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INTRODUCTION

Viral infections can lead to acute or chronic demyelinating diseases of the central or peripheral nervous systems in animals and humans. These diseases can be acute or chronic with progressive or relapsing-remitting courses. The loss of myelin with relative sparing of axons may or may not be associated with inflammation or gliosis, and oligodendrocytes or Schwann cells may or may not be altered. The mechanisms of demyelination are varied.

Natural infections of rodents (mouse hepatitis and Theiler’s viruses), canines (canine distemper virus), and ruminants (visna of sheep and caprine arthritis-encephalitis viruses) have been associated with central nervous system (CNS) demyelination; see Chapter 44. This chapter focuses on three human CNS demyelinating diseases: acute disseminated encephalomyelitis (ADEM), also known as post-infectious encephalomyelitis, progressive multifocal leukoencephalopathy (PML), and multiple sclerosis (MS).

These three diseases have very different clinical courses and distinctive pathological features, although all share the essential element of demyelination. ADEM and PML have antithetic modes of pathogenesis; the former is a predominantly extraneural infection resulting in a virus-induced host autoimmune response, and the latter is a direct lytic infection of oligodendrocytes in an immunocompromised host. The role of infections in MS is unclear; epidemiological studies implicate an early life exposure in the genesis of the disease that could represent an infection. Exacerbations of disease more often follow viral-like illnesses and patients with MS have abnormal immune responses to viruses, including the intrathecal generation of antibodies to measles and a variety of other agents.

PROPOSED MECHANISMS OF VIRUS-INDUCED DEMYELINATION

Several mechanisms have been proposed to explain the demyelination seen with viral infections, and in MS when an infectious etiology has been postulated (Tab. 40.1). The most straightforward mechanism to explain CNS myelin loss is the selective destruction of the myelin maintaining cells, oligodendrocytes. This mechanism is responsible for the loss of myelin in PML, a disease characterized by infection and lysis of oligodendrocytes (discussed later).

In the immunocompetent host, immune responses are often proposed to explain myelin destruction. Most agents associated with CNS demyelination are enveloped viruses. Viral envelopes are formed by insertion of virus-coded proteins into the lipid bilayer of the cell membrane. The core or nucleocapsid of the virus then acquires the envelope by budding through the modified membrane. Hypothetically, immune responses against viral proteins within the membrane could caused myelin membrane damage in situ. Alternatively, virus
replicating in oligodendrocytes could transport sequestered myelin antigens into the systemic circulation.

Infection of macrophages, microglia or astrocytes may release soluble cytokines, chemokines or viral proteins, which are toxic to other uninfected CNS cells. Visna virus infections of sheep have a long incubation period followed by either a progressive or remitting and relapsing course, accompanied by patchy demyelinated lesions simulating MS. However, infection is limited to cells of macrophage origin. In the brain, macrophages and microglia appear to release a cytokine or similar soluble substance that results in demyelination (Kennedy et al., 1985). A related lentivirus, human immunodeficiency virus (HIV), has subsequently been shown to infect the same restricted cell population in the human brain. Viral proteins as well as cytokines released from infected macrophages and microglia have also been implicated in the pathogenesis of HIV encephalopathy (Power and Johnson, 2001).

Demyelination can also result from immune responses against myelin in the absence of nervous system infection. Molecular mimicry, in which an immune response to an environmental agent cross-reacts with a host antigen, has been a popular postulated mechanism. A similar amino acid sequence contained in both viral protein and myelin proteins might allow systemic virus replication to induce an immune response against an epitope on the CNS myelin. Searching sequence databases for commonality of encephalitogenic sequences of myelin basic protein and viral proteins turned up a sequence within the P protein of hepatitis B virus. Inoculation of this synthesized viral sequence into rabbits resulted in an inflammatory response in the brain (Fujinami and Oldstone, 1985). In natural infections, Camphylobactor infections followed by the axonal form of Guillain-Barre syndrome are probably due to similarities between bacterial and axonal proteins (Moran and Prendergast, 2001), and cellular damage in HTLV-1-associated myelopathy (tropical spastic paraparesis) appears related, in part, to homology between nuclear ribonucleolar neuronal protein and the tax protein of HTLV-1 (Levin et al., 2002).

Finally, infection of lymphoid cells may disrupt normal cellular immune responses. Activated T cells normally traffic through the CNS, but only those recognizing an antigen remain (Irani and Griffin, 1996). Thus, in systemic infections causing lymphocyte activation, CNS traffic of T cells is increased. If normally suppressed responses to self-antigens are disrupted by the infection, autoimmune disease can occur such as ADEM associated with measles virus infections (discussed later).

For over half a century, the focus on experimental autoimmune (allergic) encephalomyelitis (EAE) as the prototype autoimmune disease biased thought on virus-induced demyelination. In recent years, studies of humoral immune responses in the pathogenesis of Guillain-Barre syndrome and focus on toxic effects of cytokines and viral proteins in studies of neurological complications of HIV infections have provided a more balanced perspective.

| TABLE 40.1 Proposed Mechanisms of Virus-Induced CNS Demyelination |
|---------------------------------------------------------------|
| CNS infection                                                |
| Infection of oligodendrocytes                                |
| Direct destruction                                           |
| Pathogenic immune response to viral antigens on cell membranes|
| Introduction of cell membranes into systematic circulation   |
| Infection of other CNS cells                                 |
| Release of cytokines or viral proteins toxic to myelin supporting cells or myelin membranes |
| Extraneural infection                                        |
| Molecular mimicry (virus proteins and myelin proteins)      |
| Disruption of immune responses                               |
ACUTE DISSEMINATED ENCEPHALOMYELITIS

Definition

ADEM is an acute, inflammatory, demyelinating disease of the brain and spinal cord. In most patients it has an abrupt onset days to several weeks after a viral exanthem or viral-like illness. But the disease is not specific to viruses and has been reported after several bacterial illnesses, immunizations, and drug and serum administration.

The nosology is confusing, since the disease has been described under a remarkable variety of names. Post-infectious, parainfectious, post-exanthematous, post-vaccinal, post-measles, and post-influenzal encephalomyelitis have been applied to describe the clinical settings. Acute disseminated encephalomyelitis, perivascular myelinolysis, perivenous encephalitis, and acute demyelinating encephalomyelitis have been coined to describe the pathological features. Allergic encephalomyelitis, immune-mediated encephalomyelitis, hyperergic encephalomyelitis, and disseminated vasculomyelinopathy imply knowledge of pathogenetic mechanisms (Johnson et al., 1985). Since the essential features for diagnosis are the neuropathological changes, ADEM will be used here except in specific cases such as post-measles and post-vaccinal encephalomyelitis.

Acute hemorrhagic necrotizing leukoencephalitis is generally regarded as a more intense, “hyperacute” form of ADEM. However, this rare acute demyelinating disease has distinct clinical and pathological features and is associated with a different spectrum of antecedent infections.

Epidemiology

ADEM was a common disease in the mid-20th century, representing about one-third of all cases of encephalitis. The most common cause of ADEM was measles, which, along with ADEM cases following rubella and mumps, has been largely eliminated in regions of the world that have adequately protected children with the measles-mumps-rubella (MMR) vaccine. The second major cause of ADEM was a vaccine, vaccinia virus, which was discontinued after the worldwide eradication of natural smallpox in 1977. More recently, the varicella vaccine has further reduced the risk of ADEM. Now in countries with active childhood vaccination programs, ADEM makes up less than 10% of the cases of encephalitis, and the most common antecedent illnesses are nonspecific respiratory infections (Johnson, 1998).

The incidence of ADEM after clinically distinctive virus illnesses is highly variable (Tab. 40.2); the incidence after Epstein-Barr (EB) virus, Mycoplasma pneumoniae, influenza, and nonspecific upper respiratory infections are uncertain. The clinical findings after specific infections show some distinctive features, and the mortality and morbidity rates are quite different. Despite the common pathology, the pathogenesis may vary. Most data on pathogenesis relate to post-measles encephalomyelitis.

Pathology

In acute fatal cases the brain may be congested and swollen. On gross sections, vessels are prominent in white matter with discoloration along the veins.

TABLE 40.2 Postinfectious Encephalomyelitis with Perivenular Demyelination Associated with Exanthematous Viral Infections

|                | Case rate     | Fatality rate | Sequelae rate |
|----------------|---------------|---------------|---------------|
| Vaccinia       | 1:63 to 1:250,000 | 10%           | Rare          |
| Measles        | 1:1000        | 25%           | Frequent      |
| Varicella*     | 1:10,000      | 5%            | 10%           |
| Rubella*       | 1:20,000      | 20%           | Very rare     |

*Estimates difficult to determine because of frequency of toxic encephalopathy or Reye’s syndrome (different pathology) and acute cerebellar ataxia (unknown pathology) and the rare documentation of perivenular demyelinating disease.
On microscopic examination mononuclear cells are prominent along the small veins. In intense acute cases, polymorphonuclear cells may also be present. Pallor and loss of myelin staining is seen along the vessels; its perivenular localization often produces flame shaped lesions. In the spinal cord, this causes a characteristic radial pattern (Fig. 40.1), a pattern distinctive from the plaques of demyelination seen in PML or MS. Patients dying later in disease show even more sharply demarcated lesions and lipid-laden macrophages (Johnson et al., 1985).

Immunocytochemical staining of myelin proteins also distinguishes ADEM from PML and MS. In ADEM, as in EAE, areas demonstrating loss of myelin basic protein and myelin-associated glycoprotein are concordant (Gendelman et al., 1984). In PML, the area of myelin-associated glycoprotein loss is distinctly larger than the area of myelin basic protein loss in demyelinated lesions. Presumably this reflects a direct attack on the myelin membranes in ADEM and EAE; whereas in PML, where disease results from oligodendrocyte infection, the myelin-associated glycoprotein, concentrated in the periaxonal, most distal extensions of the myelin membrane, is lost first. Thus, in PML lesions, areas of decreased myelin-associated glycoprotein staining are two to three times larger than areas of myelin basic protein loss. Demyelinated plaques in MS show a mixture of patterns, suggesting different pathogenic mechanisms accounting for myelin loss (Gendelman et al., 1985).

In acute hemorrhagic necrotizing leukoencephalitis, the brain is usually strikingly swollen with evidence of herniation. Gross hemorrhages are evident. Microscopically both veins and arterioles show fibrinoid necrosis. This vascular necrosis is associated with transudates of fibrin into the tissue, extravasation of red blood cells, and tissue

**FIGURE 40.1**
ADEM following varicella. This 12-year-old girl developed paraparesis abruptly 2 weeks after the onset of uncomplicated chickenpox. Over the next 3 days the disease evolved with arm weakness, blindness, respiratory distress, seizures, and death. Section of spinal cord shows the characteristic pattern of perivenular demyelination.
necrosis. The inflammatory infiltrate is predominantly polymorphonuclear. These findings are most intense in the white matter; and despite the large necrotic areas, there are regions where myelin loss with relative sparing of axons is evident.

Pathogenesis

The pathological changes in ADEM resemble those seen in the “neuroparalytic accidents” reported after post-exposure vaccination for rabies using killed virus prepared in animal brain and spinal cord. Indeed, the similarity between the demyelinating encephalomyelitis after vaccination with vaccinia virus to prevent smallpox and the complications of rabies vaccine led Rivers and Schwentker (1935) to studies in monkeys. Animals repeatedly inoculated with emulsions of normal brain developed neurological signs and had perivascular demyelination. This was the discovery of EAE. Subsequently Kabat and colleagues (1947) found that in some species, a single injection of brain could induce the disease if brain inoculum was emulsified in adjuvant. Others showed that specific sequences of myelin basic protein and proteolipid protein could cause disease, and that disease could be passively transferred with sensitized lymphocytes (Paterson, 1960) (see Chapter 43).

EAE mimics ADEM and post-rabies vaccine encephalitis (Tab. 40.3). Lymphocytes from patients with post-rabies vaccine encephalomyelitis (Hemachudha et al., 1988), post-measles encephalitis, post-varicella cerebellar ataxia, and encephalomyelitis following respiratory infections have been shown to proliferate when cultured in the presence of myelin basic protein. The gap in these parallels is that patients with ADEM have not been injected with myelin proteins.

Studies of the pathogenesis of ADEM have focused primarily on measles because the clinical diagnosis is easy, the incidence of ADEM is high compared to other infections, and the neurological complications of measles are homogeneous. Generalization of these studies to ADEM and other post-infectious complications of other viruses is cautioned, since the systemic pathogenesis and effects on the immune system by the other agents are variable.

TABLE 40.3 Comparisons of Experimental Allergic Encephalomyelitis with Encephalomyelitis after Rabies Vaccine and Viral Infections

| Inducing event | Experimental allergic encephalomyelitis | Post-rabies vaccine encephalomyelitis | Postinfectious encephalomyelitis |
|----------------|----------------------------------------|---------------------------------------|----------------------------------|
| Inoculation with CNS tissue or myelin basic Protein | Inoculation with CNS tissue | Infection with enveloped viruses |
| Latent period | 10–21 days | 7–42 days | 10–40 days* |
| Clinical forms | | | |
| Acute onset | + | + | + |
| Monophasic disease | + | + | + |
| Occasional chronic or relapsing forms | + | + | + |
| Pathologic findings | | | |
| Perivascular lymphocytes | + | + | + |
| Perivascular demyelination | + | + | + |
| Immunological studies | | | |
| Lymphocytes stimulated in vitro by myelin basic protein | + | + | + |
| In vitro demyelination by lymphocytes | + | ? | + |
| Anti-myelin protein antibodies | + | + | – |

*From beginning of incubation periods. From Johnson (1998).
Measles was the first virus shown to cause immunosuppression. von Pirquet (1908) demonstrated that children had a conversion of positive tuberculin reactions during measles, and for weeks thereafter. Subsequent studies showed inhibition of lymphocyte responses to mitogens for up to 4 weeks after uncomplicated measles virus infection. The magnitude of inhibition of lymphocyte proliferation was the same in children with uncomplicated measles, in those with pneumonitis related to immunodeficiency, and in those with ADEM thought to be due to an autoimmune response (Hirsch et al., 1984). In contrast, spontaneous proliferation of CD4, CD8, and B lymphocytes was found, as well as signs of immune activation, which included lymphoproliferation of lymphocytes in the presence of myelin basic protein in 15% of cases of uncomplicated measles and in 47% of those with ADEM (Johnson et al., 1984).

The mechanisms underlying the profound suppression of cell-mediated immunity accompanying measles is still not fully understood. In vitro infection of human monocytes specifically down-regulates IL-12 production, a cytokine critical in cell-mediated immunity (Karp et al., 1996), however disruption at other sites in the complex cytokine network is likely.

In a subsequent study of ADEM after varied infections, T-cell lines were established from patients. The frequency of cell lines reactive to myelin basic protein was ten times higher in patients with ADEM than patients with encephalitis or controls. IL-4 was the prominent cytokine secreted by T-cell lines from patients with ADEM during the recovery phase (Pohl-Koppe et al., 1998). This finding supports a more general relevance of T-cell responses to myelin proteins in the pathogenesis of ADEM.

Clinical Features

ADEM is best defined by its unique pathology, because many of the causative agents are associated with multiple post-infectious syndromes and many of the clinical syndromes and imaging studies overlap with other disease processes. The two most clearly defined cases of ADEM are those following vaccinia and measles virus infections. Each follows a clinically distinct exanthem and presents a similar clinical course and consistent pathology. In contrast, neurological syndromes associated with rubella, varicella, mumps, and influenza may include direct encephalitis, Reye’s syndrome, and acute cerebellar ataxia—all of which may have different mechanisms of pathogenesis not characterized by demyelination (Tab. 40.2).

The common clinical features are a lag of 3 days to 3 weeks after the exanthem or respiratory disease, an abrupt onset of headache, fever, and impaired consciousness, and the finding of focal neurological signs. The disease reaches a nadir within days, and recovery is variable, depending, in large part, on the causative agent. The spinal fluid usually shows a modest pleocytosis and mild protein elevation, but can also appear normal. The myelin basic protein content may be elevated, particularly early in disease. In some cases, magnetic resonance imaging has proved an effective method of differentiation from acute encephalitis or Reye’s syndrome. Multifocal white matter lesions of similar age are found, which may or may not enhance. When enhancement is seen in all lesions simultaneously (Fig. 40.2), the image is characteristic of ADEM.

Acute ADEM not associated with viral exanthems can be difficult to differentiate from acute viral encephalitis or, in some cases, the initial attack of MS. A viral prodrome, high lesion load on magnetic resonance imaging involving deep gray matter, and the absence of oligoclonal bands in the spinal fluid favor a diagnosis of ADEM (Hynson et al., 2001). Followup studies of patients diagnosed with ADEM have shown a subsequent diagnosis of MS in some patients, raising the question of whether ADEM might be a part of an “MS spectrum” (Hartung and Grossman, 2001). MS is not, however, an outcome of classical ADEM following measles or vaccinia infections, and ADEM has a very distinctive neuropathology.

Measles

Measles probably remains the most common cause of ADEM worldwide. Although indigenous measles has been eliminated from the Western Hemisphere and Western
Europe, it still causes over 1 million childhood deaths each year, largely in developing countries. Measles continues to rank as the third most common infectious cause of childhood death worldwide, following diarrheal illnesses and malaria. The most frequent fatal complications of measles, pneumonitis, gastroenteritis, and secondary bacterial infections, result from a depression of cell-mediated immune responses extending for 1 to 4 weeks after the rash. Prior to the emergence of HIV, measles virus was the most important and lethal causes of a virus-induced immunodeficiency syndrome. However, the major neurological complication of measles infection, ADEM, was assumed to be an allergic or autoimmune disease. Although this initially appeared to be a paradox, studies of measles and HIV have shown that immune activation can suppress immune responses as well as release autoimmune responses.

The incidence of post-measles encephalitis is reasonably constant, at about 1 per 1000 cases, and is age dependent, since children under 2 years of age seldom develop encephalitis. It does not appear to be nutritionally dependent, as are some opportunistic infections. Thus, populations such as those of West Africa tend to suffer high rates of measles mortality from opportunistic infections and infant deaths. On the other hand, more affluent countries without adequate immunization programs have greater mortality and morbidity from post-measles encephalitis, because older children become infected. Although polyneuritis, toxic encephalopathy and acute hemiplegia of possible vascular etiology have been reported with measles, over 95% of the neurological illnesses are represented by ADEM.

**FIGURE 40.2**
ADEM after primary Epstein-Barr virus infection. This college student had classical monospot positive infectious mononucleosis. Two weeks after onset, she developed multifocal neurological signs and coma. The enhanced MRI at that time shows widespread white matter lesions with intense enhancement. She subsequently recovered and returned to school with minimal sequelae.
The incubation period of measles is 10 to 14 days. The prodrome and period of infectivity is marked by coryza, conjunctivitis, cough, and the pathognomonic Koplik spots on the buccal mucosa. Coincident with the antibody response and the end of infectivity, a maculopapular rash develops on the face and trunk, later spreading to the extremities. Virus can be recovered from the rash, and for up to 5 days after the appearance of the rash, viral antigen or RNA can be found in epithelioid cells of the lung, gut, bladder, and lymphoid organs (Moench et al., 1988). On rare occasions, viral antigen and RNA can also be detected in cerebrovascular endothelial cells (Esolen et al., 1995). Although there is no evidence of infection of neural cells, approximately 50% of children have an abnormal electroencephalogram during the rash (Gibbs et al., 1959), and approximately 30% have a pleocytosis (Ojala, 1947).

Post-measles encephalitis typically develops 4 to 5 days after the onset of the rash, but may precede the rash or be delayed until 3 weeks after. Typically the child is afebrile, the rash is fading and the child is returning to normal activities, when fever returns with headache. Obtundation is frequent and may progress to coma. Generalized or focal seizures occur in about half of the children. Multifocal neurological signs may include cranial nerve abnormalities, pyramidal tract signs, abnormal movements, and ataxia. Sensory deficits are infrequent. The spinal fluid may show a modest mononuclear cell pleocytosis but is acellular in about one-third of the patients. Protein elevations are variable, but many have high levels of myelin basic protein in spinal fluid, particularly early in the course of the encephalitis. Elevated IgG synthesis and oligoclonal bands are usually absent (Johnson et al., 1984). Mortality and morbidity are high. Between 10 and 40% mortality are reported, and neurological sequellae are found in the majority of survivors. Prognosis has been linked to length of stupor or coma (Tyler, 1957), but remarkable recoveries can be seen after prolonged coma (Johnson et al., 1984).

The measles vaccine is an attenuated live virus, which has led to the question of whether or not the vaccine may, on rare occasions, cause ADEM. The vaccine virus can produce fever, but in early tests of the vaccine, no abnormalities were found on electroencephalograms that are common with wild-type virus infections. Post-liscencing studies reported one case of encephalitis per million children following vaccination, a number lower than the observed background of two per million cases of encephalitis or encephalopathy per month, suggesting a coincidental relationship between vaccine and disease. Further analysis, however, showed some clustering of cases during the 6 to 15 days after immunization, suggesting a possible relationship (Landrigan and Witte, 1973). Since no histopathological studies have been reported in post-vaccine illnesses, whether or not a few of these cases represent rare cases of ADEM remains unknown.

**Vaccinia**

Until recently, the neurological complications of smallpox and vaccinia were of only historical interest; the last case of natural smallpox was observed in 1977, and within a few years vaccinia inoculation had been abandoned worldwide. Recent threats of bioterrorism have led to consideration of renewed vaccinia virus inoculation, and the risks of ADEM must be reconsidered.

In retrospect, ADEM accompanied smallpox but was hidden under the devastating systemic disease (Marsden and Hurst, 1932). In the 1920s, ADEM became appreciated as a complication of immunization with vaccinia virus. The incidence of complications after vaccination is extraordinarily variable; an incidence of 1 in 63 is cited for one Dutch vaccine program (but a variety of types of illness were included) (DeVries, 1960). During World War II, an incidence of post-vaccinal encephalomyelitis in England was estimated 1 per 175,000 (Miller, 1953), a subsequent retrospective analysis in the United States estimated 1 per 200,000; during the sidewalk vaccination of 5 million people in New York in 1947 a similar incidence of about 1 per 100,000 was estimated, and during the more recent mass vaccination during the smallpox outbreak in the United Kingdom in 1962, passive reporting estimated neurological complications in 1 per 20,000 (Spillane and Wells, 1964). The variation in incidence may be due to different ethnic populations, different patches of virus, and certainly variable and often poor data collection. ADEM
is clearly more common with primary immunization, and some data suggest that incidence increases with age.

At the time of maximal cutaneous reaction or shortly thereafter, patients with ADEM develop fever, nuchal rigidity and obtundation. Movement disorders including tremor, ataxia, trismus, and myoclonus are specifically mentioned in several reports (Miller and Stanton, 1954; Spillane and Wells, 1964). Fatality rates are as varied as incidence rates, but are generally lower than those for post-measles encephalomyelitis, and sequelae are less frequent.

The pathogenesis is assumed to resemble that of measles, but vaccinia virus usually replicates only in the dermis. In some patients a viremia is found, and this is more prolonged in patients with ADEM. Virus has been recovered from brains and spinal fluids of patients with ADEM (Brooks, 1979). Which hematogenous cells are infected and the effect on immune responses has not been studied.

In considering the resumption of vaccination some have recommended (1) vaccination of those likely to encounter victims (first-responders, family health care providers, and clinic and emergency room personnel), (2) vaccination of all who request vaccine, or (3) mandatory universal vaccination. In any of these scenarios, most of those receiving vaccine would be over age 2 and a majority would be receiving primary vaccinations, two factors that presumably increase the risk of ADEM. If the incidence of ADEM were 1 per 20,000 vaccinees (some would consider that figure high; others low) in the United States, we might anticipate 10,000 cases of ADEM with 1000 deaths if universal immunization were the option chosen.

Varicella

Of the neurological complications accompanying chickenpox, fully 50% are acute cerebellar ataxia, which complicates 1 in 4000 chickenpox cases. Typical ADEM is quite rare but does occur (Fig. 40.1). The most dreaded complication is Reye's syndrome, in which fatty degeneration of the liver is accompanied by life threatening brain edema, but in this disease both inflammation and demyelination are absent.

Post-varicella cerebellar ataxia has a good prognosis and the absence of fatalities leave the histopathology undefined. In several cases of acute ataxia with chickenpox, lymphoproliferative responses in the presence of myelin basic protein have been reported, suggesting a pathogenesis similar to post-measles encephalomyelitis (Johnson et al., 1984; Lisak et al., 1977). Antibodies reacting with sections of cerebrum and cerebellum were reported in 3 of 8 children with post-varicella cerebellar ataxia, suggesting that humoral immune responses may also be involved (Adams et al., 2000).

The typical ADEM resembles post-measles ADEM; disease onset is abrupt at 4 to 15 days after the onset of rash. Fever, headache, obtundation, seizures, and focal neurological signs are common (Gollomp and Fahn, 1987). Neuropathological studies are similar.

Rubella

Rubella is the fourth exanthem classically associated with ADEM. It is estimated that an incidence of 1 per 20,000 cases develop ADEM. The onset is typical; during the week after the rash, fever, headache, and obtundation develop often with seizures. Focal neurological signs are usually not found, but a pleocytosis is. Fatalities do occur, but most have failed to show the typical hallmarks of ADEM; a minority has shown characteristic perivenular inflammation and demyelination suggesting varied types of post-rubella encephalopathies. In a single child, a lymphoproliferative response to myelin basic protein was documented (Johnson et al., 1985).

Human Immunodeficiency Virus

HIV has been associated with a remarkable spectrum of neurological diseases. Acute meningitis and acute demyelinating polyneuritis (Guillian-Barre syndrome) have often been seen about the time of initial seroconversion. These are assumed to be autoimmune disorders associated with the initial activation of CD4 cells. A small number of newly
infected individuals have developed a fatal encephalomyelitis, and pathological studies have shown ADEM (Narciso et al., 2001; Silver et al., 1997).

Years later with onset of AIDS different central and peripheral nervous system diseases are prominent associated with intense immunodeficiency. HIV dementia, which develops in 20 to 40% of AIDS patients, can be characterized by “diffuse myelin pallor,” visualized on magnetic resonance imaging as a hypodense lesion of white matter, primarily in the cerebral hemispheres, and in pathological sections by a pallor of myelin staining. Initially thought to represent demyelination, immunocytochemical staining for myelin proteins has failed to show myelin loss. The abnormal signal on imaging and pallor of staining seem to reflect a breakdown of the blood-brain barrier (Power et al., 1993). In addition to the prominent diffuse pallor of myelin, small flame-shaped areas of demyelination without inflammation are occasionally seen along vessels; these may represent the residua of minor or subclinical demyelinating encephalitis that occurred at the time of seroconversion.

Other Agents
Several viruses have been associated with ADEM that are also associated with apparent acute encephalitis or meningitis. In neurological complications of mumps virus, EB virus, and influenza A and B virus infections, virus has been recovered from brain or spinal fluid and pathological findings have been varied; some suggest direct effects of virus replication in neural cells and some histopathologically are ADEM (Hart and Earle, 1975).

Mumps was the single most common viral cause of viral meningitis, but over 90% of these illnesses are now prevented in countries with adequate MMR vaccine administration. Indeed, mumps may be the most neuroinvasive virus, since examination of spinal fluid in patients with uncomplicated parotitis showed that fully 50% had a pleocytosis. Fortunately, mumps is not highly neurovirulent, and the common neurological complication is benign meningitis. In patients with mumps meningitis, virus can readily be isolated from spinal fluid, and viral nucleocapsids can be visualized by electron microscopy within ependymal cells found in spinal fluid (Herndon et al., 1974). This suggests a similar pathogenesis of CNS invasion in humans as seen in hamsters, where mumps virus selectively infects ependymal cells (Johnson, 1968). Serious CNS complications of mumps virus infections are rare. Even prior to immunization, when mumps virus infection was universal, only four to five deaths from mumps encephalitis were reported to the Centers for Disease Control each year. Of those cases, about half showed the histological findings of perivenular demyelination (Schwarz et al., 1968).

The neurological complications of EB virus infections pose an even more confusing spectrum of disease. Typically these disorders arise 1 to 2 weeks after the onset of clinical infectious mononucleosis. About 1% of patients have neurological complications, but these vary from Guillain-Barre syndrome and acute cerebellar ataxia (usually associated with autoimmune responses) to meningitis, encephalitis, and myelitis typical of direct infections (Gautier-Smith, 1965). The finding of viral DNA by PCR is not meaningful, since EB virus is latent in B lymphocytes and a single B cell traversing through cerebral circulation or drifting into spinal fluid could give a positive signal. As with mumps virus infections, intrathecal antibody synthesis and oligoclonal bands of IgG in the spinal fluid may be found; this is in contrast to post-measles encephalomyelitis but does not exclude an immunopathological mechanism. Again, the diagnosis of ADEM is pathology-based. In the rare fatal cases of encephalitis, a necrotizing polioencephalitis is found; but in others perivenular demyelination has been reported (Paskavitz et al., 1995).

Both influenza A and B have been, on rare occasions, related to Reye’s syndrome, acute transverse myelitis, and Guillain-Barre syndrome. The association with pathologically verified ADEM is very rare (Hoult and Flewett, 1960). Mycoplasma pneumonia is also associated with a variety of neurological complications including the occasional case of ADEM (Riedel et al., 2001). A diagnosis of ADEM is often entertained clinically, but autopsy studies of fatal cases have generally shown brain edema or perivascular inflammation without convincing demyelination. Recent anecdotal reports of probable ADEM associated with hepatitis C virus (Sacconi et al., 2001), attenuated polio vaccine virus (Ozawa et al., 2000), and acute herpetic gingivostomatitis (Ito et al., 2000) may be coincidental.
Acute Hemorrhagic Necrotizing Leukoencephalopathy

This acute hemorrhagic disease has been regarded as a more intense form of ADEM and as a distinct entity. The clinical setting is similar. Usually 1 to 20 days following a virus-like illness, the disease develops with fever, obtundation, seizures, and focal neurological signs. Although reported in individual cases after measles and chickenpox, most follow a non-specific upper respiratory infection. In Asia, a number of cases have been associated with influenza virus infections (Voudris et al., 2001); recently several cases were reported with Mycoplasma pneumoniae infections (Pfausler et al., 2002). In contrast to ADEM, the illness is fulminant with signs suggesting an expanding mass lesion, and the majority of patients die within 5 days. The spinal fluid shows polymorphonuclear cells and red cells; in addition, a peripheral leukocytosis and proteinuria are usually found.

The arguments linking acute hemorrhagic necrotizing leukoencephalitis to ADEM are (1) the similar clinical setting despite a distinct spectrum of precipitating illnesses, (2) an apparent continuum of pathology with cases clearly showing features of both diseases, and (3) the animal model of hyperacute EAE that resembles hemorrhagic necrotizing leukoencephalitis (Levine and Wenk, 1965). In a single case, a proliferative response of the patient’s lymphocytes was demonstrated when cultured with myelin basic protein (Behan et al., 1968).

Prevention and Treatment

Few arenas of medicine have celebrated the extraordinary success in disease prevention at an astonishing cost-benefit ratio as in the prevention of infections with vaccines. The measles vaccine alone prevents between 2 million and 3 million deaths each year, reduces the burden of permanently neurologically impaired by even greater numbers, has reduced childhood deafness by 10%, and has virtually eliminated subacute sclerosing panencephalitis from countries with sustained vaccine programs. The cessation of vaccination to prevent smallpox and the introduction of vaccine programs to prevent mumps, rubella, and chickenpox have decreased the incidence of ADEM dramatically.

Treatment is less effective. Although the literature abounds with anecdotal claims of the benefits of corticosteroids, no randomized placebo-controlled study supports their value. Several studies of sequential patients with measles encephalitis who did or did not receive steroids or ACTH showed no difference in mortality or morbidity (Ziegler, 1961). These studies may be applicable only to measles, and empiric treatment with steroids remains common in ADEM, particularly when there is any evidence of increased intracranial pressure. One retrospective study of post-infectious encephalomyelitis showed higher mortality and morbidity rates among those who had received corticosteroids. On the assumption that the more seriously ill would tend to be treated, the data were reanalyzed to include only patients admitted in coma. Even in this reevaluation the mortality was higher in treated patients (Boe et al., 1965).

Supportive treatment is important since children can have remarkable recoveries after prolonged coma. Therefore lowering of fever, management of seizures, careful management of fluids, reducing increased intracranial pressure, and prevention of urinary and respiratory tract and skin infections are paramount. Mechanical ventilation is often necessary.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

Definition

PML

In the 1950s, a rapidly progressing neurologic syndrome was observed in a patient suffering from chronic lymphatic leukemia (CLL). Although the cause was ruled to be a result of leukemic cells that had infiltrated the brain, post-mortem examination revealed bizarre, enlarged nuclei and demyelination, which was not consistent with the diagnosis. A few
years later, another patient with CLL developed similar neurologic symptoms. Again, autopsy revealed demyelinated plaques populated by cells with enlarged, dense nuclei, which were hypothesized to be oligodendrocytes with an unusual, undescribed pathology. The results were published in 1958 as the first clinical and neuropathological description of a new disease, progressive multifocal leukoencephalopathy (PML), also known as Richardson’s disease after E. P. Richardson, who was responsible for the initial description (Astrom et al., 1958). There were early suspicions that PML was caused by a virus; however, the etiologic agent was not identified until 1971 as the human polyomavirus, JC Virus. Like other polyomaviruses, JCV was named after the initials of the patient from whom virus was first isolated (Padgett et al., 1971)

Initially the disease was mainly associated with patients suffering from lymphoproliferative and myeloproliferative diseases such as CLL, Hodgkin’s disease, and sarcoidosis (Richardson, 1974). Following the description of PML, retrospective review of the literature uncovered several similar accounts in patients with dementia suffering from a variety of underlying immunosuppressive disorders. The accounts dated back as far as 1930 with pathologic features consistent with PML (Hallervorden, 1930). PML is almost exclusively associated with an underlying cellular immunodeficiency. Prior to the AIDS epidemic, the association was most frequently observed in patients with Hodgkin’s disease and CLL. In the decades following, the exponential rise in the incidence of AIDS resulted in a much larger and rapidly growing population of immunosuppressed individuals. Presently, PML has become an increasingly common complication in AIDS patients, and has been the AIDS defining illness in approximately 1% of the cases. It is expected that approximately 4 to 5% of all AIDS patients will eventually develop this disease (Berger and Major, 1999).

**JC Virus**

JCV is a small, nonenveloped virus, approximately 45 nm in diameter. The viral capsid is icosahedral in symmetry, and made up of the 3 viral capsid proteins encoded by the late region of the JCV genome. After translation and transcription in the cytoplasm, nuclear localization signals located on the amino terminal region transport the proteins to the nucleus, where the virion particles are assembled (Moreland and Garcea, 1991). Capsid assembly is governed by the major structural protein, Vp1, which accounts for more than 70% of the entire viral protein content. Vp1 also contains the antigenic epitopes to which a specific antibody response is mounted, and it is responsible for the ability of JCV to agglutinate human type O erythrocytes (Shah et al., 1977; Wang et al., 1999).

Located inside the viral capsid are the JCV minichromosomes, each of which is a single molecule of viral DNA complexed with cellular and nuclear histones. The complete genome is a closed, circular supercoiled structure, approximately 5.13 kb in length (Frisque et al., 1984). Transcription occurs in both directions starting from the highly conserved origin of replication, marked by a single EcoR1 restriction site. Extensive sequencing data has revealed that the genome can be functionally divided into three regions: the early region, encoding two nonstructural proteins; the late region, encoding three capsid proteins; and the regulatory region. The early region, located on the proximal side of the origin, is transcribed and expressed early after viral entry. Counterclockwise transcription of this region starting from the EcoR1 site, followed by differential splicing, produces two major mRNA species encoding the Large T and small t antigens. It is known that Large T is a nonstructural, DNA binding protein with multiple functions (Fanning, 1992), one of which is the initiation of JC viral DNA replication by the unwinding and separation of the two strands of DNA so that the polymerases can function. Furthermore, it autoregulates to prevent transcription of the early genes during the later stages of infection, when the structural genes are being produced. Large T also plays a part in the malignant transformation of cells by binding to cell cycle regulatory proteins as well as tumor suppressors such as p53 and pRB, resulting in cellular malignant transformation.

The late region, located on the distal side of the origin, is encoded in the strand of DNA complementary to the strand that encodes the early genes. Clockwise transcription from the origin of replication yields the capsid proteins VP1, VP2, and VP3, as well as the Agnoprotein. VP1, VP2, and VP3, as discussed earlier, are the structural proteins that make up the
viral capsid. Agnoprotein in related viruses has been implicated in DNA binding and localization of VP1 to the nucleus (Cole, 1996, Carswell and Alwine, 1986). A recent report describing the presence of JCV agnoprotein in the cytoplasm of infected cells, suggests that protein shuttles freely between the cytoplasm and the nucleus and is important for JCV proliferation (Okada et al., 2001). Early gene expression must take place before viral DNA replication can proceed, since the early products, Large T and small t, are necessary for the expression of the late gene products. Thus, efficient expression of those late structural proteins occurs only after viral transcription and translation have taken place. The noncoding sequences located between the early and late genes contain the origin of replication, the JCV promoter, and enhancer sequences. Collectively, this area is also known as the viral regulatory region (RR), thought to control host range for lytic infection.

Studies of JCV infection in various cell types have demonstrated the extremely narrow host range of this virus. It has been shown that in cell culture systems, human glial cells are the most susceptible to infection as well as the most conducive to virion production (Wroblewska et al., 1980). It was originally hypothesized that the Large T viral protein defined the neurotropism of JCV, since the virus was shown to be able to replicate in nonglial cell types only in the presence of JCV or SV40 Large T protein, both of which share significant homology (Feigenbaum et al., 1987). Subsequent studies interchanged the JCV promoter with other viral promoters and used the constructs to infect various cell types. The results have suggested that the cell type specificity of JCV may also be a function of the viral promoter (Feigenbaum et al., 1992). The restricted growth in cell types from extra neural tissue such as kidney and tonsil has led to questions regarding the nature of JCV susceptibility. Is JCV host cell restriction at the level of binding and entry or is it at the molecular level? The recent identification of the specific cell surface receptor for JCV has provided some answers to these questions (Liu et al., 1998). The receptor, an α 2-6 linked sialic acid, is a commonly expressed ganglioside found on the surface of various cells. Indeed, binding studies of JCV have shown that the virus can bind to and enter numerous cell types via this receptor, both susceptible and nonsusceptible.

Thus, unlike other viruses, viral susceptibility to JCV is not a function of specific receptor binding and entry. The focus has now shifted to intracellular factors that may contribute to the specificity of this virus. The regulatory region of JCV has several binding sites for the NF1 family of transcription factors. A comparison of relative NF1 expression between highly susceptible cells from the human fetal brain (HFB) and a nonsusceptible epithelial cell line (HeLa), demonstrated that one class in particular, NF1-D, was elevated in the HFB cells (Sumner et al., 1996). Further studies have shown that overexpression of NF1-D in a normally nonpermissive cell line can render the cells susceptible to JCV infection, as determined by early and late gene production (Monaco et al., 2001). The regulatory region of this virus has been of much interest, because it is becoming increasingly clear that despite effective virus binding or the presence of viral DNA in the cells, productive infection only occurs in the cell types that express the appropriate transcriptional factors. The very narrow host range and the cell type specificity of JCV are unique properties of this virus.

**Epidemiology**

It would be expected that the incidence of PML would be higher in the geographic areas coinciding with a large HIV infected population. However, since the technology required to make a definitive diagnosis of PML may not readily be available in such areas, the actual incidence of this disease may be masked to some extent. As such, the entire worldwide incidence of PML has not yet been determined. However, serological studies of human polyomaviruses in geographically isolated regions have yielded much information on the natural distribution of JCV in human populations. The first study published in 1973 described a random sampling of 406 individuals from the state of Wisconsin, screened for the presence of JCV antibodies. The results showed that approximately 70% of assayed individuals had significant antibody titers to JCV in their blood. However, the percentage dropped dramatically in very young children, suggesting that initial infection
and seroconversion most likely occurs during early childhood (Padgett and Walker, 1973). Much larger studies were conducted shortly thereafter to study the worldwide distribution of human polyomaviruses. Again, JCV infection was prevalent worldwide, as demonstrated by the presence of antibodies in infected individuals. The majority of adolescents and a much higher percentage of adults (80%) showed elevated levels of JCV-specific antibodies (Brown et al., 1975, Walker and Padgett, 1983).

A more recent epidemiological study of the incidence of PML in the United States was conducted from 1979 to 1994. The survey reported the incidence of PML among HIV infected individuals as 1.6%. However, since the data only included PML cases that were diagnosed ante mortem, and not new cases discovered at autopsy, the authors felt that the numbers were an under-representation of the actual prevalence of the disease. It was also reported that 89% of the PML cases were attributed to HIV infection during this time period (Holman et al., 1991). A recently concluded seroepidemiological study examining the circulation of human polyomaviruses in the population determined that 80% of the population exhibited antibodies to JCV (Maher, D. et al., in press).

Pathogenesis
The exact route of JCV transmission, initial infection and pathogenesis continues to be investigated. Seroepidemiological studies have proven that the majority of the healthy, human population has antibodies against the virus, although the percentage drops in very young children. Thus, it is postulated that initial infection and seroconversion occurs within the first 6 years of life with no associated clinical symptoms. The initial route of infection remains elusive, although one study has reported JCV replication in human tonsillar tissue, implicating a site of latency and also a primary infection route via inhalation (Monaco et al., 1998). Trafficking B lymphocytes (Tornatore et al., 1992) may then carry the virus from the tonsillar and stromal tissue to other latent sites, including the bone marrow, tonsils, colon epithelial cells, and kidney (Jensen and Major, 1999; Laghi et al., 1999) (Fig. 40.3). It is well documented that the virus is excreted in the urine of healthy individuals as well as patients with PML (Agostini et al., 1996). Transmission via this route is unlikely, however, because the predominant genotype found in urine isolates has not been shown to be able to cause a robust infection in any type of human cell (Ault, 1997). Systemic circulation of the virus to these different compartments is thought to be via a hematogenous route, most likely involving infected B lymphocytes. Lymphocytes may shuttle the virus across the blood brain barrier during a period of severe immune deficiency, which sets the stage for the onset of PML when JCV infection is passed from the lymphocytes to the highly susceptible glial cell population.

Pathology
The initial descriptions of PML were focused on the histopathological features associated with the disease, such as the enlarged oligodendrogial nuclei and bizarre, giant astrocytes. The swollen nuclei of infected oligodendrocytes usually display a change in chromatin pattern. They often appear more homogenous or will have the chromatin concentrated at the periphery, near the nuclear membrane. The nuclei have also been described as having “ground glass” appearance, which is due to the presence of numerous inclusion bodies. Electron microscopy has revealed that these inclusion bodies are actually a dense, crystalline, or filamentous array of JC virion particles (Aksamit, 1995).

Upon gross examination, demyelinated plaques and lesions can be visible to the naked eye. The foci of demyelination are initially few and randomly distributed. They can range in size from millimeters to centimeters in diameter, occasionally coalescing to form even larger lesions. The lesions have been identified throughout the white matter of the brain, including the cerebral hemispheres, cerebellum, medulla, and even spinal cord. However, lesions are most commonly located in the subcortical white matter, near the gray-white matter junction, which is an area of increased cerebral blood flow. This lends support to the hypothesis of viral entry into the CNS via hematogenous dissemination. However,
lesions do not follow the cerebral vasculature of the brain. Microscopic examination of the plaques shows lytic destruction of oligodendrocytes, myelin degradation, but sparing of the associated axons. Active viral replication and capsid formation in infected oligodendrocytes is followed by cytolysis and viral release to surrounding cells. Susceptible cell types will follow the same pattern, resulting in a lesion where infected oligodendrocytes are concentrated at the periphery, encroaching outwards as the lesion grows. Thus, JCV is disseminated by cell-to-cell contact. Oligodendrocytes can be found throughout the lesion, often with enlarged, basophilic nuclei. Astrocytes found in this area may be hypertrophied and severely enlarged, while neurons are typically spared. In some cases, lipid-laden macrophages have been identified in the center of the lesions as well, evidence of active myelin degradation.

The mechanism behind JCV induced demyelination is primarily through the lytic infection of oligodendrocytes. However, there is some evidence that JCV large T protein

FIGURE 40.3
Pathogenesis of progressive multifocal leukoencephalopathy. Primary infection is followed by an extended period of latency in several anatomical compartments. Current data implicate the kidneys and bone marrow as potential sites for JCV latency (*). Active JCV replication has been demonstrated in tonsillar tissue as well as in kidney (†). Following viral activation, infected B lymphocytes may traffic the virus across the blood brain barrier and into the parenchyma of the brain, where the infection is then passed to the highly susceptible glial cell population, ultimately resulting in the pathological and clinical symptoms associated with PML.
may interfere with the production of myelin. Inflammatory infiltrates are rare in PML, although there is a pronounced active astrocytosis in the lesions.

Radiographic Findings

Besides the onset of clinical symptoms, one of the first indicators of PML is a lesion visualized by CT or MRI. Lesions are often difficult to attribute to a specific disease because, radiologically, they can appear very similar. As a general rule, PML lesions are multifocal and noncontrast enhancing. The location and extent of lesioning can vary, however. As such, radiographic imaging cannot be used alone to diagnose PML.

Neuroimaging has remained one of the most important tools in studying the PML patient (Thurnher et al., 1997). The noninvasive visualization of lesions is helpful for a preliminary diagnosis, especially in cases where suspected lesions are in areas where the risk of biopsy is too high. By computerized tomography (CT) scan, the demyelinated lesions appear as asymmetrically distributed subcortical areas of decreased signaling intensity. Contrast enhancement is very rarely seen, indicating an intact blood brain barrier. CT scans are limited in detecting the extent of lesions occurring deeper in the brain, such as in the cerebellum or brainstem.

Magnetic resonance imaging (MRI) is extremely sensitive in determining not only the number of lesions, but the extent as well. In fact, MRI has been shown to be more sensitive than CT in both aspects, often revealing lesions where the CT scan previously appeared normal. As such, MRI is the preferred diagnostic technique for the evaluation of potential PML cases. In contrast to the hypodense lesions seen in CT scans, demyelinated lesions appear as patchy areas of increased signal intensity by T2 weighted MRI. T1 weighted images exhibit a decrease in signal intensity, as opposed to the hyperintense T2 weighted images. T1 related decrease in signal intensity is consistent with demyelination.

Metabolic assays generally have not been of much use in studying PML because lesions tend to occur either in the myelinated white matter or at the gray white matter junction.

Clinical Features

Viral reactivation and lytic JCV infection of oligodendrocytes can cause demyelination in any white matter tract located throughout the brain. The clinical symptoms seen in PML patients are consistent with the extent and location of subcortical white matter destruction, with no inflammatory changes in the CSF. Furthermore, the spectrum of symptoms in HIV-associated PML patients is nearly identical to that of PML associated with other underlying immune deficiencies. The most common presenting symptoms are known collectively as the “triad,” which include a progressive deterioration of visual, motor, and cognitive functions. In HIV associated PML cases, the onset of neurological signs and symptoms may actually precede a diagnosis of AIDS in HIV infected patients and has been added to the list of AIDS defining illnesses.

The most common presenting ailments are motor abnormalities. By the time of diagnosis, the majority of patients will exhibit signs of moderate to severe weakness, which typically affects limbs on one side of the body (hemiparesis). Gait abnormalities (Berger and Major, 1999) or difficulty performing routine motor tasks are often accompanied by complaints of lethargy or impaired movement of the arms and legs. Visual deficits also account for a significant percentage of presenting symptoms. The severity of the symptoms often correlate with the extent of lesioning. Hemianopsia, or blindness in one-half the visual field in each eye is common.

Predictors of longer survival time with PML include lack of clinical progression during the first 2 months of treatment (De Luca et al., 2001), contrast enhancement and mass effect (Berger, 2000) in HIV patients, concomitant treatment with HAART and importantly, low JCV virus levels in the CSF (Yiannoutsos et al., 1999).
There is no established therapy, as yet, for the effective treatment of PML. Although there have been isolated reports in individual patients, large-scale studies have been difficult to conduct, due to the limited number of possible test subjects. Attempts to treat the disease have traditionally been aimed at curing the underlying immune deficit, thereby alleviating symptoms of opportunistic infections. Therapies aimed specifically against JCV have also been investigated, but with mixed results. Such antiviral therapies are difficult due to the fact that the drugs not only interfere with the virus but also with the normal functioning of the host cell.

Cytarabine, or Ara-C, is a nucleoside, well established as a chemotherapeutic agent to treat various malignant disorders, but has also been reported to be beneficial in the treatment of PML patients. Positive case reports, in addition to preliminary results suggesting that the drug may have some activity against JCV in primary human cells in vitro (Hou and Major, 1998), prompted the undertaking of ACTG 243, the largest clinical trial, as yet, for any opportunistic infection associated with AIDS. HIV infected, biopsy proven PML patients were administered Ara-C, either intravenously or intrathecally, with or without concomitant antiretroviral therapy. Patients were monitored for clinical signs and viral load during treatment. The results from this trial showed that the administration of Ara-C resulted in no statistical difference between the outcome of treated patients to untreated controls (Hall et al., 1998). However, this and other trials investigating Ara-C resulted in data showing the prognostic value of JC viral load in the CSF. Patients with a reduction in the levels of virus present in the CSF had a significant increase in survival time (De Luca et al., 1999; Yiannoutsos et al., 1999).

Cidofovir is an antiviral agent that is active against a broad spectrum of human DNA viruses, best documented in the treatment of cytomegalovirus (CMV) retinitis, a significant complication in AIDS patients, or genital herpes. There have been several reports of success in treating AIDS-related PML with the drug, particularly in European countries. Although failures have been reported, the addition of cidofovir to preexisting antiretroviral therapy in AIDS patients seemed to have improved the symptoms of PML, even in cases where the course of the disease worsened despite highly active antiretroviral therapy (Portilla et al., 2000). Significant radiological improvements, marked by decreases in the extent of lesions, have also been reported in response to cidofovir therapy (Cardenas et al., 2001). In contrast, the results from the only clinical trial specifically using cidofovir in relation to PML, ACTG protocol 363, were far less encouraging. Reported at the 8th Conference on Retroviruses and Opportunistic Infections, the preliminary statistical analysis revealed no difference in prognosis between patients receiving cidofovir and those without (Marra et al., 2002).

Since the beginning of the AIDS pandemic, the most common underlying immune deficit resulting in PML has been HIV infection. Combination retroviral therapy also known as highly active anti-retroviral therapy or HAART has been frequently reported to increase the immune status, clinical and radiological symptoms, and survival times in some PML patients (Inui et al., 1999). The presence of JCV in the CSF or the initiation of a JCV-specific humoral intrathecal response have been used as markers for viral replication and immune status, both of which may possess prognostic value for the progression of PML. This is particularly true with the advent of HAART therapy, which in multicenter analyses has been successfully correlated with longer survival.

However, despite prolonged survival, HAART did not always halt the rapid neurological deterioration of PML, suggesting that early intervention with a drug specific for JCV will be necessary to stabilize the destruction of the white matter (Gasnault et al., 1999). Similar case reports have also shown that patients can develop PML while on HAART therapy, and that a decrease in HIV viral load and increase in CD4+ cell counts does not always correlate with resolution of neurological symptoms, again emphasizing the need for a definitive antiviral treatment (Tantisiriwat et al., 1999). It also appears that the initiation of HAART therapy in patients with a high JC viral load at the time of diagnosis does not have a significant effect on the survival of the patients following treatment, whereas
patients with median or low viral loads at diagnosis do have prolonged survival on HAART (Taoufik et al., 2000).

One recently completed study retrospectively analyzed a series of PML patients being treated with HAART in three northern Italian neurological clinics. The trial revealed that although approximately 50 percent of the patients did show disease stabilization and longer survival in response to HAART, the rapid immune reconstitution could speed the progression of PML as a result of the circulation of lymphocytes that are actively infected by JCV (Cinque et al., 2001).

Conclusions

PML is the only human demyelinating disease with a known direct viral infection of oligodendrocytes. It remains a challenge to understand how a virus with such a widespread presence in the normal human population can be targeted so specifically to oligodendrocytic destruction in the brain of immune compromised individuals. Once considered exceedingly rare, PML has been given much attention recently due to the continual rise in the incidence of AIDS and other immunosuppressive disorders due to chemotherapy in cancer patients and preventative measures against graft rejection in transplant recipients. Since the majority of the population has already been infected with JCV, and a significant percentage of AIDS patients will develop the disease, it is critically important to establish an effective treatment regimen and develop methods to screen severely immunocompromised patients for the potential risk of developing PML.

MULTIPLE SCLEROSIS

Definition

Although the plaques of MS on gross brain and spinal cord specimens had been previously described, the clinical complex of multifocal remitting and relapsing disease and pathological correlation with plaques of sclerosis was made by Charcot in 1868. In keeping with his times, he postulated that exposure to cold, physical injury or emotional stress caused the disease. Over the next 15 years, Koch and Pasteur laid the foundations of microbiology and immunology, so it is not surprising that in 1884 Pierre Marie, a student and successor of Charcot as Professor of Neurology in Paris, proposed a microbial cause of MS. Indeed, in the afterglow of Pasteur’s discovery of a post-exposure vaccine to protect against rabies, Marie prematurely predicted that a vaccine would soon be available for MS.

Speculation concerning an infectious agent as a cause of MS has recurred over the past century but has gained greater credence over the past 50 years because of three areas of investigation: (1) epidemiologic studies have suggested that MS results, in part, from a childhood environmental exposure followed by a long latency; (2) studies of viral diseases of animals and humans have documented that infections can have long incubation periods, give rise to relapsing and remitting courses, and cause demyelination; (3) studies of patients with MS have consistently shown abnormal immune responses to viral antigens, particularly the intrathecal synthesis of antibodies to viral antigens.

MS is defined as a demyelinating disease of the CNS with lesions separated in space and time and in which other diagnoses have been ruled out. Before the recovery of Borellia burgdorferi or the human T-cell lymphotropic virus, some cases of Lyme disease and tropical spastic paraparesis fell within the definition of MS. We may view these observations from two perspectives: (1) in the past we made erroneous diagnoses now corrected by greater knowledge or (2) what we define as MS may have multiple causes and manifestations, and with more specific diagnoses the syndrome will become better and more narrowly defined.

The epidemiology, pathology, and clinical features of MS are comprehensively covered in other chapters. Discussion in this chapter will focus on features suggesting possible infectious causes.
Epidemiology

Geographic and Ethnic Distribution

The prevalence of MS is highly variable, dependent on geography and ethnicity. The prevalence of over 200 per 100,000 in the Shetland and Orkney Islands of the North Atlantic contrasts with prevalences approaching 1 per 100,000 in areas of Africa. This variance is not explained by clinical sophistication or quality of health care as once believed. Even within Europe and North America a north/south gradient or zones exist with higher incidence related to higher latitude. This appears to be reflected in the Southern Hemisphere, where the incidence of MS is higher in southern than northern areas of Australia and higher on the south than the north island of New Zealand (Kurtzke, 1993).

In Northern Europe, Canada, and the Northern United States, prevalence rates are high and range from 30 to 80 cases per 100,000. In Southern Europe and the Southern United States, moderate rates of 6 to 29 are usual. Low prevalence rates are defined as those below 5 per 100,000 and prevail in Northern South America and all known areas of Africa and Asia. Remarkable exceptions to the general correlation of latitude to prevalence are exemplified by the absence of MS in Eskimos, and a prevalence in excess of 150 per 100,000 in Sardinia (Montomoli et al., 2002). The incidence of MS is clearly tied to geography, but whether this reflects immigration routes of Northern Europeans carrying susceptibility genes, or whether it reflects regional exposure still provokes controversy.

MS is more common in women than men in all regions, which supports the postulated immune-mediated pathogenesis. Distribution among racial groups is also unequal; it is more common in whites than Asians, and more common in Asians than African Americans. This supports genetic factors in causation, but it is difficult to accredit this solely to genetic factors since the prevalence in non-whites in the United States increases with increasing latitude, implicating the importance of environmental agents (Lowis, 1988). MS prevalence also correlates to a lesser extent with higher socioeconomic class and urbanization.

Little data are available to determine whether the prevalence of MS has increased over time. The increasing identification of occasional cases in those ethnic group previously thought to be spared from the disease (native-born Andeans residents, black South Africans, Eastern European gypsies, etc.) may represent better medical care and availability of imaging or may represent a genuine spread of MS to previously unaffected populations. In Rochester, Minnesota, a stable rate was noted for many years and a more recent survey showed a striking rise (Wynn et al., 1990). Prevalence data are hard to compare, since the earlier diagnosis and longer survival increase prevalence rates and incidence rates are less accessible.

Familial Aggregation and Genetics

The risk of MS is significantly increased in the siblings and progeny of MS patients. This is striking in twins where the Canadian study has shown monozygotic twins had a concordance rate of 30.8% and dizygotic sex- alike twins had a concordance rate of 4.7% (Sadovnick et al., 1993). This strengthens the long recognized genetic component of causation; however, 70% of the monozygotic twins were discordant suggesting important nongenetic factors. The Canadian study subsequently evaluated adopted, nonbiological relatives and could find little effect of shared environment (Ebers et al., 1995).

Migration Studies

Studies of populations migrating from high-risk regions to low-risk regions and studies of patterns of disease on North Atlantic islands have provided the strongest support for the role of a childhood exposure followed by a long incubation period. These investigations began after World War II when Dean (1970) observed that the majority of patients with MS in South Africa were immigrants from the United Kingdom or Northern Europe, even though they made up less that 10% of the population. He determined a prevalence of 50 per 100,000 in this population, compared with 11 per 100,000 in native born English-speaking South Africans, 3 per 100,000 in white Africaans speaking natives, and absence of MS in
black, native-born South Africans. Further analysis showed that migration prior to age 15 years led to a risk similar to that of native born English South Africans; and migration as an adult resulted in a risk similar to that of the country of origin (Dean and Kurtzke, 1971). Similar risk of early life exposure for migrants have been reported from Israel, Australia, Hawaii, and the Antilles. Recent studies of migrants from the United Kingdom and Ireland to varied latitudes in Australia showed prevalence in migrants was considerably less than their country of origin, but did not show a shift at age 15, suggesting that environmental factors may operate over a period of many years and not only in childhood (Hammond et al., 2000). Studies of migrants from low risk regions to high risk regions suggest a similar phenomenon but are less complete.

Similar evidence of early life exposure is provided by studies of World War II veterans. In the United States, residence at birth and military induction showed a sharp north to south differential in risk for MS. Residence after induction and at the onset of disease showed no geographic correlation indicating that risk was acquired prior to conscription (Beebe et al., 1967).

**Apparent Epidemics**

Continental incidence rates appear rather stable, but studies of island populations of Norse ancestry in the North Atlantic have shown fluctuations suggesting epidemics. Prevalence rates on the Shetland and Orkney Islands repeatedly showed the highest rates worldwide, but the breakdown of incidences of new cases per year show an abrupt upsurge in the late 1930s, and an equally sudden decline in new cases in 1971, suggesting an epidemic during the intervening 3 decades (Kurtzke, 2000).

The data from the Faeroe Islands are even more dramatic. No cases of MS were documented prior to 1943; then 16 patients had onsets between 1943 and 1949 with three subsequent waves of cases. The onset of the initial outbreak coincided with the British occupation of the islands from 1940 to 1945, and detailed analysis showed a spatial relationship between villages where MS patients lived and where British troops had been quartered. The studies in the Faeroes have concluded that MS is not only an acquired disease but that it is a transmissible disease (Kurtzke, 2000).

MS has long been recognized in Iceland, but a reexamination of their rates show a rise in incidence in 1922, which plateaued until 1945 when a rise in incidence occurred over the subsequent decade. Again this followed the occupation by American, Canadian, and British military forces.

**Epidemiological Evidence of a Specific Virus**

Although late childhood or adolescent infection with a virus, followed by a long incubation has been suggested by epidemiological investigations, very little data implicate a specific agent. A number of reports confirm that measles and other common childhood infections occur at a more advanced age in MS patients than in controls (Bachmann and Kesselring, 1998). A history of infectious mononucleosis is more frequent in MS patients, indicating later infection, since infections with the ubiquitous EB virus are generally asymptomatic in children and manifest with mononucleosis only when infections is delayed until adolescence and young adult life. These findings favoring late acquisition of common infections simply may reflect that persons who develop MS may have had more sheltered childhoods.

An unexplained north to south gradient of varicella-zoster virus infection occurs with chickenpox being an almost universal disease of elementary school children in temperate zones and an infrequent disease of children in the tropics. In the tropics more adult cases, and even adult epidemics, occur (Ross, 1998). An excess of spring births of persons who develop MS has been cited to implicate an infectious etiology, but this correlation would implicate maternal or neonatal infection, which is not consistent with the migration studies.

Animal exposures have been investigated extensively. Initially, the Faeroe Island outbreak was postulated to be related to canine distemper virus, a viral infection thought to have been imported by the British officers’ dogs. Subsequently a cluster of MS cases in
Sitka, Alaska, was noted to have followed a canine distemper outbreak 4 to 5 years previously (Cook and Dowling, 1982). Studies of MS patients have not confirmed antibody responses to canine distemper specific polypeptides.

**Viral Infections and Exacerbations**

Patients often relate exacerbations of MS to psychological stress, physical trauma or physical fatigue, but prospective studies quite consistently show a relationship only with symptoms of respiratory infections (Casetta and Granieri, 2000; Marrie et al., 2000). A recent study extended the findings to show that exacerbations in the contest of a systemic infections lead to more sustained damage (Buljevac et al., 2002).

A number of studies have also examined activation of human herpesviruses with exacerbations, particularly EB virus and human herpesvirus 6 (HHV6), but also active replication of herpes simplex type 1 has been associated with exacerbations (Ferrante et al., 2000). The obvious dilemma in these studies is the precise timing of the onset of the exacerbation and the activation of the latent virus—that is, determining which came first.

**Pathology**

Electron microscopic studies in 1964 identified papovavirus particles in inclusion bodies of PML and in 1965 identified structures resembling morbillivirus nucleocapsids in the inclusions in subacute sclerosing panencephalitis. Amid the exuberance over finding viruses by electron microscopy of poorly fixed autopsy and biopsy tissues, a number of studies reported “viruslike” particles in MS brains. The ovoid membrane bodies of 30 to 200 nm in diameter are now thought to represent myelin breakdown products, the dense intracytoplasmic granules of 60 to 80 nm diameter probably represent nonspecific changes in reactive astrocytes, and the intranuclear structures in inflammatory cells originally identified as myxovirus nucleocapsids are now believed to be nonspecific alterations in nuclear chromatin. None of these or other structures have been definitively identified as viral in nature (Johnson and Herndon, 1974).

Although the neuropathology of MS in covered in Chapter 31, one perspective of the pathology that may be relevant to causation is the heterogeneity of lesions in MS. Lucchinetti, et al. (2000) analyzed acute demyelinating lesions in 51 biopsies and 32 autopsies from MS patients. All cases contained at least one active lesion with inflammation, macrophages with myelin debris, and demyelination. CD3 T cells, macrophages, and occasional plasma cells characterized all patterns, but in some cases the demyelination was periventricular and in others not. Some had plaques with sharp margins, others were ill-defined. In some, oligodendrocytes persisted in demyelinated foci and remyelination was evident, in others apoptosis of oligodendrocytes was associated with wider loss of myelin associated glycoprotein. Despite this heterogeneity there was homogeneity among active lesions in the same patient. They divided the cases into four patterns, but the parallels of some to ADEM and others to PML are very suggestive of different modes of pathogenesis.

In viral diseases, consistency of a clinical-pathological syndrome does not imply a single causative agent. When lymphocytic choriomeningitis virus was recovered from spinal fluid it was regarded as the cause of benign aseptic meningitis, yet subsequent studies have implicated more than 100 different viruses in that syndrome. Conversely a single virus can evoke varied clinical-pathological responses such a varicella-zoster virus which can cause ADEM, Reye’s disease, acute myelitis, vasculitis, and postherpetic neuralgia.

Among clinical laboratory tests, the intrathecal synthesis of IgG and the presence of spinal fluid oligoclonal bands are hallmarks of MS. Other diseases that consistently show these abnormalities are infectious processes—neurosyphilis, neuroborreliosis, subacute sclerosing panencephalitis, HTLV-1 associated myelitis, and other chronic infections. In these diseases, intrathecal antibodies can be shown to react with viral antigens; the antigen(s) in MS are unknown but the analysis of IgG heavy chain sequences in MS brains suggest an antigen driven response rather than a nonspecific B-cell activation (Smith-Jensen et al., 2000).
Virological Studies

Studies of patients with MS have implicated poxviruses, herpesviruses, rhabdoviruses, orthomyxoviruses, paramyxoviruses, coronaviruses, flaviviruses, picornaviruses, retroviruses, and a variety of unclassified or mythical agents. Several parasites and bacteria have also been implicated. Assays of antibodies in serum and in spinal fluid have consistently shown higher titers of various antibodies in MS patients than in controls, inoculations of patient fluids or tissues into cell cultures or laboratory animals have shown changes interpreted as evidence of virus replication, or finally the change in quantity or topography of an agent known to persist in humans has been interpreted as suggesting a causal relationship to MS.

Serological Studies

In 1962, the first report appeared that titers of antibodies to measles virus were higher in the serum of MS patients than controls. The same antibodies were detectable in the spinal fluids of 75% of MS patients and not in spinal fluids of controls (Adams and Imagawa, 1962). Initially, these findings were regarded with skepticism, but study after study confirmed these odd findings; more than 30 confirmations have been published. Subsequently other studies showed higher titers and intrathecal synthesis of antibodies to a variety of other viruses, but never with the magnitude or consistency of measles antibodies (Tab. 40.4). In one study, 23% of patients with MS had disproportionately high antibodies to 2 or more viruses in the spinal fluid (Norrby et al., 1974); in another study one patient was reported who had evidence of intrathecal synthesis of antibody to 11 different viruses (Salmi et al., 1983). In general, twin studies have shown higher levels in antibody in the serum or spinal fluid of the affected twin than of the healthy twin (Kinnunen et al., 1990).

The range of responses has suggested a nonspecific activation of B cells, but responses against one protein of a virus and not another (Nath and Wolinsky, 1990), and studies of the antibody variable regions suggest a more specific response. The finding of higher serum antibody titers to measles is not specific to MS. Similar higher titers have been reported in systemic lupus erythematosus, chronic hepatitis, and Reiter syndrome. In MS, as in these other diseases, individual levels of antimeasles antibody are not remarkable, as they are in subacute sclerosing panencephalitis, but the mean titer of large group of patients is consistently higher than the mean of a group of matched controls. Furthermore, measles infection is not a prerequisite to MS, since cases of MS have been observed, presenting prior to the acquisition of measles.

| TABLE 40.4 Higher Antiviral Antibodies in Multiple Sclerosis Than in Controls |
|------------------------------------------|
| Serum | CSF |
|---|---|
| **Measles** | **Measles** |
| Parainfluenza 3 | Parainfluenza 1, 2, 3 |
| Influenza C | Influenza A, B |
| Varicella | Varicella |
| Herpes simplex | Herpes simplex |
| Human herpes virus—6 | Human herpes virus 6 |
| Epstein-Barr | Epstein-Barr |
| Rubella | Rubella |
| | Mumps |
| | Respiratory syncytial |
| | Coronaviruses |
| | Adenoviruses |
| Borna disease virus | Borna disease virus |
| HTLV-I (gag) | HTLV-I (gag) |
| HTLV-II | Simian Virus-5 |

Modified from Johnson (1998).
**Isolation Reports**

Recovery of agents from MS has a colorful history. During the first half of the 20th century extensive interest was given to a putative spirochete. In 1917, the agent was claimed to have been recovered from the spinal fluid of patients with MS inoculated into guinea pigs and rabbits. In 1952, direct staining of the agent in brain and spinal cord led to its naming as *Spirochaeta myelophthora* (Steiner, 1952). Interest was rekindled in 1957 with further claims of cultivation of spirochetes from spinal fluids (Ichelson, 1957), claims that later reports failed to confirm. This controversy was finally settled by extensive negative results using the precise methods recommended for cultivation but substituting autoclaved water (Kurtzke et al., 1962).

In the 1930s in England, an organism tentatively named *Spherula insularis*, possibly a Mycoplasma, was reported to have been isolated from spinal fluid of 176 of 189 patients with MS (Chevassut, 1930). A vaccine was made, and more than 100 patients were given the vaccine prior to an abrupt retraction (Purves-Stewart, 1931). In 1956, *Toxoplasma gondii* was alleged to have been isolated from spinal fluid and blood of MS patients, but that too went unconfirmed. A number of claims of transmission to primates and other animals were made, but agents were not characterized and results remained unconfirmed (Johnson, 1985).

The first recovery of a virus that evoked serious consideration was the 1946 Soviet claim of recovery of a virus in mice inoculated with spinal fluid and brain tissue of two patients with MS (Margulis et al., 1946). The virus was shown independently to be rabies virus; whether the patient diagnosis was wrong or the agents were laboratory contaminants was unknown. No laboratories confirmed the isolations, although the original laboratory reported subsequent similar isolations.

In 1964, herpes simplex virus was recovered from the spinal fluid of a patient with MS (Gudnadottir et al., 1964). This virus subsequently was shown to be a type 2 herpes simplex strain; the type that has subsequently be isolated frequently from spinal fluid in recurrent meningitis. This may also represent the first isolation of “normal flora” from MS specimens and the beginning of the persistent question of cause or effect.

Some of the viruses on Table 40.5 have subsequently been retracted as laboratory contaminants (scrapie and measles), some are thought to represent animal viruses recovered from inoculated laboratory animals (chimpanzee cytomegalovirus and corona-

| TABLE 40.5 Viruses Recovered from Patients with Multiple Sclerosis (MS) |
|-------------------------------------------------------------|
| Rabies virus | 1946 |
| Herpes simplex virus, type 2 | 1964 |
| Scrapie agent | 1965 |
| MS-associated agent | 1972 |
| Parainfluenza virus 1 | 1972 |
| Measles virus | 1972 |
| Simian virus 5 | 1978 |
| Chimpanzee cytomegalovirus | 1979 |
| Coronavirus | 1980 |
| SMON-like virus | 1982 |
| Tick-borne encephalitis flavivirus | 1982 |
| HTLV-I | 1985 |
| LM7 (retrovirus) | 1989 |
| Herpes simplex virus, type 1 | 1989 |
| Human herpesvirus 6 | 1994 |
| Endogenous retroviruses | 1998 |

HTLV, human T-cell lymphotrophic virus, SMON, subacute myelo-optico-neuropathy.
virus), and some probably do not represent viruses but only laboratory observations interpreted as representing viral activity not verified in independent laboratories (MS-associated agent and SMON virus). A critique of these reported isolations previously has been published (Johnson, 1998).

During the National Institutes of Health studies of slow infections, tissues from MS patients were inoculated into chimpanzees. No evidence of transmission has been observed over the subsequent 30 years. This is not definitive evidence against a viral cause, however. During those studies tissue from PML and subacute sclerosing panencephalitis, diseases now known to be caused by viruses, were similarly inoculated into chimpanzees with negative long-term observations.

**Agents of Current Interest**

Over the past 5 years the literature on infectious agents and MS has been dominated by studies of the herpesviruses, EB and human herpesvirus 6 (HHV6), endogenous retroviruses, and a bacterium, *Chlamydia pneumoniae*. In contrast to prior attempts to recover a unique MS virus, these all represent ubiquitous agents that persist in humans, and studies have focused on quantitation and cellular sites of infection. In each case the difficult question is whether changes are related to causation or whether replication and host cells changes are secondary to the immunological changes in MS.

**Epstein Barr virus**  Interest continues in the long postulated role of EB virus in MS. As mentioned earlier, the age of acquisition determines disease in this infection. Early life infections, as occur in tropical climes and impoverished communities, lead to immunity but no clinical illness; delayed infections in adolescence and young adult life often lead to the syndrome of infectious mononucleosis. Furthermore, even in case-controlled studies MS patients report a greater frequency of preceding infectious mononucleosis. Prevalence of antibodies to EB virus in MS patients is greater than in controls, and in most studies 100% have antibodies against EB, an extent of seropositivity unique to EB virus. A number of authors have suggested that EB infection is a prerequisite to development of MS (Ascherio and Munch, 2000; Munch et al., 1998; Myhr et al., 1998) Longitudinal studies have also found an association between EB virus activation and disease activity in MS patients (Wandinger et al., 2000).

There have been several reports of patients with neurological complications of primary EB virus infections who went on to develop progressive or relapsing disease subsequently diagnosed as MS (Bray et al., 1992; Shaw and Alvord, 1987). One 6-year-old had 11 episodes of relapsing disease with high titers of EB antibodies. At death, the neuropathological diagnosis was typical MS, and PCR of the brain showed EB virus sequences (Pedneault et al., 1992).

EB virus maintains latency in B cells, which are not present, or at least very rare, in normal nervous tissue. B cells are a feature of the inflammatory response in MS and other inflammatory and infectious diseases. Therefore, the presence of EB DNA determined by PCR may only reflect the presence of B cells; detection of viral proteins in cells or infectious virus in brain or spinal fluid are evidence of active infection, but again this could represent nonspecific activation during the attack of MS.

**Human herpesvirus 6**  HHV6 is a recently recovered human herpesvirus. The virus is ubiquitous with a 70 to 100% seroprevalence in adult populations worldwide. Two variants have been distinguished, and the B variant is the predominant cause of exanthem subitum in childhood. Encephalitis has long been a recognized complication of exanthem subitum. After primary infection, HHV6 remains latent primarily in T cells, but the virus is pleiotropic, with latency in B cells and CNS glial cells having been reported (Soldan et al., 2001).

Similar with many other viruses, higher levels of serum antibodies and presence of spinal fluid antibodies to HHV6 were reported in many MS patients. In addition, HHV6 DNA was detected in spinal fluid by PCR (Wilborn et al., 1994). The report by Challoner and colleagues in 1995 made HHV6 a serious candidate as the cause of MS. They found
HHV6 DNA in the majority of MS and control brains, but protein expression was primarily in MS brains. Furthermore, immunocytochemical staining showed positive meningeal cells in both groups, but in MS lesions there was staining of cells adjacent to the plaques thought to be neurons and oligodendrocytes. Many conflicting publications have followed. HHV6 IgM in serum and spinal fluid, higher titers of antibodies in serum, and higher frequency of antibody in spinal fluid have all been reported in MS patients compared to controls; and all of these findings have been refuted in other studies. More frequent detection of HHV6 DNA in peripheral blood mononuclear cells, serum, spinal fluid, and brain of MS patients have been reported; and again others have failed to confirm the claims. In one study of lymphoproliferative responses to HHV6 antigens by MS patients, more frequent responses of patients was found to the A variant, although most data has implicated the B variant (Soldan et al., 2000).

Several reported results raise a need for confirmation or extension. One group reported immunocytochemical staining in 90% of sections showing active demyelinating lesions and only 13% in tissue sections free of active disease (Knox et al., 2000); this level of sensitivity and specificity is unique among reports. Recently elevated serum and spinal fluid levels of membrane cofactor protein CD46 were reported in MS patients; this is important because CD46 is a receptor for HHV6 (as well as measles, the traditionally most implicated virus) and is a regulator of the complement cascade involved in antibody mediated immunopathology (Soldan et al., 2001). This provocative finding may lead to new mechanisms by which viruses might evoke in immune-mediated diseases.

***Endogenous retroviruses*** Human endogenous retroviruses (HERVs) are DNA sequences present within human chromosomes and make up about 2% of the human genome. The characteristic presence of long terminal repeats followed by gag, pol, and env genes identify their retroviral origins; they are thought to represent ancestral infections in which integrated DNA is now passed on in Mendelian fashion. Comparisons to ERVs of apes and old world monkeys suggest that some entered our genome 25 million years ago (Voisset et al., 1999). HERVs are defective in that they do not code infectious particles or transmit horizontally. Some do encode functional proteins, and complementation may result in virion formation.

No endogenous retroviruses have been convincingly associated with human disease. The potential to enhance downstream cellular genes has led to speculation that they might be involved in autoimmune disease. They have been proposed as factors in the pathogenesis of MS, systemic lupus erythematous, Sjogren’s disease, and type 1 diabetes (Perron and Seigneurin, 1999).

In 1991 a retrovirus was reported budding from a cell line of meningeal cells established from the spinal fluid of a patient with MS (Perron et al., 1991). Subsequently, C-type retrovirus particles were found in peripheral blood mononuclear cells cultured from several patients with MS, but this proved to be a different HERV (Christensen et al., 1998).

Several recent studies suggest that increased expression of these viruses is a consequence of immune activity rather than the cause. Johnston et al. (2001) showed that levels of HERV RNA increased in cultured macrophages nonspecifically stimulated in vitro; several HERVs were also expressed in brain tissue of patients with human immunodeficiency virus infections and with MS, correlating with tumor necrosis factor expression and macrophage activation. In another study (Dolei et al., 2002), analysis of blood for HERV reported detection in all MS patients, most patients with other inflammatory neurological disease and rarely in healthy donors. The role of these viruses as cofactors rather than simply secondary responders remains unclear.

***Chlamydia pneumoniae*** C. pneumoniae is an obligate intracellular Gram-negative bacterium. It is a common respiratory pathogen causing pharyngitis, bronchitis and atypical pneumonia. Seroprevalence is 40 to 70% in adults, with most seroconversions occurring during adolescence. Numerous attempts to relate C. pneumoniae to chronic disease have been made, most notably coronary artery disease and atherosclerosis. Because
the bacteria persist in human macrophages, PCR studies of tissues with macrophage infiltrates often are positive.

After observing a single patient with apparent acute MS from whom C. pneumonia was recovered from spinal fluid and who improved with antibiotic therapy, the Vanderbilt group undertook an extensive study. From spinal fluid of MS patients, they cultured C. pneumoniae from 64% but recovered bacteria from only 11% of spinal fluids from patients with other diseases. PCR was positive in 97% compared to 18% in control patients. Spinal fluid IgG directed against the bacterium was found in 97%, compared to 18% with other neurological diseases (Sriram et al., 1999). Subsequently they reported that oligoclonal bands in spinal fluids of patients with MS not only reacted with C. pneumoniae antigens (116 of 17 patients) but could be partially or completely adsorbed by antigens (Yao et al., 2001). A large number of contradictory reports have followed. Some have been confirmatory to some facets, but none with the high percentages of the initial report; the majority have been negative. A recent report, for example, using PCR detected C. pneumoniae in spinal fluid of 21% of MS patients, in 43% of patients with other neurological diseases, and in no healthy controls (Gievers et al., 2001). This would suggest that inflammatory responses that recruit macrophages into the spinal fluid, where under normal circumstances they are not found, can carry in C. pneumonia. Better standardization of both cultivation and PCR methods are needed before fair comparisons of studies can be made.

SUMMARY

It is clear that a diverse array of viruses can infect the human central nervous system. The resulting viral infections can result in a wide variety of clinical and pathological symptoms. While some viruses may cause widespread inflammation and neurodegeneration, other viruses may remain latent in the CNS and only produce pathological changes during reactivation. Viral induced demyelination can occur both from direct infection of the myelin-producing oligodendrocytes, as in PML, or by indirect mechanisms that have yet to be determined. The final outcome of a viral CNS infection will depend not only on viral characteristics, but also the interaction between virus and host cell. Understanding factors such as immune modulation and host cell regulation of viral gene expression could improve current methods of diagnosis or even lead to innovative methods for therapeutic intervention.

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