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Chapter 7

Infections and multiple sclerosis

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INTRODUCTION

Infectious causes of multiple sclerosis have been proposed for over 100 years. During the past half-century three areas of investigation have given greater credence to the role of infectious agents, particularly viruses, in the initiation and/or exacerbations of multiple sclerosis: (1) epidemiologic studies indicate that multiple sclerosis is related, in part, to environmental exposure followed by a long latency period, and exacerbations of disease often follow virus-like illnesses; (2) studies in animal and other human demyelinating diseases have demonstrated a variety of mechanisms by which infectious agents can have long incubation periods, induce relapsing and remitting disease, and cause myelin destruction with relative preservation of axons; and (3) studies in patients with multiple sclerosis have consistently shown enhanced immune responses, particularly to viral antigens.

Genetic, autoimmune, or infectious causation had partisan advocates in the past, but a multifactorial etiology is now generally accepted. Genetic factors are clearly important in multiple sclerosis, but monozygotic twins have only a 14–30% concordance, suggesting a major role for environmental factors. Assuming the pathogenesis of lesions is immunologically driven, what evokes the response remains unknown. Infections remain plausible explanations for the initiation of these responses and for the clinical and pathological features of the disease.

Since the earlier edition of this chapter in the *Handbook of Clinical Neurology* over 25 years ago (Johnson, 1985), hundreds of additional papers have been published on infectious agents in multiple sclerosis. Nevertheless, the arguments for or against a virus or bacteria in the cause of multiple sclerosis seems not one whit stronger or weaker than it was more than two

decades ago. A shift has occurred, however, both in methodology and goals. New methods of detection, localization, and quantitation of infectious agents are now available, and the focus of studies has shifted to known agents rather than the search for a unique “multiple sclerosis virus.”

EARLY OBSERVATIONS IMPLICATING INFECTIONS

Jean Martin Charcot described the clinical and pathologic features of multiple sclerosis in a series of lectures in 1868 (Murray, 2005). Since theories of humors causing diseases were still in vogue, he proposed that exposure to cold, physical injury, or emotional stress might bring on the disease. Over the subsequent 15 years, the work of Koch and Pasteur established microbial causes for many diseases. Thus, in historical perspective it is predictable that in 1884 Charcot’s student and successor, Pierre Marie, proposed infections as the cause for multiple sclerosis (Marie, 1884). Indeed, in the afterglow of Pasteur’s widely hailed discovery of postexposure immunization against rabies virus, Marie prematurely predicted a vaccine would soon be available for multiple sclerosis.

In the first half of the 20th century, reports of spirochetes began with the study of Kuhn and Steiner (1917), who claimed to have recovered them from the spinal fluid of patients with multiple sclerosis by the inoculation of guinea pigs and rabbits. Subsequently, Steiner (1952) reported similar organisms by direct staining of brains and spinal cords of patients. He named the putative agent *Spirochaeta myelophthora*. In 1957 another laboratory reported the cultivation of spirochetes from spinal fluids from patients with multiple sclerosis (Ichelson, 1957). This claim was confirmed and denied in subsequent reports but was finally

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settled by an extensive negative study using Ichelson's methods, except for the use of autoclaved water (Kurtzke et al., 1962).

In the 1930s a putative virus (possibly a *Mycoplasma*) was reported to have been isolated from the spinal fluids of 176 of 189 patients with multiple sclerosis (Chevassut, 1930). This organism was named *Spherula insularis*, a vaccine was produced against the agent, and over 100 patients were treated. Enthusiasm ended when an oddly uninformative retraction was published by the clinician involved in the studies (Purves-Stewart, 1930, 1931; Murray, 2005). On the basis of serologic tests Le Gac (1960) proposed a *Rickettsia* in multiple sclerosis. Even protozoa were implicated, with the reported recovery of *Toxoplasma gondii* from the spinal fluid and blood of patients (Bequignon, 1956). In addition, there were a number of reports of the transmission of neurologic disease to a variety of animals inoculated with the tissues and spinal fluid of patients with multiple sclerosis (Innes and Kurland, 1952). None withstood scrutiny or independent confirmation.

An important early advance in the understanding of immune responses in demyelinating diseases came from experimental virology. Following measles and vaccinia virus infections, an acute encephalomyelitis occasionally develops that is characterized by perivenular demyelination of the brain and spinal cord. The neuroparalytic complications of rabies virus vaccines prepared in neural tissues bore a striking histopathologic resemblance to these virus-induced diseases. Thomas Rivers, the father of American virology, was intrigued by this similarity, and in the course of his studies with vaccinia virus, he inoculated monkeys with homogenates of normal neural tissue. After 6 months of repeated injections, several monkeys developed inflammatory demyelinating encephalitis simulating postinfectious encephalomyelitis and the neuroparalytic complications of rabies vaccines (Rivers and Schwentker, 1935). This was the first induction of experimental allergic encephalomyelitis. The subsequent claim by Schaltenbrand (1943) of transmission of multiple sclerosis to monkeys presumably represented an independent finding of this phenomenon, since the inflammation and demyelination that he induced in monkeys followed repeated injections of human neural tissue.

Experimental allergic (autoimmune) encephalomyelitis became a practical disease model after 1947, when Kabat and colleagues found that the experimental disease could be reliably reproduced in some species by a single injection of brain tissue if emulsified in Freund's complete adjuvant. The antigens proved to be specific amino acid sequences of myelin proteins, the immunopathologic response was found to be cell-mediated, and the disease could be passively transferred with

sensitized lymphocytes. Experimental autoimmune encephalomyelitis became the prototype experimental autoimmune disease, and it simulates acute disseminated encephalomyelitis in humans (Johnson et al., 1984; Johnson, 1998). Since the 1950s it has also been presented as an experimental model of multiple sclerosis, but this analogy seems overdrawn (Subramaniam and Steiner, 2005).

After mid-century the landscape changed with renewed interest in viruses as causes of chronic neurologic diseases. Between 1967 and 1971 the transmission of kuru and Creutzfeldt–Jakob disease and the recovery of viruses from subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy led to widespread anticipation that multiple sclerosis would soon be transmitted or prove to yield consistent viral recovery (Johnson, 1998). The epidemiologic studies have continued to suggest an environmental factor, studies in animals and humans delineated more mechanisms by which viruses can lead to acute and chronic demyelinating diseases, and new methodologies have incriminated different groups of agents that may play a role in the pathogenesis of multiple sclerosis. The optimism of the 1970s, however, has waned.

EPIDEMIOLOGIC STUDIES IMPLICATING INFECTION

Geographic distribution

National and regional death rates and over 300 prevalence studies show an uneven geographic distribution of multiple sclerosis (Kurtzke, 2005). The prevalence of over 200 per 100 000 in the Shetland and Orkney Islands of the North Atlantic contrasts with a prevalence approaching 1 per 100 000 in areas of Africa. These differences are not entirely due to variations in clinical sophistication or healthcare delivery, as once was assumed.

Data collected largely since World War II have demonstrated in Europe and North America a north/south difference in prevalence, with rates of 30–80 cases per 100 000 in northern Europe, southern Canada, and the northern United States, in contrast to rates of 6–20 per 100 000 in most of southern Europe and southern United States. The southern hemisphere is less well defined, but multiple sclerosis reaches moderate prevalence rates in South Africa, appears more common in southern than in northern Australia, and is more prevalent in the southern than in the northern island of New Zealand. The rate is higher for women than for men and half as frequent in African Americans as in white North Americans, although the same north/south gradient in the United States is seen in both races. Multiple sclerosis is a rare disease in Asians (Kurtzke, 1983, 2005).

This distribution has been interpreted as a latitude gradient, but it can also be viewed as three high-risk zones, including northern Europe and southern Scandinavia, northern United States and southern Canada, and New Zealand and south-east Australia. Each of these regions appears to be bounded by areas of medium frequency, with prevalence rates between 5 and 29 per 100 000, suggesting that multiple sclerosis may have risen in northern Europe, with subsequent establishment of foci among the descendants of migrants in the other locations (Kurtzke, 1983). Striking exceptions to the correlation of prevalence with latitude exist, such as the apparent absence of multiple sclerosis in Eskimos and a prevalence in excess of 150 per 100 000 in Sardinia (Montomoli et al., 2002). Whether prevalence in high-incidence areas has increased over time is uncertain. On the other hand, multiple sclerosis is now being reported in populations previously thought to be exempt from the disease such as American Indians, South African black populations, and European gypsies.

Migration studies

Following World War II, the majority of patients with multiple sclerosis in South Africa were immigrants from the United Kingdom or northern Europe, even though they made up less than 10% of the total population. Dean (1970) determined a prevalence rate for white Afrikaans-speaking natives of 3 per 100 000, for white English-speaking natives of 11 per 100 000, and for migrants from northern Europe of 50 per 100 000 – the same order of magnitude seen in northern Europe. Further analysis indicated that migration prior to 15 years of age led to the same risk as that of native-born white South Africans, whereas migration after that age carried the risk of the birthplace (Dean and Kurtzke, 1971).

Similar studies in Israel indicated migrants from areas of high prevalence and low prevalence carried with them the risk of their country of origin, if they immigrated after the age of 15 (Alter and Kurtzke, 1968). Studies of immigration to Australia, Hawaii, and the Antilles all show similar high rates of disease of immigrants from high-risk areas (Kurtzke, 1983). Recent studies in French West Indies showed a higher prevalence in return immigrants from France if they had preadolescent “exposure” in France (Cabre et al., 2005).

In a matched-control study of veterans of World War II, residence of birth and at induction into the military showed a sharp north to south differential in risk of multiple sclerosis. Residence after induction and at onset of disease showed no significant correlation (Beebe et al., 1967). In a parallel case-control study of veterans of Vietnam and subsequent military service, interesting changes were found. Prevalence in women increased,

prevalence in black men increased in comparison to white men, and the north–south gradient persisted but was less marked. Attenuation of the latitudinal gradient over time was also observed in the Nurses’ Health Study, which assessed multiple sclerosis incidence among female nurses in the United States born between 1921 and 1946. Although the initial study demonstrated that women born north of 41–42° north latitude had a 3.5-fold higher risk of multiple sclerosis compared to women born south of 37°, there was no such association in the follow-up Nurses’ Health Study II, which included nurses born between 1947 and 1964 (Hernan et al., 1999). A systematic review of over 38 studies from Europe, Australia, and the United States also found strong evidence for a recent diminution of the latitudinal gradient (Alonso and Hernan, 2008). Overall, these changes in gender, race, and geography over a short interval can be interpreted as strong evidence for environmental factors rather than genetic factors in the cause or precipitation of multiple sclerosis (Wallin et al., 2004).

Familial aggregation

Genes play an important role in multiple sclerosis. The most consistent association across all populations has been with the HLA-DRB1 gene, although an increasing number of genes of modest effect are being identified through genome-wide association studies (McElroy and Oksenberg, 2011). First-degree relatives of patients have a 3% lifetime risk. Twin studies have shown a higher concordance rate, between 14 and 25% in monozygotic twins and 2–5% in dizygotic twins (Willer et al., 2003; Hansen et al., 2005; Ristori et al., 2006). This suggests that up to 70% of determinants may be nongenetic. Recent analysis of adoptees, half-siblings, and step-siblings in the Canadian Network of Multiple Sclerosis Clinics showed no increase in prevalence in these family members, suggesting the environmental factor or factors were not in the familial microenvironment (Sadovnick, 2006). This would be an argument against microbes that spread from human to human but not those that spread by vectors or zoonoses.

Apparent epidemics

Rates of multiple sclerosis in Europe and North America have remained stable or increased gradually over years, but rates among inhabitants of north Atlantic islands of Norse ancestry have shown fluctuations, suggesting small epidemics. As mentioned above, the prevalence rates on the Orkney and Shetland Islands are the highest in the world, but analysis of cases suggests an abrupt increase in cases in the 1930s and an equally abrupt decline in cases in 1971 (Kurtzke, 2000).

By contrast, the neighboring Faroe Islands had been reported as having almost no multiple sclerosis. No cases were diagnosed before 1943; then 16 cases had onsets between 1943 and 1949, with several subsequent small waves of disease (Kurtzke et al., 1995). This gives the appearance of a point-source epidemic. The most unique antecedent event prior to 1943 was the 1940 occupation of the Faroes by the British, which leads to speculation that the disease was introduced by the British or their baggage. Detailed analysis showed a spatial relation between the homes of patients and the site of quartering of troops (Kurtzke, 2000). Notably, a recent examination of Danish National Health Service records of notifiable diseases in the Faroe Islands demonstrates a temporal association between acute gastrointestinal illness and the arrival of British troops (Wallin et al., 2010).

Multiple sclerosis has long been recognized in Iceland, but in view of the Faroe data, the year of onset of cases was re-examined. The disease appeared to occur at a steady low level until a sudden rise in 1923, which then plateaued until 1944, when there was a second rise that now is beginning to decline. Iceland was heavily occupied during World War II by Canadians and Americans as well as British military forces (Kurtzke et al., 1982).

Epidemiologic evidence of a specific virus

Human viral infections can be divided into three epidemiologic groups: those transmitted from human to human by enteric, respiratory, or venereal contact; those injected by hematophagous arthropods such as mosquitoes, ticks, or biting flies; and zoonoses, transmitted by the bite, exposure to, or consumption of animals that are the natural host of the virus.

The age of acquisition of an infection may influence the clinical manifestations. For example, poliomyelitis viruses seldom cause disease in infants, but cause more frequent and more severe paralysis at older ages. Thus, improved sanitation, that delays acquisition of this enteric infection, increases the risk of paralysis. Parallels between the epidemiology of multiple sclerosis and poliomyelitis have been made (Poskanzer et al., 1963). However, travel from high-risk to low-risk areas increases incidences of paralytic poliomyelitis, a phenomenon that is not seen with multiple sclerosis. Disease associated with improved sanitation should have a later and later onset of disease over the decades. Similar analogies with varicella-zoster virus have been made with its opposite distribution; early infection is prevalent in temperate zones, and infections are less common and of later onset at more tropical latitudes (Ross and Cheang, 1995). A more convincing argument has been made for Epstein-Barr virus (EBV), where early childhood infection in developing countries is not associated

with clinical disease, whereas infection in adolescence and young adults is related to the clinical syndrome of infectious mononucleosis. Later but uniform infection with the virus and a more frequent history of infectious mononucleosis have been found in multiple sclerosis patients (see section on EBV, below). In several studies patients with multiple sclerosis were found to have a history of measles and other childhood diseases at later ages (Sullivan et al., 1984; Bachmann and Kesselring, 1998), but these findings were not confirmed in a more recent study that minimized recall bias (Bager et al., 2004). The notion that lack of certain infections owing to improved sanitation may lead to multiple sclerosis via inappropriate responses to innocuous substances or subsequent infections later in life has been formally conceptualized as the “hygiene hypothesis,” and has been proposed by some to underlie the higher incidence of multiple sclerosis in industrialized countries (Leibowitz et al., 1966; Fleming and Fabry, 2007). In support of this hypothesis, a small study demonstrated that parasitic infection can ameliorate the clinical course of multiple sclerosis and induce beneficial immune responses (Correale and Farez, 2007).

Arthropod-borne viruses show distinct geographic limitations, but these are even more sharply circumscribed than multiple sclerosis because of the localized distribution of the vectors. Furthermore, arboviral infections are highly seasonal, as are many of the viruses that are spread from human to human. If a virus had a latency from 3 to 30 years after infection, however, seasonality in initial exposure would be washed out by time.

Animal exposures have been evaluated in a number of studies. In the past there was an interest in a possible relationship to sheep. One retrospective epidemiologic study suggested an association with household dogs prior to the onset of symptoms of multiple sclerosis (Cook and Dowling, 1977). The authors linked their hypothesis to the Faroe data and postulated that British troops brought in canine distemper virus with their dogs (Cook et al., 1978). Similar distemper epidemics in Iceland were thought to correspond to increases in the incidence of multiple sclerosis, and cases of multiple sclerosis in Sitka, Alaska, 4–5 years after an outbreak of canine distemper, were reported (Cook and Dowling, 1984). Although apparent antibody to canine distemper virus can be found in human sera, this virus is closely related to measles, and reactions probably represent cross-reacting antibodies.

Viral infections and exacerbations

Patients with multiple sclerosis often relate exacerbations of disease to psychologic stress, physical trauma, or severe fatigue. Prospective studies have quite

consistently shown a more robust relationship with banal respiratory infections and exacerbations of multiple sclerosis (Buljevac et al., 2002). Most studies have not implicated a specific respiratory virus, and most regard these data as evidence that nonspecific antigenic stimulation can precipitate attacks. One serologic study did correlate adenovirus antibody increases with attacks but not influenza viruses (Anderson et al., 1993). Another study compared years of onset of multiple sclerosis to epidemic influenza data and found a correlation of increased disease onset with influenza virus activity (Kazmierski et al., 2004).

VIRUS-INDUCED DEMYELINATING DISEASES OF ANIMALS AND HUMANS

Several naturally occurring infections of animals are characterized by demyelination (Table 7.1). Laboratory infections of animals with human viruses or infections with adapted or genetically modified viruses have also shown variable amounts of demyelination, but these will not be addressed in this review. Two human demyelinating diseases clearly are caused by viruses, acute disseminated encephalomyelitis and progressive multifocal leukoencephalopathy, and the mechanisms of demyelination could not be more dissimilar. Other human viral infections, such as subacute sclerosing panencephalitis, human immunodeficiency virus (HIV) encephalopathy, and even herpes simplex encephalitis, are associated with variable amounts of myelin loss but are not considered primary demyelinating diseases.

Some of these virus-induced demyelinating diseases show how long latency periods can intervene between exposure to virus and the onset of clinical disease (lentiviruses in sheep and goats and progressive multifocal leukoencephalopathy in humans), some show mechanisms for relapsing and remitting courses of demyelination (lentiviruses in sheep and goats, Theiler's virus in mice, and neurotropic mouse hepatitis virus), and all show different mechanisms of myelin loss.

Table 7.1

Natural animal models of acute and chronic viral demyelinating diseases

Lentivirus	Visna virus in sheep Caprine arthritis- encephalitis virus
Picornavirus	Theiler's virus in mice
Coronavirus	JHM strain mouse hepatitis virus
Papovavirus	SV40 in macaque monkeys
Paramyxovirus	Canine distemper virus

SV40, simian virus 40.

Animal diseases

VISNA AND CAPRINE ARTHRITIS-ENCEPHALITIS VIRUSES

These natural virus infections of sheep and goats probably represent the best animal models for multiple sclerosis. They have an incubation period of months to years, may run a subacute or relapsing-remitting course of weeks to months, and give rise to focal areas of demyelination. They are caused by a subfamily of retroviruses called lentiviruses (the same subfamily that includes HIV) (Clements and Zink, 1996). Lentiviruses are unique in their ability to integrate their proviral DNA into post-mitotic cells, particularly monocytes and macrophages. Antibodies appear to downregulate virus production, but frequent mutations of the virus cause relapses of disease. The same group of agents is also associated with chronic pneumonitis and arthritis (Narayan and Cork, 1985).

Visna is a progressive paralytic disease of sheep first recognized in Iceland (Sigurdsson and Palsson, 1958). It was experimentally transmitted by inoculation of the brains of affected sheep into other sheep with an incubation period of months to several years. The clinical course is progressive, but can occur in waves with static periods before later worsening. During this clinical disease, the animals are afebrile, but their spinal fluids show a pleocytosis and elevated protein (Nathanson et al., 1985). Leukoencephalomyelitis of goats is caused by a related virus, but this disease has a more rapid clinical course (Cork and Narayan, 1980). The encephalitis usually occurs in kids less than 6 months of age, and paralysis or death may ensue within weeks. Again, the animals are afebrile during the disease with a pleocytosis and elevated spinal fluid protein. Pathology in both diseases is characterized by multifocal white-matter lesions that are most prominent in the periventricular and subependymal areas. Demyelination occurs with axon preservation, but in severely involved areas there is leukomalacia with cavitation.

Visna viruses isolated late in the course of the incubation period or disease are not neutralized by the antibody formed against the initial infecting virus (Narayan et al., 1978). Virulent mutants arise within the persistently infected host that is not inactivated by neutralizing antibody formed against previous virus or mutants (Clements et al., 1980). Only cells of macrophage lineage are infected in the nervous system. The demyelination appears associated with an interferon-like molecule (a cytokine or lymphokine) released in the nervous system by infected macrophages and microglia (Narayan et al., 1983; Kennedy et al., 1985). The management of sheep and goats in laboratories and the lack of ruminant reagents for immunological studies have limited investigations of these models.

THEILER'S VIRUS

In 1934, Theiler recovered a virus from mice with naturally occurring acute flaccid paralysis. The virus is an enterovirus similar morphologically and epidemiologically to human polioviruses. These murine viruses, however, persist in the nervous system, and demyelination was found in chronically infected mice (Daniels et al., 1952). In 1975, Lipton reported that, after intracerebral inoculation of young mice, many developed acute paralytic disease with typical pathology of poliomyelitis, and virus antigen was shown in neurons; but about half of these animals survived. After intervals of 1–5 months, these survivors developed a progressive inflammatory myelopathy characterized by demyelination.

This unique biphasic disease has been widely studied. The acute paralytic disease is clearly associated with the infection and destruction of motor neurons. The mechanism of the late demyelination involves persistent infection, and both macrophages and oligodendrocytes are infected (Brahic et al., 2005; Lipton et al., 2005). Immunosuppression with cyclophosphamide or antithymocyte serum prevents the late demyelination, however, suggesting that demyelination is immune-mediated (Lipton and Dal Canto, 1976). Indeed, *in vivo* depletion and neutralization studies have suggested important roles for both CD4+ and CD8+ T cells in mediating Theiler's murine encephalomyelitis virus-induced demyelination (Tsunoda and Fujinami, 2010).

The phenomenon of epitope spreading was found in studies of chronic Theiler's virus infections in specific strains of mice. In the course of glial cell infection, cell-mediated immune responses develop against myelin epitomes. Thus, although the initial response is directed against the virus, the response broadens with myelin proteins becoming targets. In this model of demyelinating disease persistence of infection would not be necessary to maintain recurrent or progressive disease (Katz-Levy et al., 1999; Tompkins et al., 2002).

NEUROTROPIC MOUSE HEPATITIS VIRUS

This virus was originally isolated from laboratory mice with spontaneous hind-leg paralysis (Cheever et al., 1949); histologic studies showed patchy areas of demyelination with sparing of axons in brain and spinal cord (Bailey et al., 1949). In 1969, Weiner obtained the virus from the original laboratory and showed that, with manipulation of dosage of virus, age of mice, and route of inoculation, demyelinating lesions could be produced consistently. Immunocytochemical staining showed that virus antigen was predominantly in glial cells of the white matter. The mechanism of demyelination did not

appear to be immunopathologic, since immunosuppression with cyclophosphamide did not prevent demyelination (Weiner, 1973). Ultrastructural studies suggested a direct lytic infection of oligodendrocytes (Lampert et al., 1973).

Although the virus was found to persist in the nervous system, the evident clinical disease was a uniphasic demyelinating process associated with selective infection of oligodendrocytes. However, in studies of remyelination it was noted that new areas of subclinical demyelination appeared to be developing concurrently with remyelination of older lesions (Herndon et al., 1975, 1977). The pathogenesis appears to be complex, with studies showing that late demyelination does not occur in mice lacking B or T cells, but does occur with transfer of virus-specific T cells or virus-specific antibodies (Stohlman and Hinton, 2001; Kim and Perlman, 2005). In addition, both monocyte-derived macrophages and microglia are present in regions of demyelination and can be observed to contact demyelinating axons (Templeton et al., 2008; Bender and Weiss, 2010). Thus, acute demyelination may be caused by direct lysis of oligodendrocytes, but persistent viral infection of glial cells may be capable of initiating humoral and cell-mediated immune responses that cause exacerbations of demyelination.

OTHER NATURAL INFECTIONS CAUSING DEMYELINATION

Progressive multifocal leukoencephalopathy morphologically and ultrastructurally simulating the human disease has been found in macaque monkeys at a primate colony (Gribble et al., 1975). All animals had underlying diseases probably leading to immunodeficiency, such as lymphoma, giant cell pneumonia, or colitis. SV40 virus, a simian polyomavirus, was recovered from brains. Experimental infection of monkeys with an immunodeficiency virus and SV40 has been associated with inflammatory encephalitis as well as demyelination (Axthelm et al., 2004).

Canine distemper is caused by a morbillivirus related to measles. It is an acute respiratory and gastrointestinal disease in dogs often complicated by central nervous system disease that may occur acutely or weeks or months after recovery from the acute illness (McCullough et al., 1974). During the acute infection the dog is immunosuppressed, and there is little inflammatory response. Viral persistence, which is thought to underlie progression of inflammatory demyelination, is achieved through noncytolytic cell-to-cell spread, as demonstrated in astrocytes (Wyss-Fluehmann et al., 2010). Initiation of demyelination was previously presumed to follow glial cell lysis,

but may in fact result from metabolic insufficiency of oligodendrocytes (Sips et al., 2007). In the late demyelination or relapses of disease, intense inflammation is found in the white matter. A paucity of viral antigen is found, but viral nucleocapsid structures have been identified in glial cells in the brain (Raine, 1976). Recent studies suggest that chronic inflammatory demyelination results from interactions between macrophages and antiviral antibodies (Vandeveldel and Zurbriggen, 2005).

Human demyelinating diseases

ACUTE DISSEMINATED ENCEPHALOMYELITIS

This is an acute perivenular demyelinating disease of the brain and spinal cord. In most patients it erupts abruptly days or several weeks after a viral exanthem or virus-like illness, but the disorder is not specific to viruses and has been seen after some bacterial illness, immunizations, and drug and serum therapies (Noorbakhsh et al., 2008). Fifty years ago it was estimated that one-third of all cases of clinical encephalitis were postinfectious. The commonest cause was measles, which, along with cases rarely associated with rubella and mumps, has been largely eliminated in regions where children are protected by the measles-mumps-rubella (MMR) vaccine. The second major cause of acute disseminated encephalomyelitis was a vaccine, vaccinia virus, which was abandoned after the worldwide eradication of smallpox in 1977. More recently the varicella-zoster vaccine has further reduced the risk of this disease. It is now estimated that less than 10% of cases of encephalitis represent acute disseminated encephalomyelitis. This exhibits a remarkable success in disease control by both introducing and eliminating immunizations (Johnson, 1994; Johnson and Major, 2004). The pathologic appearance is remarkably similar to that in the neuroparalytic complication in postexposure rabies vaccination and experimental allergic encephalomyelitis (Johnson et al., 1985). All three disorders have an abrupt monophasic clinical disease, similar perivenular inflammation and demyelination, and immunologic findings indicating sensitization to myelin proteins (Lisak and Zweiman, 1977; Johnson et al., 1984).

After measles, this complication occurs in about 1 per 1000 cases, with a mortality rate of about 20% and a high rate of sequelae in survivors. Measles virus is known to infect peripheral blood leukocytes and to alter immune responses. The frequent secondary infections are thought to result from immunosuppression, yet the encephalomyelitis is assumed to represent a hypersensitivity response. Antibody response to measles virus is normal in both uncomplicated measles and measles encephalomyelitis, but lymphocytes from patients with

measles show prolonged suppression of lymphoproliferative responses to mitogens. This occurs to an equal degree in uncomplicated measles, measles complicated by secondary infections, and measles with encephalomyelitis (Hirsch et al., 1984). The failure of mitogen responses and normal immune regulation appear to result from immune activations by the infection (Griffin et al., 1989). One result of this activation is the finding that a large percentage of patients with encephalomyelitis have lymphocyte proliferative responses in the presence of human myelin basic protein (Johnson et al., 1984), a finding analogous to that in experimental allergic encephalomyelitis. Thus, it appears that measles may interrupt the normal regulatory mechanisms that prevent responses to self antigens.

In measles encephalomyelitis, induction of autoimmune disease may occur without virus invasion of the nervous system. During acute measles virus can be found in a number of organs (Moench et al., 1988) but only in endothelial cells in the central nervous system (Esolen et al., 1995). The virus is cleared by the time encephalomyelitis develops, and virus cannot be isolated from the brain or spinal fluid, intrathecal synthesis of immunoglobulins against measles virus is not found (Johnson et al., 1984), and immunocytochemical staining fails to demonstrate measles virus antigen in the brains of patients dying at various intervals after measles encephalomyelitis (Gendelman et al., 1984). Thus, in this acute demyelinating disease, viral infection of peripheral immunocompetent cells appears to be the primary event; this leads to deregulation of normal suppression of responses against myelin proteins, and demyelination occurs without direct viral infection of neural cells (Johnson et al., 1985).

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

Progressive multifocal leukoencephalopathy is a subacute multifocal demyelinating disease usually complicating disease of the reticuloendothelial system or immunosuppression administered to maintain organ allografts or to treat other diseases. It was an exceedingly rare disease until the epidemic of HIV began in the 1980s. By the mid-1990s approximately 4% of patients dying of acquired immunodeficiency syndrome (AIDS) had progressive multifocal leukoencephalopathy. With the routine use of highly active antiretroviral therapy in 1996, incidence and death rates of progressive multifocal leukoencephalopathy have gradually declined, though not reaching pre-epidemic levels (Christensen et al., 2010). The demyelinated foci show a loss of oligodendrocytes. Surrounding the demyelinated areas, the oligodendrocytes are enlarged and contain intranuclear

inclusion bodies filled with papovavirus particles, often forming pseudocrystalline arrays (ZuRhein, 1969). Astrocytes within the lesions are often abnormal, multinucleated, and may contain mitotic figures. Astrocytes may express tumor antigen of JC virus but are not classically transformed, since some virus particles are found in astrocytes. No evidence of neuronal infection is found, with the possible exception of cerebellar granule cell neurons (Wuthrich et al., 2009).

The cause is a human papovavirus, JC virus (Padgett et al., 1976), a common human agent that produces widespread subclinical human infection. Most people develop antibodies before adolescence, and the virus remains latent in kidney, bone marrow, and B cells (Johnson and Major, 2004). With activations in the immunodeficient it is thought the B cells transport JC virus to the brain (Tornatore et al., 1992). In this demyelinating disease it is not an aberrant immune response that causes demyelination, as in postinfectious encephalomyelitis, but a failure of immune responses that contain a ubiquitous latent infection. The activated virus spreads to the brain, where it selectively destroys oligodendrocytes, leading to loss of the myelin sheaths.

Immunocytochemical staining of demyelinating lesions for myelin basic protein and myelin-associated glycoprotein shows different patterns of loss between postmeasles encephalomyelitis and progressive multifocal leukoencephalopathy. In progressive multifocal leukoencephalopathy, where there is a direct infection of oligodendrocytes, myelin-associated glycoprotein is lost over a wider perimeter than myelin basic protein (Itoyama et al., 1982). In postmeasles encephalomyelitis and in experimental allergic encephalomyelitis, where there is a direct attack of immune cells on myelin, concordant loss of the two myelin proteins is seen (Itoyama and Webster, 1982; Gendelman et al., 1984). In multiple sclerosis, both concordant and discordant losses of the two myelin proteins are found in acute lesions; this suggests that the mechanism of demyelination is not directly analogous to the other diseases (Gendelman et al., 1985), but may indicate that several mechanisms are at play in multiple sclerosis with some affecting oligodendrocytes and others affecting myelin directly.

Mechanisms of virus-induced demyelination

Viruses can cause demyelination by a variety of mechanisms (Table 7.2). Some of these are documented; some remain theoretical. Virus replication in oligodendrocytes can produce acute or chronic demyelinating disease, as seen in the acute lesions due to neurotropic mouse hepatitis virus or the subacute demyelination in progressive multifocal leukoencephalopathy. Theoretically,

Table 7.2

Possible mechanisms of virus-induced demyelination

Direct viral effects

Virus infection of oligodendrocytes causing demyelination through cell lysis or an alteration in cell metabolism

Myelin membrane destruction by viral proteins

Virus-induced immune-mediated reactions

Antibody or cell-mediated reactions to viral antigens on cell membranes

Sensitization of host to myelin antigens (epitope spreading)

Breakdown of myelin by infection with release into the circulation

Incorporation of myelin antigens into virus envelope

Modification of antigenicity of myelin membranes

Cross-reacting antigens between virus and myelin proteins (molecular mimicry)

Cytokine or protease demyelination (bystander effect)

Viral disruption of regulatory mechanisms of the immune system

Modified from Johnson (1985).

toxic effects of virus proteins alone might cause demyelination without immune responses or selective infection of oligodendrocytes.

Most virus-induced demyelinating diseases appear to involve immune responses, but in lentivirus, Theiler's virus, and murine hepatitis virus infections, each has a different twist in the mechanism of demyelination. In viral infections with most enveloped viruses, virus-specified polypeptides were inserted in the plasma membrane of the host cell. If the oligodendrocytes are infected, antibody or cell-mediated immune reactions against these antigens could lead to oligodendrocyte lysis and demyelination. Alternatively, virus infection of the myelin-supporting cells could release myelin antigens into the circulation or myelin antigens could be incorporated into the virus envelope, and the virus would act as a vehicle to induce autoimmunity. The latter mechanism may initiate epitope spreading and recurrent autoimmune demyelination.

Molecular mimicry, in which a cross-reaction occurs between an environmental factor (such as a virus) and a host antigen (such as a myelin protein), has been a popular hypothesis. In this scenario demyelinating disease could result from systemic or other organ system infections without involvement of the nervous system. This has not been incriminated in the diseases above, but sequence homologies between the nuclear ribonuclear neuronal protein in human nervous system and the tax protein of human T-lymphotropic virus-1 (HTLV-1) virus has been implicated in cellular damage in HTLV-1-associated myelopathy (Levin et al., 2002).

In support of this hypothesis in the pathogenesis of multiple sclerosis, a single T-cell receptor from a multiple sclerosis patient was found to recognize peptides from myelin basic protein and EBV complexed with common multiple sclerosis-associated major histocompatibility complex (MHC) class II alleles (Lang et al., 2002). Similar cross-reactivity has been observed between human herpesvirus-6 (HHV-6), influenza, and human coronavirus peptides and myelin epitopes, emphasizing the promiscuity of T cell-peptide interactions that may promote autoimmunity following infection (Tejada-Simon et al., 2003; Markovic-Plese et al., 2005; Boucher et al., 2007).

Demyelination due to soluble molecules released by inflammatory cells (cytokines and lymphokines or proteases) has been called the “innocent bystander effect.” This was originally described with the demyelination surrounding an intracerebral tuberculin reaction and related to proteases released by activated lymphocytes or macrophages (Cammer et al., 1978). In visna virus leukoencephalitis the myelin destruction appears related to infected macrophages and microglia with release of a toxic signal molecule (Kennedy et al., 1985). Many glial-derived molecules have been implicated in inflammatory demyelination and neuronal injury, including tumor necrosis factor- α , interleukin-1, interleukin-6, and nitric oxide (Gandhi et al., 2010).

Finally, virus-induced demyelination may not necessitate viral infection of the nervous system or involve molecular mimicry. In acute disseminated encephalomyelitis, infection of lymphoid cells disrupts normal immune regulation. In the state of normal health lymphocyte traffic through the central nervous system is limited and restricted largely to activated cells. In measles two factors increase the risk of autoimmune disease: first, the lack of downregulation of self-reacting cells and second, the activation of lymphocytes increasing traffic into the nervous system.

STUDIES OF PATIENTS IMPLICATING VIRUSES

Studies of patients with multiple sclerosis by serologic and virus detection methods have implicated poxviruses, herpesviruses, rhabdoviruses, orthomyxoviruses, paramyxoviruses, coronaviruses, togaviruses, flaviviruses, adenoviruses, picornaviruses, papovaviruses, retroviruses, and a variety of uncharacterized putative agents. Thus, almost the full gamut of viruses has, at one time or another, been suspected in multiple sclerosis. Occasional bacterial and parasitic agents have been implicated, but these claims have been sparse by comparison. Viruses have been incriminated by immunologic studies of patients, electron microscopic examination of brain lesions, reports of virus isolation, demonstration of viral

nucleic acids by polymerase chain reaction (PCR), or demonstration of laboratory effects on tissue cultures or animals thought to indicate the presence of a virus.

Antibody studies

In 1962, Adams and Imagawa reported that antibody titers against measles virus were higher in patients with multiple sclerosis than in controls. Measles antibodies were detected in cerebrospinal fluid of over 75% of the patients with multiple sclerosis and not in controls. These startling findings soon were confirmed in over 30 subsequent studies. These confirmatory studies also found higher levels of serum antibodies against many other viruses, but the results were less consistent and less robust than the findings of measles antibodies (Norrby, 1978; Bray et al., 1983). Studies comparing levels of antibody in spinal fluid with those in serum rather consistently showed intrathecal synthesis of antibodies against measles virus, but abnormalities in the serum/spinal fluid antibody ratios have also been found for a wider spectrum of agents (Table 7.3). In one study, 23% of patients with multiple sclerosis had disproportionately high spinal fluid antibodies to two or more viruses (Norrby et al., 1974); in another study, simultaneous intrathecal synthesis occurred to as many as 11 different agents in the same patient (Salmi et al., 1983). In discordant twin studies higher serum and spinal fluid levels of antibody are usually found in the affected twin (Kinnunen et al., 1990).

Table 7.3

Higher antiviral antibodies in multiple sclerosis than in controls

Serum	Cerebrospinal fluid
Measles	Measles
Parainfluenza 2, 3	Parainfluenza 1, 2, 3
Influenza A, C	Influenza A, B
Varicella	Varicella
Herpes simplex	Herpes simplex
Human herpes virus-6	Human herpesvirus-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
Human herpesvirus-6	Human herpesvirus-6

HTLV, human T lymphotropic virus.

These findings might suggest that multiple viruses can cause multiple sclerosis or that preprogrammed B cells are nonspecifically activated or released from immune regulation after entering the brain (Cremer et al., 1980). Studies of responses to one protein of a virus and not another and studies of antibody-variable regions suggest a more specific response (Nath and Wolinsky, 1990). It is important to note that the finding of higher serum antibody titers to measles is not disease-specific; similar findings have been reported in lupus erythematosus, Reiter's syndrome, and chronic hepatitis. Also measles infection is not a prerequisite for disease, since measles has been reported to occur after the onset of multiple sclerosis.

The studies of antibody levels have uniformly showed enhanced responses to viral antigens in multiple sclerosis patients and the intrathecal synthesis of antiviral antibodies. The preponderance of data implicate measles virus, but this finding is neither uniform nor specific to multiple sclerosis.

Electron microscopic studies

In the 1970s a number of electron microscopic studies of autopsy and biopsy tissue of patients with multiple sclerosis were reported to show "virus-like" particles. These included: (1) ovoid membrane bodies 30–200 nm in diameter, which are now thought to represent myelin breakdown products; (2) dense intracytoplasmic osmiophilic granules of 60–80 nm in diameter surrounded by membrane, which are thought to be nonspecific changes in reactive astrocytes (Andrews and Andrews, 1973; Kirk, 1979); and (3) intranuclear structures in inflammatory cells, which were initially thought to represent myxovirus nucleocapsids and are now believed to be nonspecific alterations of nuclear chromatin (Lampert and Lampert, 1975). None of these particles is specific for, or consistent in, multiple sclerosis, and none has been identified as viral in nature.

Virus isolations

Interest in specific viruses in multiple sclerosis was stimulated by the report in 1946 from the Soviet Union that viruses had been isolated in mice from 2 patients with multiple sclerosis: one from the blood of the patient and the other from cerebral tissue (Margulis et al., 1946). These viruses, however, were identified in other laboratories as rabies virus, and antibody was not found against the agent in the sera of patients (Dick et al., 1958). Nevertheless, the same laboratory later reported further isolates of similar agents (Bychkova, 1964) (Table 7.4). The isolation of a herpes simplex virus from brain tissue of a patient was reported from Iceland (Gudnadottir et al., 1964), but this isolate was subsequently characterized as

a type 2 herpes simplex virus, a virus known to reactivate intermittently in the nervous system, causing recurrent meningitis.

The report of the induction of scrapie in Icelandic sheep 16–20 months after the intracerebral inoculation of multiple sclerosis brain now appears to have resulted from the contamination of a shipping container. A further link between scrapie and multiple sclerosis was raised by the report of the diminution of mouse polymorphonuclear cells and inhibition of cell culture growth by inoculation of homogenates of tissue of scrapie and multiple sclerosis. These findings were not verified in controlled studies (Brown and Gajdusek, 1974; Carp et al., 1977).

The isolation of parainfluenza type 1 virus from tissue culture derived from 2 patients dying of multiple sclerosis was pursued extensively and unsuccessfully. The recovery of measles from a single patient was interpreted in the original report as a probable laboratory contamination, and tests for measles viral proteins and genetic material by other methods have failed to show evidence of measles in brains of multiple sclerosis patients (Stevens et al., 1980; Hall and Choppin, 1982).

Cytopathic agents have been reported from bone marrow and from cerebrospinal fluids of patients by inoculation of MRC-5 human diploid cells. In the first of these studies, the cells were found to be contaminated with *Mycoplasma* (Mitchell et al., 1979), which might explain the cytopathology. Subsequently the authors reported that there was a virus that they identified as simian virus 5. However, serologic studies showed no indication of an etiologic relationship between simian virus 5 and multiple sclerosis (Goswami et al., 1984). Another putative agent was reported in MRC-5 cells inoculated with spinal fluid from 3 patients with multiple sclerosis and one with amyotrophic lateral sclerosis (Melnick, 1982). Attempts at confirmation have been unsuccessful.

A simian cytomegalovirus was recovered from a chimpanzee inoculated neonatally with brain cells from a patient, but the clinical disease that developed in the chimpanzee suggested an acute polyneuropathy that may or may not have been related to the simian agent. The recovery of coronaviruses from mice inoculated with brains of patients dying of multiple sclerosis was of interest in view of the observations that a murine coronavirus can cause remitting and relapsing demyelinating disease in mice. Subsequent studies of these isolates, however, have shown that they share extensive nucleotide homology with murine coronaviruses, suggesting that they are of mouse rather than of human origin (Weiss, 1983). The recovery of a flavivirus, tick-borne encephalitis virus, in mice also can be assumed to be a contaminate, since hundreds of similar mouse inoculations have not yielded similar results (Vagabov et al., 1982).

Table 7.4

Viruses recovered from multiple sclerosis patients

Virus	Isolation method	Reference
Rabies virus	Encephalitis in mice inoculated with brain or blood	Margulis et al. (1946) Bychkova (1964)
Herpes simplex virus	Cytopathic changes in cell culture inoculated with homogenate of brain	Gudnadottir et al. (1964)
Scrapie agent	Scrapie developed in sheep 16–21 months after inoculation with brain	Palsson et al. (1965)
Multiple sclerosis-associated agent	Decrease in polymorphonuclear cells in mice inoculated with multiple sclerosis tissue	Carp et al. (1972)
Parainfluenza virus 1	Cell cultures of brain tissue of 2 patients fused with other cells and virus recovered	ter Meulen et al. (1972)
Measles virus	Cytopathic changes in monkey kidney cells inoculated with homogenate of brain biopsy	Field et al. (1972)
Simian virus 5	Syncytia formed in MRC5 cell cultures inoculated with patients' bone marrow cells	Mitchell et al. (1978)
Chimpanzee cytomegalovirus	Neonatal chimpanzee inoculated with brain cells of patient developed paralysis 3 years later	Wrobleiska et al. (1979)
Coronavirus	Fresh unfrozen brains inoculated into mice and grown in cultured yielded virus	Burks et al. (1980)
SMON-like virus	Cytopathic changes on MRC5 inoculated with CSF	Melnick et al. (1982)
Tick-borne encephalitis virus	Blood from 2 patients inoculated intracerebrally into mice	Vagabov et al. (1982)
HTLV-I	RNA sequences in CSF cells of 4 of 8 patients	Koprowski et al. (1985)
LM7 (retrovirus)	Found in leptomeningeal cell line from CSF	Perron et al. (1989)
Herpes simplex virus, type 1	Isolated from CSF during first attack	Bergstrom et al., 1989
Human herpesvirus-6	Viral DNA in CSF of several patients	Wilborn et al. (1994)
JC virus	PCR detection of DNA in CSF	Ferrante et al. (1998)

SMON, subacute myelo-optical neuropathy; HTLV, human T lymphotropic virus; CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

HTLV-I virus sequences were reported in cells cultured from spinal fluids of patients with multiple sclerosis (Koprowski et al., 1985), but a number of attempts to confirm these studies failed. Sequences of JC virus were reported by direct PCR on spinal fluid in 11 of 121 multiple sclerosis patients (Ferrante et al., 1998). In a more recent study, however, virus was rarely detected and, when present, viral copy numbers were low (Iacobaeus et al., 2009). PCR examination of spinal fluid for JC has become a routine procedure for diagnosis of progressive multifocal leukoencephalopathy, where it has proved highly specific, although of variable sensitivity (Ryschkewitsch et al., 2004).

The recovery of endogenous retroviruses and herpesviruses is still being actively pursued and is discussed in more detail below. There have also been extensive negative studies, including the inoculation and long-term holding of chimpanzees (Gajdusek, 1978). No single test, however, comprises a definitive negative result. It must be remembered, for example, that the neural tissue from

patients with subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy was also inoculated into primates without producing disease. As Freeman Dyson (1982), a physicist, has cautioned, "Absence of evidence is not the same thing as evidence of absence."

Agents of recent interest

Over the past two decades, since the prior *Handbook* edition (Johnson, 1985), virologic interest has shifted to the pathogenic role of endogenous or latent human agents rather than an "exogenous multiple sclerosis agent." Recent reports have focused on herpesviruses (particularly EBV and HHV-6), endogenous retroviruses, and a bacterium, *Chlamydia pneumoniae*. All represent ubiquitous agents that persist in humans and might be regarded as "normal flora." Therefore, rather than seeking a novel agent, epidemiologic, virologic, and immunocytochemical studies have focused on the timing and

prevalence of acquisition and on the quantitation and cellular localization of these infections. In each case the difficult question is whether changes are related to causation or are secondary to immunologic changes inherent in multiple sclerosis (Johnson and Major, 2004).

EPSTEIN–BARR VIRUS

Infection with this virus is almost universal, and the virus persists in latent form in B cells. In tropical and impoverished areas of the world, where multiple sclerosis prevalence is low, infection occurs in childhood and is essentially asymptomatic. In temperate zones and developed countries infection is delayed in many until adolescence or young adult years, and in these cases primary infection may be manifest by the clinical syndrome of infectious mononucleosis. In numerous cohort and case-control studies a history of infectious mononucleosis was more frequent in patients with multiple sclerosis, and a recent meta-analysis estimates that infectious mononucleosis doubles the risk of multiple sclerosis (Handel et al., 2010). In many serologic studies multiple sclerosis patients are virtually uniformly positive for antibodies to EBV before onset of neurologic symptoms, although prior infection is not a prerequisite for development of disease in the pediatric population (Matyn et al., 1993; Haahr et al., 1995; Ascherio and Munch, 2000; Levin et al., 2003; Pohl et al., 2006; Banwell et al., 2007). A prospective study showed that persons who will develop multiple sclerosis exhibit an altered immune response against EBV with a high IgG activity to Epstein–Barr nuclear antigen (EBNA-1) in the absence of high activity to virus capsid antigen (VCA), and this is pronounced in the 5 years before clinical onset of multiple sclerosis (Sundstrom et al., 2004). In many other studies, serum antibodies to both VCA and EBNA1 are elevated. Notably, both seroprevalence and titers of antibodies to EBNA1 and VCA are increased in pediatric multiple sclerosis cases, indicating that infection precedes multiple sclerosis even in cases of early-onset multiple sclerosis (Pohl et al., 2006; Banwell et al., 2007). A recent meta-analysis that included numerous case-control studies of antibodies to EBV antigens found odds ratios of over 12 for EBNA-1 and over 5 for VCA seropositivity in multiple sclerosis patients compared to controls (Santiago et al., 2010).

Although serologic studies have consistently demonstrated evidence of past infection, there is little evidence for active infection or reactivation of EBV in patients with multiple sclerosis. Neither the presence nor quantity of viral DNA in plasma, peripheral blood-derived mononuclear cells, or whole blood has been consistently increased in individuals with multiple sclerosis (Santiago et al., 2010; Lucas et al., 2011). Although some

longitudinal studies have found an association between virus activation and disease activity in multiple sclerosis patients, others have not confirmed this, finding instead an association between titers of EBNA-1 and clinical and radiologic markers of disease activity (Wandinger et al., 2000; Torkildsen et al., 2008; Farrell et al., 2009).

In many nervous system infections antibodies are generated intrathecally, giving rise to oligoclonal bands of antibody in spinal fluid; these bands are typically present in multiple sclerosis, but the target antigen remains a mystery. In some multiple sclerosis patients these bands have been reported to bind to EBNA (Rand et al., 2000), while more recent studies suggest that, when present, oligoclonal EBV-specific responses are often systemic in origin and are found in a wide variety of neurologic disorders (Franciotta et al., 2010).

Several provocative reports have described patients with neurologic complication of acute EBV infections who went on to develop progressive or relapsing disease, diagnosed as multiple sclerosis (Shaw and Alvard, 1987; Bray et al., 1992). Another describes a 6-year-old with 11 episodes of relapsing disease and high titers of Epstein–Barr antibodies. At autopsy typical multiple sclerosis lesions were found, and PCR of brain showed EBV sequences (Pedneault et al., 1992). Laser capture microdissection of meninges and white-matter lesions from multiple sclerosis brains followed by reverse transcription PCR has demonstrated the presence of EBV latency-associated genes in inflamed tissue (Serafini et al., 2010). In addition, EBV-encoded RNAs (EBERs), the small noncoding RNAs which are abundantly expressed in lately infected cells, have been found in multiple sclerosis lesions via *in situ* hybridization, although they are also present in cases of stroke (Tzartos et al., 2012). These findings in brain or spinal fluid are difficult to interpret, since EBV is latent in B cells. B cells are rare in normal brain or spinal fluid but do enter in inflammatory diseases (and are present in the lumen of vessels in brain sections). Demonstration of the virus in neural cells would provide stronger evidence for a causal relationship between this virus and multiple sclerosis.

HUMAN HERPESVIRUS-6

This herpesvirus was recently recognized and associated with exanthem subitum (roseola infantum). Between 70 and 100% of adults have serologic evidence of past infection. The virus is latent primarily in T lymphocytes, but is more pleiotropic than other herpesviruses and has been reported in B cells and central nervous system glial cells in people without neurological disease (Soldan et al., 2001). Human herpesvirus-6 isolates have been subdivided into type A and type B. Type B variants have

been the predominant isolates from childhood exanthems, and the isolates in multiple sclerosis studies have been predominantly type A variants.

Initial observations of higher antibody levels in serum and presence of antibodies in spinal fluid were similar to other viruses. However, DNA was detected in spinal fluid by PCR (Wilborn et al., 1994), and the following year herpesvirus-6 DNA was reported in the majority of multiple sclerosis and control brains (Challoner et al., 1995). However, immunocytochemical staining showed a change of distribution: in normal brains virus antigen was found primarily in meningeal cells, but in multiple sclerosis lesions, adjacent cells, thought to represent neurons and oligodendrocytes, were positive. Subsequent studies have shown remarkable variance; some have correlated virus in blood or spinal fluid with disease activity (Alvarez-Lafuente et al., 2004), whereas others have not. Different IgG and IgM responses have been reported, but other reports have failed to confirm this finding (Virtanen and Jacobson, 2012). The frequency of detectable virus in brain and the correlation with disease have been extreme, from reports of lack of detectable virus in diseased or normal brain tissue, to a claim that 90% of active multiple sclerosis lesions were positive for viral antigen while positive staining was found in only 13% of brain sections without active disease (Knox et al., 2000). Utilizing laser capture microdissection followed by PCR, Cermelli and colleagues (2003) found that herpesvirus-6 DNA was detected far more frequently in multiple sclerosis plaques as compared to normal-appearing white matter from multiple sclerosis patients or from controls. Using *in situ* PCR, Goodman and colleagues (2003) showed herpesvirus-6 genome in numerous oligodendrocytes as well as lymphocytes and microglia in acute multiple sclerosis lesions. Also with *in situ* PCR, Opsahl and Kennedy (2005) have shown herpesvirus-6 gene transcription in oligodendrocytes in multiple sclerosis patients' normal-appearing white matter, lesional tissue, and normal control brain sections. Viral mRNA was higher in white matter and lesions of patients, and viral gene expression was higher in samples from lesions.

The presence of this and other herpesvirus in nervous system cells seems well established. Activation of herpes simplex viruses, varicella-zoster, and human herpesviruses-7 and 8 have also been correlated with disease activity (exacerbations) in multiple sclerosis (Sanders et al., 1996; Tomson et al., 2001; Ordonez et al., 2004; Pietropaolo et al., 2005; Kang et al., 2011). Therefore, the activation of human herpesviruses lacks specificity. Activation or spread coincident with attacks of multiple sclerosis may represent a trigger or cause of the attack or may represent a secondary, nonspecific activation of latent herpesviruses.

ENDOGENOUS RETROVIRUSES

Human endogenous retroviruses (HERVs) are DNA sequences within human chromosomes; they comprise up to 8% of the human genome. The characteristic presence of long terminal repeats followed by gag, pol, and env genes identifies their retroviral origins. These sequences 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). No endogenous retrovirus has been convincingly linked to human disease, but the potential to enhance downstream cellular genes has led to speculation that they might be important in autoimmune diseases such as multiple sclerosis, Sjögren's disease, systemic lupus erythematosus, and type I diabetes (Perron and Seigneurin, 1999).

In 1989 Perron and colleagues reported evidence of a retrovirus in a cell line of meningeal cells grown from spinal fluid of a patient with multiple sclerosis. Subsequently his group analyzed monocytes from patients and controls and reported evidence of retroviral activity or retrovirus particles in the majority (Perron et al., 1991). Recognition that the viral elements were endogenous, as opposed to an exogenous retrovirus, resulted in coinage of the name "multiple sclerosis-associated retroviral element" (MSRV). Several other laboratories have found similar HERVs in blood and spinal fluid of multiple sclerosis patients, and the presence of MSRV in the cerebrospinal fluid of patients with multiple sclerosis has been associated with a greater rate of disease progression (Sotgiu et al., 2010). Although similar activity has also been found in other inflammatory neurologic diseases and schizophrenia, the frequency of HERV detection has generally been more frequent in multiple sclerosis (Dolei et al., 2002; Dolei, 2005). Of the over 30 HERV families named according to the one-letter code of the transfer RNA used to prime reverse transcription, HERV-W, HERV-K, and HERV-H have been most frequently associated with multiple sclerosis (Antony et al., 2011). Much recent attention has focused upon HERV-W, of which MSRV is a member. In addition, the protein syncytin-1, a glial protein that induces neuroinflammation and is expressed more frequently in multiple sclerosis than control brains, is derived from an envelope gene of HERV-W (Antony et al., 2004). Retroviral protein and virion production has been postulated to result from interactions with other viruses, including herpesviruses (Christensen, 2005). Specificity was further questioned in studies that showed HERV RNA levels increased with stimulation of macrophages in culture, and in brain in patients with HIV encephalopathy as well as in multiple sclerosis (Johnston et al., 2001). This study suggests that

immune activity enhances viral expression rather than being a cause of the immune activation.

CHLAMYDIA PNEUMONIAE

C. pneumoniae is an obligate intracellular Gram-negative bacterium. It is a common respiratory pathogen; 40–70% of adults have antibody, with most seroconversion occurring during adolescence. The bacteria persist in macrophages, and the attempts to relate the bacterium to chronic diseases such as atherosclerosis and multiple sclerosis are confounded by the possibility that the agent is simply carried into inflammatory lesions by infiltrating macrophages.

After the recovery of *C. pneumoniae* from the spinal fluid of a patient with multiple sclerosis, [Sriram and coworkers \(1999\)](#) carried out an extensive study using culture and PCR methods. They reported positive cultures in 64% of multiple sclerosis patients and only 11% of control patients, and positive PCR results in 97% of multiple sclerosis patients and only 18% of patients with other neurologic diseases. They subsequently reported that oligoclonal bands in spinal fluid could be partially or completely adsorbed by *C. pneumoniae* antigens ([Yao et al., 2001](#)). These reports have been followed by a plethora of reports partially confirming or refuting the results, but none with the robust results of the initial report. In a subsequent report, for example, PCR detected *C. pneumoniae* in 21% of multiple sclerosis patients, 43% of patients with other neuroinflammatory diseases, and none in healthy controls ([Gieffers et al., 2001](#)). A prospective serologic study of several large US populations showed that neither seropositivity to *C. pneumoniae* nor level of antibody predicted risk of developing multiple sclerosis ([Munger et al., 2004](#)). However, a meta-analysis examining over 70 studies found that multiple sclerosis patients were more likely to have detectable levels of *Chlamydia* DNA in their cerebrospinal fluid and intrathecally synthesized immunoglobulins compared to other patients with neurologic diseases ([Bagos et al., 2006](#)). A recent small study found that *C. pneumoniae*-specific intrathecal immunoglobulins were found more frequently in patients with progressive, as compared to remitting-relapsing, forms of multiple sclerosis ([Fainardi et al., 2009](#)). Overall, these reports support the hypothesis that the bacteria are passively carried into the nervous system when host macrophages traffic into inflammatory responses.

Infections in the context of host genetics

It has long been recognized that multiple sclerosis may arise as a result of interactions between environmental triggers and host genetic factors, the combination of which may influence disease susceptibility or expression.

Much interest has focused on interactions between the strongest identified genetic risk factor for multiple sclerosis, HLA-DRB1*15, and viral exposure. The presence of high EBNA1 antibody titers was associated with a higher risk for multiple sclerosis among HLA-DRB1*15-positive individuals as compared with DRB1*15-negative individuals ([Sundstrom et al., 2008](#)), although several subsequent studies have not confirmed a direct interaction ([De Jager et al., 2008](#); [Waubant et al., 2011](#)). Another major histocompatibility gene locus, HLA B7, was associated with increased anti-EBV VCA levels and worse radiologic markers of disease ([Zivadinov et al., 2009](#)). These studies support the idea that HLA genes may influence the risk of multiple sclerosis in part through the immune control of EBV infection. Notably, remote infection with herpes simplex virus 1 was associated with a decreased risk of multiple sclerosis in DRB1*15-positive children, while the reverse was observed in those who were DRB1*15-negative ([Waubant et al., 2011](#)). Active replication of human herpesvirus-6 in the serum of patients with multiple sclerosis was associated with a polymorphism of the MHC2TA gene, which encodes a transcription factor involved in expression of MHC class II genes, including HLA DRB1*15 ([Martinez et al., 2007](#)).

The interplay between multiple infections is likely to contribute to multiple sclerosis risk and disease activity. Although prior infection with herpes simplex virus 1 was not an independent risk factor in a pediatric multiple sclerosis cohort, children seropositive for both herpes simplex virus and EBV were more likely to have multiple sclerosis as compared with children seropositive only for EBV ([Banwell et al., 2007](#)). Recent recognition of the vast numbers of commensal organisms that reside within an individual, termed the human microbiome, suggests that the manner in which microbes interact with each other and with the human genome to influence risk of multiple sclerosis and other autoimmune diseases is likely to be extremely complex ([Ochoa-Reparaz et al., 2011](#)).

CONCLUSIONS

The role of infections in multiple sclerosis remains a riddle. Infectious agents, particularly viruses, are the conspicuous candidates for the environmental factors in multiple sclerosis, but conclusive data are missing. Miscellaneous “virus-like” illnesses often precipitate attacks, but this may have little relevance to the more important issue of causation. Viruses can during a transient infection initiate an autoimmune disease, as seen with epitope spreading in animal experiments. Viral infection of nonneural tissues can set up demyelination by molecular mimicry or immune deregulation. Viruses

that activate coincident with exacerbations of disease may be important or nonspecific. Virus, though causal, may not be recoverable at the time of disease, as in acute disseminated encephalomyelitis. Studies have not provided the desired answers but have defined the complexity of the questions. Finally, it may be that the childhood exposure is not to an infectious agent at all, but to some other factor that leads to both demyelinating disease and abnormal production of viral antibodies.

The delineation of different pathologic changes in acute multiple sclerosis lesions (Lucchinetti et al., 2000) raises the question of multiple mechanisms of pathogenesis in multiple sclerosis and consequently multiple causes. Historically a syndrome (signs and symptoms “that run together”) was assumed to have a common cause, but advances in both genetics and infectious diseases repeatedly provide contrary evidence. In genetics one phenotype often proves to have many genotypes, and single genotypes can be expressed as different phenotypes. In virology the neurologic syndrome of “aseptic meningitis” has proved to be caused by over 100 different infectious agents, and conversely single agents such as HIV, varicella-zoster virus and *Treponema pallidum* can cause a spectrum of clinically and pathologically distinct diseases. Golden (2005) and Lipton et al. (2007) in recent reviews support the idea of a “single multiple sclerosis virus”; but from a different vantage point one could consider some cases of tropical spastic paraplegia and Lyme encephalopathy with white-matter lesions dispersed in time and space. Before the discovery of HTLV-1 virus or *Borrelia burgdorferi* spirochete, these patients’ diseases fulfilled Schumacher’s criteria for multiple sclerosis. With present knowledge do we now regard these as disorders where, in our ignorance, we erred in diagnosis, or should we consider these as a small portion of that multiple sclerosis syndrome for which we now know the cause?

Finally, whether as a fundamental cause, as a trigger of attacks, or simply as an activation marker of activity, viruses appear related to multiple sclerosis. To maintain perspective we must recognize that the great majority of microbial agents in the world still defy current cultivation methods, but new technologies are being developed to identify them at a rapid pace (Relman, 2011). Within that panoply of potential human pathogens may lie the agent or agents responsible for multiple sclerosis.

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