infections, biologically these infections are significantly different in terms of their incidence, risk factors, and clinical expression. It has been suggested that late posttransplantation infections be further subdivided into postprophylaxis HCMV infection and disease that occurs within the first 6 months following discontinuation of antiviral prophylaxis, and truly late-onset infection and disease that occurs over 6 months after prophylaxis is discontinued.[277] Regardless of the terminology employed to describe different populations of SOT recipients with HCMV infections, it appears that HCMV infection in these two groups differs fundamentally in its mechanisms of disease, and therefore in risk factors for infection and clinical expression of HCMV infection in each group. As an example, the conventional paradigm suggesting increased clinical risk for HCMV infection in D+/R− SOT recipients has been reported as a risk factor for infection and disease shortly following discontinuation of prophylaxis, whereas this category of SOT recipients was not suggested to be at higher risk for late-onset disease.[274,277,302–307] Importantly, both categories of late infections have been attributed to the effective suppression of HCMV replication by antiviral therapy, which in turn is thought to result in delayed development of adaptive immunity, specifically T-lymphocyte responses to HCMV, whereas some investigators have suggested that ganciclovir could inhibit T-lymphocyte proliferation directly and result in a more global deficit in T-lymphocyte responses.[127,308–311] It is of interest to note that the role of antiviral prophylaxis in the development of late-onset disease is unclear.[307,312] Lastly, the role of T-lymphocyte–depleting antibodies in the induction of immunosuppression is also thought to be a clinically significant risk for the development of late HCMV infections and disease.

The clinical manifestations of HCMV infection in the posttransplantation period can be protean, and symptomatic HCMV infections are not infrequent, occurring in perhaps as high as 20% of high-risk allograft recipients even with antiviral prophylaxis. Together with the presence of comorbid conditions and other infections in these patients, the diagnosis of infection based solely on clinical presentation is unreliable, with the possible exception of HCMV retinitis, which can present with characteristic ophthalmologic findings. The most commonly described clinical manifestation of HCMV in the SOT recipient has been referred to as the "CMV syndrome."[313] The clinical criteria for the diagnosis of CMV syndrome have varied among transplantation centers, which has made the comparison of results from clinical trials in different centers difficult to interpret. Recently in an attempt to establish common criteria, Ljungman and colleagues suggested criteria for CMV syndrome that include detection of HCMV in the blood together with at least two of the following criteria: (1) fever >38°C for >2 days, (2) new or increased fatigue/malaise, (3) leukopenia or neutropenia, (4) >5% reactive lymphocytes, (5) platelet counts <100,000 cells/μL or <20% of platelet counts if starting platelet count is <115,000 cells/μL, and (6) elevation of hepatic aminotransferases (Table 137.3).[313]

In contrast to the CMV syndrome in SOT recipients, end-organ disease has been well described in both SOT and HSCT HCMV-infected allograft recipients. Clinical manifestations of HCMV infection that have been described in these patients include pneumonia, gastrointestinal disease, hepatitis, retinitis, nephritis/cystitis, pancreatitis, myocarditis, and CNS disease, with gastrointestinal tract infection and disease being the most common clinical manifestation of HCMV infection (see Table 137.3). In each case, with the exception of retinitis, a definitive diagnosis requires demonstration of HCMV in tissue specimens from the affected organ and, if possible, quantitation of the amount of virus. The level of HCMV in the blood can serve to alert to the possibility of end-organ disease, but diagnosis of organ involvement requires detection of HCMV in the organ system. In cases of suspected HCMV pneumonia, quantitation of virus in bronchoalveolar lavage (BAL) fluid has been suggested to be of considerable value in the evaluation of SOT (and HSCT) recipients with pneumonia, and may lead to withholding of treatment from patients who may only be shedding virus and who do not have invasive infection. Considerable effort to establish levels of HCMV DNA in BAL specimens that are predictive of invasive HCMV disease is ongoing, and, although variable, values of HCMV DNA that are predictive of pneumonia in patients have been reported for lung transplant recipients, other SOT recipients, and HSCT recipients.[314–317] However, as illustrated in one case report, caution has been suggested in the

TABLE 137.3 Clinical Expression of HCMV Infections in Solid-Organ Transplant Recipients

CLINICAL MANIFESTATION	CLINICAL AND LABORATORY FINDINGS
CMV syndrome	Fever New or increased fatigue/malaise Leukopenia or neutropenia Reactive lymphocytes Thrombocytopenia Elevation of hepatic aminotransferases
End-organ disease	Gastrointestinal disease (colitis, hepatitis, esophagitis) Pneumonitis (CMV+ BAL) CNS (retinitis, encephalitis)
Allograft dysfunction	Allograft rejection (chronic)

BAL, Bronchoalveolar lavage; *CMV,* cytomegalovirus; *CNS,* central nervous system.

interpretation of a low or absent HCMV DNA signal in a BAL specimen when other clinical and laboratory findings are consistent with the diagnosis of HCMV pneumonia.[318]

Of note, these clinical manifestations of HCMV disease in transplant recipients reflect end-organ disease associated with high levels of virus replication and usually present clinically in the early posttransplantation period or within months after discontinuing antiviral prophylaxis. In contrast, the clinical manifestations of end-organ disease and CMV syndrome can be more variable in late-onset disease, and in SOT recipients, late-onset HCMV disease may not be associated with significant viremia and evidence of disseminated infection.[307,319,320] Furthermore, routine care of SOT recipients late after transplantation may be provided by community physicians who may not have high index of suspicion for atypical presentations of HCMV infection, and thus patients may be diagnosed late in the course of the infection.[277] In SOT recipients, late-onset HCMV infection has been correlated with allograft loss and increased all-cause mortality in some studies, whereas these associations have not been reported in the analyses of other SOT populations.[302,321–323,324–326]

Finally, HCMV infections in the posttransplantation period have long been associated with an increased incidence of fungal and bacterial infections in allograft recipients.[327] Whether the increased risk for nonviral infections in these patients represents an indirect effect of HCMV on the capacity of the host to mount protective immune responses, or merely represents a role of HCMV as sentinel of status of immunity in these patients, continues to be debated.[148]

HCMV Infection and Allograft Survival and Function in Solid-Organ Transplantation

An important but yet unanswered question in the biology of HCMV infection in allograft transplantation is the role infection with this virus plays in the function and survival of the allograft. As was noted previously, an extensive literature has argued that HCMV infection can impact graft function acutely as well as altering long-term graft function and survival.[157,276,328–340] The association between HCMV infection and graft dysfunction, graft loss, or both has been most extensively described in cardiac and renal allograft recipients.[149,276,331,341–347] Cardiac allograft vasculopathy (CAV) is a well-described condition associated with rejection of cardiac allografts, displaying characteristic histopathologic finding in the coronary arteries of the allograft that include intimal fibromuscular thickening with concentric narrowing of the vessel lumen that is often associated with inflammation in all layers of the coronary arteries.[348–350] Development of CAV is associated with graft dysfunction and loss, and often requires cardiac retransplantation. Although the mechanisms that induce CAV have not been defined, a pathway associated with endothelial damage followed by migration and proliferation of smooth muscle cells and ultimately fibrosis and thickening of the intima has been suggested.[351,352] Damage to the endothelium is thought to involve a combination of innate and adaptive immune effector functions, including antibodies that could mediate graft rejection, although the precise definition and composition of these effectors remains to be precisely determined.[353–361] Thus HCMV infection in the donor or recipient has been associated with the development of CAV, but a unifying mechanism that can account for the role of this virus infection and the development of CAV remains to be defined. Because of the limitations of invasive studies in humans, the role of HCMV infection in the development CAV cannot be easily studied with sufficient precision to define a mechanism(s) of disease, particularly in a complex population such as transplant recipients with underlying disease and exposure to immunosuppressive agents. Perhaps even more challenging is the task of dissecting the role of HCMV in CAV development in the face of an ongoing alloreaction that may include both T-lymphocyte and antibody-mediated rejection of the cardiac allograft. Studies utilizing antiviral drugs and analysis of HCMV-specific T-lymphocyte responses have suggested that control of HCMV replication can limit CAV in cardiac allograft recipients, thus providing some insight into potential mechanisms of disease in these patients that can be explored in relevant models.[149,342,362–365] Fortunately, small animal models of cardiac allograft transplantation have been developed in which CMV infection results in decreased graft survival with histopathologic changes that resemble findings reported in human cardiac allografts with evidence of rejection.[155,352,366–368] Ideally, these models will allow direct hypothesis testing of mechanisms that contribute to CAV that cannot be carried out in a clinical population.

Although the control of episodes of acute rejection of a renal allograft has improved significantly, chronic renal allograft rejection resulting in the loss of a functioning graft continues to represent a major clinical issue in renal allotransplantation. Results from decades-old studies have linked HCMV infection with renal allograft loss, although as with CAV in cardiac allografts, the mechanism(s) through which HCMV contributes to allograft loss remains undefined. Chronic rejection in renal transplant recipients is histologically characterized by tubulointerstitial nephritis and tubular atrophy leading to the loss of proximal tubules.[369] HCMV infection has been proposed to contribute to both the development and progression of chronic renal allograft rejection. Importantly, the findings of chronic renal allograft rejection have also been modeled in animal systems, thus allowing for more controlled studies of the role of CMV in this process.[158,370–378] However, it should also be noted that even though HCMV infection in renal allograft recipients has been repeatedly associated with an increased incidence of graft dysfunction in both the early and late posttransplantation period, whether HCMV infection has a direct role in this chronic inflammatory process remains somewhat contentious.[152,156,157,329,379–382] Inflammation remains linked to graft rejection, and it has been proposed that viral gene expression contributes to the inflammatory responses in renal allografts undergoing chronic rejection. Interestingly, studies in humans have demonstrated HCMV-encoded proteins in >90% of failing renal allografts.[164,383] Thus the presence of HCMV in the allograft could influence not only the intensity, but the nature of chronic inflammation associated with this alloreaction, as has been demonstrated in small animal models of HCMV-associated graft dysfunction.[154] Lastly, CMV infection of allografts in small animal models has been shown to induce increased angiogenesis as well as transcription programs that resemble that of wound healing.[150,180,384] Thus several lines of evidence point to HCMV as a proximal cause of graft dysfunction and tubulointerstitial nephritis and tubular atrophy in renal allografts. Hopefully, informative animal model systems that have been developed to investigate the role of HCMV infection in allograft rejection will help define mechanisms associated with this process.[158,372,376–378]

HCMV Infections in Hematopoietic Stem Cell Transplant Recipients (See Also Chapter 307)

The importance of HCMV infection in the outcomes of HSCT recipients has long been recognized.[14] HCMV infection in the early posttransplantation period has been associated with a decrease in survival, including decreased survival unrelated to relapse of the underlying malignancy following transplantation for treatment of leukemias and lymphomas.[338,339,385,386] With very intensive monitoring, HCMV reactivation occurs in about 40% to 50% of all recipients of HSCTs, depending on the risk factor for HCMV reactivation or acquisition of HCMV. In contrast to SOT recipients, among whom the high-risk group for HCMV infection and disease is defined as seronegative recipients of a donor-seropositive organ (D+/R−), this combination has been defined as an intermediate-risk group in HSCT recipients (see Table 137.2).[387,388] The remarkable difference between HSCT and SOT allograft recipients was illustrated by comparison of the incidence of viremia in D+/R− HSCT recipients (3.7%) and D+/R− SOT recipients (78%) when results in a single center were analyzed.[388] A potential explanation for the difference between HSCT and SOT recipients is that in HSCT D+/R− transplant recipients, the efficiency of HCMV transmission appears to be low and has been correlated with the total number of nucleated cells in the allograft.[389] Thus the HCMV serostatus of the recipient is a major determinant of the risk of HCMV reactivation and disease in HSCT recipients. Additional characteristics of the donor and recipients have been described that identify HSCTs with an increased risk for HCMV infection and disease. These include (1) a related donor with one HLA-A, -B, or -DR mismatch or an unrelated donor with one HLA-A, -B, -C, or -DR mismatch; (2) use of T-lymphocyte–depleting strategies, including antithymocyte globulin or alemtuzumab; (3) umbilical cord stem cell HSCT in seropositive recipients; (4) HLA-haploidentical HSCT;

and (5) graft-versus-host disease requiring steroid therapy (see Table 137.2).[390–394]

Clinical expression of HCMV infections in HSCT recipients can range from asymptomatic virus shedding to end-organ disease and death, particularly in patients with HCMV pneumonia. Thus the differentiation between patients with HCMV reactivation and asymptomatic shedding versus those patients who will progress to end-organ disease cannot be dependent on clinical assessments and can only be accomplished utilizing algorithms that incorporate frequent virologic monitoring that includes quantitative measures of virus replication. Overall, in HSCT recipients, HCMV shedding/infection occurs in over 50% of patients at risk for HCMV reactivation in the early posttransplantation period, with a median onset between 40 and 60 days posttransplantation.[339,386–389] End-organ disease in these patients included a spectrum of manifestations similar to that observed in SOT recipients, with gastrointestinal disease being most frequently described. In contrast to SOT recipients, HCMV pneumonia remains a significant source of morbidity and mortality in HSCT recipients. HCMV pneumonia has been reported to account for about 30% of total cases of end-organ disease in HSCT recipients; however, as compared to disease in the gastrointestinal tract, HCMV pneumonia is accompanied by higher rates of morbidity and mortality, with a fatal outcome reported in nearly 50% of some patient populations.[140,395,396] Although this case fatality rate remains unacceptably high, it must be contrasted with mortality rates from HCMV pneumonia of nearly 100% prior to the introduction of virologic monitoring and preemptive antiviral therapy.[141,397,398]

With improved control of HCMV infections in the early posttransplantation period provided by effective antiviral therapies, late-onset HCMV infection has become increasingly important and accounts for significant morbidity and mortality in HSCT recipients. Although HCMV infections late after transplantation are relatively infrequent (on the order of 5%), these infections are associated with decreased overall survival in this population, and mortality rates of nearly 50% have been described in some centers.[140,387,399–401] Clinical manifestations of late-onset disease can include end-organ dysfunction that has been catalogued for HCMV infections early after transplantation and similarly appears to be related to deficits in adaptive immunity, specifically HCMV-specific T-lymphocyte responses, as has been reported early after HSCT.[402] Attempts to monitor reconstitution of HCMV-specific T-lymphocyte responses utilizing class I MHC tetramer assays and interferon responses following ex vivo stimulation have been reported.[139,301,403–408] Although these reports suggested potential utility of this strategy in monitoring reconstitution of HCMV-specific T-lymphocyte responses, there has been only limited introduction of these approaches into posttransplantation monitoring of HSCT recipients. Finally, there are reports that late-onset fungal infections are associated with HCMV reactivation in the early posttransplantation period.[409]

Congenital HCMV Infection

HCMV is the most common cause of congenital (present at birth) viral infection. The prevalence of this intrauterine infection ranges from 6 in 1000 in the United States to over 10 in 1000 in South America, Asia, and Africa.[195,202–205] Although a comprehensive discussion of the natural history of this important perinatal infection is beyond the scope of this chapter, a brief description of characteristics of maternal HCMV infections during pregnancy and the outcomes of pregnancy can serve to highlight significant aspects of this perinatal infection.

Similar to some other perinatal infections such as rubella and toxoplasmosis, HCMV can cross the placenta during pregnancy and establish an intrauterine infection. Natural history studies in urban populations in the Southeastern United States defined many of the salient features of congenital HCMV infections. In these populations, maternal HCMV infections were categorized as primary infections during pregnancy (*de novo* appearance of HCMV-specific immunoglobulin G [IgG] during pregnancy) or recurrent infections in women with preconceptional HCMV-specific IgG antibodies (nonprimary) that have been argued to result from reactivation of HCMV in persistently infected women. Although almost all maternal HCMV infections during pregnancy are subclinical, extensive natural history studies that prospectively monitored women during pregnancy determined that intrauterine transmission occurs in about 30% of women undergoing primary HCMV infection during pregnancy.[204,410,411] This rate of transmission has been confirmed in several maternal populations, although underlying deficits in maternal immunity, such as those associated with HIV/AIDS infections, can be associated with increased rates of intrauterine HCMV transmission.[412–414] The rate of intrauterine transmission associated with nonprimary infection is unknown but commonly has been suggested to be on the order of 1% to 2%, although this number represents an extrapolation of data from natural history studies and not from prospective studies.[415] From these early studies, it was proposed that the most severely affected infants with congenital HCMV infection were born to women with primary infection during pregnancy.[416] In addition, the intrauterine transmission rate of HCMV was proposed to be about 20- to 30-fold more frequent in women with primary infection during pregnancy as compared to women with nonprimary infections. Thus the paradigm that preconceptional maternal immunity to HCMV can limit intrauterine transmission and also modify the severity of the intrauterine infection was established, although a number of more contemporary observations have challenged this long-held but unverified concept.[415,417]

The severity of the intrauterine infection, and more importantly the degree of CNS damage, associated with congenital HCMV infection have been correlated with neurodevelopmental status of the fetus such that infections in the late first trimester or early second trimester are more commonly associated with more severe infection and CNS damage. However, this observation has also been described in congenital infection following maternal rubella or toxoplasma infections.[418–420] From existing natural history studies, about 10% to 15% of congenitally infected infants will present in the newborn period with some evidence of congenital infection and be classified as having a symptomatic infection. This identification of infants with symptomatic congenital HCMV infection is of clinical importance because it allows stratification of congenitally infected infants in terms of their risk for long-term neurodevelopment sequelae. Some 30% will have long-term neurologic sequelae as a result of infection during neurodevelopment.[410,421] Infants with evidence of congenital HCMV infections (symptomatic infection) can exhibit characteristic findings initially described in infants with cytomegalic inclusion disease. These include micrencephaly, jaundice, hepatosplenomegaly, petechial rashes, evidence of neurologic disease (e.g., seizures or abnormal tone and posture), chorioretinitis, and, rarely, evidence of extramedullary hematopoiesis.[422,423] More recently, investigators have also included evidence of intrauterine growth restriction as a clinically apparent manifestation of symptomatic congenital HCMV infection.

Laboratory abnormalities can include elevated liver transaminases and elevated bilirubin, anemia, and thrombocytopenia.[410,421] Cerebrospinal fluid (CSF) pleocytosis has been described in infants with symptomatic congenital HCMV infections and more commonly, CNS involvement has been diagnosed by neuroimaging. Case series have described intracranial calcifications and altered brain development such as cerebellar hypoplasia, and deficits in cortical migration, including pachygyria and even lissencephaly.[424–430] It should be noted that such severely affected infants represent only about 30% of the cases of symptomatic congenital HCMV infections and less than 5% of all cases of infants with congenital HCMV infection.[421] Prior to the availability of antiviral therapies, the mortality rate in the newborn period was approximately 10% in infants with severe symptomatic congenital HCMV infections.[431] Well over 90% of infants with congenital HCMV infections will not exhibit clinical findings in the newborn that can be attributed to this infection, and thus can only be identified if a newborn screening program is in place that can detect HCMV shedding in the newborn period. Long-term neurodevelopmental sequelae are a hallmark of congenital HCMV infections. Sequelae resulting from damage to other end-organ systems, such as the liver, are rarely observed.

Hearing loss is the most frequently identified sequela.[432–439] It has been estimated that hearing loss associated with congenital HCMV infections may account for approximately 25% of all cases of hearing loss in children.[440] An important characteristic of hearing loss associated with congenital HCMV infection is that only about 50% of infected infants will have hearing loss in the newborn period, and therefore only a fraction of infected infants at risk for hearing loss will be identified during newborn auditory screening programs. The remaining 50% can

develop hearing loss during childhood, and existing hearing loss may progress during this time.[441] Neurodevelopmental delays have been well documented in infants with symptomatic congenital HCMV infections, particularly those with CNS structural abnormalities, but several studies have failed to demonstrate neurodevelopmental delays in infants with asymptomatic infections.[442–445]

In dramatic contrast to the protection from congenital infection that is afforded by preconceptional immunity to rubella and toxoplasmosis, maternal immunity to HCMV that is present prior to pregnancy does not prevent transmission of the virus to the fetus or symptomatic infection in the newborn. This observation was made over 3 decades ago and has been confirmed in a number of different studies and maternal populations.[203,416,446–455] As was previously noted, there remains an ongoing debate about the level of protection provided by preexisting maternal immunity both in terms of protection from transmission and prevention of severe symptomatic infections.[415,417] However, several unique observations in the natural history of congenital HCMV infections suggest that it will be difficult to quantify the protection provided by maternal immunity, because this effect could be unique to a maternal population. As an example, the incidence of congenital HCMV infection increases with increasing maternal seroprevalence, such that maternal populations with the highest seroprevalence have higher rates of congenital HCMV infection in their offspring.[456] It is of considerable interest that in contrast to other perinatal virus infections, such as rubella, the rate of congenital HCMV infection does not reach a plateau when plotted against the maternal HCMV seroprevalence. This finding, when modeled, suggests that the incidence of congenital HCMV infections that result from primary maternal infections will never approach the number of congenital HCMV infections that follow nonprimary infections.[457] Furthermore, it is well documented that symptomatic congenital HCMV infections can follow nonprimary infections, further demonstrating that not only is maternal immunity incompletely protective in terms of intrauterine transmission, but it may also not prevent the development of symptomatic congenital infections.[68,70,203,435,458,459] This potential shift in the paradigm that maternal immunity is protective and can prevent significant intrauterine infections and long-term sequelae has implications that HCMV vaccines might be able to prevent the sequelae of congenital HCMV infection. Lastly, the source of nonprimary maternal infections has long been believed to be the reactivation of a persistent infection or a recurrence. However, with the availability of newer technologies coupled with findings from basic studies of HCMV, investigators have been able to demonstrate that immunocompetent adults with immunity to HCMV, including pregnant women, can be reinfected with new strains of HCMV that differ both antigenically and genetically from strains of virus in the persistently infected host.[68–72,460] Thus the current understanding of the relationship between maternal immunity and the outcome of maternal infections with HCMV during pregnancy, whether congenital HCMV infection follows primary or nonprimary maternal infections, continues to be incomplete.

DIAGNOSIS OF HCMV INFECTIONS

Although HCMV-specific serology and, in some centers, isolation of HCMV remain important components in the diagnosis of HCMV infection, viral antigen detection and nucleic acid amplification technologies (NAATs) have replaced these older methodologies and now represent the mainstay of diagnostic approaches for detection of HCMV in clinical specimens (Table 137.4). A major hurdle to the optimization of care of allograft transplant recipients and other immunocompromised patients has been the standardization of both NAATs and antigen detection between transplant centers. When the variability in quantitation of diagnostic assays is combined with the inherent variability of patient populations and individual center-specific protocols for management of transplant recipients, the translation of findings from different centers into coherent management strategies has been difficult. More recently, several steps have been taken to remedy this issue, including standardization of results from NAATs and reporting quantified results from antigen detection assays. These steps have provided a foundation for integration of results from different transplant centers, and thus provide a larger number of evaluable patients for comparative analysis of outcome data, as discussed in the following sections.

TABLE 137.4 Diagnostic Approaches for Detection of HCMV Infections

ASSAY	APPLICATIONS	PERFORMANCE CHARACTERISTICS
Nucleic Acid Detection		
In situ hybridization	Detection of HCMV nucleic acids in tissue specimens	Sensitive detection of HCMV in tissues specimens Technically demanding
Nucleic acid amplification technologies	Quantitative determination of HCMV nucleic acids in blood, body fluids, and tissue specimens	Standardized assays Can quantify HCMV genomes in tissues and body fluids Rapid turnaround Can be used to guide therapy and detect development of antiviral resistance
Antigen Detection		
Immunological detection of immediate-early antigen	Rapid detection of HCMV in assays of virus infectivity	Increased sensitivity of detection of infectious HCMV in clinical specimens Requires tissue culture facilities
Immunohistochemistry	Detection of HCMV in tissue specimens	Increased sensitivity of detection of HCMV in tissue specimens Only semiquantitative
Antigenemia	Quantitative determination of HCMV in blood	Can be used in preemptive treatment protocols and for monitoring treatment Requires technical expertise Limited value in patients with leukopenia
Virus isolation	Determination of virus shedding or presence of virus in tissue	Sensitivity dependent on quality of specimen and viral load in specimen Requires tissue culture facilities Prolonged period of culture can be necessary (up to 28 days)
Serology		
Virus-specific immunoglobulin G (IgG)	Determination if infected with HCMV; detection of seroconversion	Standardized assays High positive and negative predictive values in immunocompetent hosts
Virus-specific immunoglobulin M (IgM)	Detection of recent HCMV infection	Standardized assays HCMV-specific IgM can persist for weeks to months, limiting value in assignment of timing of acquisition of infection
Virus-specific IgG avidity	Estimation of interval from IgG seroconversion	Useful to estimate timing of infection in immunocompetent hosts Value uncertain in allograft recipients
Histopathology	Detection of HCMV in tissues	Characteristic findings Insensitive in comparison to immunohistochemistry and nucleic acid amplification technologies

Quantitative Nucleic Acid Amplification Technologies

Quantitative NAATs have become the methodology of choice for the detection and quantitation of HCMV in specimens from both normal and immunocompromised individuals. In general, NAATs for HCMV detection are primarily quantitative PCR (qPCR) amplification of DNA using different primer sets. NAATs-based assays that are based on the amplification of HCMV RNA have also been utilized in monitoring both SOT and HSCT recipients.[461–464] The detection of HCMV RNA was proposed to be a more relevant measure of HCMV replication than assays employing qPCR amplification of blood because several studies demonstrated that HCMV DNA can be amplified from leukocytes obtained from normal, immunocompetent individuals with past HCMV infection secondary to the presence of latently infected myeloid cells. This finding raised the possibility that results from PCR amplification of HCMV DNA in whole blood would fail to quantify the level of virus replication.[465,466] Although this concern is well founded, practical experience with qPCR-based assays of whole blood or plasma have been successfully employed to quantify virus replication, primarily through serial measurements. Thus qPCR amplification of HCMV DNA has largely replaced assays of HCMV RNA and qualitative PCR assays for the detection of HCMV in transplant recipients.

Multiple formats for qPCR quantification of HCMV DNA have been developed, and multiple protocols for DNA extraction and preparation of PCR analysis have been reported. As could have been anticipated from such laboratory-specific approaches, the major hurdle for implementation and interpretation of screening and monitoring strategies utilizing qPCR has been the lack of standardization of sample preparation and PCR assays, and perhaps most importantly, the standardization of the quantity of HCMV DNA detected in each assay format.[467–469] Harmonization of sample preparation and of qPCR assays represents a significant challenge, since the preparation of tissue specimens for PCR analysis in many laboratories is generic and not specific to a single infectious agent. Similarly, the experience of the laboratory with different PCR formats will dictate the preference of the laboratory for a particular qPCR assay. However, a very simple solution was provided by supplying reference standards for HCMV DNA that allowed calibration of each assay system and thus enabled comparison of results from different laboratories.[470,471] Utilizing these calibration standards, interlaboratory variability could be controlled.[469,472] Lastly, even with HCMV DNA calibration standards, the variability of results among different formats, particularly different commercial systems, can be observed in the extremes of the performance characteristics of each assay.[469] Thus until PCR protocols are standardized to produce similar performance in characteristics, such as the limit of detection of input HCMV DNA, seamless integration of qPCR findings from different transplant centers will remain problematic.

A second issue surrounding a universally accepted NAATs-based approach for detection and monitoring HCMV infections in immunocompromised hosts is the selection of a blood component for assay. There is little debate about the value of other fluids, such as BAL fluid, CSF, urine, or tissue, but the choice between using whole blood or plasma for the detection of HCMV by qPCR remains unsettled. Multiple laboratories have reported that assays using whole blood are more sensitive than those using plasma, although there is good concordance between the results from whole blood and plasma.[473–477] Lastly, simplicity of sample preparation favors the use of whole blood, since an accurate amount of sample can be processed prior to an additional centrifugation step required to prepare plasma.

Antigen Detection

The generation and characterization of HCMV-specific monoclonal antibodies led quickly to the development of assays for detection of HCMV antigens. These early assays became the standard approach for rapid detection of HCMV in SOT and HSCT recipients, until the widespread introduction of NAATs. Many of these assays continue to be used in diagnostic laboratories and in the management of HCMV infections in allograft recipients, particularly in situations that require the identification of HCMV in body fluids and tissue specimens (see Table 137.4). One of the initial assays utilized monoclonal antibodies to identify the protein product of a viral immediate-early gene (*IE-1*) that is expressed within hours after infectious virus is seeded onto permissive human primary cell monolayers. This assay has been further modified by a low-speed centrifugation–enhanced inoculation to increase the efficiency of infection and therefore the sensitivity of the assay. Specimens of urine, saliva, BAL fluid, genital secretions, and, rarely, minced tissue have been assayed using this approach. The assay is relatively rapid, requiring about 12 to 16 hours from receipt of specimen to reporting of results; has minimal interobserver error in its interpretation; and compares favorably in sensitivity to tissue culture isolation of HCMV. Older studies have suggested that this assay may more be sensitive than standard tissue culture methods for detection of HCMV, although similar comparative studies were not performed by other laboratories.[478] However, this assay is labor intensive and less amenable to high-throughput screening unless adapted to microtiter plate formats, requires tissue culture facilities, and requires relatively expensive instrumentation depending on the method for detection of the HCMV monoclonal antibody. Additional applications for HCMV antigen detection include use of HCMV-specific antibodies in immunohistochemistry protocols to detect HCMV in tissue sections, both frozen and paraffin-embedded tissue (Fig. 137.3). These assays can complement NAATs approaches to demonstrate tissue-invasive HCMV infection and importantly can provide direct evidence of end-organ infection and disease. In contrast to NAATs-based assays for detection of HCMV in tissue, antigen detection assays are extremely labor intensive and require technical expertise to optimize the assay for each tissue and fixation condition.

In the early 1990s, the antigenemia assay was introduced as an approach for monitoring HCMV in the blood of immunocompromised patients. This assay was based on the detection of the HCMV tegument protein pp65 (ppUL83) in circulating leukocytes, primarily polymorphonuclear leukocytes (PMNs).[479,480] Detection of HCMV infection in patients is based on the detection of this viral protein in samples of peripheral blood that have been enriched for PMNs and spotted onto glass slides. The slides are processed using an anti-HCMV pp65 antibody followed by detection of the pp65 protein within PMNs. The assay is technically straightforward, and has a rapid turnaround time as compared to culture or even IE-1 detection in cell monolayers inoculated with HCMV, and the results can be quantitatively expressed as the number of HCMV-positive cells per 50,000 to 200,000 PMNs. Quantitation of the results from this assay has been and continues to be used in some transplant centers to guide institution of preemptive antiviral therapy,

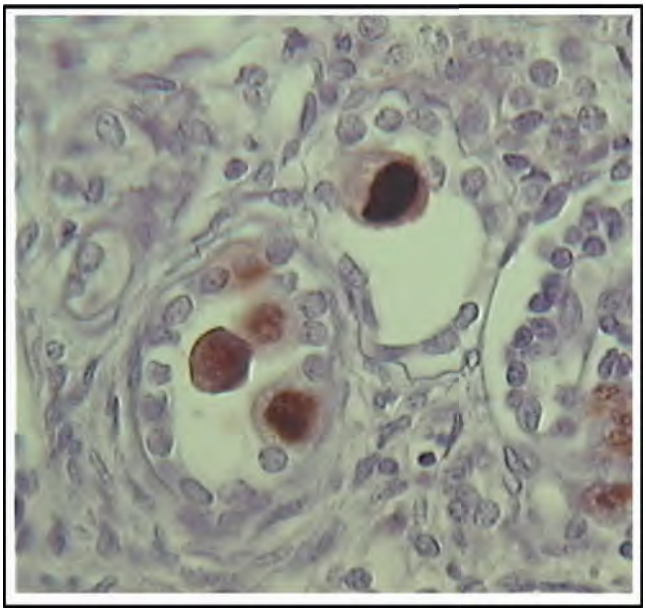

FIG. 137.3 Immunohistochemical detection of HCMV infected cells. Viral nuclear antigen (immediate-early gene *IE-1*) present in renal epithelium is indicated by brown-stained nuclei (magnification ×33).

to detect HCMV infection after discontinuation of prophylaxis, and to monitor the response to antiviral therapy. The clinical utility of this assay compared favorably to early-generation NAATs-based assays in monitoring HCMV infections in SOT and HSCT recipients, perhaps because results from the antigenemia assay offered a high positive predictive value of clinically significant levels of HCMV replication in these populations.[481–485] However, the assay also suffered several drawbacks, such as the requirement to have sufficient numbers of circulating leukocytes for valid test results, inherent difficulties in standardizing results across different centers secondary to limited dynamic range of the assay, operator dependence, and inability to process large numbers of samples concurrently; also, in some centers the antigenemia assay had only a moderate negative predictive value.[484,486–489] Because of these limitations, most large-volume transplant centers have transitioned to newer-generation quantitative NAATs-based assays for monitoring HCMV infections in both SOT and HSCT recipients. However, it must be noted that comparative studies within single institutions have provided evidence for the concordance of findings from the antigenemia assay and NAATs.[395,490]

Virus Culture

Isolation of replicating HCMV from tissue specimens or blood requires significant time and effort as well as facilities for maintaining primary human cells (see Table 137.4). As a result, this approach to the diagnosis of HCMV is utilized primarily in centers with dedicated diagnostic virology laboratories. HCMV grows slowly, and primary isolates are highly cell associated and require prolonged culture. A culture must be held for 4 weeks before it is considered negative. Identification of infected cells utilizes recognition of characteristic cytopathology induced by HCMV and is usually verified by either antigen detection within cells from the infected culture or NAATs-based assays of the culture. Recovery of infectious virus from tissue specimens can also be challenging, secondary to microbial contamination of the specimen and toxicity of tissue homogenates. In most cases, tissue specimens are subjected to antigen detection technologies such as immunohistochemistry or NAATs.

Serology

Serologic demonstration of previous and current HCMV infection is of value, particularly in identifying previously infected individuals as part of pretransplantation screening of donors and recipients (see Table 137.4). Several formats for detection of HCMV IgG antibodies are available commercially. Although serial samples can demonstrate either *de novo* IgG seroconversion or increasing antibody titers in acutely infected individuals, in most cases laboratories must rely on avidity determinations to estimate the chronicity of an HCMV infection. These assays utilize avidity maturation of HCMV-specific IgG antibodies to estimate the duration of an infection. Low-avidity antibodies that are bound to an immobilized antigen can be readily released by exposure to a low concentration of urea or to a chaotrope such as guanidine. These antibodies have been shown to develop early in infection, whereas high-avidity antibodies that require higher concentrations of the chaotrope to be released from the antigen are found later in infection following maturation of the antibody response. In most individuals, this follows a relatively predictable time course that can then be extrapolated to estimate the chronicity of the infection. These assays are widely used in serologic studies of pregnant women who also have HCMV-specific immunoglobulin M antibodies as an approach to estimate the chronicity of HCMV infections during pregnancy.[491–495] The detection of higher-avidity HCMV-specific IgG antibodies following allograft transplantation has been described, but its value in management of HCMV infections in either SOT or HSCT recipients has not been defined.[496]

Diagnosis of Congenital Infections

The diagnosis of congenital HCMV infections contrasts with the diagnosis of HCMV infections in immunocompromised patients, primarily because it is qualitative. Although quantitation of the level of infection (viral load) has been correlated with severity of infection and long-term outcome, the wide variance in outcome as a function of viral load has limited the clinical value of quantitative results from NAATs in the management of congenitally infected infants.[410,497–500] Diagnosis of congenital HCMV requires the demonstration of HCMV or HCMV DNA in urine, saliva, or blood from an infant within the first 2 weeks of life by isolation of replicating virus, detection of viral antigens, or NAATs. Detection of HCMV DNA or HCMV after 4 weeks of life cannot be unequivocally defined as congenital (present at birth) HCMV infection. Newborn infants can excrete HCMV following exposure to genital tract secretions containing HCMV during vaginal delivery and from ingestion of breast milk from a seropositive mother. Contemporary diagnostic approaches include qPCR analysis of saliva and urine, because large amounts of HCMV can be found in these fluids in congenitally infected infants.[202,501–504] Finally, fetal infection can be diagnosed by characteristic findings of imaging studies, including ultrasound and magnetic resonance imaging, and by the detection of HCMV in amniotic fluid, usually by NAATs.[505,506] Importantly, false-negative results have been reported in amniotic fluid when testing takes place before 17 weeks of gestation, or too early (<7 weeks) following maternal seroconversion.[505–509] Finally, in contrast to the limited clinical value of quantitation of viral load in congenitally infected infants, investigators have suggested that quantitation of the viral load in amniotic fluid can be correlated with the severity of the fetal infection, and thus with the potential long-term damage associated with this intrauterine infection.[510–512]

THERAPY OF HCMV INFECTIONS (see also Chapter 46)

Antiviral drugs with activity against HCMV have been available for nearly 3 decades, yet the treatment of clinically significant infections caused by this virus remains challenging. This is secondary to the modest efficacy of available drugs and to their toxicity, which limits their use in specific patient populations and often requires dose modifications that further limit their activity. In addition, clinically significant resistance to single agents can develop during treatment. Currently licensed agents include (1) ganciclovir (GCV), a deoxyguanosine nucleoside analogue that can inhibit the viral DNA polymerase and also function as a chain terminator; (2) foscarnet, a pyrophosphate analogue that directly inhibits the viral DNA polymerase; (3) cidofovir, a cytidine deoxynucleoside analogue that also inhibits the viral DNA polymerase; and (4) letermovir, the newest antiviral for HCMV, an agent that inhibits function of UL51 and UL56 of the HCMV terminase complex (UL51, UL56, and UL89). Additional agents include maribavir, an inhibitor of UL97, and brincidofovir, an analogue of cidofovir that has less renal toxicity; both are currently in late clinical development.

GCV and valganciclovir, its L-valyl prodrug with improved oral bioavailability, are currently the first-line agents for treatment and prophylaxis of HCMV infections. GCV requires phosphorylation by the HCMV-encoded kinase UL97 as an initial modification, followed by additional phosphorylations by host kinases to produce the triphosphate form of GCV that functions as an inhibitor of the viral DNA polymerase and DNA polymerization.[513–515] The toxicity of GCV is almost exclusively bone marrow suppression, resulting in reversible neutropenia and less often thrombocytopenia. Because it is not metabolized to any extent and is excreted almost unchanged into the urine, dosing can depend on renal function. Foscarnet is an inhibitor of many herpesviruses and is often used when myelosuppression limits GCV use or GCV resistance develops during treatment, or both. This agent has been shown to provide clinical benefit in immunocompromised patients, including patients with HIV/AIDS and HCMV end-organ disease, and in HSCT recipients requiring preemptive therapy for HCMV infections.[397,516,517] It is excreted almost unchanged into the urine, and its major toxicity is reversible renal impairment.[517] Cidofovir has been shown to be a potent inhibitor of several different viral polymerases other than HCMV, including poxviruses, polyomaviruses, and adenoviruses. Its clinical use is limited by significant nephrotoxicity that can result in irreversible acute kidney injury.[518–522] The most recently licensed agent, letermovir, has recently been shown to be useful in the prophylaxis of high-risk HSCT recipients, since myelosuppression has not been associated with its use.[394] Its use in SOT recipients remains to be demonstrated, but it appears to have limited significant toxicity at least in these initial trials, suggesting that it may have more widespread indications.

Maribavir has been shown to directly inhibit the HCMV viral kinase UL97, and it has been shown to inhibit critical steps in virion

morphogenesis and, potentially, in the function of the viral polymerase.[523–526] Extensive preclinical studies led many investigators to suggest that this agent would be active in treatment of HCMV infections. This enthusiasm was further supported by the demonstration of its activity in a limited trial in HSCT recipients.[527] However, a pivotal clinical trial failed to show that maribavir improved outcome of HSCT recipients over placebo.[528] Importantly, there remains some controversy over the end points that were utilized to estimate the clinical benefit provided by maribavir in this later trial and in a subsequent trial that suggested some benefit.[529,530] Lastly, brincidofovir is an oral analogue of cidofovir that has been shown to have significantly less nephrotoxicity than the parent drug, although case reports of brincidofovir-associated acute kidney injury have been reported.[531,532,533] In a small trial, this agent was shown to be active in suppression of HCMV infection in HSCT recipients; however, treatment with this agent was associated with diarrhea that in some cases was dose limiting.[532]

Prophylaxis and Preemption

The treatment of HCMV infections with antiviral agents has been most well studied in allograft recipients, although there is an extensive literature on the use of antivirals to treat HCMV infection in HIV/AIDS patients prior to the extensive use of combination antiretroviral therapies. Two approaches have been described in transplant recipients: preemptive therapy and prophylaxis. Prophylaxis protocols often incorporate a preemptive strategy after discontinuation of prophylaxis. The selection of one of these approaches over the other is often dictated by the preference and experience of the transplant center, the type of transplantation, and the risk for HCMV infection and disease in the allograft recipient. Preemptive treatment of HCMV infection was first deployed in HSCT recipients and utilized serial quantitative measures of HCMV in various body fluids as early indicators of HCMV infection. Results from virologic monitoring were combined with predetermined criteria for significant levels of HCMV, and if indicated, antiviral therapies were initiated until resolution of the HCMV infection, or significant reduction in the quantity of virus. This approach evolved from early studies that demonstrated that monitoring HSCT recipients for HCMV shedding in the posttransplantation period, followed by GCV treatment and prophylaxis until day 100 posttransplantation, significantly reduced the incidence of HCMV pneumonia and improved overall outcome.[534,535] The initial preemptive therapy approaches were followed by protocols incorporating the presence of antigenemia as an indication for initiating GCV treatment.[536]

Of interest is that the results from these very early studies could not definitively demonstrate a clear advantage of antiviral prophylaxis or preemptive therapy in patient outcome, since each approach had unique advantages as well as limitations. Even today, there continues to be discussion as to which is the most effective approach. In SOT recipients, reports of studies in renal, liver, and heart allograft recipients argue that universal prophylaxis results in more favorable outcomes, whereas other studies have suggested that outcomes in patients managed by preemptive strategies are similar to those in patients given prophylaxis.[537–539,540–545] In contrast to SOT recipients, prophylaxis with GCV in HSCT recipients is associated with delayed engraftment, and some 20% to 30% of patients who received GCV prophylaxis in the first 100 days posttransplantation developed neutropenia.[546] An increased risk of bacterial and fungal infections is observed in patients with prolonged cytopenias secondary to delayed engraftment and neutropenia.[536,547] Thus universal prophylaxis in HSCT recipients utilizing a myelosuppressive agent such as GCV cannot be viewed similarly to that in SOT recipients, in whom reversible neutropenia can be managed by dose adjustments. Perhaps the availability of an effective antiviral such as letermovir that can be used in prophylactic protocols in HSCT recipients will lead to hybrid prophylaxis–preemptive treatment strategies in these patients.

Two major disadvantages of universal prophylaxis strategies that continue to invite discussion are (1) development of late-onset disease secondary to suppression of antigen stimulation during reconstitution of T-lymphocyte responses, and (2) the selection of antiviral drug–resistant viruses. The longstanding controversy surrounding the impact of early suppression or treatment of HCMV infections and the risk of late-onset disease has not been resolved. Similarly, concerns regarding the development and selection of antiviral drug–resistant viruses by widespread use of antiviral drugs as prophylaxis for HCMV infections in allograft recipients are valid. There are numerous reports of the development of clinically significant resistance in HCMV in patients treated with antivirals. Reports from studies in SOT recipients have described rates of GCV resistance ranging from approximately 5% to 30% in patients treated with GCV in the posttransplantation period.[548–551,552] Several risk factors were noted for development of GCV resistance. These include higher viral loads; high-risk (D+/R–) SOT recipients, including those receiving multivisceral allografts; and interestingly, suboptimal dosing of GCV prophylaxis.[548,550–552] A study from a reference laboratory reported that of over 500 submitted specimens, some 30% had mutations in UL97 or UL54 that have been associated with antiviral resistance.[553] Antiviral resistance has also been well described in HSCT recipients, although perhaps not as frequently as in SOT recipients, because of the less frequent use of GCV prophylaxis in these patients.[554,555,556,557]

Viral Resistance

Identification of antiviral drug–resistant viruses traditionally employed the demonstration of a resistant phenotype, that is, reduction in the yield of infectious virus in an in vitro assay in which varying concentrations of antiviral drug are added to the culture. The results are generally reported as the effective concentration of a drug at which a 50% decrease in virus production is observed. Such assays are of limited value for clinical use because (1) adaptation of a potential virus swarm to in vitro propagation may occur, (2) weeks of virus propagation are required to perform the assay, (3) there may be an inability to capture resistance patterns of minor populations, and (4) there are difficulties in standardization of assays between laboratories. These assays have been replaced with genotyping assays, which can rapidly identify mutations in the targets of antiviral drugs that confer resistance. These assays rely on PCR amplification of HCMV genes targeted by antivirals such as viral kinase UL97 (confers GCV resistance), viral DNA polymerase UL54 (confers resistance to GCV, foscarnet, cidofovir, and brincidofovir), and the terminase complex (UL51, UL56, UL89), and on Sanger sequencing of the products to identify mutations that have been associated with drug resistance.[558,559,560] Next-generation sequencing of these products can be very informative and can identify minor populations of variant sequences associated with drug resistance, although the clinical relevance of minor virus populations remains incompletely defined.[561–563] Clinical observations have also demonstrated the importance of antiviral resistance testing of relevant patient specimens, since compartmentalization of antiviral resistance has been shown for specimens from one compartment (aqueous fluid of eye or CSF) but not another (blood).[564,565]

Therapy of Congenital Infections

Treatment of congenital HCMV infections with currently licensed antiviral agents cannot be accomplished in utero, a time point in the infection that would likely provide the greatest benefit because of the assumed toxicity of these agents on the developing fetus. However, there are scattered case reports describing administration of GCV to pregnant women as a treatment for fetal HCMV infection.[566] Thus the current understanding of the potential role of these agents in modifying the natural history of congenital HCMV infections has been derived from studies of exclusively GCV and its oral analogue, valganciclovir, in the treatment of newborns with clinical stigmata of congenital HCMV infections. In two large trials, one utilizing parentally administered GCV and the second utilizing oral valganciclovir, treatment of newborn infants with symptomatic congenital HCMV infections demonstrated that both approaches could suppress virus replication and limit end-organ dysfunction, and were generally well tolerated except for dose-limiting myelosuppression, which required dose reductions.[567,568] Outcomes from these initial trials were encouraging, since the trials demonstrated a reduction in the number of infants with hearing loss compared to historical controls.[567] In the second study, infants were treated with oral valganciclovir for a 6-month period, and although the primary end point of differences in hearing loss at 6 months was not met, overall there were benefits from GCV treatment during limited follow-up.[568] Together, results from the first and the second trial suggested an improvement

in long-term hearing outcomes of treated infants with symptomatic congenital HCMV infections, particularly in the maintenance of functional hearing.[568] Other trials using GCV treatment of infants with symptomatic congenital HCMV infections with different durations of therapy but all beginning therapy in the newborn period also demonstrated a benefit of treatment.[569,570] A current trial is underway that will utilize the second strategy of treatment with oral valganciclovir of infants with asymptomatic congenital HCMV infections. A number of case reports and small series have described the outcome of GCV treatment of infants with congenital HCMV infections, including infants with hearing losses associated with congenital HCMV infections. Data from these reports are encouraging, but without well-controlled studies with adequate follow-up it will be difficult to determine the value of GCV treatment in infants with asymptomatic congenital HCMV infections, since only 15% could potentially benefit from antiviral therapy, and the remaining 85% will have a low risk of exhibiting neurodevelopmental sequelae from this intrauterine infection. Lastly, about 20% of infants will develop neutropenia that will require dose modifications during treatment, and the long-term consequences of treatment with this nucleoside analogue that can be incorporated in host DNA must be weighed against presumed benefits of treatment.

Passive Immunotherapies for the Prevention and Treatment of HCMV Infections

The development of immunoprophylaxis and treatment of HCMV infection in immunocompromised patients, including fetuses infected in utero, has been an active area of research for over 2 decades. Early studies of HCMV demonstrated the capacity of anti-HCMV antibodies to neutralize infectious virus in in vitro assays, and small animal models of HCMV infections also demonstrated the in vivo protective activity of virus-neutralizing antibodies. With the development of technologies to isolate human immunoglobulins from donor plasma and to formulate preparations for intravenous infusion, there was great interest in these pooled blood products for the prophylaxis and treatment of infectious diseases. CMV hyperimmune globulins (CMV-IGs) were prepared from donor plasma screened to contain high titers of anti-HCMV antibodies and not from immunized donors. In addition, donor pools were identified using antigen-binding assays that had been described to correlate with in vivo protection but were not selected based on activities in virus neutralization assays. However, this donor selection strategy increased the relative HCMV-specific antibody-binding activity as well as the virus-neutralizing activity in these products. It is interesting that comparison of two commercially available products (Cytotect CP and Cytogam) revealed little difference in functional activity between the products.[571] Initial studies using high-titer anti-HCMV immunoglobulins provided evidence of the value to this approach in SOT recipients, as initially shown in renal and liver allograft recipients.[572–574] More recently, the therapeutic value of CMV-IG when combined with GCV has been shown in thoracic SOT and multivisceral SOT recipients.[575,576,577–580] In contrast to the utility of CMV-IG in both prophylaxis and treatment of SOT recipients, trials in allogeneic HSCT failed to provide equivocal evidence of benefit from intravenous immune globulins.[581] In agreement with older studies, more recent meta-analyses of studies of the value of CMV-IG in HSCT have suggested that this approach has little benefit for outcome following HSCT.[582,583]

Studies in small animal models of congenital HCMV infections provided evidence that serum from immune animals could prevent or ameliorate intrauterine infection with HCMV.[584,585] Thus it was proposed that HCMV antibodies transferred to pregnant women undergoing a primary HCMV infection could potentially modify the maternal infection and, following transplacental passage, achieve a therapeutic effect in the infected fetus. Nigro and colleagues reported that treatment of pregnant women undergoing a primary HCMV infection resulted in both a decreased transmission rate and improved outcomes.[586] This study was not controlled and included significant biases in the selection of women who would be treated. A subsequent study that in was controlled, used the same CMV-IG product, and avoided the biases present in the first study failed to demonstrate any reduction in mother-to-fetus transmission in women undergoing primary infection.[587] Although there were no significant differences in the outcomes of infected newborns born to treated and untreated women, there were more adverse outcomes in control, noninfected women treated with CMV-IG.[587] A current large, multicenter study sponsored by the National Institutes of Health will hopefully define the role of CMV-IG treatment in women undergoing primary HCMV infection during pregnancy.

In addition to CMV-IG, several human or humanized monoclonal antibodies have been studied in immunocompromised individuals. These are antibodies against the major virion envelope glycoproteins, including the pentamer complex (UL75, UL115, UL128, UL129, UL131) and glycoproteins gB and gH. Results from early studies in immunocompromised hosts were not encouraging, at least for one of the products.[588,589] More recently, very potent virus-neutralizing monoclonal antibodies directed at the pentamer complex and individual components of this viral envelope complex (gH, gL, UL128) and gB have been characterized, and at least a combination of two of these have been studied in early-phase clinical trials, which showed some beneficial effects on reduction of frequency of CMV-associated disease.[590]

Vaccines for the Prevention and Treatment of HCMV Infections

The search for prophylactic and therapeutic vaccines for HCMV infections has been ongoing for over 4 decades.[591] The first candidate vaccine that provided some evidence that vaccination against HCMV could modify the course of HCMV infections in SOT recipients was reported by Plotkin and colleagues.[592] This vaccine (Towne) was derived from a clinical isolate extensively passaged in tissue culture and described as attenuated, although at the time there was little understanding of determinants of viral virulence. The Towne vaccine protected vaccinated volunteers from low-dose wild-type, low-passage virus (clinical isolate) challenge, but when the challenge dose was increased only minimally, protection induced by the vaccine waned.[593] This vaccine has subsequently been tested in HCMV-seronegative women and failed to prevent virus acquisition in vaccinated women.[594] Several reasons have been proposed for the limited activity of this vaccine, but most argue that is overly attenuated and replicates poorly in vaccinated individuals. However, the development of replication-attenuated HCMV vaccines remains an important strategy in the quest for an efficacious vaccine.[595–598] More recently, a replication-defective HCMV that expresses a complete set of viral genes associated with in vivo pathogenicity is in advanced preclinical development.

In contrast to limited studies of replication-competent or replication-defective HCMV vaccine candidates, or both, subunit (viral protein) and DNA-based candidate vaccines have been evaluated in clinical trials. The most widely studied was an adjuvanted viral envelope glycoprotein (gB) vaccine. An initial evaluation of this candidate vaccine in seronegative women suggested transient efficacy in prevention of infection.[599] A follow-up study in normal adolescent females failed to provide evidence of prevention of infection as compared to placebo.[600] At best, the results from these studies demonstrated immunogenicity of the vaccine formulation, but limited evidence of protective immunity. Several explanations have been proposed for the limited protective activity of this candidate vaccine, including the use of a nonnative viral protein as an immunogen, the potentially transient nature of the protective responses, and the lack of a meaningful surrogate marker of protection in these populations that could be correlated with responses to this vaccine. The same formulation has also been studied in SOT recipients with perhaps more encouraging results.[601] In SOT (renal and liver) recipients, pretransplantation vaccination with the adjuvanted HCMV gB vaccine resulted in increased antibody titers to gB in both HCMV-seronegative and HCMV-seropositive recipients, and led to a decreased duration of HCMV viremia and use of GCV as compared to recipients of the placebo.[601] Further development of this candidate vaccine has not progressed, however. A second candidate vaccine based on DNA immunization has been tested in clinical trials. This candidate vaccine includes plasmid DNA encoding two immunogenic HCMV proteins, gB and pp65, and has been shown to be immunogenic in normal individuals and in HSCT recipients. Results from early-phase trials suggested some protective activity in HSCT.[602–604] Other candidate vaccines currently under study

include modified vaccinia Ankara virus–vectored HCMV glycoprotein-based and peptide-based vaccines.[605–607]

Vaccines that can modify the natural history of congenital HCMV infection and reduce morbidity and mortality, including long-term graft loss associated with HCMV infections, must be viewed as a priority for both government and industry support. Significant hurdles in our understanding of the biology of this virus remain, and it can be argued that the empiricism of previously successful approaches for vaccine development may not be applicable to HCMV. Perhaps this is most clearly reflected in the variability of definitions of protective efficacy that have been suggested in published studies. Hopefully, clinically meaningful end points of protection can be established for the evaluation of vaccines in each target population for future clinical trials, such as those recently described to aid in antiviral drug development and evaluation in transplantation patients.[313]

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138 Epstein-Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus–Associated Malignant Diseases, and Other Diseases)

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SHORT VIEW SUMMARY

Definition
- Infectious mononucleosis is a clinical syndrome characterized by pharyngitis, fever, lymphadenopathy, and the presence of atypical lymphocytes on a peripheral blood smear. Primary Epstein-Barr virus (EBV) infection is the most common cause of this syndrome.

Virology and Epidemiology
- EBV is a herpesvirus that establishes lifelong latent infection in B lymphocytes.
- Replication occurs in oral epithelium, and infectious EBV is frequently present in the saliva of asymptomatic seropositive individuals.
- EBV is transmitted predominantly through exposure to infected saliva, frequently as a result of kissing.
- Seroprevalence approaches 95% in adults, and EBV is distributed throughout the world.
- In childhood primary EBV infection is usually asymptomatic or a nonspecific illness.
- Frequency of presentation as infectious mononucleosis increases with age to about 50% of primary infections by adolescence.
- EBV is tightly linked with several malignancies, including endemic Burkitt lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disease (LPD).

Microbiology
- EBV is a gamma-1 herpesvirus, genus *Lymphocryptovirus*.
- EBV is a double-stranded DNA virus that is enveloped.
- EBV is also known as human herpesvirus 4.

Clinical Manifestations
- Infectious mononucleosis is generally a self-limited, spontaneously remitting syndrome.
- Complications may occur, including splenic rupture, neurologic manifestations such as encephalitis, autoimmune hemolytic anemia, and mild hepatocellular enzyme elevations.

Diagnosis
- The appearance of nonspecific, heterophile antibodies (immunoglobulin M [IgM] reacting with sheep or horse red blood cells) can distinguish primary EBV infection from other causes of infectious mononucleosis.
- The presence of IgM viral capsid antigen antibodies is closely correlated with acute EBV infection. Heterophile antibodies in a person with clinical infectious mononucleosis is sufficient to establish the diagnosis.
- EBV serology may be helpful in atypical cases and in children, who are frequently heterophile negative.
- Primary human immunodeficiency virus infection is the most important differential diagnostic consideration.
- Serial measurement of EBV viral loads may be useful in the detection of EBV-associated malignancies in immunosuppressed individuals, especially for LPD.

Therapy
- Treatment of mononucleosis is primarily supportive.
- Corticosteroids may be helpful in managing mononucleosis complications such as airway impingement from tonsillar enlargement.
- Antiviral therapy is of no proven benefit in infectious mononucleosis.
- EBV-associated LPD may be treated with decrease of immune suppression, rituximab, or adoptive immunotherapy, depending on the cause (see later).

Prevention
- There is currently no EBV vaccine, but this is an area of active investigation.

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus. Infection with EBV is common, worldwide in distribution, and largely subclinical in early childhood. EBV has been established as the causative agent of heterophile-positive infectious mononucleosis, which occurs most frequently in late adolescence or early adulthood. In addition, EBV is causally associated with the development of malignant diseases, including Burkitt lymphoma, lymphoproliferative disease (LPD), Hodgkin lymphoma, primary central nervous system (CNS) lymphomas in acquired immunodeficiency syndrome (AIDS), and nasopharyngeal carcinoma (NPC), based on seroepidemiologic data and the detection of EBV genomes in these tumors. Some epidemiologic studies describe an association between EBV and autoimmune diseases, particularly multiple sclerosis; however, a causal relationship is not established.

HISTORY

Historical accounts of infectious mononucleosis often attribute the initial description of the disease to Filatov or Pfeiffer, who nearly simultaneously at the end of the 19th century described an illness characterized by malaise, fever, hepatosplenomegaly, lymphadenopathy, and abdominal discomfort.[1,2] This illness came to be known as Drusenfieber (glandular fever); however, without specific techniques with which to establish the diagnosis, the concept of Drusenfieber as a clinical entity fell into disrepute. Between 1910 and 1920 a number of observers reported cases of apparent spontaneous remission of leukemia, with a clinical course that is consistent with resolution of infectious mononucleosis.[3,4] The establishment of infectious mononucleosis as a clinical entity is credited to Sprunt and Evans,[5] who in 1920 described six cases of fever, lymphadenopathy, and prostration that occurred in previously healthy young adults. The authors pointed out the mononuclear lymphocytosis that developed in each of the patients and contrasted the pathologic appearance of these lymphocytes with the uniform lymphocyte morphology observed in children with other infections. Two years later Downey and McKinlay[6] described additional cases of infectious mononucleosis and provided a more detailed morphologic description of the atypical lymphocyte. The recognition of atypical lymphocytosis as a hematologic marker for the disease led to more accurate descriptions of the clinical manifestations of this illness.

A major advance occurred in 1932, when Paul and Bunnell,[7] investigating immunologic mechanisms in serum sickness, unexpectedly encountered high titers of spontaneously occurring sheep red blood cell (RBC) agglutinins (heterophile antibodies) in the sera of patients with infectious mononucleosis.

During the 1940s and 1950s substantial efforts were made to detect a causative agent for infectious mononucleosis. Attempts to culture etiologically related bacteria and viruses from patients with infectious mononucleosis proved unsuccessful. The disease could not be transmitted to animals. Interpretation of experimental attempts to transmit the disease to humans was hindered by the failure to appreciate the widespread occurrence of asymptomatic infection in preadolescents and the absence of a serologic marker of immunity.[8–10]

The identification of EBV followed the description by Burkitt[11] in 1958 of an unusual lymphoma with a predilection for the head and neck. The geographic distribution of this tumor paralleled that of certain mosquito-borne diseases in Africa, and a search for an etiologically related arbovirus was undertaken. Epstein and associates[12] in 1964 described the presence of particles that resembled herpesviruses in tissue cultures of biopsy specimens from patients with Burkitt lymphoma. An indirect immunofluorescent assay for detecting anti-EBV antibodies was developed by Werner and Gertrude Henle,[13] and high titers were detected in patients with Burkitt lymphoma. Additional studies revealed that 90% of American adults had demonstrable EBV antibodies as well.[13] The development of infectious mononucleosis in a technician in the Henles' laboratory, from whom sequentially obtained sera were analyzed for EBV antibody, suggested that acute EBV infection may be associated with this illness.[14] Large-scale epidemiologic studies[15–18] showed that heterophile-positive infectious mononucleosis occurred in patients without preexisting EBV antibody, and conversely, heterophile-positive infectious mononucleosis was always accompanied by acquisition of EBV antibodies. These epidemiologic studies indicated that subclinical EBV infection also occurred. With specific antibody tests for EBV, it became apparent that 10% to 20% of the cases of mononucleosis, of which most were heterophile negative, were caused by other agents, the most frequent of which was cytomegalovirus (CMV). This chapter deals primarily with EBV-induced infectious mononucleosis and EBV-associated malignancies.

DESCRIPTION OF EPSTEIN-BARR VIRUS

Physical Properties

EBV, or human herpesvirus 4, is a gamma-1 herpesvirus. Like the other members of the Herpesviridae family, EBV has a double-stranded DNA genome encased in an icosahedral protein nucleocapsid surrounded by a lipid envelope embedded with viral glycoproteins. Herpesviruses also have an amorphous protein layer, the tegument, which lies between the capsid and envelope. The B95-8 laboratory strain of EBV, the first herpesvirus genome sequenced, was found to have a 12-kilobase (kb) deletion and the wild-type EBV genome, which is approximately 172 kb in size and encodes about 90 proteins and 25 microRNAs (miRNAs).[19,20]

Life Cycle

Primary infection with EBV results from exposure to the oral secretions of seropositive individuals through kissing, sharing of food, or other intimate contact. It is generally accepted that EBV infection spreads to B lymphocytes after initial productive (lytic) infection of oral epithelial cells. Direct evidence of epithelial infection in immunocompetent hosts has been difficult to obtain. Tonsillar biopsies from patients with primary EBV infection did not reveal any infected epithelial cells, but infected lymphocytes were readily seen.[21,22] EBV undoubtedly has clinically significant tropism for epithelial cells, as is seen in NPC and oral hairy leukoplakia. It remains possible that significant infection of oral epithelial cells occurs in nontonsillar sites or that an initial round of lytic replication precedes spread to the B-cell compartment and the onset of symptoms.[23,24] Indeed, there is evidence that many herpesviruses transiently infect epithelial cells on multiple occasions before they establish lifelong infection within the appropriate latency cell type.[25] Studies using banked samples have demonstrated that during EBV's 30- to 50-day incubation period, the virus is detectible in low levels in the blood. Symptoms of infectious mononucleosis coincide with a rapid rise in serum EBV viral loads and increased detection in saliva.[26] Infected B lymphocytes incite an intense cytotoxic T-cell response, and these T cells constitute the atypical lymphocytosis characteristic of primary EBV infection.[27,28] In healthy individuals most infected B lymphocytes are cleared through immune surveillance, but between 1 and 50 B cells per million remain quiescently infected and serve as the reservoir for lifelong infection of the individual.[29,30] Thus EBV shares the properties of lifelong latency and persistence with other members of the herpesvirus family. In contrast to that of alphaherpesviruses (herpes simplex virus [HSV] and varicella-zoster virus [VSV]), shedding of infectious EBV particles into the saliva from periodic reactivation of latently infected cells is entirely asymptomatic. This shedding occurs in otherwise healthy persons but is more frequent in immunosuppressed hosts (Table 138.1).

TABLE 138.1 Frequency of EBV Shedding

POPULATION DESCRIPTION	OROPHARYNGEAL SHEDDING RATE (RANGE)	REFERENCE
EBV-seronegative individuals	0	89
Seropositive healthy adults	12%–25%	85–87, 89–91
Solid-tumor patients	27%	86, 87
HIV-1–infected individuals	50%	88
Renal transplant recipients	56%–70%	85, 87
Infectious mononucleosis patients	50%–100%	89–91, 348
Critically ill leukemia or lymphoma patients	74%–92%	86, 87

EBV, Epstein-Barr virus; *HIV,* human immunodeficiency virus.

The host range of the virus is limited. In vitro cultivation of the virus has been described primarily in B lymphocytes and also in nasopharyngeal epithelial cells of humans and certain nonhuman primates.[31] EBV binds to its receptor, the cluster of differentiation 21 (CD21) molecule, through an interaction with its major envelope glycoprotein, gp350. CD21 or complement receptor 2 transduces signals important for B-lymphocyte proliferation and can also be expressed by follicular dendritic cells and nasopharyngeal epithelial cells.[32–38] Another EBV glycoprotein, gp42, binds class II major histocompatibility complex (MHC) molecules, which serve as coreceptors for infection of B cells.[39–43] Gp42 also promotes B-cell infection by forming a heterotrimeric complex with the gH/gL EBV glycoproteins, masking a motif on gH that is important for infection of epithelial cells.[44] EBV virions released from infected B lymphocytes contain lower levels of gp42 and infect epithelial cells more efficiently than virions derived from epithelial cells. This reciprocal tropism is proposed to enhance EBV shuttling between B lymphocytes and the oral epithelium.[45]

Latent Infection and Growth Transformation

Epstein-Barr Virus Drives B Cells to Proliferate

After infection with EBV, B lymphocytes enter the cell cycle and proliferate continuously in a process termed *transformation* or *immortalization;* these cells can be propagated in vitro indefinitely.[46] This ability of EBV to convert peripheral blood B cells into immortalized lymphoblastoid cell lines is widely used in genomic studies as a means of preserving DNA samples from volunteer donors for future use.[47] In vivo EBV-driven B-cell proliferation is observed during infectious mononucleosis, in which it probably serves to expand rapidly the pool of infected B lymphocytes. These B lymphocytes are usually rapidly cleared from the circulation.[48–51] However, in the absence of an intact immune response, EBV infection can result in life-threatening LPD.[52,53] The growth-transforming properties of EBV can act in concert with genetic and environmental cofactors to cause malignant diseases in immunocompetent hosts as well.[54,55]

Epstein-Barr Virus Genome Is Circularized in Latent Infection and Expresses a Subset of Viral Genes

EBV infection of B lymphocytes is characterized by a state of viral latency in which the genome circularizes in the nucleus and is replicated

TABLE 138.2 Patterns of EBV Latent Gene Expression

				EBV-ASSOCIATED MALIGNANT DISEASES				
		Acute Infection	Healthy Carrier	Latency III	Latency II		Latency I	
EBV GENE PRODUCT	FUNCTION	*IM*	*PBB*	*LPD*	*HL*	*NPC*	*GC*	*BL*
EBNA1	EBV genome maintenance	+	?	+	+	+	+	+
EBNA2	Activates expression of EBV/host genes	+	—	+	—	—	—	—
EBNA3s[a]	Represses p16/INK4A tumor suppressor expression	+	—	+	—	—	—	—
EBNALP	Coactivates with EBNA2	+	—	+	—	—	—	—
LMP1	Mimics CD40 signaling	+	—	+	+	+	—	—
LMP2	Mimics BCR signaling	+	+	+	+	+	±	—
miRNAs	Block expression of host RNAs	+	?	+	+	+	+	+
EBERs	Noncoding, highly expressed RNAs	+	+	+	+	+	+	+

[a]Includes EBNA3A, EBNA3B, and EBNA3C.
BCR, B-cell receptor; *BL,* Burkitt lymphoma; *EBERs,* EBV-encoded RNAs; *EBNA,* EBV nuclear antigen, *EBV,* Epstein-Barr virus; *GC,* gastric cancer; *HL,* Hodgkin lymphoma; *IM,* infectious mononucleosis; *LMP,* latent infection membrane protein; *LPD,* lymphoproliferative disease; *miRNAs,* micro–RNAs; *NPC,* nasopharyngeal carcinoma; *PBB,* peripheral blood B cell; *PCNSL,* primary central nervous system lymphoma.
Note: Because EBV-positive gastric cancers sometimes express LMP2 and, rarely, LMP1, these tumors have been classified as latency I or II by different experts.

as an episome in concert with host chromosomes by cell enzymes. The infection is latent in the sense that viral particles are not being produced, but it is anything but quiescent. Limited viral gene expression persists, and these genes exert effects on the infected cell. In vitro latent infection of B lymphocytes with EBV is characterized by the expression of latent infection membrane proteins 1 and 2 (LMP1 and LMP2), six EBV nuclear antigens (EBNAs), and two small, nuclear, noncoding RNAs (EBV-encoded RNAs [EBERs]) that are transcribed by RNA polymerase III (Table 138.2).[56] Additional EBV transcripts have been detected in latent infection and are termed *complementary strand transcripts* or *Bam*H1 A rightward transcripts. Translation of these transcripts into proteins has not been shown, but they appear to serve as precursors for two of three clusters of EBV-encoded miRNAs whose role in EBV biology remains unclear.[57,58] Recombinant reverse genetic analysis has determined that only LMP1, EBNA1, EBNA2, EBNA3A, EBNA3C, and EBNALP are critical for B-cell growth transformation.[56] The mechanisms by which these EBV gene products promote B-lymphocyte growth have been the subject of intense investigation.

Functions of Epstein-Barr Virus Genes Expressed in Latent Infection

After the virus gains entry to susceptible B lymphocytes, EBNA2 and EBNALP are the first proteins expressed. EBNA2 is an acidic transactivator that acts as the major switch to turn on latent virus gene expression and several B-cell gene products (including c-MYC, c-FGR, CD21, and CD23). It has no intrinsic sequence-specific DNA binding capacity but rather is targeted to promoters by binding to a host DNA binding protein RBP-Jκ (also called CBF1 or CSL), a downstream component of the Notch signaling pathway.[59,60] By an incompletely understood mechanism, EBNALP cooperates with EBNA2 to activate expression of the remaining nuclear proteins and LMP1 and LMP2.[61] *LMP1* is the major EBV-encoded oncogene, and its expression in transgenic mice results in B-cell lymphomas.[62,63] It constitutively activates signaling pathways that mimic the growth and survival signals given to B cells by $CD4^+$ T lymphocytes through the CD40 surface glycoprotein. LMP1 sends this signal through its cytoplasmic tail, which binds a set of second-messenger proteins similar but not identical to those used by CD40.[64,65] Unlike CD40, LMP1 does not require the presence of ligand to form patches in the cell membrane but self-associates constitutively, approximating its cytoplasmic tails to activate signaling.[66] This results in the activation of nuclear factor κB (NF-κB), c-JUN, upregulation of adhesion molecules (intercellular adhesion molecule 1, lymphocyte function-associated antigen 1 [LFA-1], and LFA-3), cytokine production, B-cell proliferation; and induction of an antiapoptotic state.[48,55] A second EBV latent membrane protein, LMP2, mimics another signal necessary for B-cell survival.[67] By interacting with signaling molecules of the B-cell receptor (BCR), LMP2 mimics BCR engagement by constitutive patching in the membrane in a manner analogous to LMP1. LMP2 probably also interferes with normal signaling through the BCR by antigenic stimulation to inhibit activation of lytic viral replication (discussed subsequently). Of interest, LMP2 is not necessary for EBV-mediated outgrowth of B cells in vitro but is probably a critical component of the viral strategy in vivo. The nuclear protein EBNA1 acts to promote the replication of the viral genome by the host machinery when the virus is in the latent, episomal state and to ensure proper segregation of the EBV genome to both daughter cells. The EBNA3 proteins also interact with the recombination signal binding protein (RBP)-Jκ DNA binding protein and promote B-lymphocyte growth and survival by silencing expression of the $p16^{INK4}$ and $p14^{ARF}$ tumor suppressor gene products.[68,69] The function of the highly expressed, noncoding EBERs is incompletely understood.

Patterns of Epstein-Barr Virus Gene Expression Vary in Different Malignancies

EBV-associated malignant diseases are exclusively associated with latent infection and latent gene expression. Three general patterns of expression of EBV-encoded proteins have been observed in association with latency (see Table 138.2).[48,49] Expression of all latent genes is seen in LPD in immunosuppressed hosts, in primary CNS lymphoma of patients with AIDS, and during primary EBV infection (infectious mononucleosis), and this program of gene expression is often referred to as *latency III*.[54] EBV-associated NPCs, Hodgkin lymphoma, and T-cell lymphomas exhibit a more restricted pattern of EBV gene expression (latency II) that includes LMP1, LMP2, EBNA1, and the EBERs and EBNA1.[70–72] In Burkitt lymphoma (latency I) only EBERs and EBNA1 are expressed.[54] The more restricted patterns of latent gene expression in some tumors are probably in part the result of the intense immune response against viral proteins.

Lytic Infection

Latent infection can be activated to lytic infection by stimulation of host B cells by certain chemicals, calcium ionophores, or antibodies to surface immunoglobulin.[73] The physiologic signals that reactivate EBV lytic replication are unknown, but signaling through the B-cell receptor after antigenic stimulation is a possible scenario. After this inciting event, two EBV-encoded transcriptional activators are expressed: *BZLF1* and *BRLF1*. Expression of these immediate early genes leads to a cascade of events that culminate in the production of early EBV gene (early antigen [EA]) products responsible for viral replication (e.g., thymidine

kinase, DNA polymerase) and late (structural) genes of the virus, including viral capsid antigens (VCAs).[74] Lytic infection produces EBV virions and can cause host cell death.

EPIDEMIOLOGY

Serum Antibody Prevalence

Antibodies to EBV have been found in all population groups studied, and most studies have shown no predilection for either gender. Antibodies are acquired earlier in life in developing countries than in industrialized countries, but by adulthood 90% to 95% of most populations have demonstrable EBV antibodies.[75,76] In the United States and in Great Britain EBV seroconversion occurs before the age of 5 years in about 50% of the population.[76–78] A second wave of seroconversion occurs midway through the second decade of life. EBV seroconversion may occur at a younger average age in the southern United States than in other areas of that country.[79] Lower socioeconomic groups have a higher EBV antibody prevalence than more affluent age-matched control groups. In the United States EBV antibody prevalence among individuals aged 6 to 19 years decreased from 72% in 2003–04 to 65% in 2009–10. The change was mainly due to decrease in EBV antibodies among non–Hispanic white individuals.[80]

Two strains of EBV have been defined on the basis of viral gene sequences expressed during latency and their ability to transform B lymphocytes.[73] The strains (type 1 [A] or 2 [B]) are not distinguishable serologically, making estimates of their distribution challenging. Available epidemiologic data show a high rate of type 1 EBV infection worldwide, whereas the prevalence of type 2 EBV infection (or coinfection) may be less than 10% in the developed world.[81] Type 2 EBV infection is more prevalent in sub-Saharan Africa and Papua New Guinea and observed more frequently in immunocompromised people.[82]

Incidence of Infection

Clinically apparent infectious mononucleosis is more common in populations in which primary EBV exposure is delayed until after the first decade of life. The disease is diagnosed most frequently among adolescents of higher socioeconomic groups in industrialized countries.[83] The incidence of infectious mononucleosis in a large epidemiologic study in the United States was 45.2 cases per 100,000 per year and was highest in the 15- to 24-year-old age group.[84] The incidence was the same for women as for men, but the peak age-specific incidence occurred 2 years earlier in women. No clear seasonal incidence has been noted.

Methods of Spread

The virus persists in the B-cell compartment for the life of the infected host and can be cultured from throat washings from 10% to 20% of healthy adults, from 50% of kidney transplant recipients, and from greater proportions of those critically ill with leukemia or lymphoma (see Table 138.1).[85–87] Approximately 50% of men with human immunodeficiency virus type-1 (HIV-1) infection who have sex with men shed EBV in oropharyngeal secretions.[88] Low titers of EBV are present in throat washings of persons with infectious mononucleosis.[89–91] Susceptible roommates of students with infectious mononucleosis or with inapparent EBV infection have EBV seroconversion no more frequently than the general susceptible college population.[17,79] Only 6% of those with infectious mononucleosis cite previous contact with another case of infectious mononucleosis.[84] EBV DNA or protein, or both, have also been identified in parotid duct and uterine cervical epithelia, although the implications of this distribution are unclear with respect to viral transmission.[92,93]

EBV, like other herpesviruses, is relatively labile in the laboratory, and the virus has not been recovered from environmental sources, including fomites. These data suggest that EBV is a widespread agent that is not particularly contagious and that most cases of infectious mononucleosis are probably contracted by intimate contact between susceptible individuals and asymptomatic shedders of EBV. Among young adults, spread of the virus may be facilitated by the transfer of saliva with kissing.[94,95] EBV infection has been linked to sexual intercourse; however, other studies have found that kissing with coitus conferred no additional risk of EBV infection compared with kissing without coitus.[96,97] Serologic evidence suggests that the virus may also be spread among susceptible individuals within families.[98,99] In malaria-endemic regions, EBV infection is usually acquired within the first 6 months of life, and this has been linked to high EBV loads in breast milk.[100] Although EBV has been detected in breast milk from other parts of the world,[101] transmission by this route appears to be very inefficient outside of sub-Saharan Africa. EBV has also been spread via blood transfusion and after open heart surgery as the postpump perfusion syndrome.[102] Most postpump perfusion infectious mononucleosis is, however, heterophile negative and attributable to CMV.

Although several apparent epidemics of infectious mononucleosis have been described, these reports have not been substantiated with EBV serologic data and have lacked rigorous epidemiologic, clinical, or laboratory support. Some of these have resulted from errors in the performance of Monospot tests.[103] On the basis of the previously discussed information, true epidemics of infectious mononucleosis are unlikely to occur.

Public Health Impact

College and military populations experience the highest morbidity from infectious mononucleosis, although cases occur in other groups as well. Infectious mononucleosis accounted for 5% of all hospitalizations of University of Wisconsin students, with an incidence rate of 450 admissions per 100,000 students per year. Other American universities have reported similar incidence rates.[104,105] Approximately 12% of susceptible college students undergo EBV seroconversion yearly.[17,18] Many of these infections are subclinical (see subsequent discussion).[17,79] Although primary EBV infection may be clinically apparent in only about 10% of military cases, infectious mononucleosis ranked fourth as the cause of days lost because of illness in Army personnel.[106,107] Detailed information about the impact of infectious mononucleosis on the general population is not available because infectious mononucleosis is not a reportable disease in most states. However, morbidity from infectious mononucleosis likely is generally underestimated because a specific diagnosis may not be made and the nonspecific illness can be attributed to a variety of other causes.

PATHOGENESIS

Host Immune Response

Immune Response Controls Epstein-Barr Virus Infection and Is the Cause of Mononucleosis Symptoms

EBV presents a formidable challenge to the immune system. At the height of acute infection up to 20% of peripheral blood B lymphocytes may express EBNA, and 0.005% to 0.5% of circulating mononuclear cells are capable of forming continuous cell lines if cultured in vitro.[108,109] The immune response to EBV-infected transformed lymphocytes is complex and involves both humoral and cell-mediated immune mechanisms.[27,110] An intact immune response is critical to prevention of the unchecked proliferation of these cells as seen in LPD but is also responsible for most of the symptoms of infectious mononucleosis. The increase in prevalence in symptomatic acute EBV infection with age of seroconversion is probably the result of differences in the immune responses of different age groups.

Cellular Response to Epstein-Barr Virus

The cellular immune response to EBV is complex and well integrated and includes $CD8^+$ and $CD4^+$ cytotoxic T-lymphocytes (CTLs) and natural killer (NK) cells.[28,111–114] The massive atypical lymphocytosis of infectious mononucleosis is composed primarily of antigen-stimulated $CD8^+$ cytotoxic T cells. In one study 40% of circulating $CD8^+$ T cells were reactive against a single EBV epitope.[115] These lymphocytes probably produce most of the signs and symptoms of infectious mononucleosis through the abundant production of cytokines, including tumor necrosis factor, interleukin-1 (IL-1), and IL-6.[116] During acute infection $CD8^+$ T cells specific for lytic antigens predominate, but with convalescence, a shift occurs toward cells that recognize latent proteins, particularly the EBNA3 proteins.[27,115,117] T cells reactive against EBV latent proteins are sufficiently numerous that unselected mononuclear cells from EBV-immune adults suppress the outgrowth of autologous EBV-infected B lymphocytes in vitro.[114] An expansion of EBV-specific $CD4^+$ lymphocytes has also been

described in infectious mononucleosis but is small in magnitude, and its significance in containing acute EBV infection is unclear.[118,119]

Humoral Response to Epstein-Barr Virus

The humoral immune response to EBV has been extensively studied, primarily as a means to diagnose EBV infection (see "Laboratory Diagnosis" section for detailed discussion). In general, specific antibodies directed against EBV lytic antigens (VCA and EA) are demonstrable in most patients with infectious mononucleosis. By contrast, antibody responses to the latency-associated EBV nuclear antigens (EBNA1, EBNA2, EBNA3s, and EBNALP) do not develop until convalescence.[27] The significance of any of these antibody responses to containment of EBV infection is not established; however, antibodies to EBV surface glycoproteins have been shown to prevent experimental EBV infection.[120–122]

Heterophile Antibodies Generated Early During Primary Epstein-Barr Virus Infection Have No Known Role in Pathogenesis

For unclear reasons, primary EBV infection is associated with the synthesis of large amounts of antibodies reactive against antigens found on sheep, horse, and beef red cells. These so-called heterophile antibodies are a heterogeneous group of predominantly immunoglobulin M (IgM) antibodies that do not react with specific EBV proteins.[123] Detection of these antibodies in sera of patients with mononucleosis syndromes predicts acute EBV infection with high specificity and adequate sensitivity and is discussed in the section "Laboratory Diagnosis." No good correlation is found between the heterophile titer and the severity of the illness, and no clearly defined role exists for heterophile antibodies in the pathogenesis of EBV disease or in immune clearance of the virus.

Epstein-Barr Virus Immune Evasion

EBV has evolved multiple strategies to elude this aggressive immune response. The EBV BCRF1 protein shares 70% homology with the cytokine IL-10. This EBV protein is functional and is thought to mimic IL-10 inhibition of interferon-γ (IFN-γ) synthesis by mononuclear cells in the peripheral blood. Thus BCRF1 expression during lytic infection is expected to promote a shift toward Th2 differentiated $CD4^+$ effectors that can provide B-cell help but do not promote the $CD8^+$ responses needed to kill EBV-infected cells.[124,125] Another EBV protein, *Bam*H1 rightward frame 1 (BARF1), can function as a soluble receptor for colony-stimulating factor 1 and may interfere with the ability of this cytokine to enhance expression of interferon-α (IFN-α) from monocytes.[126] EBV also encodes a Bcl2 homologue that is expressed during lytic replication and may act to prevent apoptosis of the host cell.[127] Finally, the virus has evolved a strategy for ensuring its persistence in the memory B-cell compartment. After acute infection resolves, most latent proteins are no longer expressed to circumvent the strong immune pressure exerted against EBV. However, in any cycling cell, EBV must express EBNA1 to ensure that its genome is replicated. To prevent targeting of this key protein, EBNA1 contains a sequence of expanded glycine-alanine repeats—not necessary for its function in genome maintenance—capable of inhibiting proteasomal processing of the protein.[128] Without this processing, EBNA1 peptides cannot be presented on class I MHC molecules, and cells that express EBNA1 may elude immune surveillance.

Histopathologic Findings

Because biopsies are rarely obtained in patients with uncomplicated infectious mononucleosis, most data come from pathologic examination of tissues obtained from fatal cases or from cases with atypical features in which biopsy specimens were obtained for diagnostic evaluation. During the acute phase of the illness lymph nodes throughout the body are moderately enlarged. Individual nodes reveal increased numbers of enlarged, moderately active lymphoid follicles. Germinal centers are also enlarged, with cores that contain blast cells, histiocytes, and lymphocytes. Although the reticulin framework remains intact, invasion by the hyperplastic pulp makes its borders less distinct.[129] In studies of spleens obtained at autopsy or at surgery after rupture, the organ is usually two to three times its normal weight.[130] The splenic capsule and trabeculae are edematous, thinned, and invaded by lymphoid cells. Most of the increased splenic size is the result of hyperplasia of the red pulp. Throughout the red pulp, pleomorphic blast cells are evident. The spleen is often congested with focal, particularly subcapsular, hemorrhages. The white pulp is relatively normal. Tonsillar biopsy specimens obtained during the course of mononucleosis reveal intense proliferation with numerous mitoses.[131] Bone marrow aspirate and biopsy specimens are often strikingly normal when compared with the florid changes noted in peripheral blood. Biopsy specimens are usually normocellular to mildly hypercellular. Small granulomas may be present, but these are not specific for mononucleosis and have no prognostic significance.[132,133]

Changes in hepatic histologic features are usually mild. Hepatocytes show minimal swelling and vacuolization. Pleomorphic lymphocytic and monocytic portal infiltration is usually evident. Bile ducts may be minimally swollen, but frank biliary stasis is rare.[134,135] A number of histopathologic changes have been reported in the nervous system in fatal cases of infectious mononucleosis.[131,136,137] These changes include neuronal degeneration, perivascular cuffing, perivascular hemorrhage, and astrocytic hyperplasia. Little mononuclear infiltration may be present despite demonstrable degenerative changes in the neurons of the cortex, basal ganglia, cerebellum, or spinal cord.

CLINICAL MANIFESTATIONS

Infectious Mononucleosis (Primary Infection)

Spectrum of Illness

EBV induces a broad spectrum of illness in humans. Classic or typical infectious mononucleosis is an acute illness characterized clinically by sore throat, fever, and lymphadenopathy; serologically by the transient appearance of heterophile antibodies; and hematologically by a mononuclear leukocytosis that consists, in part, of atypical lymphocytes (Table 138.3). An individual case may have most but not necessarily all the aforementioned characteristics. Specific serologic tests for EBV infection indicate that infection results in a spectrum of clinical manifestations. Attempts to exclude cases that fail to meet the classic criteria for infectious mononucleosis result in artificial and often misleading distinctions.

The age of the patient has a profound influence on the clinical expression of EBV infection. In children primary EBV infection is often asymptomatic. Young children may be more likely to exhibit rashes, neutropenia, or pneumonia than individuals with primary EBV infection at an older age.[138] Clinically apparent infections in very young children are heterophile negative in about one-half of the cases.[139] The proportions of clinically apparent disease and of heterophile-positive cases increase with age. By 4 years of age 80% of children with primary EBV infection are heterophile antibody positive.[140] During the course of the illness 90% of the adolescents with clinically apparent infectious mononucleosis should be heterophile positive.

In patients of college age the ratio of clinically apparent to inapparent EBV infection ranges from 1:3 to 9:1.[17,79,96] In two prospective series a much higher rate (89%) of symptomatic infection was observed when students were evaluated at 8-week intervals compared with 25% when students were evaluated 3 years after enrollment.[96,97] In military recruits this ratio has been as low as 1:10.[107] Because of previously existing immunity, the disease is less common in older patients. When it does occur, however, clinical and serologic manifestations are similar to those found in adolescents.[141] In general, EBV infection is inapparent or is a self-limited illness that lasts 2 or 3 weeks. In rare cases the disease can be devastating and can be accompanied by severe prostration, major complications, and even death,[142] as discussed subsequently.

Symptoms and Signs

Most cases of infectious mononucleosis consist of the clinical triad of sore throat, fever, and lymphadenopathy (Table 138.4). Epidemiologic studies suggest that the incubation period of acute infectious mononucleosis is 30 to 50 days. Viral shedding in oral secretions has been observed for up to 36 days before onset of symptoms,[90,96,143] although a recent study found that oral virus could be detected only 1 week before symptoms.[26] The onset may be abrupt, but often several days of prodromal symptoms can be elicited, including chills, sweats, feverish sensations, anorexia, and malaise. Retro-orbital headaches, myalgias, and feelings of abdominal fullness are other common prodromal

TABLE 138.3 Manifestations of Epstein-Barr Virus–Induced Infectious Mononucleosis

Clinical
Fever
Sore throat
Lymphadenopathy
Hematologic
>50% mononuclear cells
>10% atypical lymphocytes
Serologic
Transient appearance of heterophile antibodies
Permanent emergence of antibodies to Epstein-Barr virus

TABLE 138.4 Signs and Symptoms of Primary EBV and Infectious Mononucleosis

	INFECTIOUS MONONUCLEOSIS[a]		PRIMARY EBV[b]
Symptom	**Percentage**	**Range (%)**	**Percentage (%)**
Sore throat	82	70–88	83
Malaise	57	43–76	59
Headache	51	37–55	42
Anorexia	21	10–27	39
Myalgias	20	12–22	36
Chills	16	9–18	
Nausea	12	2–17	
Abdominal discomfort	9	2–14	8
Cough	5	5	
Vomiting	5	5	
Arthralgias	2	2	
Sign	**Percentage**	**Range (%)**	**Percentage (%)**
Lymphadenopathy	94	93–100	68
Pharyngitis	84	69–91	
Fever	76	63–100	38
Splenomegaly	52	50–63	
Upper eyelid edema	50	<20–50	
Hepatomegaly	12	6–14	
Palatal enanthem	11	5–13	
Jaundice	9	4–10	
Rash	20	0–23	

[a]Data for Infectious Mononucleosis from references 104, 144, 145, 147, 487.
[b]Primary EBV infection refers to new EBV infection and includes asymptomatic cases, those cases with symptoms but not meeting criteria for infectious mononucleosis, and those meeting criteria (77%) for infectious mononucleosis. From prospective study of primary EBV infection by Balfour.[96]
EBV, Epstein-Barr virus.

symptoms. The most frequent symptom is sore throat, which may be the most severe the patient has experienced.[144,145] Other patients seek medical attention because of prolonged fever or malaise and less frequently because of incidentally encountered lymphadenopathy. Rarely, the first manifestation of illness is one of the complications of infectious mononucleosis described subsequently.

The signs of infectious mononucleosis are summarized in Table 138.4. Fever is present in greater than 90% of patients with infectious mononucleosis. The fever usually peaks in the afternoon with temperatures of 38° to 39° C, although a temperature as high as 40° C is not uncommon. In most cases fever resolves over a 10- to 14-day period. Bilateral upper eyelid edema (the Hoagland sign) occurs only during the first few days of illness and has been reported in up to 50% of cases in some series[145,146] but less frequently in others.[147] Tonsillar enlargement is usually present, occasionally with tonsils meeting at the midline. The pharynx is erythematous, with an exudate in about one-third of cases. Palatal petechiae may be seen in 25% to 60% of cases but are not diagnostic of infectious mononucleosis. The petechiae are usually multiple, are 1 to 2 mm in diameter, occur in crops that last 3 to 4 days, and are usually seen at the junction of the hard and soft palate.[148] However, palatal petechiae are not unique to infectious mononucleosis and can also be observed in rubella or accompanying group A streptococcal pharyngitis. Cervical adenopathy, usually symmetrical, is present in 80% to 90% of patients. Posterior adenopathy is most common, but submandibular and anterior adenopathies are quite frequent as well, and axillary and inguinal adenopathies also occur. Individual nodes are freely movable, are not spontaneously painful, and are only mildly tender to palpation. The results of examination of the lungs and heart are usually normal. Abdominal examination may detect hepatomegaly in 10% to 15% of cases, although mild tenderness to fist percussion over the liver is present somewhat more frequently.[144,147] Jaundice is present in approximately 5% of cases.[145] Splenomegaly is present in about one-half of cases if sought carefully over the course of the illness. The splenomegaly is usually maximal at the beginning of the second week of illness and regresses over the next 7 to 10 days. The results of neurologic examination are generally normal, although occasional complications may occur (see subsequent discussion).

Complications

Most patients with infectious mononucleosis recover uneventfully. Complications that occasionally occur have been extensively reported in the literature. Even these complications have generally resolved fully, although rare fatalities have been reported.

Dermatologic Complications

Rash may accompany infectious mononucleosis and may be macular, petechial, scarlatiniform, urticarial, or erythema multiforme–like. Historically, rash was felt to be rare, occurring in about 5% of patients, whereas the administration of antibiotics, particularly ampicillin was observed to result in a pruritic, maculopapular eruption in 90% to 100% of patients (Fig. 138.1); the rash could appear either during or after cessation of the antibiotic.[149,150] The ampicillin-related rash does not necessarily predict future intolerance to ampicillin.[151,152] More recently, reports have indicated a higher rate of rash of 20% in infectious mononucleosis in the absence of antibiotics, with perhaps little or no further increase in rash incidence after the administration of antibiotics.[153,154] The reasons underlying these differences in rash incidence are unclear.

Acute, painful genital ulcers, typically involving the labia minora (Lipschutz ulcers) can occur in up to 30% of prepubertal or adolescent females during infectious mononucleosis.[146] These lesions are often greater than 1 cm diameter with characteristic purple edges and a necrotic base. They are not sexually transmitted but due to their appearance and location can prompt evaluations of sexual abuse, which can be distressful for patients and families. These lesions may be misdiagnosed as HSV or Behçet syndrome. Lipschutz ulcers remit spontaneously within 6 weeks without scarring. Treatment is supportive.

Primary EBV infection is the most common cause of Gianotti-Crosti syndrome (papular acrodermatitis of childhood).[146] Lesions, which can be asymptomatic or slightly pruritic, are papular and located in a symmetrical distribution on the cheeks, buttocks, and extensor surfaces of extremities and usually resolve within several weeks. Gianotti-Crosti syndrome typically occurs in children age 6 years or younger but can occur in adolescents. Due to the younger age of these patients, many of the typical symptoms of mononucleosis may be absent.

Hematologic Complications

Autoimmune hemolytic anemia occurs in 0.5% to 3% of the patients with infectious mononucleosis.[155,156] Cold agglutinins, almost always of the IgM class, are present in 70% to 80% of cases.[157] Anti-I specificity has been reported in 20% to 70% of cases.[158,159] Most, but not all, cases

FIG. 138.1 Patient with infectious mononucleosis and ampicillin-induced rash. Maculopapular rash extends over the trunk and extremities. Rash frequently has a violaceous hue and is often accompanied by pruritus. *(Courtesy Dr. Stephen Gellis.)*

of autoimmune hemolytic anemia in infectious mononucleosis are mediated by antibodies of this specificity.[160–163] The hemolysis usually becomes clinically apparent during the second or third week of illness and subsides over a 1- to 2-month period.[164] Corticosteroids may hasten recovery in some cases. Hemophagocytic syndrome, a rare complication of EBV infection, is discussed in a subsequent section.

Mild thrombocytopenia is common in infectious mononucleosis. Platelet counts less than 140,000/mm^3 were noted in 50% of patients with uncomplicated infectious mononucleosis in one series.[165] Profound thrombocytopenia with bleeding occurs rarely,[166] but platelet counts less than 1000/mm^3 and deaths from intracerebral bleeding have been reported.[167,168] The mechanism for the thrombocytopenia is not known. The presence of normal or increased numbers of megakaryocytes in the marrow, coupled with reports of antiplatelet antibodies, suggests that peripheral destruction of platelets may occur, possibly on an autoimmune basis.[158,162,169] Corticosteroids have been reported to be beneficial for the thrombocytopenia in some, but not all, cases.[166–168,170] For refractory cases splenectomy may be indicated.[169]

Neutropenia is seen rather frequently in uncomplicated infectious mononucleosis. The neutropenia is usually mild and self-limiting, although deaths associated with bacterial sepsis or pneumonia, or both, have been reported.[171–178] Anaerobic sepsis without associated granulocytopenia, presumably of pharyngeal origin, has also been reported.[179]

Splenic Rupture

Splenic rupture is a rare but dramatic complication of infectious mononucleosis. Lymphocytic infiltration of the capsule, trabeculae, and vascular walls, coupled with rapid splenic enlargement, predisposes the organ to rupture. The incidence of rupture is highest in the second or third week of illness but may be the first sign of infectious mononucleosis. Abdominal pain is uncommon in infectious mononucleosis,[180] and splenic rupture must be strongly considered whenever abdominal pain occurs. The onset of this pain may be insidious or abrupt. Pathologic examination of some ruptured spleens has revealed subcapsular hematomas that suggest that rupture may be preceded by intermittent subcapsular bleeding. The pain, usually in the left upper quadrant, may radiate to the left scapular area. Left upper quadrant tenderness to palpation, with or without rebound tenderness, is usually present along with peritoneal signs or shifting dullness. In rare cases splenic rupture is unaccompanied by pain and is manifested as shock. Laboratory findings include a falling hematocrit and in some cases an elevated left hemidiaphragm. The abdominal catastrophe may reverse the usual differential count of infectious mononucleosis and evoke a neutrophilia. Confirmatory findings should not be awaited if splenic rupture is suspected. Prompt splenectomy is the treatment of choice, although nonoperative observation and splenorrhaphy have a role in the management of selected patients with subcapsular splenic hematoma.[181,182] Because a history of trauma may be elicited in about one-half the cases of splenic rupture,[183] elimination of contact sports, attention to constipation, and caution in splenic palpation are prudent measures for at least the first month after diagnosis (see "Therapy" section).

TABLE 138.5 Neurologic Complications of Infectious Mononucleosis

Encephalitis[185–189]
Meningitis[185]
Guillain-Barré syndrome[189]
Optic neuritis[191]
Retrobulbar neuritis[192]
Cranial nerve palsies[189]
Mononeuritis multiplex[193]
Brachial plexus neuropathy[194]
Seizures[185,189]
Subacute sclerosing panencephalitis[195]
Transverse myelitis[196]
Psychosis[197]
Demyelination[198]
Hemiplegia[199]

Neurologic Manifestations

Neurologic complications, which occur in less than 1% of the cases, can dominate the clinical presentation (Table 138.5).[184–199] On occasion, these neurologic signs can be the first or only manifestation of infectious mononucleosis. In many cases the heterophile antibody determination is negative, atypical lymphocytes may be low in number or delayed in appearance, and the diagnosis must be made by changes in EBV-specific antibodies.[184,185,190] The encephalitis seen with infectious mononucleosis may be acute in onset and rapidly progressive and severe but is usually associated with complete recovery. The encephalitis is commonly manifested as a cerebellitis but may also be global.[186–188] The clinical presentation may also resemble that of aseptic meningitis. In both encephalitis and meningitis changes in the spinal fluid are mild. The opening pressure is normal or slightly elevated. A predominantly mononuclear pleocytosis may be present, with most cell counts much less than 200/mm.^{3} Atypical lymphocytes have been seen in the cerebrospinal fluid (CSF) in a number of cases. The protein level is usually normal to mildly elevated, and the glucose concentration is usually normal. Low titers of EBV VCA can be found in the CSF.[189] Cases of Guillain-Barré syndrome, Bell palsy, and transverse myelitis have been reported in primary EBV infection.[190] Although neurologic complications are the most frequent cause of death in infectious mononucleosis, the benign outcome of most of these episodes should be emphasized.[200] Eighty-five percent of the patients with neurologic complications recover completely.[184]

Hepatic Manifestations

Hepatic manifestations consist largely of self-limited elevations of hepatocellular enzyme levels, which are present in 80% to 90% of the cases of infectious mononucleosis.[201] Fulminant hepatitis is rarely seen in primary EBV infection and suggests an underlying immunodeficiency. In such cases hepatitis appears to result from infiltration of the liver by EBV-infected lymphocytes and reactive cells rather than EBV infection of hepatocytes.[202,203]

Renal Manifestations

Abnormal urinary sediment is common in acute infectious mononucleosis.[204,205] Microscopic hematuria and proteinuria are the most frequently

noted abnormalities.[206] Overt renal dysfunction is, however, extremely rare, although sporadic cases of acute renal failure in association with infectious mononucleosis have been reported.[207] The renal manifestations of infectious mononucleosis have been hypothesized as usually attributable to interstitial nephritis from renal infiltration by activated T lymphocytes.[207] Renal dysfunction in association with EBV-associated rhabdomyolysis has also been reported, although not all cases of rhabdomyolysis are accompanied by renal dysfunction.[208]

Cardiac Manifestations

Clinically significant cardiac disease is uncommon. Electrocardiographic abnormalities, usually confined to ST-T wave abnormalities, were reported in 6% of the cases in one series.[209] Pericarditis and fatal myocarditis have also been observed.[210,211]

Pulmonary Manifestations

Pulmonary manifestations of infectious mononucleosis are rare.[212–215] Early studies reported the presence of interstitial infiltrates in 3% to 5% of the cases. However, systematic examination for other causes of nonbacterial pneumonias (e.g., *Mycoplasma*) was not carried out in these studies, and whether these infiltrates were related to EBV infection is not clear. Severe pneumonia has, however, been reported, and in at least one instance EBERs, which indicate EBV infection of cells, were found in pulmonary tissue.[216,217] The attribution of pulmonary lesions to EBV infection should be made only after other pathogens have been carefully excluded.

Death

Death from infectious mononucleosis is rare.[200,218] Death may occur as a result of overwhelming EBV infection or from complications of the disease. Neurologic complications of the illness, splenic rupture, and upper airway obstruction are the most frequent causes of death from infectious mononucleosis in previously healthy persons. Deaths from complications associated with granulocytopenia, thrombocytopenia, hepatic failure, and myocarditis have also been reported.[a]

Clinical Course

Most cases of infectious mononucleosis resolve spontaneously over a 2- to 3-week period. The sore throat is usually maximal for 3 to 5 days and then gradually resolves over the course of a week to 10 days. Patients remain febrile for 10 to 14 days, but in the last 5 to 7 days the fever is usually low grade and associated with little morbidity. The prostration associated with infectious mononucleosis is generally more gradual in its resolution. As the illness resolves, patients often have days of relative well-being that alternate with recrudescence of symptoms.

Genetic Disorders Associated With Severe Epstein-Barr Virus Disease

EBV is the only virus to induce B-cell proliferation, which is primarily controlled by NK and T cells. Certain genetic disorders disrupt NK- or T-cell function, resulting in uncontrolled EBV infection. Some of these disorders affect only EBV disease, whereas others affect EBV and other infections.[221] Immunodeficiencies specific for EBV can be either X-linked or autosomal. Genetic testing should be considered in patients with severe EBV disease in the absence of known risk factors; for instance, EBV LPD in the absence of known immune suppression.

X-linked Genetic Predispositions to Severe Epstein-Barr Virus Disease

An X-linked syndrome has been described in which boys, without other evidence of immunodeficiency, develop overwhelming primary EBV infection with demonstrable virus in lymph nodes, spleen, thymus, and other organs.[222,223] This syndrome has been designated *X-linked lymphoproliferative disease 1* (XLP1) and is sometimes referred to as Purtilo syndrome or Duncan disease. Affected boys develop a large proliferation of polyclonal B and T cells in response to primary EBV infection that frequently results in fulminant hepatitis and hemophagocytic syndrome (discussed subsequently). Patients who survive primary EBV infection frequently develop progressive agammaglobulinemia, or they may develop lymphoma within several years after initial infection.[224–229] This disorder was linked to mutations in the signaling lymphocyte activation molecule (SLAM)–associated protein (SAP) gene in 1998.[230] SAP is an important mediator of signal transduction of SLAM and SLAM-related receptors found on T and NK cells.[231,232] Elegant studies of female XLP carriers has demonstrated that the critical defect responsible for EBV susceptibility arises from the inability of $CD8^+$ T cells to recognize antigens presented on B lymphocytes.[233] Thus the specificity of SAP mutation for EBV results from the propensity of this virus to infect and transform B cells.

Mutations in the X-linked inhibitor of apoptosis *(XIAP)* gene results in XLP2; this syndrome is a distinct clinical entity with a high incidence of hemophagocytosis and minimal risk of LPD.[234]

The X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) syndrome is caused by mutations in the magnesium transporter 1 (MAGT1), and can result in B-cell lymphoma.[235] Preliminary data in two patients with XMEN suggests that magnesium supplementation reduces the numbers of EBV-infected cells in blood and therefore may be of clinical benefit in this disease.[236]

Autosomal Genetic Predispositions to Severe Epstein-Barr Virus Disease

Several rare autosomal-recessive genetic disorders have been linked with severe EBV infection.[221] Mutations in the genes responsible for these disorders include *ITK* (IL-2–inducible T-cell kinase), *CD27*, *CORO1A* (coronin actin-binding protein 1A), and *LRBA* (lipopolysaccharide [LPS]-responsive beige-like anchor protein). These disorders can lead to EBV-associated lymphoproliferative syndrome. *CD27* or *ITK* mutations can also lead to *hemophagocytic lymphohistiocytosis* (HLH) (see later).

Genetic Disorders That Predispose to Severe Epstein-Barr Virus Disease and Other Infections

A number of autosomal genetic disorders can result in severe EBV disease and a predisposition to other infections, including those caused by bacteria or other viruses.[221] These include mutations in *PIK3CD* (phosphoinositide-3-kinase [PI3K] catalytic subunit 110delta), *CTPS1* (cytidine triphosphate [CTP] synthase 1), *STK4* (serine threonine kinase 4), *GATA2* (GATA binding protein 2), *MCM4* (minichromosome maintenance complex component 4), *FCGR3A* (Fc gamma receptor 3A [CD16a]), *CARD11* (caspase recruitment domain-containing protein 11), or *ATM* (ataxia telangiectasia mutated). Severe combined immunodeficiency (SCID) can be due to mutations in multiple genes. Mutation of *WAS* (Wiskott-Aldrich syndrome protein) can lead to severe EBV disease or other infections and is X-linked, rather than autosomal.

Chronic Active Epstein-Barr Virus Infection

Persistent EBV infection has been suggested as a frequent cause of fatigue and malaise in young and middle-aged adults.[237–240] This speculation has arisen from reports of a syndrome characterized by fatigue, sore throat, mild cognitive dysfunction, and myalgias initially noted in association with an apparent increase in antibody titers to the EBV EA complex (see "Laboratory Diagnosis" section).[237,238] These reports have included primarily young adults, usually with a female preponderance, who report a nonspecific symptom complex more reminiscent of the prodrome of infectious mononucleosis than of the syndrome itself (often known as *chronic mononucleosis syndrome* or *chronic fatigue syndrome*). These patients have been noted either sporadically[237,238,241] or in epidemic clusters.[240] The initial suggestion that the syndrome is attributable to EBV has become untenable on the basis of serologic and epidemiologic observations.[241,242] Investigation of the syndrome has been hampered by the vagueness of the symptoms and the absence of objective laboratory diagnostic criteria. A consensus case definition that focuses on fatigue rather than on EBV as the central feature of the syndrome has emerged.[243,244] The chronic fatigue syndrome is discussed in more detail in Chapter 130.

[a]References 163, 176, 200, 211, 219, 220.

In contrast to the nonspecific syndrome just noted, patients in whom EBV appears to play a direct role in ongoing objective organ system dysfunction have been identified.[245–249] These cases have been termed *chronic active EBV* (CAEBV) infection and are extremely rare in the United States. CAEBV is more frequent in Asia and South America, where, in striking contrast to other EBV LPDs, it has been associated with EBV infection of NK or T cells.[250–252] To distinguish CAEBV from other nonspecific syndromes, diagnostic criteria have been proposed.[253–255] First, patients have severe illness that lasts more than 6 months, began as primary EBV infection, and is associated with markedly elevated titers to EBV lytic antigens (VCA immunoglobulin G [IgG] ≥ 1:640 or EA IgG ≥ 1:160) or EBV DNA level in the blood (>300 copies/μg DNA). Second, histologic evidence of major organ involvement, such as interstitial pneumonia, hemophagocytosis, uveitis, lymphadenitis, or persistent hepatitis, is present. Third, affected tissues should contain elevated amounts of EBV DNA, RNA, or proteins by in situ hybridization or immunohistochemical staining. The prognosis for these patients is poor, with most dying of progressive pancytopenia and hypogammaglobulinemia or NK-/T-cell nasal lymphoma within a few years, although survival for more than 10 years after diagnosis has been observed.[248] Presentation before age 8 years, without thrombocytopenia, or with an NK-cell phenotype is associated with an improved prognosis.[256] Antiviral therapy with acyclovir or ganciclovir is of no proven benefit, but case reports of adoptive immunotherapy and bone marrow transplantation for patients with CAEBV have been found.[247,257–261] The pathogenesis of CAEBV is not well understood but is probably the result of an immune defect that permits the proliferation of EBV-infected T or NK cells.[262] Certain genetic defects have been linked to CAEBV and hemophagocytic lymphohistocytosis (described later). The limited number of cases of CAEBV diagnosed in the United States often includes disease associated with EBV infection of B cells.[261] Whether B-cell CAEBV represents a distinct clinicopathologic entity is an intriguing but academic question because the prognosis is similarly poor and treatment options are no different than NK- or T-cell CAEBV.

Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis

The hemophagocytic syndrome is characterized by excessive lymphocyte and macrophage (histiocyte) activation and infiltration of bone marrow, lymph nodes, spleen, and liver, with prominent phagocytosis of erythrocytes and nucleated cells.[263] Although the hemophagocytic syndrome can occur as a consequence of XLP syndrome or CAEBV, it can present as a distinct clinical entity in the absence of these diseases and has been called HLH. Children are primarily affected, usually before the age of 3 years, with high fevers, pancytopenia, liver dysfunction, and coagulopathy.[263,264] HLH usually develops as the sequela of a viral infection, most commonly primary EBV infection. Most, if not all, cases of HLH are associated with a monoclonal proliferation of T cells that are usually $CD8^+$.[264–266] In EBV-associated HLH most infiltrating T lymphocytes are monoclonally infected with EBV.[267–269] These unregulated proliferating T cells are thought to account for the markedly elevated levels of tumor necrosis factor (TNF)-α, IFN-γ, macrophage-specific colony-stimulating factor, IL-6, IL-10, IL-18, and soluble IL-2 receptor that typify HLH and drive macrophage activation.[270,271] The disease group familial hemophagocytic lymphohistiocytosis (FHL) comprises rare diseases that result from mutations in genes responsible for the maturation or release of CTL- or NK-cell cytotoxic granules, or for target cell entry of these granule proteins. These genes exhibit autosomal-recessive inheritance and are due to mutations in *PRF1* (perforin), *UNC13D* (MUNC13-4 protein), *STX11* (syntaxin 11), or *STXBP2* (MUNC18-2). These genes are responsible for FHL2, FHL3, FHL4, and FLH5, respectively.[221] FHL2, 3, and 5 are also associated with CAEBV. Mutations in *ITK* or *CD27* (described earlier) can also lead to HLH. Untreated, the prognosis of EBV-associated HLH is poor. However, treatment with the etoposide, dexamethasone, and cyclosporine-based HLH-94 protocol has been associated with survival rates of approximately 75%.[272] A newer protocol, HLH-2004, has been published, which confirmed the efficacy of etoposide and dexamethasone, but the benefit of cyclosporine has been questioned.[273,274]

Oral Hairy Leukoplakia

As previously stated, reactivation of lytic EBV replication with viral shedding in the saliva is usually entirely asymptomatic. An important exception to this rule is *oral hairy leukoplakia* (OHL), which arises as a corrugated or "hairy" white lesion usually on the lateral surface of the tongue but sometimes elsewhere. This nonmalignant lesion is seen in AIDS and other states of immunosuppression and is caused by unchecked lytic replication of EBV.[275,276] The diagnosis of OHL is based on the typical appearance of the lesions in the appropriate clinical setting. The differential diagnosis includes oral candidiasis, which, unlike OHL, can be removed with gentle scraping of the tongue. Alternatively, thrush may be diagnosed with a potassium hydroxide wet mount or should respond to an empirical trial of antifungal therapy. Biopsy for histology and in situ hybridization or immunofluorescence staining for EBV is rarely necessary but confirms the diagnosis. Polymerase chain reaction (PCR) detection of EBV in "oral scrapes" is neither sensitive nor specific for OHL.[277]

Epstein-Barr Virus–Associated Malignant Diseases

EBV is an extremely well-adapted parasite that establishes lifelong latent infection without lasting adverse effects in about 95% of the human population. However, in immunosuppressed hosts, the growth-transforming properties of EBV can result in malignant disease. EBV in conjunction with environmental or genetic factors, or both, can rarely result in malignant disease in immunocompetent hosts (Table 138.6).

TABLE 138.6 Prevalent EBV-Associated Malignant Diseases

MALIGNANT DISEASE	EBV ASSOCIATION	POPULATION AT RISK	COFACTORS
Lymphoproliferative disease	~90%	Transplantation patients	Immunosuppression
Hodgkin lymphoma	~50%, depending on histologic subtype	Children (developing countries) Young adults (western countries)	Unknown
Diffuse large B-cell lymphoma	~10%	?Elderly	Unknown
Nasopharyngeal carcinoma	100% undifferentiated	Southern Chinese, Inuit	Genetic predisposition and dietary factors
Gastric cancer	~4%[a] 20% 30%[a] 100% squamous	Unknown	Unknown
Burkitt lymphoma	>95% endemic ~20% sporadic ~40% HIV associated	African children Independent of $CD4^+$ count	*c-myc* translocations (all) Malaria (endemic only)

AIDS, Acquired immunodeficiency syndrome; *CNS,* central nervous system; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus.
Modified from Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: Knipe D, Howley P, Griffin D, et al, eds. Fields Virology. *5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:2603–2654.*

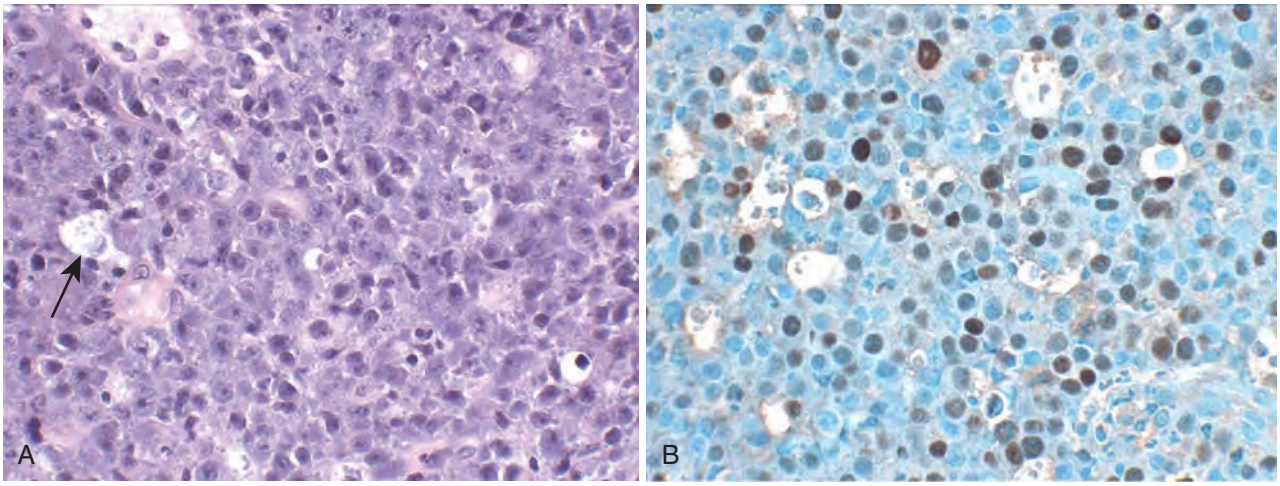

FIG. 138.2 Posttransplant lymphoproliferative disease involving the colon. (A) Tumor is composed of large, atypical lymphoid cells (hematoxylin and eosin). Scattered macrophages *(arrow)* are seen, producing "starry-sky" appearance. (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows variably intense nuclear staining in most tumor cells, indicating EBV infection. (Original magnification, ×400.) *(Courtesy Dr. Jeffery Kutok.)*

Lymphoproliferative Disease

In the absence of effective immune surveillance, uncontrolled proliferation of EBV-infected B lymphocytes may occur. This disorder is referred to as *LPD* and represents the in vivo equivalent of the immortalized B-cell lines seen with EBV infection in vitro. Proliferating B cells in LPD express all EBV latent proteins (latency III), including the EBNA3 proteins that are normally strong targets for $CD8^+$ cytotoxic T cells (see Table 138.2).[27,56] Patients with LPD typically present with symptoms similar to those of infectious mononucleosis or with fever and lymphomatous infiltration of lymph nodes, spleen, liver, bone marrow, kidney, lung, CNS, or intestine (Fig. 138.2). The frequency of this disease in solid-organ and bone marrow transplant recipients has led to the designation *post-transplant lymphoproliferative disease* (PTLD), but it can be seen in any patient receiving high-dose immune suppression or in those with inherited disorders that affect T-cell immunity. Patients with more severe cellular immune impairment, such as those receiving T-cell–depleted bone marrow transplants, cord transplants, haploid identical stem cell transplants, or antithymocyte globulin, are at increased risk for PTLD, as are those with primary EBV infection after transplantation.[52,53] Notably, the timing of risk for PTLD differs in stem cell versus solid-organ transplant recipients because of differences in immune suppression. In stem cell transplants the overall risk is 1%, and this risk is greatest within the first 5 months after transplantation when the immune suppression is severe and before immune reconstitution.[278] In solid-organ transplantation the risk is more prolonged because of the need for long-term immunosuppression. PTLD is most common in multivisceral transplantation (up to 33%); least common in renal, liver, and heart transplantation (≈1%–2.5%); and intermediate in lung (≈6%) or intestinal (≈10%) transplantation.[279–281] Up to half of PTLDs occur in the setting of primary EBV infection. These may arise early after transplantation and often occur in children, who are more likely to be EBV seronegative.[282] The cells of origin for PTLD may be either host derived or donor derived. Most often, PTLD occurs in host-derived cells in solid-organ transplantation and in donor-derived cells in allogeneic stem cell transplantation.[283–286]

Burkitt Lymphoma

Burkitt lymphoma is a high-grade lymphoma with characteristic small, noncleaved B cells and is endemic in equatorial Africa. Endemic Burkitt lymphoma is geographically associated with *Plasmodium falciparum* malaria and usually arises as a tumor of the jaw. Although the fact that greater than 90% of Burkitt lymphomas are EBV associated has been long appreciated, the role of the virus in its pathogenesis is unclear because most of the EBV transforming genes are not expressed. In fact, viral gene expression is restricted to *EBNA1* and the EBERs (latency I; see Table 138.2).[49,54,55] It is unlikely that EBV is merely a passenger because terminal repeat analysis of EBV genomes has confirmed that the viral infection occurred before expansion of the tumor.[287] Also, persons in endemic regions with elevated titers to EBV lytic antigens are at high risk for Burkitt lymphoma.[288] Children from an endemic region with high risk of Burkitt lymphoma were shown to be infected with EBV earlier in life and had elevated viral loads during infancy compared with those from a nonendemic area.[289] In addition to EBV association, virtually all Burkitt lymphomas contain a chromosomal translocation that involves the *c-myc* oncogene and an immunoglobulin heavy- or light-chain locus. The unregulated expression of this potent oncogene probably supplants the need for expression of many of the EBV-transforming genes that otherwise would serve as targets for immune surveillance. In addition to the endemic form of the disease, sporadic Burkitt-like lymphomas are seen that typically arise as abdominal masses. These lymphomas also contain *c-myc* translocations but are less consistently associated with EBV (only about 25% of cases).[290] Persons with HIV are at increased risk for Burkitt-like lymphoma, independent of degree of immunodeficiency.[291]

Hodgkin Lymphoma

Hodgkin lymphoma is an unusual malignant disease in that the malignant Hodgkin and Reed-Sternberg (HRS) cells constitute as little as 1% of the tumor. The balance of the tumor mass is composed of an infiltrate of reactive mononuclear and stromal cells. An infectious etiology for Hodgkin lymphoma was proposed as early as 1966 on the basis of the epidemiology of the disease, but definitive evidence was slow to evolve because of technical difficulties presented by the scarcity of the HRS cells.[292–294] Subsequently, EBV DNA and protein expression were shown in HRS cells from some forms of Hodgkin lymphoma.[295,296] Furthermore, symptomatic EBV mononucleosis increases the risk (relative risk, 4.0) for EBV-positive Hodgkin disease as was shown in a Scandinavian study; Hodgkin disease occurred a median of 4 years after the mononucleosis syndrome.[297] The strongest associations are with the mixed cellularity (Fig. 138.3) and lymphocyte-depleted histologic subtypes.[70] No association with the lymphocyte-predominant subtype could be proved, and this is now considered a distinct, non–EBV-associated entity. There is, however, general agreement that in EBV-associated Hodgkin lymphoma, the malignant HRS cells represent postgerminal center B cells that express a latency II EBV gene pattern (*LMP1, LMP2, EBNA1,* and EBERs; see Table 138.2). EBV genomes, when present in HRS cells, are monoclonal with terminal repeat analysis, which suggests that EBV infection preceded the development of the malignant disease.[296] Many HRS contain "crippling mutations" in their immunoglobulin genes or fail to express surface immunoglobulins and thus lack a critical anti-apoptotic signal normally transmitted by the B-cell receptor (BCR).

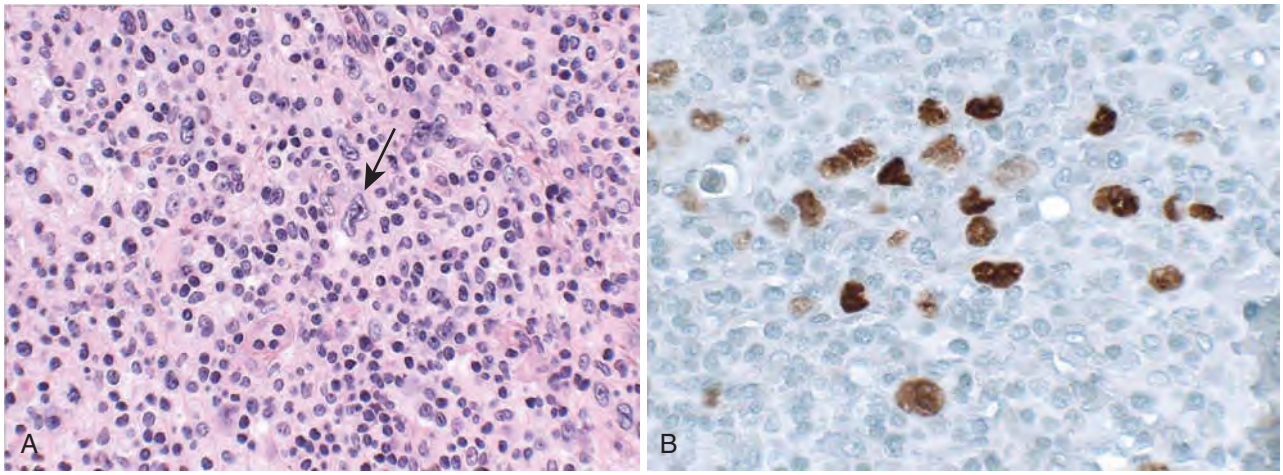

FIG. 138.3 Mixed cellularity classic Hodgkin lymphoma. (A) Lymph node architecture is effaced by infiltrate composed of small lymphocytes, epithelioid histiocytes, plasma cells, eosinophils, and Hodgkin and Reed-Sternberg (HRS) cells (*arrow;* hematoxylin and eosin). (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows EBV infection in malignant HRS cells. (Original magnification, ×400.) *(Courtesy Dr. Jeffery Kutok.)*

Expression of EBV *LMP2A* can serve as a surrogate BCR signal and may allow the survival of cells otherwise destined to undergo apoptosis from failure to express functional immunoglobulin.[298] Activation of NF-κB signaling is also typical of HRS cells, which suggests activation of this pathway by LMP1.[299] In some EBV-negative HRS cells, inhibitor of kappa Bα *(IκBα)* gene mutations have been reported that could serve as an alternative means of constitutively activating the NF-κB pathway.[300] Speculation is tempting that EBV gene expression can serve as one step in the malignant transformation of HRS cells that is circumvented by other mutational events in EBV-negative forms of the disease.

Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common type of high-grade non-Hodgkin lymphoma, accounting for about 40% of cases worldwide.[301] These lymphomas are diffuse in the sense that they lack the normal architecture of more differentiated lymphomas (e.g., follicular). Lymphomas exhibiting DLBCL morphology are quite heterogeneous and are thought to represent multiple distinct diseases. An important advance was the use of gene expression profiling to subdivide DLBCL into germinal center B-cell–like (GCB) and activated B-cell–like (ABC) subtypes, with the latter carrying a worse prognosis.[302] About 10% of DLBCL are EBV positive, most exhibiting the ABC phenotype. EBV-positive DLBCLs were originally reported to be most prevalent in elderly patients and attributed to waning cellular immunity with advancing age.[303] In the setting of HIV infection, the frequency of EBV positivity in DLBCL increases to about 60%.[290] However, EBV-positive DLBCL are increasingly appreciated to occur in younger immunocompetent persons and have most recently been reclassified as EBV+ DLBCL-NOS.[301,304] The NOS (not otherwise specified) is to ensure that tumors that can already be classified as well defined, rare EBV-associated lymphomas (see Table 138.7) are not included in this category. One rare lymphoma meriting special mention is primary CNS lymphoma. This subtype of DLBCL bears a striking resemblance to PTLD and is highly associated with EBV, with rates approaching 90%. As with PTLD, it occurs in the setting of profound immunosuppression; those with the lowest $CD4^+$ counts for the longest time are at greatest risk. The prognosis is poor, and excluding more treatable conditions such as CNS toxoplasmosis is essential.

Nasopharyngeal Carcinoma

NPC is a rare disease in most western countries, but its prevalence rate approaches 50 per 100,000 in southern China and among the Inuit in Alaska.[305] An association between EBV and NPC was first suggested with the observation that patients with this malignant disease had elevated IgG and immunoglobulin A (IgA) titers to EBV lytic antigens (VCA and EA).[306] The undifferentiated form (Fig. 138.4) is EBV associated in nearly 100% of cases, whereas squamous NPCs are inconsistently EBV associated, particularly outside endemic regions. The undifferentiated form bears some resemblance to Hodgkin lymphoma in that the tumor consists of EBV-positive cells (of epithelial origin in this case) that express a latency II gene pattern infiltrated with reactive, nonmalignant lymphoid cells.[71,307,308] Terminal repeat assays have confirmed that these epithelial cells contain monoclonal EBV genomes, placing EBV infection early in the genesis of the malignant disease as seen in EBV-associated B-cell neoplasia.[309] In addition to EBV, evidence indicates that genetic and environmental factors may have roles in tumor development.[310–312] Recent reviews of epidemiologic data indicate that the incidence of NPC is gradually declining and that mortality has fallen substantially. Potential explanations for this improvement include expanded population

TABLE 138.7 Rare EBV-Associated Cancers or Proliferative Disorders

DISEASE	% EBV POSITIVE	REFERENCE
EBV Infection of B Cells		
Lymphomatoid granulomatosis	100	488
Primary effusion lymphoma	75–90	489
Pyothorax-associated lymphoma	70	490
Plasmablastic lymphoma	75–90	491
EBV-positive mucocutaneous ulcer	100	492
Angioimmunoblastic T-cell lymphoma	100[a]	493
Primary CNS lymphoma	10–90[b]	494
EBV Infection of T Cells		
Nasal NK-/T-cell lymphoma	100	495
Chronic active EBV	100	496
Hemophagocytic lymphohistiocytosis	75	497
Nonhepatosplenic gamma-delta T-cell lymphoma	50	498
Hydroa vacciniforme–like lymphoma	100	324
EBV Infection of Nonlymphoid Cells		
Leiomyosarcomas (associated with immunosuppression)	100	499

[a]This T-cell lymphoma is characterized by infiltration of EBV-positive B cells.
[b]~10% in HIV-negative patients, ~90% in HIV-positive patients.
CNS, Central nervous system; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus.

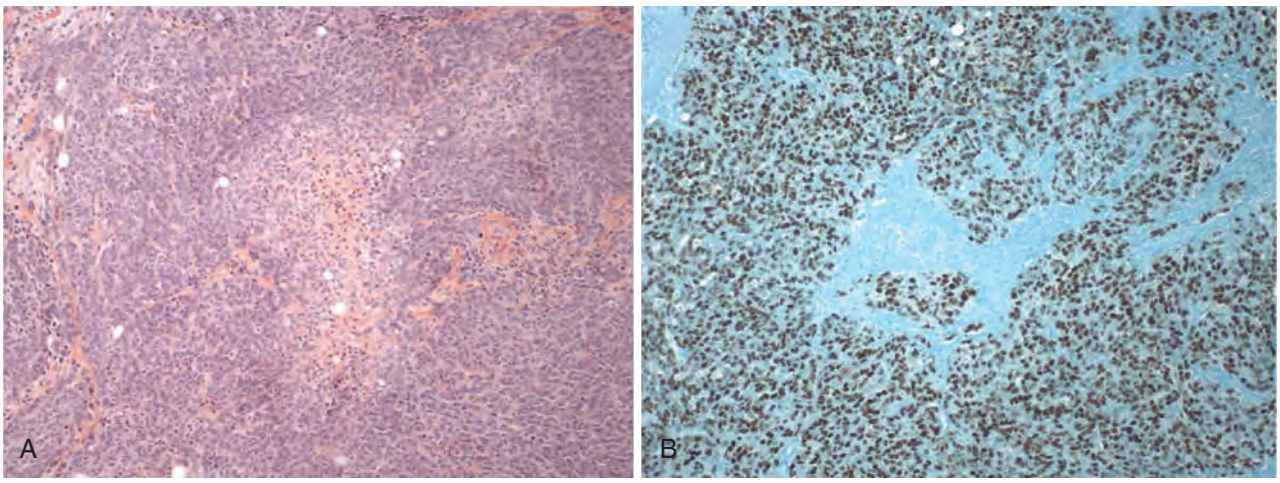

FIG. 138.4 Nasopharyngeal carcinoma (NPC). (A) Nests of metastatic undifferentiated NPC in a fibrous stroma in lymph node (hematoxylin and eosin). Metastases often lack infiltrating lymphocytes. (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows EBV infection in most cells in the same area of tissue. (Magnification, ×100.) *(Courtesy Dr. Miguel Rivera.)*

screening and advances in radiotherapy and treatment with systemic agents.[313]

Gastric Carcinoma

Since the first report of an EBV-positive gastric carcinoma in 1990, numerous studies have confirmed that approximately 10% of gastric carcinomas worldwide are EBV associated.[314,315] Lymphoepithelioid gastric cancers in which malignant epithelial cells are surrounded by lymphoid stroma are EBV positive in up to 80% of cases, as are a small fraction of gastric cancers with typical morphology.[316] The proportion of EBV-positive tumors also varies inversely with gastric cancer incidence, ranging from approximately 17% in the United States and Germany to 4% in China.[317] EBV genomes are monoclonal by terminal repeat analysis consistent with EBV infection occurring as an early event in tumorigenesis.[316] EBV latent gene expression in gastric carcinomas consists of *EBNA1,* EBERs, EBV miRNAs, and variable *LMP2A* expression, and rarely LMP1, consistent with either a latency I or II pattern.[318] The role(s) played by these EBV gene products in gastric cancer tumorigenesis remains to be defined.

Other Malignant Diseases

Nasal NK-/T-cell lymphomas (Table 138.7) are angiocentric lymphomas that typically present as a midline facial destructive disease (lethal midline granuloma) but can also arise at other extranodal sites.[319] They are highly associated with EBV, and, like NPC and Hodgkin lymphoma (HL), malignant cells typically express a latency II gene pattern.[320] As discussed previously, persons with CAEBV are at high risk of development of this subtype of peripheral T-cell lymphoma.[321,322] A similar angiocentric malignant disease, *lymphomatoid granulomatosis* (LG), is now known to be a distinct clinical entity caused by EBV-infected proliferating B lymphocytes with an exuberant reactive T-cell infiltrate. Patients with LG typically present with pulmonary lesions and synchronous brain, skin, kidney, or liver lesions that can be easily mistaken for disseminated fungal infections.[323] Another rare EBV-associated cutaneous NK-/T-cell LPD is hydroa vacciniforme–like lymphoma, seen primarily in children in Central and South America and Asia.[324] An EBV association has also been reported in some breast cancers, hepatocellular cancers, and smooth muscle tumors, but the contribution of EBV to the pathogenesis of these malignant diseases remains to be established.[325,326]

In addition to the Burkitt-like lymphomas and DFCL, persons with HIV (or other immunosuppression) are at increased risk for an unusual EBV-associated lymphoma, *primary effusion lymphoma* (PEL).[327,328] These human herpesvirus 8 (Kaposi sarcoma–associated herpesvirus)–related lymphomas are often coinfected with EBV. PELs derived their name from a tendency to arise within potential body cavities, such as the pleural, pericardial, or peritoneal spaces, and frequently follow an aggressive clinical course. *Pyothorax-associated lymphoma* is sometimes confused with PEL but differs in that it is strictly EBV (not Kaposi sarcoma–associated herpesvirus)-associated, forms an identifiable mass lesion, arises in patients with long-standing pleural-based inflammation, and is seen in patients without HIV.[329] In pediatric patients with AIDS, EBV has been reported in leiomyosarcomas in HIV-infected individuals and also in transplant recipients.[330] Another study reported EBV in leiomyosarcomas in both HIV-infected and also transplant recipients.[331]

Multiple Sclerosis and Other Autoimmune Diseases

Viruses have long been suspected as environmental triggers for autoimmune diseases in genetically predisposed individuals. EBV has been a candidate on the basis of seroepidemiologic studies that link it to systemic lupus erythematosus, rheumatoid arthritis, and *multiple sclerosis* (MS).[332–335] Although definitive proof is lacking, patients with MS are more likely to be EBV seropositive than age-matched control subjects.[334] This difference is most notable in pediatric patients in whom the rate of EBV seropositivity of control subjects is much lower.[336] Patients with MS have higher titers to EBV antigens, whereas elevated titers are not observed in other viruses, including CMV, VZV, and HSV.[336–340] Furthermore, prospective studies have shown that antibody titers, particularly to EBNA1, are elevated more than 10 years before the onset of MS symptoms.[341–343] In addition, a history of symptomatic infectious mononucleosis is associated with a twofold increased risk of development of MS relative to asymptomatic primary EBV infection.[344] Finally, Cepok and associates[345] determined that the two most frequent MS-specific oligoclonal IgG bands in the CSF of MS patients recognized peptides present in the EBV proteins EBNA1 and BRRF2. Thus a body of data supports an association of EBV infection with MS. Whether these observed differences in immune responses to EBV cause MS or are merely an epiphenomenon that results from the same immunologic dysregulation that causes MS remains to be seen.

LABORATORY DIAGNOSIS

Infectious Mononucleosis

Hematologic Findings

The central hematologic manifestation of the illness is a circulating lymphocytosis. At presentation, a relative and absolute mononuclear lymphocytosis is found in about 70% of the cases. The lymphocytosis peaks during the second or third week of illness, and monocytes and lymphocytes account for 60% to 70% of the total white cell counts of 12,000 to 18,000/mm.^{3} However, higher white cell counts are not uncommon, and occasional patients manifest 30,000 to 50,000 leukocytes/mm.^{3} Atypical lymphocytes are the hematologic hallmark of infectious mononucleosis and account for about 30% of the differential count at

their zenith.[145,147] The wide range in the atypical lymphocytosis is well recognized, and some cases show none or only a few atypical lymphocytes, whereas 90% or greater of the circulating lymphocytes may be atypical in other cases. These atypical lymphocytes are composed largely of reactive $CD8^+$ cytotoxic T cells, and their degree of elevation correlates with symptom severity.[96] Atypical lymphocytes are not pathognomonic for infectious mononucleosis and can be observed with CMV infection, primary HIV infection, viral hepatitis, toxoplasmosis, rubella, mumps, and roseola and in drug reactions (Table 138.8).[346,347] The atypical lymphocyte is generally larger than the mature lymphocyte encountered in peripheral blood. The cytoplasm is often vacuolated and basophilic, and its edges have a rolled-up appearance with a tendency to flow around adjacent RBCs on a peripheral smear. Nuclei are often lobulated and are eccentrically placed. Although the cells may appear quite immature, the heterogeneity of morphologic and tinctorial characteristics of such cells helps to distinguish atypical lymphocytes from the more uniform lymphoblasts of acute lymphocytic leukemia.[6,346]

A relative and absolute neutropenia is evident in 60% to 90% of the cases, and neutrophils that remain in circulation exhibit a mild left shift.[172,173] In most cases the neutropenia is mild, with total granulocyte counts of 2000 to 3000/mm,[3] although profound granulocytopenia has also been reported.[171,174–178,225,348] The neutropenia is usually self-limited, and counts rise gradually toward normal by a month after presentation.[172]

Thrombocytopenia is also common, and 50% of the patients in one series manifested platelet counts of less than 140,000/mm^3.[165] Although cases of profound thrombocytopenia with bleeding have been reported,[166–170] these cases are rare and contrast markedly with the generally benign course of the common mild thrombocytopenia.

TABLE 138.8 Differential Diagnosis of Atypical Lymphocytosis

Epstein-Barr virus primary infection (infectious mononucleosis)
Cytomegalovirus primary infection (heterophile-negative mononucleosis)[a]
Human herpesvirus 6 primary infection (roseola)
Primary human immunodeficiency virus infection
Toxoplasmosis
Acute viral hepatitis
Rubella, mumps
Drug reactions (e.g., phenytoin, sulfa)

[a]Cytomegalovirus is the most common cause of heterophile-negative mononucleosis.

Heterophile Antibodies

Heterophile antibodies are low-affinity IgM antibodies with broad specificity for predominantly carbohydrate antigens that can react with molecules found on the surface of a number of nonhuman erythrocytes (hence heterophile = other loving). Originally described by Paul and Bunnell[7] as sheep erythrocyte agglutinins, they play no role in EBV immunity but may be a consequence of polyclonal B-cell infection by the virus. Various technical improvements, such as preadsorption of serum against other antigens or use of purified beef erythrocyte extracts as the detection reagent, have increased the specificity of this test to around 95% to 99% for primary EBV infection. Commercial spot kits such as the Monospot test are available and generally equivalent to traditional heterophile antibody assays based on erythrocyte agglutination.[349] Occasional false-positive heterophile tests have been reported in patients with lymphoma or hepatitis, but the rarity of this event makes confirmation of a positive Monospot test result with EBV-specific serology unnecessary.[350–352] Three cases of false-positive Monospot tests in the setting of primary HIV infection have been reported.[353] One study of 132 patients with positive Monospot test results found no instances of primary HIV infection.[354] However, the exact rate of false-positive heterophile results among patients with primary HIV infection is not known. Heterophile antibodies are, however, relatively insensitive for diagnosing primary EBV infection, with sensitivities ranging from 70% to 90% for adults and adolescents and less than 50% in children.[349] When absent at the onset of illness, heterophile antibodies may appear later in the course. Thus, in the appropriate clinical setting, a positive heterophile test is sufficient to confirm the diagnosis of infectious mononucleosis, but a negative test does not exclude it.

Epstein-Barr Virus–Specific Antibodies

In addition to the transient heterophile antibodies, infection with EBV results in the development of virus-specific antibodies. Antibodies are formed to structural proteins or VCAs, nonstructural proteins expressed early in the lytic cycle or EAs, and nuclear proteins expressed during latent infections or EBNAs. A determination of EBV-specific antibodies is rarely necessary for the diagnosis of infectious mononucleosis because 90% of the cases are heterophile positive, and few false-positive results are obtained if the test is properly performed (see previous discussion). For heterophile-negative cases and for diagnosis in atypical cases, a determination of EBV antibodies may help to establish a cause (Table 138.9).[355]

TABLE 138.9 Antibodies to Epstein-Barr Virus

ANTIBODY SPECIFICITY	TIME OF APPEARANCE IN INFECTIOUS MONONUCLEOSIS	PERCENTAGE OF EBV-INDUCED MONONUCLEOSIS CASES WITH ANTIBODY	PERSISTENCE	COMMENTS
Viral Capsid Antigens				
IgM VCA	At clinical presentation	100	4–8 wk	Highly sensitive and specific; major diagnostic utility
IgG VCA	At clinical presentation	100	Lifelong	High titer at presentation and lifelong persistence make IgG VCA more useful as epidemiologic tool than as diagnostic tool in individual cases
Early Antigens				
Anti–EA-D	Peaks at 3–4 wk after onset	70	3–6 mo	Correlated with severe disease; also seen in nasopharyngeal carcinoma
Anti–EA-R	2 wk to several mo after onset	Low	2 mo to >3 yr	Occasionally seen with unusually severe or protracted illness; also seen in African Burkitt lymphoma
Latent Antigen				
EBV nuclear antigen	3–4 wk after onset	100	Lifelong	Late appearance helpful in diagnosis of heterophile-negative cases

EA, Early antigen; *EBV,* Epstein-Barr virus; *IgG,* immunoglobulin G; *IgM* immunoglobulin M; *VCA,* viral capsid antigen.

Viral Capsid Antigen Antibodies

Antibodies to VCA as measured with immunofluorescence arise early in the course of the illness and are seen at presentation in most cases. IgG antibodies to VCA are usually present at titers of 80 or greater on the first visit to a physician. Because these initially detected levels are close to peak VCA titers, a fourfold rise in titer is seen in only 10% to 20% of the cases. After recovery, detectable titers of VCA IgG antibody are maintained for life. Thus IgG VCA antibody titers may be of little help in the diagnosis of infectious mononucleosis. Conversely, IgM antibodies to VCA are sensitive and specific for infectious mononucleosis. IgM antibody titers are present in about 75% of patients at the onset of illness, and 95% will eventually develop them.[356] Titers fall rapidly thereafter, and in only 10% of the cases are titers greater than 5 retained by 4 months after diagnosis.[357,358] IgM VCA antibodies are not seen in the general population; thus their presence is virtually diagnostic of acute EBV infection.

Early Antigen Antibodies

Serum antibodies to EAs are also seen with indirect immunofluorescence, and two distinct patterns of fluorescence emerge.[355,358] Certain sera stain both nuclei and cytoplasm diffusely (anti–EA-D), whereas the staining of other sera is restricted (anti–EA-R) to cytoplasmic aggregates. Anti–EA-D antibody is found in about 70% of patients with acute infectious mononucleosis (see Table 138.9). Anti–EA-D titers arise later in the course of illness than those to VCA and disappear after recovery. Anti–EA-D antibodies may be found in the sera of patients with advanced NPC but are absent from the general population. The appearance of anti–EA-D antibodies in a patient with IgG VCA antibodies suggests recent EBV infection. Unfortunately, only 70% of EBV-induced cases manifest anti–EA-D antibodies. The presence and titer of anti–EA-D antibodies correlate with the duration and severity of clinical illness.[358] Anti–EA-R antibodies are only occasionally seen in infectious mononucleosis (see Table 138.9). They are present more often in protracted or atypical cases, arise after the anti–EA-D antibodies peak, and remain detectable for up to 2 years.[359] Anti–EA-R antibodies are also present in higher titers in patients with African Burkitt lymphoma and occasionally in healthy persons who also have high VCA titers.[360] Currently, commercial laboratories typically do not differentiate anti–EA-R and anti–EA-D.

Epstein-Barr Nuclear Antigen Antibodies

Antibodies to EBNAs appear late in the course of all cases of infectious mononucleosis and persist for life.[361] The appearance of EBNA antibodies in a patient who was previously VCA positive and EBNA negative is strong evidence of recent EBV infection. These antibodies may be reactive against any of the six nuclear proteins expressed during latent infection. Neutralizing antibodies to EBV also appear late in the course of infectious mononucleosis and reach maximal levels 6 to 7 weeks after the onset of illness.[362] Neutralizing antibodies persist at stable titers (mean, 40) for life. The appearance or a rise in titer of neutralizing antibodies to EBV also indicates recent EBV infection. Neutralizing antibodies are, however, difficult to measure, and tests for them are not routinely available.

Culture of Epstein-Barr Virus

EBV may be cultured from oropharyngeal washings or from circulating lymphocytes of 80% to 90% of patients with infectious mononucleosis.[90,91,94,108,363] Cultivation of the virus is, however, not routinely available in most diagnostic virology laboratories. This, coupled with the ubiquity of virus shedding in both healthy persons and in those with unrelated illnesses, renders cultivation of the virus of little clinical use (see Table 138.1). Rapid diagnostic techniques based on DNA hybridization or monoclonal antibody techniques have also been developed but for similar reasons are not helpful in the diagnosis of mononucleosis.[364–366] Of interest, up to 50% of memory B cells are infected with EBV during infectious mononucleosis, compared with 1 in 10^4 to 1 in 10^6 memory B cells that contain virus in healthy individuals.[367,368]

Epstein-Barr Virus Viral Load

EBV DNA can be detected in lymphocytes and plasma early in the course of infectious mononucleosis. Detection of viral DNA in plasma is otherwise infrequent in healthy individuals.[369] Low levels of EBV DNA can be detected in blood up to 3 weeks before onset of infectious mononucleosis symptoms in some individuals, and levels increase rapidly close to the onset of illness.[26] EBV viral load in blood is initially high in mononucleosis but then rapidly declines.[370]

The detection of EBV DNA from blood is being increasingly used for a number of EBV-associated diseases. In its most modern form DNA is quantitated with real-time PCR to determine a specific copy number. Although the use of EBV loads holds much promise, the technique is currently hampered by a lack of standardization across different centers. For instance, different sample types are used to assay viral loads and can include whole blood, plasma, or peripheral blood mononuclear cells. EBV is a cell-associated virus and therefore typically is found in the peripheral blood mononuclear cell component; but in certain disease states, notably NPC, it can be found in high levels in plasma, likely because of cell death and release of episomal DNA into the circulation. Further, extensive variability in quantitation can be found as a result of a lack of standardized DNA extraction techniques and gene amplification targets.[371,372] Taken together, these factors have resulted in a lack of validated data from multicenter trials. Thus individual centers often have their own protocols for monitoring viral load and decision points for intervention or preemptive strategies, such as for prevention of LPD (see discussion of PTLD later). A significant advance has been the establishment of WHO International Standard for EBV nucleic acid amplification techniques.[373] This has improved but not entirely eliminated the interlaboratory variation with these assays. At present, quantitative tracking of individual patients using a single reference laboratory remains the most accurate assessment of trends.[371,372]

Other Laboratory Abnormalities

Liver function test results are abnormal in almost all cases of infectious mononucleosis.[201,374,375] Levels of the hepatocellular enzymes aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase are most commonly elevated, and one of the three is abnormal in about 90% of the cases. Elevations are usually mild, with individual values in the range of two to three times the upper limit of normal. Elevation to greater than 10 times the upper limit of normal necessitates a search for another diagnosis.[201] The alkaline phosphatase level is elevated in about 60% of the cases.[374,375] Mild elevation of the bilirubin level is noted in approximately 45% of cases, although frank jaundice occurs in only about 5%. Elevations are maximal in the second week of illness and decline gradually over a 3- to 4-week period.

Cryoproteins are present in modest amounts in 90% to 95% of patients.[159,376] The cryoproteins are generally mixed cryoglobulins of IgG and IgM classes. When the cryoglobulins are dissociated, antibody of anti-i or anti-I, or both, specificities is usually seen.[376,377]

Posttransplant Lymphoproliferative Disease

The use of EBV loads has been intensively studied in PTLD. Although standard protocols are not available and controlled trials have not been performed, a number of studies report that the EBV viral load can be predictive of PTLD, especially in the setting of T-cell–depleted stem cell transplants or in umbilical cord blood transplantation, because the incidence of PTLD is higher for these patients.[369,378–380] The higher incidence is due to the importance of T-cell immunity in controlling EBV infection. Umbilical cord blood is EBV naïve, and because most transplant recipients are EBV positive, these patients are at particularly high risk for PTLD. Many transplantation centers therefore now monitor EBV loads and view elevated values as evidence for increased risk of PTLD in stem cell transplantation. However, the approach to monitoring EBV loads is not standardized and varies among institutions. One international leukemia working group recommended weekly monitoring for at least 3 months in posttransplantation in high-risk hematologic stem cell transplantation recipients.[381] Some centers now routinely treat

high EBV loads with rituximab, an anti-CD20 antibody that kills B cells and rapidly lowers EBV load, or with other approaches such as adoptive immunotherapy (discussed subsequently).[382,383] Rituximab lowered the rate of PTLD compared with historical control subjects in one study.[384]

The use of EBV viral loads is less clear after PTLD in solid-organ transplantation, and large studies are necessary to define protocols. After solid-organ transplantation, EBV viral loads tend to remain consistently high, without a clear risk for development of PTLD.[385–388] A rapid increase in viral loads may be of concern for development of PTLD. EBV load monitoring is particularly important after transplantation in individuals who are EBV seronegative, especially if the donor is EBV positive because these patients are at higher risk for PTLD after primary infection.[282] Similar to the situation with hematologic stem cell transplantation, there is no standardized approach to monitoring EBV loads. For instance, one renal transplant group recommends monitoring EBV loads monthly for the first 3 to 6 months after transplantation, followed by every 3 months for the first year, whereas another group states there are insufficient data to recommend routine monitoring and that testing should be individualized. Those with increasing EBV loads are often managed with reduced immunosuppression and sometimes with rituximab.[389,390]

Nasopharyngeal Carcinoma

NPC is difficult to diagnose in its early stages; therefore patients typically present with advanced disease. The most common initial presenting symptom is a neck mass (Fig. 138.5). Diagnosis requires endoscopy to visualize the nasopharynx and histologic examination of biopsy tissue.[391] Radiologic studies are helpful in revealing the extent of disease (Fig. 138.6). Patients with NPC have elevated levels of serum IgA directed against EBV VCA and EA.[392–394] The elevation of the IgA antibodies may occur several years before the onset of NPC. In light of this finding, a program of screening individuals for elevated EBV IgA VCA and EA titers has been instituted in southern China, where NPC is one of the leading malignant diseases. Individuals with elevated IgA titers are then observed closely for the development of disease. This screening program enhanced the diagnosis of NPC in earlier as opposed to more advanced stages of disease.

Detection of EBV DNA in nasopharyngeal brush biopsies has also been proposed in one study as a possible screening mechanism in high-risk populations. This study detected EBV DNA in 19 of 21 brush biopsies from patients with recently diagnosed NPC but only in 1.3% of control subjects.[395]

Cell-free EBV DNA in plasma is commonly detected in patients with NPC.[391] Cell-free EBV DNA is postulated to be released into the circulation on tumor cell death. In two studies by the same authors[396,397] of patients with NPC, quantitative analysis of the concentration of DNA in plasma was useful in monitoring patients for recurrence of disease. Further, patients with NPC presenting with higher plasma level viral loads have poorer outcomes and higher rates of early recurrence or metastasis after radiotherapy.[398,399] In a study from China the presence of persistent EBV DNA in plasma over a 4-week period was a useful screening tool to identify asymptomatic individuals with NPC. NPC was present in 11% of those with persistently positive EBV DNA in plasma. The carcinoma was identified at an earlier stage of disease and resulted in better patient outcomes compared with historical control subjects. However, only 1% of those with EBV DNA in plasma in a US study had NPC, indicating the positive predictive value for this approach is much lower in nonendemic regions.[400,401]

FIG. 138.5 Patient with nasopharyngeal carcinoma and neck mass *(arrow).*

Central Nervous System Lymphoma in Acquired Immunodeficiency Syndrome

PCR detection of EBV DNA in CSF has been useful in the diagnosis of CNS lymphoma in patients with HIV.[402–405] Nearly all primary CNS lymphomas in HIV disease are EBV associated, as discussed previously. Whereas patients with HIV without CNS lymphoma rarely have detectable EBV DNA in CSF, EBV DNA is frequently detected when CNS lymphoma is present. Therefore CSF PCR for EBV used in conjunction with radiologic studies may reduce the need for brain biopsy in certain instances. Quantification of EBV DNA in CSF may also be useful for monitoring the effects of CNS lymphoma therapy.[406]

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

In most cases the diagnosis of infectious mononucleosis is straightforward. The clinical manifestations of sore throat, fever, lymphadenopathy, and malaise coupled with atypical lymphocytosis and a positive heterophile test result establish the diagnosis of EBV-induced infectious mononucleosis.[407] Difficulties arise, however, when the clinical manifestations are less striking, particularly when the heterophile test results are negative.

Heterophile-Negative Infectious Mononucleosis Caused by Epstein-Barr Virus

Heterophile-negative infectious mononucleosis may be caused by several different agents, including EBV. As previously noted, the heterophile test is highly specific for primary EBV infection but not sensitive, especially in the pediatric age group.[139,140] On these occasions the diagnosis rests on the demonstration of appropriate changes in specific EBV serologic tests (see Table 138.9). Because the preclinical phase of EBV infection is over 30 days, most patients will have developed EBV antibodies at the time of clinical presentation. Detection of EBV DNA in blood can also be used to confirm the diagnosis in the appropriate clinical scenario but is rarely necessary in practice.

Cytomegalovirus as a Cause of Heterophile-Negative Infectious Mononucleosis

The most frequent cause of heterophile-negative infectious mononucleosis in most populations is CMV.[408] Although differentiation of individual cases of EBV-induced versus CMV-induced infectious mononucleosis may be difficult, certain features are more common in CMV infections. CMV more frequently follows transfusion and is more often manifested as a typhoid-like syndrome without sore throat and lymphadenopathy. Splenomegaly may be slightly more prominent with CMV-induced disease, whereas the atypical lymphocytosis is usually less intense in CMV-induced infectious mononucleosis. In age-matched control subjects the results of liver function tests are less elevated when the agent is

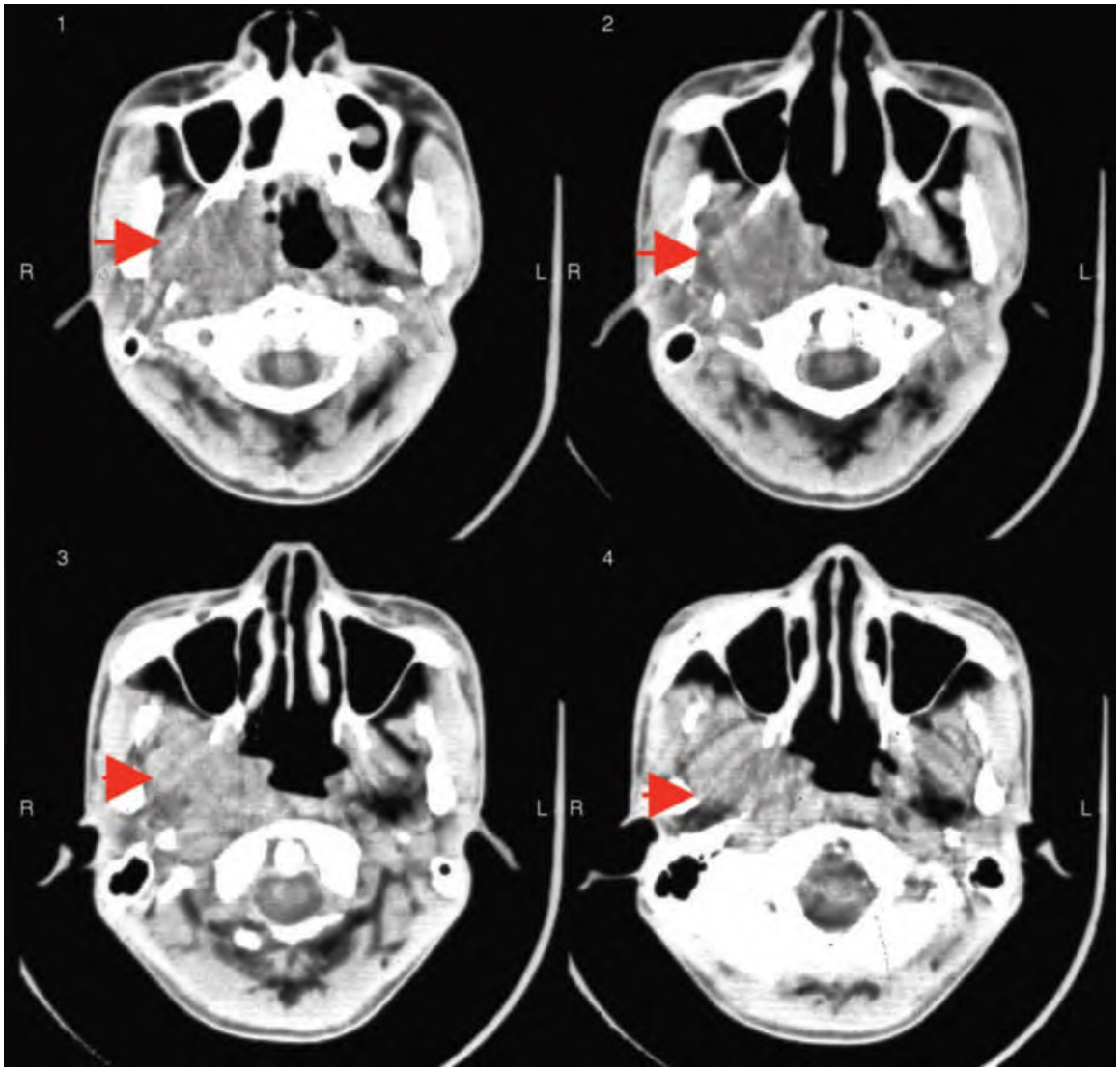

FIG. 138.6 Computed tomographic scan images of 25-year-old man with nasopharyngeal carcinoma *(arrows).* Tumor involves right parapharyngeal space. *(Courtesy Dr. Yi Zeng.)*

CMV. The illness may be attributed to CMV with serologic evidence of acute CMV infection and no evidence of acute EBV infection.

Primary Human Immunodeficiency Virus Infection

Patients with primary HIV infection may also present with fever, lymphadenopathy, and pharyngitis.[409–411] Such patients may also have a maculopapular rash and signs of aseptic meningitis. Patients with primary HIV infection are typically heterophile negative; however, rare cases of heterophile-positive primary HIV infection have been reported.[353] Thus serum or plasma should be sent for HIV RNA (viral load) as part of the evaluation of heterophile-negative infectious mononucleosis and may even be appropriate in heterophile-positive patients at high risk (see Chapter 120). Patients with primary HIV infection typically have negative or indeterminate HIV serology but can usually be diagnosed by combined antibody/antigen (fourth generation) tests.

Group A Streptococcal Pharyngitis

A streptococcal sore throat may also mimic infectious mononucleosis clinically. Adenopathy is generally submandibular and anterior cervical, and splenomegaly is absent in streptococcal sore throat. Culture of group A β-hemolytic streptococci from the throat is supportive but not conclusive evidence for this diagnosis because colonization with the organism can be common in this population of patients. If infectious mononucleosis is suspected in a patient with group A streptococci cultured from the pharynx, a positive heterophile, or positive EBV serologies can be used to confirm the diagnosis.

Hepatitis A, B, or C

Viral hepatitis may result in fever, lymphadenopathy, malaise, and an atypical lymphocytosis. In general, the atypical lymphocytosis is of lesser magnitude and accounts for less than 10% of the leukocytes. In viral hepatitis hepatocellular enzyme levels are usually markedly elevated at the initial visit, whereas in infectious mononucleosis the results of liver function tests are only mildly elevated initially and rise gradually over a 1- to 2-week period. In addition, specific serologic tests are currently available for the detection of infection with hepatitis A, B, and C viruses.

Toxoplasmosis and Other Infections

Acute toxoplasmosis may also give rise to an infectious mononucleosis-like illness. Usually the degree of the lymphocytosis is mild, and a diagnosis can be made with serologic tests for *Toxoplasma.* Rubella may also occasionally be manifested by fever, lymphadenopathy, and a mild atypical lymphocytosis, but the appearance of the exanthem and the clinical course of the illness are generally not confused with those of infectious mononucleosis. A serologic diagnosis of recent rubella infection can be obtained if the diagnosis remains in doubt. Anaplasmosis can

give rise to fever and an atypical lymphocytosis, and atypical lymphocytes can account for greater than 10% of the white blood cell differential.[412] These patients will typically lack the pharyngitis and lymphadenopathy that is present in EBV infection. In addition, *Anaplasma* infection is limited to regions where its tick vector is endemic. Diagnosis of *Anaplasma* infection can be made by PCR detection of *Anaplasma* nucleic acid in blood. Infectious lymphocytosis of childhood is a disease of uncertain cause that is characterized by fever, lymphadenopathy, occasionally diarrhea, and a lymphocytosis that consists almost exclusively of small mature lymphocytes. The disease is most common in the pediatric age group, may occur in epidemics, and is not associated with EBV infection.[413]

THERAPY

Infectious Mononucleosis

Supportive

Treatment of infectious mononucleosis is largely supportive because greater than 95% of the patients recover uneventfully without specific therapy. The level of activity is generally tailored to what the individual patient can tolerate comfortably. To avoid trauma to the spleen, contact sports or heavy lifting should be avoided during the first month of illness and until any splenomegaly has resolved. Ultrasound scan examination can be used to monitor spleen size. If constipation is present, it should be treated with a gentle laxative. Acetaminophen or nonsteroidal antiinflammatory agents can be helpful in relieving the sore throat and in suppressing the fever. Sore throat may be further alleviated with gargling with warm salt water.

Antiviral Agents

Acyclovir, ganciclovir, and foscarnet inhibit EBV replication in vitro.[414–416] However, these agents target the viral DNA polymerase, which is expressed only during lytic infection. Because EBV infection is predominantly latent, it is not surprising that these agents are ineffective in treatment of infectious mononucleosis. Further, the clinical symptoms and signs of infectious mononucleosis are largely the result of the vigorous immune response directed against EBV. A meta-analysis of five randomized controlled trials showed no significant benefit of acyclovir in the treatment of infectious mononucleosis. These trials included patients with mild, moderate, and severe mononucleosis. As expected, viral shedding from the oropharynx, where lytic replication commonly occurs, was reduced, but inhibition of shedding was lost 3 weeks after withdrawal of the antiviral agent.[417–421]

Corticosteroids

Corticosteroids should not generally be used in uncomplicated infectious mononucleosis. A double-blind, placebo-controlled trial showed that the combination of acyclovir and prednisolone did not reduce the duration of symptoms or result in an earlier return to work.[420] Other studies with corticosteroids have indicated that corticosteroids decrease the period of febrility and hasten the resolution of tonsillopharyngeal symptoms but do not reproducibly affect lymphadenopathy or liver and spleen involvement.[422–426] One particular reason to avoid corticosteroids in uncomplicated disease is that they have rarely been linked with complications such as encephalitis and myocarditis.[48,426] In addition, there is a theoretical risk that corticosteroids may inhibit the host immune response, resulting in a larger reservoir of latently infected cells that could potentially put patients at risk for EBV-associated malignant disease.

Corticosteroids may be helpful in cases of complicated infectious mononucleosis.[426–428] Tonsillar enlargement that causes airway compromise may respond rapidly to corticosteroids, eliminating the need for tracheostomy. Corticosteroids may also be helpful in autoimmune hemolytic anemia, severe thrombocytopenia, and aplastic anemia. Some investigators also advocate the use of corticosteroids for CNS involvement, myocarditis, or pericarditis. In selected cases of severe or prolonged prostration, corticosteroids may be of benefit. If corticosteroids are administered in these situations, treatment should be initiated in doses equivalent to 60 to 80 mg of prednisone per day given in a split daily regimen. The response is usually rapid, and the dosage can be tapered over a 1- to 2-week period.

Lymphoproliferative Disease

The overall mortality rate remains high, at about 50%, for LPD. Outcome appears to be enhanced with early diagnosis and treatment.[429] Multiple methods are used in the treatment of LPD, but large trials are still necessary to determine the best approaches.[52,53,282,430] The approach to therapy differs for monoclonal versus polyclonal LPD and depending on whether LPD arises in hematopoietic stem cell or solid-organ transplantation. The mainstay of LPD therapy in solid-organ transplantation is reduction of immune suppression. This strategy is logical because LPD most likely results from ineffective immune surveillance of EBV-infected B cells. Reduction of immune suppression leads to regression of tumors in up to 50% of cases. However, this approach is usually ineffective in stem cell transplantation because these patients receive high-dose chemotherapy and radiation to ablate the immune system and are dependent on engraftment of donor immune cells.[429] Reduction of immunosuppression can increase the risk of graft rejection. Surgical resection and radiotherapy are often used in localized LPD and can be combined with reduction of immune suppression in solid-organ transplant recipients with good results.

Rituximab, a monoclonal antibody directed against the CD20 antigen, found on most B cells, has become a mainstay of LPD treatment and can be given as a single agent or in combination with cytotoxic chemotherapy.[52,53,430] Binding of this antibody to B cells produces cell death through complement fixation or antibody-dependent cell-mediated cytotoxicity. Response rates range from approximately 70% to 100% with rituximab in different studies, and these differences may be a result of the timeliness of diagnosis.[429] At some centers rituximab is administered preemptively to patients without overt LPD when rising EBV DNA levels are observed in the blood.[431] Rituximab is occasionally given prophylatically in other settings but must be used judiciously as it does result in further immunosuppression due to profound B-cell depletion for up to 8 months.

Antiviral therapy is not of proven efficacy and not recommended for LPD.[432,433] Most LPD cells are latently infected by EBV and therefore do not express the EBV DNA polymerase. Experimental approaches aiming to induce lytic infection, and therefore induce expression of the EBV DNA polymerase, in LPD cells with treatment with arginine butyrate followed by ganciclovir have been reported.[434] By contrast, administration of acyclovir or ganciclovir prophylactically to high-risk pediatric solid-organ transplant recipients (donor EBV positive, recipient EBV negative) has been observed to reduce the risk of subsequent LPD development.[435,436]

Adoptive immunotherapy is another approach to LPD treatment. It is generally reserved for relapsed or refractory cases due to expense and limited availability of this therapy. It is particularly useful in allogeneic stem cell recipients, where LPD usually arises from donor cells and the donor is often available to harvest CTLs. This strategy is based on reconstitution of a cellular immune response against EBV to treat the infected tumor cells. Allogeneic stem cell recipients with LPD have been treated with unselected, donor mononuclear cells.[437,438] This approach results in response rates of up to 90% but also results in a high rate of graft-versus-host disease (GVHD) because of the presence of the infused alloreactive T cells. To avoid GVHD another approach has been to infuse selected, donor-derived, EBV-specific CTLs.[439–441] Marking of transferred T cells has shown that they persist up to 9 years.[442] This approach has also been used with success as prophylaxis for LPD in hematopoietic stem cell transplant recipients. In one study none of the 101 patients who received EBV-specific T-cell infusions as prophylaxis developed PTLD, and 11 of 13 patients with PTLD infused with EBV-specific T cells achieved complete remission.[443]

Adoptive immunotherapy can also be used in solid-organ transplant recipients. The organ recipient's CTLs can be expanded in vitro and then infused back into the patient.[444] PTLD during the first year after transplantation tends to exhibit type III latency, whereas PTLD that occurs in later years tends to have type I or II latency. Types I and II latency have programs of expression of fewer EBV latent genes, so these tumors are less immunogenic for CTL infusion and also tend to be more aggressive.[443] The infused CTLs do not expand as robustly as

in hematopoietic stem cell transplantation and only exhibit transient persistence, perhaps at least partially because of continued immunosuppression.[429] A limitation to this method occurs in primary EBV infection, in which rapid-onset LPD may not allow time to generate EBV-specific CTLs. Newer approaches to enhance the speed of CTL preparation from the typical 8 to 12 weeks with traditional expansion methods include direct selection of T cells using specific viral peptides in the context of class I human leukocyte antigen (HLA) molecules (tetramers) and isolation of IFN-γ–secreting T cells in response to EBV antigens.[443] Rapid expansion of virus-specific T cells has also been performed using antigen-presenting dendritic cells that have been engineered to express specific viral antigens with isolation of specific CTL cells in 10 days.[445,446] Another approach in solid-organ transplants has been to use closely matched allogeneic CTLs. A bank of about 100 CTLs from healthy donors has been used on a best-possible HLA match basis to treat LPD.[447] In a phase II multicenter trial, 33 patients with LPD who had failure with conventional therapy were treated with these allogeneic CTLs.[448] A response rate of 52% was seen at 6 months, and 14 patients achieved complete remission. Long-term follow-up at 4 to 9 years after the last CTL infusion showed that 12 of the 14 patients remained in complete remission, whereas two died—one of recurrent PTLD and the other from an unrelated infection.[449]

Measurement of the EBV DNA load in the blood may be helpful for prediction of response to treatment of LPD. In one study a decrease in the EBV DNA load within 72 hours correlated with response to therapy in seven responders, and all nonresponders had an increased EBV load at 72 hours.[450] Of note, however, after treatment with rituximab, peripheral blood mononuclear cell EBV DNA levels can fall even in the setting of tumor progression.[451]

Epstein-Barr Virus Targeted Therapy in Associated Malignant Diseases

A comprehensive discussion of multimodality therapy for all EBV-associated malignant diseases is beyond the scope of this chapter. The success of EBV immunotherapy in LPD has prompted its investigation in other EBV-associated malignant diseases. These malignant diseases, however, present challenges for immunotherapy that LPD does not. First, EBV gene expression is more limited in other tumors and, unlike LPD, they do not express the immunodominant EBNA3 proteins. Second, because other EBV-associated malignant diseases arise in the setting of an apparently intact immune response, it is not obvious that immunotherapy should work. Several small clinical trials of CTL infusions have shown they are generally well tolerated and have some efficacy for EBV-positive Hodgkin lymphoma and NPC.[452–460] The development of immune checkpoint inhibitors targeting the inhibitory programmed death protein 1 (PD-1) receptor found on lymphocytes, or its ligands PD-L1 and PD-L2, is an important new avenue for cancer immunotherapy.[461] Checkpoint inhibitor therapy appears to be particularly effective in tumors expressing "neoantigens" due to high somatic DNA mutation rates.[462] EBV-associated cancers upregulate the PD-L1 ligand and by definition express foreign antigens, making them attractive candidates for checkpoint inhibitor therapy.[463,464] Preliminary animal model studies have demonstrated checkpoint inhibitors can inhibit growth of EBV-associated lymphomas.[465] In summary, limited data suggest that EBV immunotherapy in patients with advanced EBV-positive Hodgkin lymphoma or NPC is well tolerated and may be beneficial. Checkpoint inhibitors, targeting the PD-1 signaling pathway, may assume an increasing role in future therapy of EBV-associated cancers. By contrast, antiviral drugs have no established role in the treatment of EBV-malignant diseases.

Oral Hairy Leukoplakia

Oral hairy leukoplakia differs from most EBV-related diseases in that the EBV infection is predominantly lytic rather than latent. In this setting of active lytic infection, agents such as acyclovir, ganciclovir, and foscarnet are effective in therapy.[466–469] Topical therapy, such as use of podophyllum resin, has also been shown to have efficacy against OHL.[470,471] In the setting of HIV-related OHL, oral lesions usually regress with the institution of effective antiretroviral therapy.

PREVENTION

Public Health Measures

Because the spread of virus requires intimate contact, isolation of patients with infectious mononucleosis is not necessary. Elevated viremia is seen for several months after recovery, so consideration should be given to postponement of blood donation by patients with infectious mononucleosis for at least 6 months after the onset of illness.

Vaccine

EBV vaccine development has been an elusive goal for many years, and substantial research activity is being devoted to this area, as recently reviewed.[472] Because EBV infection does not cause severe disease in most instances, a vaccine must be particularly safe.[50] However, there are about 125,000 cases of infectious mononucleosis in the United States each year and approximately 200,000 new cases of EBV-associated malignancy across the world.[473] Development of a vaccine that could reduce or eliminate this burden of disease remains a significant goal. The only herpesvirus vaccine currently licensed by the US Food and Drug Administration is a live-attenuated varicella-zoster vaccine. Because of EBV's associations with malignant diseases, acceptance of a live-attenuated vaccine is highly unlikely.

The goals of an EBV vaccine are not yet clearly defined[282] and likely will be determined based on the levels of protection provided by different vaccine formulations. Complete protection from infection, at first glance, appears to be the primary goal, but its attainment may be limited by the biology of the virus (see subsequent discussion). Another potential goal is prevention of symptomatic infection of infectious mononucleosis, without necessarily prevention of lifelong latent viral infection. In such a case determination is critical of whether the vaccine also provides any protection against complications of EBV, such as EBV-related malignancy or fulminant primary infection in XLP. Notably, symptomatic infectious mononucleosis is associated with a 3.4-fold increased risk of EBV-related Hodgkin lymphoma, so a vaccine that prevents symptomatic infection without necessarily preventing infection could be of significant benefit.[474] In addition, because elevated EBV viral loads in blood can predict development of EBV-associated malignancy in transplant recipients, and because EBV viral loads are elevated at the onset of NPC, an EBV vaccine that induces better immune control of infection and prevents increased viral loads may potentially prevent EBV malignancies.[473] To address these issues a meeting was held at the US National Institutes of Health in 2011. The group concluded that the goals of future EBV vaccine research should be the prevention of infectious mononucleosis and EBV-associated malignancies. Another recommendation was to identify surrogate markers that predict EBV-associated malignancies, to be able to more rapidly assess the effectiveness of vaccines before the advent of malignancy.[474]

Two major approaches have been taken to EBV vaccine development. The most common approach taken with EBV vaccines is to induce EBV neutralizing antibody directed against the viral glycoprotein gp350, the most abundant glycoprotein on the virus, and which binds to the EBV cellular CD21 receptor.[475] Immunization with gp350 protects against EBV-induced lymphomas in an animal model.[476] Of interest, despite initial expectations, cell-mediated immunity appears to play an important role in the gp350 vaccination prevention of lymphoma in this model.[477] A small trial in China with recombinant vaccinia virus expressing gp350 protected six of nine children from EBV infection at 16 months compared with none of 10 control subjects.[478] However, the use of live vaccinia-based vaccines is unlikely to become widespread.[282] Of importance, phase I and II studies of a purified gp350 vaccine have recently shown protection from symptoms of infectious mononucleosis but did not prevent asymptomatic infection with EBV, consistent with the potential goal of reducing cases of symptomatic mononucleosis. Of note, one initially EBV-seropositive participant experienced an oligoarthritis reaction, which may have been related to the vaccine.[479–481] A recent discovery found that presenting the CD21 binding component of gp350 in a symmetrical array on self-assembling nanoparticles induced potent neutralizing antibodies in mice and nonhuman primates and improved protection in a mouse model.[482] It is possible that such an approach may enhance the effectiveness of gp350 vaccines.

The second approach has been to develop a vaccine with known EBV class I–restricted MHC CTL epitopes.[483–485] Although such a vaccine would not necessarily be designed to prevent primary infection, it is expected to ameliorate the symptoms of mononucleosis.[483] Another important potential use of such a vaccine would be to boost the CTL response to avoid development of, or possibly treat, EBV-associated malignant diseases. A significant number of EBV epitopes recognized by CTLs have now been identified. A phase I trial has been completed in Australia with a single EBNA3 EBV epitope. In this trial two placebo recipients became EBV infected, and one had symptomatic mononucleosis; four vaccine recipients acquired EBV infection but none were symptomatic.[486] To generate a broad-based CTL response, a vaccine containing multiple EBV epitopes is necessary. In addition, because CTLs from individuals with different HLA alleles recognize different EBV epitopes, inclusion of relevant epitopes in a vaccine is important. Therefore current efforts have fused multiple peptide epitopes together for use in vaccines.[50,483]

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139 Human Herpesvirus Types 6 and 7 (Exanthem Subitum)

Jeffrey I. Cohen[a]

SHORT VIEW SUMMARY

Definition
- Human herpesviruses 6 and 7 (HHV-6 and HHV-7) cause exanthem subitum or febrile seizures in young children and reactivate frequently in highly immunocompromised hosts. They can also cause encephalitis in immunocompromised hosts.

Epidemiology
- Most adults are seropositive for HHV-6 and HHV-7.
- The average age of infection with HHV-6 is about 1 year, and for HHV-7 is 2 years.
- About 50% of hematopoietic transplant recipients and 20% to 33% of organ transplant recipients have HHV-6 and HHV-7 DNA in the blood.
- HHV-6 DNA is integrated in the chromosomes of 1% to 2% of persons and is transmitted in the germline DNA.

Microbiology
- HHV-6 and HHV-7, like cytomegalovirus, are betaherpesviruses.

Diagnosis
- Exanthem subitum is usually diagnosed clinically, but seroconversion to HHV-6 or HHV-7 antibody positivity can be used.
- Diagnosis of HHV-6 or HHV-7 disease in immunocompromised persons is difficult owing to the high frequency of asymptomatic reactivation and the finding that up to 2% of persons have HHV-6 DNA integrated in their chromosomes.
- HHV-6 limbic encephalitis is diagnosed based on clinical signs and symptoms and HHV-6 DNA in the cerebrospinal fluid.
- Detection of HHV-6 protein or RNA in tissues is more specific than HHV-6 DNA for diagnosing virus-associated disease in immunocompromised persons.

Therapy
- No therapy has been shown to be effective for treatment of HHV-6 or HHV-7, but both viruses are sensitive to ganciclovir, foscarnet, and cidofovir in vitro.
- Ganciclovir, foscarnet, or both have been used to treat immunocompromised persons with HHV-6 or HHV-7 disease, especially with limbic encephalitis.

Human herpesviruses 6 and 7 (HHV-6 and HHV-7) are both members of the Betaherpesvirinae subfamily. Both viruses infect T cells, are present ubiquitously, and can cause exanthema subitum (or roseola infantum). In addition, both viruses frequently reactivate in highly immunocompromised patients but rarely cause serious disease in these patients.

HUMAN HERPESVIRUS TYPE 6

History

HHV-6 was discovered by Salahuddin and colleagues[1] in 1986 in patients with lymphoproliferative disorders and human immunodeficiency virus (HIV). Subsequently, two variants of HHV-6 were described: HHV-6A and HHV-6B.[2] HHV-6B was shown to be an etiologic agent of exanthema subitum,[3] whereas HHV-6A has rarely been associated with disease.[4]

Description of the Virus

HHV-6 is a member of the *Roseolovirus* genus of betaherpesviruses and shares a number of features with cytomegalovirus (CMV), including numerous homologous viral proteins and similar genomic structures.[5] HHV-6A and HHV-6B, which share 90% nucleotide sequence identity, are sufficiently different in their sequences and in their cell tropism that they could be classified as separate species of herpesviruses. The receptor for HHV-6A is CD46 and for HHV-6B is CD134, both of which interact with a complex consisting of viral glycoproteins gH, gL, gQ1, and gQ2. The HHV-6 genome contains about 165 kilobase pairs of DNA.

Epidemiology

More than 95% of adults are seropositive for HHV-6. Maternal antibody to HHV-6 declines during the first 5 months of life. About 40% to 50% of children are infected by 1 year of age, and 77% to 82% are infected by 2 years of age (Fig. 139.1).[6,7] The peak of infection occurs at 9 to 21 months. Approximately 90% of infections in children are symptomatic; in one study, 40% of infants with HHV-6 were seen by a physician.[6] There is no seasonal peak for primary HHV-6 infection.[6] HHV-6 is usually transmitted horizontally, presumably by infected saliva from close contacts with children. Outbreaks of HHV-6 infection have been reported at daycare centers.

HHV-6 infects peripheral blood mononuclear cells (PBMCs) and cells in the liver, salivary glands, endothelial cells, and central nervous system (CNS). HHV-6 DNA was detected in PBMCs from 22% and in cervical swabs from 7.5% of pregnant women.[8] HHV-6 was also detected in about 1% of cord blood samples and in fetal blood, indicating a potential for fetal disease. Congenital infection with HHV-6 is most often due to chromosomally integrated HHV-6 (ciHHV-6) passed in the germline DNA. In one study, 86% of congenital infections were from ciHHV-6, and 14% were due to transplacental infection.[9] These infants have 10^5 or 10^6 copies of HHV-6 DNA per microgram of cellular DNA. HHV-6 DNA persists in the blood intermittently after primary infection in most children and can reactivate in healthy children without apparent illness.[10] HHV-6 has been transmitted by organ transplantation[11] and has been transmitted to hematopoietic stem cell transplant (HSCT) recipients by ciHHV-6 from donor cells.[12]

HHV-6 frequently reactivates in immunocompromised patients. About 50% of HSCT and about 33% of solid-organ transplant recipients reactivate HHV-6 as defined by detection of viral DNA in the peripheral blood.[13,14] More than 95% of HHV-6 reactivations in HSCT recipients are due to HHV-6B. Reactivation of the virus usually occurs within the first month after transplant, and reactivation is increased with reduced cellular immunity, particularly in patients receiving anti-CD3 antibody or corticosteroids, and in those who have undergone allogeneic or cord blood transplants.[14,15] Half of adults who receive cord blood transplants have reactivation lasting a median of 4 years after transplant.[16] Reactivation has also been reported in 54% of critically ill patients who are not otherwise immunocompromised[17] and frequently occurs in patients with drug reaction with eosinophilia and systemic symptoms (DRESS).

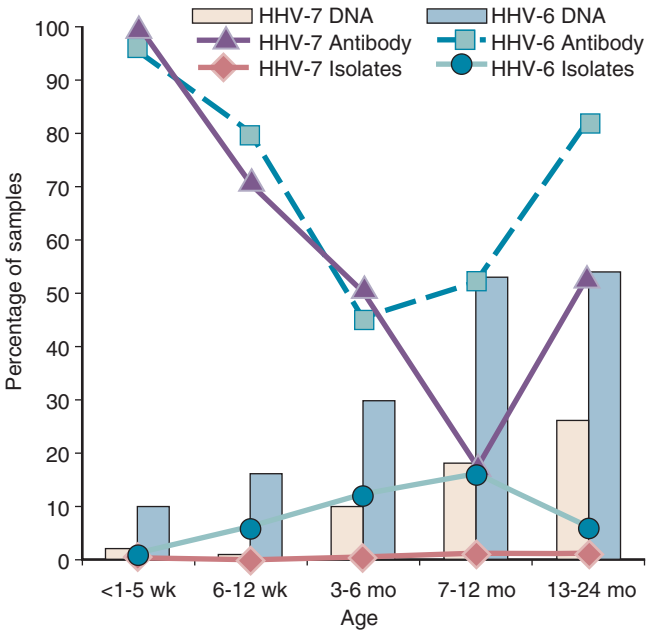

FIG. 139.1 Percentages of blood samples from children 2 years of age or younger with antibody, virus culture, and virus polymerase chain reaction for human herpesvirus *(HHV)*-6 and HHV-7. *(From Hall CB, Caserta MT, Schnabel KC, et al. Characteristics and acquisition of human herpesvirus [HHV] 7 infections in relation to infection with HHV-6.* J Infect Dis. *2006;193:1063–1069.)*

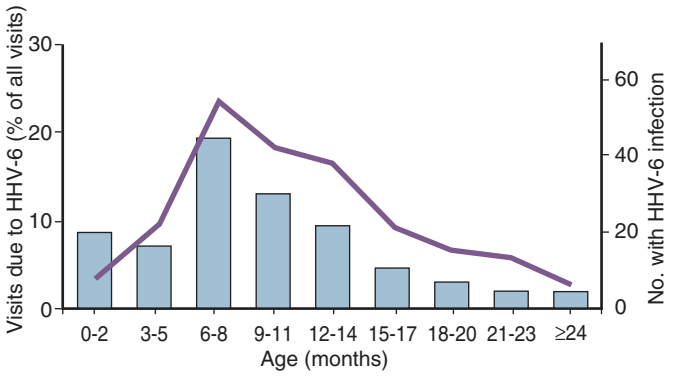

FIG. 139.2 Percentage of visits to the emergency department for febrile illnesses associated with human herpesvirus 6 *(HHV-6) (line)* and the number of children with primary HHV-6 infection *(bars).* *(From Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation.* N Engl J Med. *1994;331:432–438.)*

Pathogenesis

In addition to infecting its primary target, $CD4^+$ T cells, HHV-6 infects other T cells, B cells, natural killer (NK) cells, monocytes, macrophages, epithelial cells, and neural cells. HHV-6A has a greater predilection to infect neural cells than does HHV-6B, whereas HHV-6B is more commonly detected in PBMCs than HHV-6A. Detection of HHV-6 DNA at high levels in the olfactory bulb or tract relative to other portions of the brain and in nasal mucous specimens suggests that HHV-6 may enter the CNS through the olfactory pathway.[18] Infection of lymphocytes with HHV-6 results in ballooning of the cells with intranuclear inclusions, followed by cell death. HHV-6 establishes a latent infection in $CD34^+$ hematopoietic stem cells, monocytes, and macrophages, and a persistent infection in salivary glands. HHV-6 is detected in saliva, but at a much lower frequency than HHV-7. Cellular immunity is more important than humoral immunity for controlling HHV-6 infection.

HHV-6 induces expression of CD4 on the surface of T cells, which can increase susceptibility to HIV infection. However, infection of cells with HHV-6 results in reduced expression of the HIV coreceptor CXCR4 on the surface of cells and increased expression of the RANTES (regulated on activation, normal T-cell expressed and secreted) chemokine, which can inhibit replication of CCR5 tropic HIV strains in HHV-6-infected cells. HHV-6A inhibits expression of major histocompatibility complex (MHC) class I on dendritic cells.

Clinical Manifestations

Infantile Fever and Seizures

The incubation time for HHV-6 infection is estimated to be 1 to 2 weeks. Infantile fever is the most common manifestation of HHV-6 infection. More than 90% of children infected with HHV-6 have symptoms, including fussiness, rhinorrhea, and fever in over half of patients; cough, diarrhea, and rash occur in about one-third of patients.[6] It is estimated that 5% to 25% of visits to emergency departments for fever in infants are due to HHV-6. Children older than 6 months are more likely to have fever than are younger children.[6] Of children younger than 3 years seen in emergency departments with fever, 10% had primary infection with HHV-6; this number increased to 20% for children 6 to 12 months old (Fig. 139.2).[19] The mean age of primary HHV-6 infection was 9.4 months, the median duration of illness was 6 days, and the mean temperature was 39.6°C. In another study of 243 children seen in the emergency department with HHV-6, more than 50% had fever of 40°C or higher, malaise, otitis, and nasal congestion.[20]

HHV-6 is responsible for about one-third of febrile seizures in children up to 2 years of age.[19] Of 160 children with acute HHV-6 infections, 13% had seizures; the median age of children with HHV-6 and seizures was 14 months. Primary infection with HHV-6 is more frequently associated with severe seizures, long seizures, and recurrent seizures than seizures not associated with HHV-6.[21] HHV-6 viremia was present in 32% of children with febrile status epilepticus; 22% of patients had primary HHV-6B infection, and 10% had virus reactivation.[22]

Exanthem Subitum (Roseola Infantum or Sixth Disease)

Exanthem subitum is caused by either HHV-6B or HHV-7. Approximately 25% of patients with HHV-6 infection in the United States have exanthem subitum at presentation[6]; in contrast, in Japan about 75% of primary HHV-6 infections result in exanthem subitum.[23] The disease begins with a high fever that usually lasts for 3 to 4 days.[6,23] At the time of defervescence, patients develop a macular or maculopapular rash that begins on the neck or trunk, spreads to the extremities, and persists for a few hours to 2 days (Fig. 139.3). The disease may be accompanied by cough, cervical and occipital lymphadenopathy, erythema of the tympanic membranes, conjunctivitis, eyelid edema, bulging fontanelles, lymphadenopathy, diarrhea, or Nagayama spots (red papules on the soft palate or base of the uvula). The median duration of symptoms is 9 days. Rare complications include febrile seizures, meningitis, and encephalitis.[24] Patients often have leukocytosis during the first day, followed by leukopenia with a relative lymphocytosis and in some cases thrombocytopenia.

Other Neurologic Symptoms Associated With HHV-6

HHV-6 is a rare cause of meningitis; the virus can also cause encephalitis in otherwise healthy children with an altered level of consciousness, seizures, psychosis, or cranial nerve deficits.[25] These children usually have a panencephalitis and can have persistent neurologic sequelae. Eight percent (13/156) of young children hospitalized in Britain and Ireland with encephalitis or fever and seizures were found to have acute HHV-6 infection.[26] HHV-6 DNA was detected in the cerebrospinal fluid (CSF) of 7% (9/138) of patients with encephalitis with a lymphocytic pleocytosis, but no specific differences were noted in patients with encephalitis attributed to HHV-6 versus that due to other causes.[27] HHV-6 may also cause encephalitis in otherwise healthy adults,[28] and HHV-6 DNA was detected in the CSF of 0.4% (4/1000) of persons in the California Encephalitis Project study.[29]

HHV-6 has been associated with multiple sclerosis based on detection of DNA in CSF and DNA and viral antigens in the brain; however, viral

FIG. 139.3 A child with exanthem subitum. *(Courtesy Professor K. Yaminishi, Osaka University Medical School, Osaka, Japan.)*

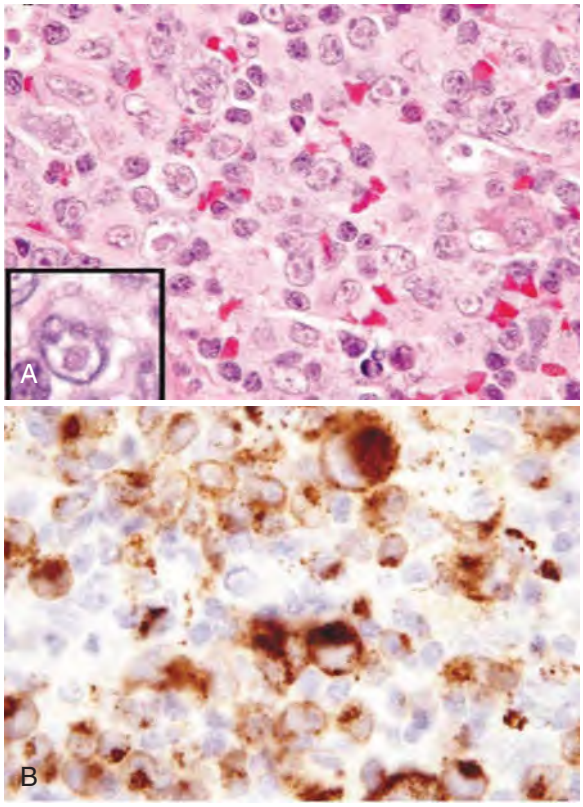

FIG. 139.4 Eosinophilic intranuclear and cytoplasmic inclusions with hematoxylin and eosin stain (A) and immunohistochemical staining with antibody to human herpesvirus 6 (HHV-6) envelope glycoprotein gp60 in viral inclusions (B), in a lymph node from a patient with human herpesvirus 6 infectious mononucleosis. *Inset* shows a cell with an intranuclear inclusion. *(From Maric I, Bryant R, Abu-Asab M, et al. Human herpesvirus-6–associated acute lymphadenitis in immunocompetent adults.* Mod Pathol. *2004;17:1427–1433.)*

DNA and proteins have also been detected in the brain of controls,[30,31] and the role of HHV-6 in multiple sclerosis is controversial.[32] HHV-6 antigen was detected in astrocytes cultured from the brain of patients with mesial temporal sclerosis (MTS).[33] A study of patients with MTS found HHV-6 DNA in 22% to 29% of studied tissues (hippocampus, amygdala, and mixed amygdala and uncus), and significantly greater levels of viral DNA were found in specimens from patients with MTS patients than from patients without MTS.[34] Markers of neuroinflammation have also been noted in association with HHV-6 infection of the CNS.[35]

Infectious Mononucleosis

Older patients who develop primary infection with HHV-6 may present with infectious mononucleosis with fever, lymphadenopathy, generalized rash, and atypical lymphocytes.[36] Lymph node biopsy specimens show intranuclear and cytoplasmic inclusions with HHV-6 antigens (Fig. 139.4).[37]

Other Complications in Healthy Persons

HHV-6 has been associated with chronic or fulminant hepatitis,[38] thrombocytopenic purpura, myocarditis,[39] and hemophagocytic syndrome in case reports. Although HHV-6 DNA has been found in some lymphomas, there is no compelling evidence that HHV-6 is associated with malignancy. HHV-6 is not a cause of chronic fatigue syndrome.

Congenital Infection

HHV-6 congenital infections (defined by detection of virus in cord blood) occurred in 1% (57/5638) of births and, unlike infections later in life, were asymptomatic.[40] In a follow-up study, neurodevelopmental scores were significantly lower at 1 year of age in infants who had HHV-6 congenital infection.[41]

Infection in the Immunocompromised Host

Because a large proportion of HSCT and organ transplant recipients reactivate HHV-6 with viral DNA in the peripheral blood (see "Epidemiology," earlier), it is often difficult to confirm that symptoms in transplant recipients are due to HHV-6.

HHV-6 is frequently associated with fever and rash early after transplant.[14,42] The virus has also been associated with delayed monocyte and platelet engraftment in HSCT recipients.[43] One study showed that high levels of HHV-6 DNA in the blood correlated with delayed platelet engraftment after transplant.[44] HHV-6 infects hematopoietic progenitor cells, inhibiting colony formation in vitro,[45] and has been associated with bone marrow suppression and delayed engraftment in some[13] but not other[46] studies. The association of HHV-6 reactivation with CMV infection and acute graft-versus-host disease[47] may make it difficult to confirm HHV-6 as a primary cause of marrow suppression or graft rejection. HHV-6 reactivation was associated with an increase in nonrelapse mortality in HSCT recipients.[47]

One of the best-documented manifestations of HHV-6 in immunosuppressed patients is limbic encephalitis.[48,49] The disease is especially common in transplant recipients receiving cord blood. In a meta-analysis, the prevalence of HHV-6 encephalitis was 8.3% in cord blood transplant recipients versus 0.5% in persons receiving another source of stem cells.[50] Other risk factors for HHV-6 acute limbic encephalitis include acute graft-versus-host disease, receipt of adult mismatched donor cells, and engraftment syndrome.[51,52] Interleukin-6 levels in the plasma were more often elevated in persons with HHV-6–associated neurologic complications compared with those without such complications.[53] In a study of 1344 HSCT recipients, 50% of cord blood recipients who developed HHV-6 limbic encephalitis died, whereas no adult donor cell recipients with HHV-6 encephalitis died.[51] Most cases of HHV-6 encephalitis in the literature were diagnosed through detection of viral DNA in the CSF[54]; HHV-6 antigen in the brain is more specific for diagnosis. Patients often present 2 to 6 weeks after HSCT with headache and confusion and nonfocal neurologic examination findings that may progress to seizures, psychosis, and cranial nerve deficits. Although the computed tomography (CT) scan is usually unremarkable initially, magnetic resonance imaging (MRI) shows abnormalities in 75% of patients with

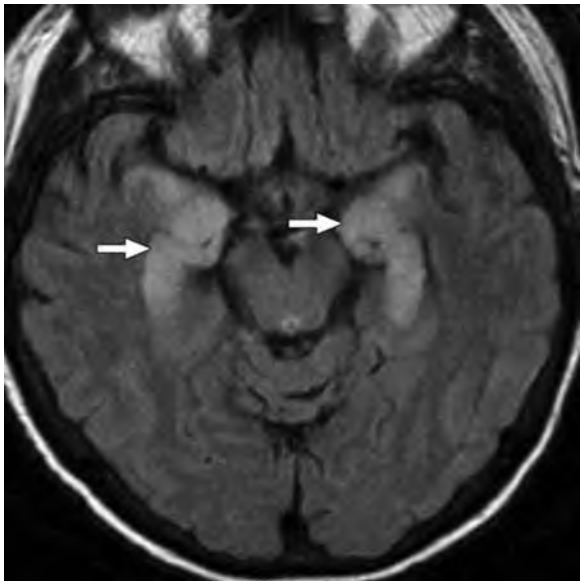

FIG. 139.5 Magnetic resonance image showing bilateral medial temporal lobe involvement *(arrows)* in a hematopoietic stem cell transplant recipient. *(Courtesy Alexander Freeman, National Institute of Allergy and Infectious Diseases.)*

changes in the gray matter of the temporal lobes, especially the medial aspect of these lobes (Fig. 139.5). The CSF shows an elevated protein in two-thirds of cases, with a mild lymphocytic pleocytosis in the minority of patients. Limbic encephalitis often manifests with short-term memory loss and insomnia, with HHV-6 proteins or RNA in the hippocampus and amygdala in HSCT recipients.[48,55,56] Nine transplant patients were reported to have limbic encephalitis with MRI changes involving the amygdala and hippocampus, inappropriate diuretic hormone secretion, and anterograde amnesia.[57] Survivors of HHV-6 often have atrophy of the hippocampus with persistent memory impairment and fatigue and frequently are unable to return to school or work.[51,58] HHV-6 reactivation in the blood is associated with delirium and cognitive decline in the first 3 months after HSCT[59] and cord blood transplantation.[60] HHV-6 myelitis has been reported during engraftment after cord blood transplantation.[61]

Although HHV-6 DNA was detected at high levels in the lungs of bone marrow transplant patients with idiopathic pneumonia compared with lower levels in the lungs of control immunocompetent patients,[62] other studies have not confirmed these findings.[46] The high frequency of virus reactivation in these patients emphasizes the importance of detecting viral proteins in tissues (see "Diagnosis," later).

Case reports have described HHV-6 associated with giant cell hepatitis[11] and other forms of hepatitis, colitis, and gastroduodenitis in transplant recipients. HHV-6 antigen was detected in PBMCs infiltrating biopsy specimens of gastroduodenal mucosa in 23% of liver transplant recipients, but also in 19% of immunocompetent patients with upper gastrointestinal symptoms; the number of HHV-6–positive cells tended to be higher in the transplant patients than in the immunocompetent patients.[63] HHV-6 proteins have been detected in tubular epithelia of kidneys undergoing rejection after transplantation.

HHV-6 has less commonly been associated with encephalitis and pneumonitis in acquired immunodeficiency syndrome (AIDS) patients. HHV-6 has generally not been shown to influence the rate of progression of HIV to AIDS.

Laboratory Diagnosis

Healthy Persons

The diagnosis of exanthem subitum is usually made clinically. The most frequently used diagnostic test for acute HHV-6 disease in children is comparison of acute and convalescent serum for seroconversion to HHV-6.[64] The observation of asymptomatic reactivations of HHV-6 in healthy persons indicates that detection of a fourfold or greater rise in titer alone may not be diagnostic of acute infection. HHV-6 immunoglobulin G (IgG) is usually present 1 week after infection, peaks in the second week, and persists for life. An alternative diagnostic test for acute disease in children is detection of HHV-6 DNA in sera or plasma at a time when antibody to the virus is absent, reflecting the transient viremia that occurs before the onset of antibody; however, this test is less specific than seroconversion.[64] Currently used serologic tests include immunofluorescent antibody, enzyme-linked immunosorbent assay (ELISA), and immunoblot assays. These tests cannot distinguish HHV-6A from HHV-6B, and there can be cross-reactivity of HHV-6 with HHV-7. HHV-6 IgM is present early in infection and persists for a few weeks; however, virus-specific IgM may not be detectable in some children, and this antibody has been detected in some adults, suggesting virus reactivation.

Culture of HHV-6 from PBMCs, serum, or plasma of patients with exanthem subitum during the febrile period is considered diagnostic[3] but is available only in research laboratories. Detection of HHV-6 DNA in plasma in children younger than 2 years is not considered sufficiently specific to differentiate infants with acute HHV-6 infection from those with serious bacterial infections.[65]

Immunocompromised Persons

Diagnosis of HHV-6 as the cause of symptoms in immunocompromised patients is often difficult. Because HHV-6 frequently reactivates in a large proportion of these patients, it is important to distinguish latent infections that are not associated with disease from productive HHV-6 infections that may cause disease. A high or a rising level of viral DNA, or detection of viral RNA (available only as a research test), in the blood is more likely to enable differentiation of productive infection associated with HHV-6 disease from latent viral DNA. In a study of HSCT recipients, the presence of more than 10^3 copies of HHV-6 DNA per 10^6 PBMCs was statistically associated with myelosuppression, pneumonitis, fever, and rash.[13] However, in a trial of liver transplant recipients, monitoring of HHV-6 viremia had no effect on outcomes.[66] Detection of viral DNA in the serum or plasma may also be more predictive of disease than the presence of HHV-6 in PBMCs. Detection of HHV-6 RNA, and especially viral proteins, in tissues is more specific for active disease than is detection of viral DNA. In addition to a rising level of HHV-6 in the blood, and preferably detection of HHV-6 in tissue, other potential causes of disease (including CMV, which is often associated with HHV-6) must be excluded before HHV-6 can be considered to have a causative role in disease.

Detection of HHV-6 in the CSF is strongly suggestive of CNS disease but is not absolutely diagnostic because some patients with HHV-6 in the CSF have had other CNS diseases documented at autopsy, and HHV-6 has been detected in the CSF of children years after acute infection.[67] In one study, HHV-6 DNA was detected in the CSF of 8/11 (73%) patients with neurologic dysfunction and 3/11 (27%) of asymptomatic HSCT recipients.[68] Although another study reported HHV-6 in the CSF of 23% of transplant recipients with encephalitis, compared with only 1% of transplant recipients without encephalitis, controls were not well matched for the level of immunosuppression.[69] Thus, other causes of encephalitis must be ruled out before diagnosis of HHV-6 encephalitis is accepted based on CSF findings. Detection of HHV-6 proteins in the brain is more specific than detection of DNA because viral DNA has been detected with polymerase chain reaction (PCR) in the brain of about one-third of healthy persons.[70]

The use of PCR for diagnosis of HHV-6 is complicated by the finding that HHV-6 is integrated into chromosomal DNA in 0.7% to 1.5% of persons.[71] Stem cells containing integrated copies of HHV-6 have transmitted viral DNA to recipients.[12] Therefore ciHHV-6, in addition to HHV-6 productive infection, must be considered when a diagnosis is based on detection of HHV-6 in leukocytes or in CSF that may contain leukocytes. A high level of HHV-6 DNA in the blood (>320,000 copies/mL in whole blood or ≥1 copy per leukocyte) that persists over time is suggestive of ciHHV-6.[72] Digital droplet PCR can enable accurate diagnosis of ciHHV-6 by showing that the HHV-6/cell ratio is 1.[73] Although ciHHV-6 can be reactivated in vitro by histone deacetylase inhibitors,[74] it is unknown if this can occur in vivo. The clinical importance of ciHHV-6 is unknown, although a large study of patients with ciHHV-6 found an increased incidence of angina pectoris but not other