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Viruses and Demyelinating Disease of the Central Nervous System

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The relation of viruses to multiple sclerosis (MS) has been a frequently discussed subject;[60] however, there is no *firm* evidence to implicate a viral infection in the pathogenesis of MS. MS remains an idiopathic disease.

There are three main issues that must be addressed in a discussion of viral infections and MS: the mechanisms by which viruses can produce demyelination, experimental demyelinating infections of animals, and the relationship of viral infections to human demyelinating diseases.

MECHANISMS OF DEMYELINATION

There are a number of ways in which a virus can lead to demyelination.

Oligodendrocyte Lytic Viral Infection. Clearly, viruses can directly infect oligodendrocytes and thereby destroy myelin. The oligodendrocyte lysis can be a selective infection of these cells, as demonstrated by JC virus infection in progressive multifocal leukoencephalopathy (PML), or can be part of a generalized encephalomyelitis, as can be seen following inoculation of mice with JHM strain of mouse hepatitis virus.

Immune-Mediated Demyelination with Oligodendrocyte Viral Infection. The immune response may contribute to demyelination when oligodendroglia are virally infected by means of several mechanisms. One such mechanism is sensitization of host to damaged oligodendrocyte or myelin constituents because of antigens released into the systemic circulation from infected white matter. Another mechanism is immune system attack against neoantigenic targets inserted into oligodendrocytes or myelin, as might occur during viral budding. Changed cellular antigens, as a result, for example, of metabolic changes from infection of budding or nonbudding viruses, could presumably also trigger a deleterious immune response. Anti-idiotypic antibody directed against oligodendrocytes and myelin is another mechanism of demyelination. This immune attack is not related

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to anti-viral antibody (which we will call idiotypic antibody) damage to oligodendrocytes or myelin as described previously but involves destruction of oligodendrocytes or myelin by anti-idiotypic antibody, which is directed against sites associated with the antigen binding site of the idiotypic antibody.[68] The possibility of immunopathologic disease caused by anti-idiotypic antibody was recently suggested by Nepom et al. during their analysis of reovirus infection.[68] In this particular example, idiotypic antibody was directed against reovirus type 3 hemagglutinin, which binds to a receptor on neuronal cells. Nepom et al. found that anti-idiotypic antibody reacted with the neuronal cell viral receptor as well as with idiotypic antibody. The reaction of anti-idiotypic antibody with the receptor of the antigenic target of idiotypic antibody has been described previously in other systems.[70] It is important to realize that anti-idiotypic antibodies are not experimental artifacts but a natural immunoregulatory mechanism.[32, 82] Whether anti-idiotypic antibodies naturally cause autoimmune diseases, however, is less clear. Presumably, a virus could infect oligodendrocytes and lead to the production of idiotypic antibodies and the secondary production of anti-idiotypic antibodies, which could then directly damage the oligodendrocyte.

Immune-Mediated Demyelination without Oligodendrocyte Infection. The immune system may also play a role in viral demyelination even though the oligodendrocyte is not infected by the virus. If there are cross-reacting antigens between the virus and the oligodendrocyte or myelin, an immune reaction directed against viral antigens may produce autoimmune demyelination. Precedence for pathogenic polyclonal cross-reacting antibodies involving a nonviral infectious agent and central nervous system (CNS) tissue comes from Husby et al.[31] who described antibody against hemolytic group A streptococci in patients with Sydenham's chorea, which cross-reacts with neurons of the caudate and subthalamic nucleus. Recent molecular virologic studies have demonstrated homology between cellular genes and genes from a number of viruses, including retroviruses[11a] and herpesviruses.[72a] Work with monoclonal antibodies has demonstrated a plethora of cross-reactions between neural tissue and non-neural antigens.[20] These commonplace monoclonal antibody cross-reactions, however, may not be relevant to naturally occurring polyclonal antibodies in vivo. Immune cells, even though they are not specifically directed against oligodendrocytes or myelin, may still produce demyelination by means of the "bystander effect." For example, animals inoculated intracerebrally with tuberculin after prior sensitization against tuberculin exhibit demyelination in association with the immune response against tuberculin.[104] Presumably proteases and other factors from activated lymphocytes or macrophages can nonspecifically damage myelin.

In many of the mechanisms of demyelination that have been proposed, the macrophage plays a key role. It is, in all likelihood, not coincidental that many viruses that cause persistent infection, including ones associated with demyelination, can infect macrophages. The infected macrophage may be important in avoiding immune clearance, delivering viruses to lesions and producing demyelination by means of a bystander effect. As in visna (see later in this article), the macrophage may also have a critical role in

determining the expression of the virus (that is, affecting its infectivity) and in producing immunopathology.[65, 100] Under certain conditions, for example, viruses may spread from a semipermissive macrophage to a more permissive oligodendroglial cell. The subsequent presentation of antigen to the immune system may trigger an immune response that contributes to viral lysis of the oligodendroglial cell.

It is clear that virus-induced demyelination can be complex and undoubtedly involve multiple mechanisms, perhaps changing with time and different host genotypes. For example, DA viral demyelination may involve both an oligodendrocyte lytic infection at one time and an important pathogenic immune response at another. Thus although viral infections are capable of producing demyelination purely by an oligodendrocyte viral lytic process, the immune system may contribute to the demyelination as well.

EXPERIMENTAL MODELS OF VIRAL DEMYELINATION

Canine Distemper Virus

Canine distemper virus (CDV) is a member of the morbillivirus subgroup of paramyxoviruses, as is measles virus (Table 1). CDV, like measles virus, has an immunosuppressive effect on the host and causes a variety of neurologic syndromes. Our understanding of CDV disease has been limited because gnotobiotic (germ-free) dogs must be used owing to the infectiousness of the virus; only a small amount of work has been performed with mouse-adapted CDV.[7, 23, 55]

At least two basic neurologic diseases of dogs are caused by CDV; the outcome of infection depends on the host age, immune status, and the viral strain employed. In one syndrome, an early acute infection is characterized by respiratory and gastrointestinal symptoms and a significant immunosuppression of the host, presumably due to lymphoid cell involvement.[56] Gray-matter lesions, as well as white-matter lesions, are seen that at least initially are associated with the presence of virus, but not with immune cells or viral antibody.[75] In another CDV syndrome, a subacute demyelinating encephalitis follows weeks or months after a mild early infection. A form of demyelination affecting old dogs, usually without a clinically apparent antecedent CDV infection, may be a variant of the subacute demyelinating encephalitis.[1] These subacute and chronic diseases have pathologic evidence of demyelination, remyelination, and inflammatory cells. The white-matter lesions are associated with virus as well as immune cell infiltrates, making the pathogenesis of the demyelination unclear.[30, 59, 96, 97] Isolates from CDV chronic demyelinated dogs have evidence of viral mutations compared with the original wild-type virus;[83a] it is unclear whether these mutations are a result of the in-vivo persistence of virus or whether they cause it. In summary, early lesions are considered secondary to direct viral lysis, whereas subacute late demyelination may have a contribution from the immune system.

Serologic and epidemiologic studies have suggested a possible relationship of CDV to the etiology of MS; however, there remains no convincing

Table 1. *Experimental Animal Models of Virus-Induced CNS Demyelination*

VIRUS	VIRUS GROUP	NATURAL HOST	EXPERIMENTAL HOST	EFFECT OF IMMUNOSUPPRESSION ON DEMYELINATION	OLIGODENDROCYTE INFECTION
Canine distemper	Paramyxovirus	Dog	Dog; mouse	?	+
JHM strain of mouse hepatitis virus type 4	Coronavirus	Mouse	Mouse; rat	No change in demyelination	+
Visna	Retrovirus	Sheep	Sheep	?	?
Theiler's	Picornavirus	Mouse	Mouse	Variable, depending on timing of immunosuppression; demyelinates Nude mice	+

evidence to prove this hypothesis. It is clear that the increased cerebrospinal fluid (CSF) antibody levels to measles virus found in patients with MS—which is part of an increased B cell activity to a variety of viruses (see article by Oger et al. in this issue)—could have confused CDV serologic studies because of the extensive cross-reactions of antibodies against these two viruses.[2, 27, 85] Some epidemiologic studies have reported an association between MS and dog-ownership;[6, 43] however, this data has been questioned by others.[39, 42, 66]

JHM Strain of Mouse Hepatitis Virus

The JHM strain of mouse hepatitis virus type 4 (see Table 1), a member of the coronavirus group of RNA-enveloped viruses, produces acute and chronic demyelinating lesions. Since its original isolation in 1947 from paralyzed mice, a number of investigators have described demyelinating encephalomyelitis following intracerebral inoculation of the virus in mice.[10, 98] Weiner produced focal demyelinated lesions in the white matter following intracerebral inoculation of four-week old Swiss mice.[101] Other inoculation schemes tended to produce no disease or to cause a more widespread fatal encephalomyelitis with only rare areas of demyelination. Later investigations with different inoculation schemes and mouse strains demonstrated varying disease courses. Balb C and C57/Bl mice surviving JHM virus inoculation develop chronic recurrent demyelination for at least 16 months.[29, 88] A temperature-sensitive (ts) mutant of JHM, ts G, causes acute, nonfatal as well as chronic demyelination in several different strains of mice[28, 37] Nagashima et al. produced demyelination in 35 per cent of rats inoculated with JHM virus after an average incubation period of 21 days.[62]

The variety of clinical pathologic syndromes that can be produced emphasize the importance of the host strain and age and the route, concentration, and strain of the virus inoculum. The genetic susceptibility of mice to JHM lethal encephalomyelitis has been attributed to an autosomal dominant gene(s)[36] or to both a dominant and a recessive gene.[86] Pickel et al. explored the age-dependent resistance of mice to JHM virus, specifically to intraperitoneal inoculation.[74] Immunologic factors altered resistance but did not completely account for the development of resistance with maturity. Stohlman et al.[87] claimed that a non-T, non-B, Ia-negative adherent cell population was responsible for age-dependent resistance of SJL mice to intracerebral inoculation with JHM virus. The part of the JHM virus genome that determines the virus' tropism and virulence is also under study. Analyses have been performed comparing the RNA from a hepatotropic strain of mouse hepatitis virus with RNA from a nonpathogenic strain by means of T_1-oligonucleotide fingerprinting analysis;[44] studies comparing demyelinating strains with nondemyelinating strains are presumably in progress.

Most studies with JHM virus have implicated a direct oligodendrocyte viral lytic infection, usually on the background of a generalized encephalomyelitis, as the cause of demyelination; that is, demyelination is not immune-mediated. For example, Weiner,[101] in his original experiments with inoculation of Swiss mice with JHM virus, demonstrated infectious virus in the brains and localized viral antigen in cells of acutely demyelinated

lesions. There was no temporal or anatomic association between inflammatory cells and demyelination. Immunosuppression with cyclophosphamide did not prevent demyelination. Lampert et al.[45] identified JHM virions in oligodendrocytes, as well as in other neural cells.

There are suggestions in the literature that the JHM infection may not always be productive. Stolhman and Weiner found evidence of viral antigen in the CNS 90 days after inoculation of JHM virus into C57/Bl mice, although no infectious virus was detected after day 14.[88] A JHM virus carrier culture with viral antigen, but without infectious virus, can be produced in vitro in the presence of antiviral antibody, suggesting that the immune response may be important in establishing a persistent defective virus infection.[88] The latter hypothesis gathers support from a report by Koga et al. concerning conversion of an acute demyelinating encephalomyelitis of JHM-virus infected suckling rats to a subacute disease if foster mothers are immunized with JHM virus.[38] Dubois-Dalcq et al. noted that ts 8 produces low levels of virus in dissociated mouse spinal cord cultures with the appearance of unusual viral inclusions, suggesting an abnormality in virus assembly;[19] in-vivo studies, however, have shown that ts 8 can be isolated in animals as long as one year following inoculation.[37]

The mechanism of JHM virus demyelination presented in the preceding paragraphs appeared almost too straightforward when compared with the confusions surrounding other models of demyelination. Not for long! A recent abstract describes a late-onset subacute demyelinating encephalomyelitis in JHM-virus-inoculated rats with lesions that resemble experimental allergic encephalomyelitis (EAE) in appearance.[99] Lymphocytes from infected rats have elevated stimulation indexes to myelin basic protein (MBP) as well as to JHM virus. Adoptive transfer of lymphocytes following stimulation in vitro with MBP produces an EAE-like disease in recipients. The study suggests that immune mechanisms are important in JHM demyelination. We await confirmation and continuation of these studies.

Visna Virus

Visna virus (see Table 1) is a member of the retrovirus group of RNA-containing viruses; members of the group possess reverse transcriptase, the enzyme that converts viral RNA to a DNA provirus intermediate that integrates into host DNA. Although viruses in the group generally have oncogenic properties, visna virus, and other agents grouped in the lentivirus sub-group, are not oncogenic.

Visna, a disease first recognized in Icelandic sheep in the 1930s, has been maintained in the laboratory since eradication of the natural disease in the 1950s.[57, 73] The experimental disease is a prototype of a slow virus infection because of its months-to-years-long incubation period and duration. Infected sheep exhibit a progressive or episodic course characterized by paralysis and CSF pleocytosis. In the first months after infection, inflammatory infiltrates are present in the periventricular areas—primarily in the white matter—and the choroid plexus. The dense infiltrates eventually become part of necrotic lesions of the white matter, suggesting the possibility of immunopathologic disease.[22] At later times, primary demyelinating lesions associated with macrophages develop.

There are several mechanisms by which visna virus can persist and avoid normal immune surveillance. (1) Since visna virus is a retrovirus, it can exist as a provirus DNA intermediate. In-situ hybridization of visna lesions demonstrated visna-virus DNA in cell nuclei, in the face of small amounts of cytoplasmic viral RNA and of fluorescent viral antigen.[4, 26] These studies suggested that visna-virus DNA provirus is present in cells in vivo but that there is a block in transcription as well as translation of viral RNA into structural proteins. (2) During disease progression in sheep, visna virus undergoes continuous mutation that enables it to avoid neutralization by each antibody formed against the new mutant; that is, the virus has continuous antigenic shifts by which it avoids neutralization by antibody.[63, 64] Similarly, visna virus cultured in vitro in the presence of viral antibody will persist and produce mutants. Visna-virus mutants that are generated in vivo and in vitro have mutations in the region of the genome thought to code for the viral envelope glycoprotein, which is at least one of the antigenic targets of neutralizing antibody.[11] (3) Narayan et al. provided insight into the visna-virus-macrophage-fibroblast relationship vis-à-vis the host immune response.[65] Although virus replicates to high titer in sheep choroid plexus cells in vitro, tissues from infected sheep do not display an exponential rise in virus titers and have only small amounts of cell-free virus throughout the disease. When tissues of infected sheep are explanted or cocultivated in vitro, however, cytopathic effect quickly develops with a rapid increase in virus titer; these changes may result from macrophages fusing with cells of other tissues (see discussion that follows). Narayan et al. studied the specific behavior of a field isolate of visna virus rather than the laboratory strain, realizing that the latter strain may have different, less biologically relevant properties.[65] Visna virus field isolates produce a slow, noncytopathic persistent infection in macrophages in vitro; virus particles are predominantly found in cytoplasmic vacuoles. The field isolates replicate poorly if at all in fibroblast cultures (sheep choroid plexus cells). Addition of macrophages to these cultures, however, causes a rapid fusion of cells and the production of virus, suggesting that macrophages are necessary for the productive infection of fibroblasts and for the accompanying expression of viral antigens on the cell surface. The implications of Narayan's study are as follows: (1) the presence of a virus in macrophages, predominantly in cytoplasmic vacuoles, may allow the virus to escape immune surveillance; and (2) the fusion of a macrophage with a fibroblast cell leads to viral antigen expression and viral growth that can result in immune-system recognition and immunopathology. One must not forget, however, that these studies of Narayan's group[63–65] involved visna in non-Icelandic sheep and that there may be differences in the disease in Icelandic animals.

What role does the immune system play in producing visna lesions? Immunosuppressive treatment with antithymocyte serum and cyclophosphamide causes a marked decrease in the early histopathologic necrotic lesions of infected sheep.[67] Limitations in the length one can carry out immunosuppression and the inability to perform cell-transfer studies in sheep make it impossible to analyze further the possible immunopathologic nature of late demyelinating lesions of visna by traditional means.

In summary, visna probably involves a persistent viral infection with little antigen expression (because of the presence of provirus and macrophage infection) or changing antigen expression (because of frequent viral mutations); however, epsiodes of productive viral infection may lead to immunopathologic lesions early in the disease. The role of immune-mediated pathology in late demyelination is not well-defined.

Theiler's Murine Encephalomyelitis Virus

Theiler's murine encephalomyelitis virus (TMEV, see Table 1) was originally isolated in 1934 from a paralyzed mouse by George Martin, a technician of Max Theiler.[90] This particular strain of TMEV became known as GDVII, signifying George's seventh disease isolate. TMEV was recognized as a common enteric picornavirus endemic in many mouse colonies.[91, 92] The virus' neurotropic properties prompted its designation as "mouse poliovirus." In 1952, Daniels et al. described an experimental demyelinating murine encephalomyelitis caused by the DA strain of TMEV.[18] The interest in this virus was reawakened by Lipton in 1975.[48]

We now know that there are two serologically related subgroups of TMEV strains that differ with respect to their biologic properties.[49] One group, typified by the GDVII strain, is highly virulent, encephalitogenic, and causes an acute lethal encephalomyelitis without virus persistence in weanling mice. The second group, typified by the DA strain, is less virulent and causes a demyelinating encephalomyelitis with virus persistence in weanling mice. Intracerebral inoculation of weanling mice with DA strain causes primary demyelination of the spinal cord that begins two to three weeks after inoculation and progresses for the life span of the mouse (Fig. 1).[13] Under certain conditions, a relapsing course can be seen with evidence of remyelination produced by Schwann cells that invade the spinal cord.[14] If the inoculum consists of a DA virus stock prepared in infected suckling mouse brain rather than in tissue-culture cells, a gray matter neuronal infection (an acute polioencephalomyelitis) antedates the white-matter disease; that is, mouse-brain-passed DA causes a biphasic disease with neuronal infection followed by demyelination, while tissue-culture-passed DA only produces demyelination.[46, 52] The reason for this difference in biologic behavior may be related to in-vivo pressures favoring the emergence of certain virus variants in the infected mouse brain.[81]

Evidence indicates that the expression of virus in chronically infected animals is not a complete and productive one. Picornaviruses are generally considered to be acutely lytic viruses, replicating to high titer. In animals chronically infected with DA, however, the virus persists and is of low titer.[46] "In-situ hybridization" studies demonstrated a small amount of viral RNA in glia from chronically demyelinated mice compared with abundant viral RNA in infected neurons earlier in the disease, suggesting a less productive infection in glial cells.[5] One wonders whether the virus that persists in the CNS mutates to a less productive form either under the pressures of a brisk immune response or because the glial cell is a less permissive cell. Biochemical studies of CNS isolates from chronically demyelinated animals showed some evidence of mutation, but generally there was a close similarity between the isolates and the inoculated wild-

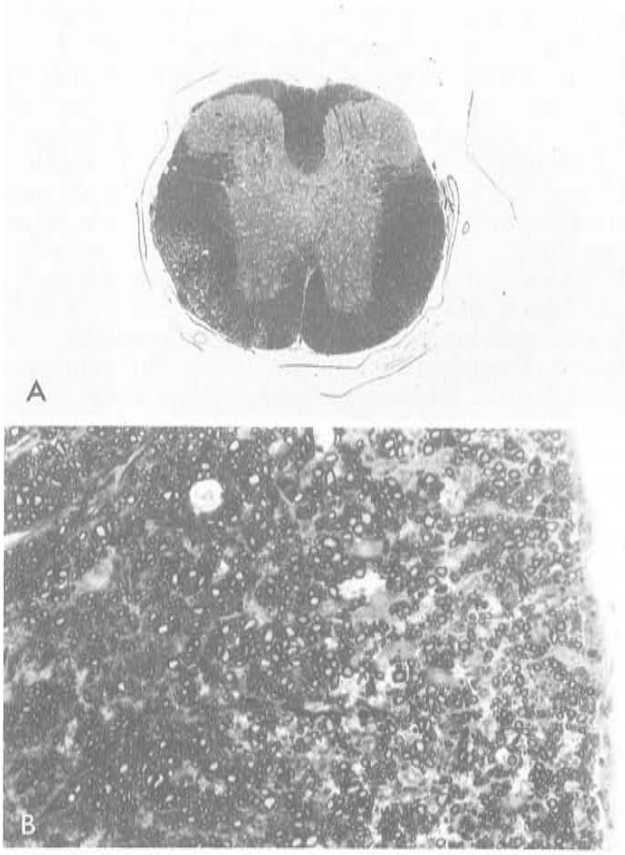

Figure 1. *A*, Demyelinated area in spinal cord from SJL/J mouse inoculated with DA virus. (Toluidine blue, × 60.) *B*, Demyelinated focus from spinal cord of SJL/J mouse inoculated with DA virus. Naked axons are seen, with preserved myelin sheaths scattered throughout the demyelinated areas. (Toluidine blue, × 640.)

type virus.[80] Of course, it is possible that a small population of productive virus amid a large population of viral mutants was inadvertantly selected for and enriched for during isolation procedures. Infection of CNS organotypic cultures and biochemical studies of viruses obtained directly from the CNS (without tissue-culture passage)[81] may elucidate this issue.

The mechanism of demyelination in DA-infected animals has been a subject of much interest. Lipton and Dal Canto[50, 51] suggested that immune factors mediate demyelination either directly or as a bystander effect for the following four reasons. (1) During demyelination, levels of virus are extremely low and virus antigen is rarely present in glias while the immune response is very brisk.[12, 15] (2) Demyelination is temporally and spatially associated with mononuclear cell infiltrates. (3) By electron microscopy, mononuclear cells are seen to strip the myelin sheaths as described in EAE.[15] (4) Immunosuppression with antithymocyte serum or cyclophosphamide prevents the appearance of demyelination.[51]

On the other hand, there are four observations that stress the importance of oligodendrocyte lytic infection in the pathogenesis of demyelination. (1) Our own studies showed that immunosuppression decreases early demyelination but does not entirely prevent it and has no effect on continuing, chronic demyelination.[79] (2) Rodriquez et al. demonstrated viral antigen in oligodendrocytes of chronically demyelinated animals.[78] (3) Brahic et al. found viral TMEV RNA in glial cells of chronically demyelinated mice by in-situ hybridization.[5] (4) Recently, we observed unequivocal ongoing demyelination in Nude mice inoculated two months previously with DA;[81a] the demyelinated lesions were not generally associated with macrophages. The Nude mouse studies indicate that a lytic infection of oligodendrocytes, without an immune-system contribution, is sufficient to produce chronic demyelination with DA virus. Our immunosuppression studies suggest that the immune system also plays a role in DA demyelination in non-Nude mice. It is unclear whether the immune system contribution is by means of a bystander effect or a more direct attack.

In many ways TMEV demyelinating disease is an excellent model of MS. As in MS, both viral and immune factors have been implicated as causes of demyelination. The reproducibility of the DA experimental disease and the ease and advantages of working in mice make this a very attractive system.

Other Demyelinating Diseases

Other viruses that produce demyelination, frequently as part of a more generalized encephalitic process include the following: ts mutants of vesicular stomatitis virus, ts mutants of Chandipura virus;[17] Venezuelan equine encephalomyelitis virus, an avirulent strain (A774) of Semliki Forest virus;[31a, 35] and strain T-48 of Ross River virus.[83] In all of these cases, inoculation of Nude mice and/or immunosuppressive treatment have demonstrated a reduction in the extent of demyelination, suggesting that the immune system plays a role in the myelin destruction.

A demyelinating encephalomyelitis has also been associated with herpes simplex virus (HSV) type 1 infection. The demyelinating potential of this virus is of special interest because it is a common virus of humans and because the ability of the virus to recur from a latent state illustrates how a virus can produce attacks and remissions. The infection involves a primary demyelination of the entry zone of the trigeminal nerve root at the junction of central and peripheral myelin following intracorneal inoculation of mice and rabbits.[93–95] Although there is intense demyelination of the central myelin, the adjacent peripheral myelin at the root entry is dramatically spared, indicating a preferential and selective destruction of CNS myelin. Townsend and Baringer[95] suggest that immune factors are important in demyelination because of the following reasons: (1) although oligodendrocytes are infected with virus, they do not undergo lysis early in the disease process; (2) infected Nude mice have only minimal demyelination;[93] and (3) immunosuppresion of infected mice with cyclophosphamide markedly decreases the extent of demyelination.[95] They postulate that an extensive, productive astrocytic infection leads to infiltration by macrophages that could demyelinate as a bystander effect; there may be an

additional later demyelination caused by a cytotoxic cell response.[94] Other experiments have demonstrated that a bystander demyelinating effect can be produced in the optic nerves of HSV-immunized rabbits following intraocular infection with inactivated HSV.[41] In conflict with experiments of Townsend and Baringer,[95] Kristensson et al. found that treatment with cyclophosphamide led to more, not less, demyelination in association with elevated HSV titers.[40] Their impression was that, at least early on, demyelination resulted from an oligodendrocyte lytic infection, although a cytotoxic immune reaction may contribute to late demyelination. It is hoped that additional studies with this system will clarify the mechanism of demyelination as well as the selectivity of CNS myelin as the target antigen.

VIRUSES AND HUMAN DEMYELINATING DISEASES

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a rare opportunistic viral infection occurring in the immunosuppressed.[77] The disease is most commonly seen on the background of lymphoproliferative disorders, but a variety of other immunosuppressive states are associated with PML. The patient manifests the subacute onset of progressive noninflammatory multifocal symptomatology, such as aphasia, hemiparesis, and ataxia, that varies depending on the location of the lesions. Death usually occurs within six months to one year.

The viral etiology of the disease was first suggested by Cavanagh et al.[8] and Richardson[76] because of the impaired host immunologic responsiveness and the rather unique histopathologic findings (Fig. 2), namely, demyelination (primarily subcortical) with inclusion bodies in the oligodendrocytes and bizarre-appearing, large astrocytes. The identification of papovavirions within the oligodendrocyte[84, 106] led to repeated efforts to isolate the virus; the initial lack of success presumably resulted from a restricted in-vitro host cell range of the virus. In 1971, Padgett et al. employed primary human fetal spongioblasts, a culture system known to be permissive for another papovavirus, to successfully isolate JC virus—a new papovavirus.[72] Weiner et al. isolated a variant of SV40 virus, another papova virus, from two cases of PML.[102] Numerous PML isolates subsequent to these reports have all been of JC virus; two subgroups of JC virus isolates have been described on the basis of their restriction enzyme patterns.[25] It is generally accepted that JC virus lytically infects oligodendrocytes (without any immunopathologic contribution), producing the demyelination of PML. The JC virus infection of oligodendrocytes is not believed to be a totally productive lytic one, as evidenced by the subacute course of demyelination; the slowness of the infection is not due to ts mutants or defective interfering particles.[24]

Serologic studies of sera have shown that about 70 per cent of adults have antibody to JC virus with seroconversion usually occurring during the first 14 years of life.[71] This finding indicates that JC virus is a common human pathogen, although the nature of the primary infection is unclear.

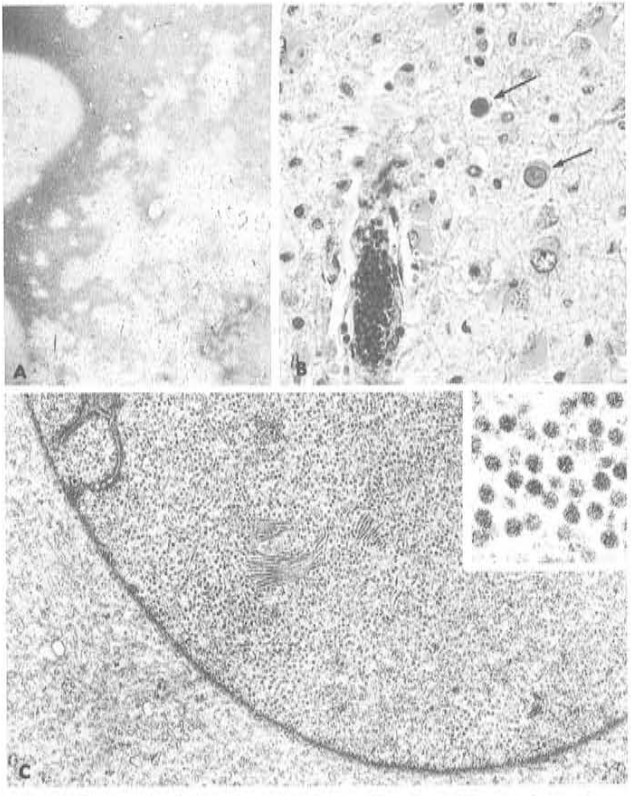

Figure 2. Progressive multifocal leukoencephalopathy. *A*, Patchy demyelination unrelated to vessels merging into confluent lesions. (LFB stain, × 8.) (Armed Forces Institute of Pathology Neg. No. 68–9405.) *B*, Oligodendrocytes with enlarged nuclei (*arrows*) occur within early demyelinated lesions. Note absence of perivascular leukocytes. (Hematoxylin and eosin stain, × 350.) (AFIP Neg. No. 68–9404.) *C*, Oligodendrocyte with enlarged nucleus filled with spheroidal and filamentous papovavirus particles. *Inset*, Magnification of virus particles in *C*. (× 20,000; *inset*, × 80,000.) (*From* Lampert, P. W.: Autoimmune and virus-induced demyelinating diseases. Am. J. Pathol., 92:176, 1978.)

It is not known whether PML is a result of a reactivation of JC virus or represents a primary infection of JC virus in an immunosuppressed host.

The finding that JC virus infection of humans is a common one is provocative because of the known oncogenic properties of papovaviruses. JC virus infection of astrocytes in PML is generally regarded as a quasi-oncogenic transformation, resulting in a bizarre appearance of the cells with frequent mitotic figures. It is known that JC virus can produce a variety of tumors in experimental animals; for example, it produces gliomas in hamsters[98] and subhuman primates.[54] However, the role of JC virus in human brain tumors is still unknown.

Postinfectious Encephalomyelitis

Investigations of postinfectious encephalomyelitis (PIE) have focused attention on the relationship of viruses to autoimmunity. The disease is

characterized by acute perivenular inflammatory demyelination associated with a preceding viral infection. Viruses that have been associated with the syndrome include: vaccinia, measles, varicella, and rubella. An autoimmune etiology of PIE has been postulated since (1) it frequently occurs following the onset of a rash in the viral exanthematous diseases; (2) the virus is rarely isolated from the CNS; and (3) the lesions histopathologically resemble EAE.

Of potential importance to our understanding of PIE have been several studies that have explored the relationship between viral infections and EAE. Caspary found that guinea pigs treated with a number of different vaccines have a more severe course of EAE.[7a] Inoculation with HSV,[30a] measles virus,[58] and Semliki Forest virus[89] has been found to exacerbate EAE in several species. Frequently the changes in EAE are dependent on rather precise timing of the viral inoculations. In all cases, the mechanism of the virus' effect on EAE has not been clarified. One wonders whether one is merely seeing the effect of damaged brain and altered blood-brain barrier on EAE.

Johnson et al.[33, 34] have recently begun to systematically investigate this syndrome from a clinicopathologic and research point of view by studying measles encephalitis (ME), a form of PIE, in Peru. The incidence of ME is 1 per 1000 infections with a 20 per cent fatality rate. The studies suggested that there is no real evidence of measles virus replication in the CNS in ME. No virus was isolated from fatal cases, and no viral antigen was present in 10 of 10 brains examined by immunoperoxidase-staining techniques.[21] There was little evidence of a CNS humoral immune response directed against measles virus; intrathecal IgG and measles virus antibody production were rarely and minimally apparent. Although there was a depression of lymphocyte mitogenic responses, there was an increased lymphocyte response to measles virus antigens and to MBP; it is unclear what the precise stimulation indexes were. MBP was present in the CSF early in the disease in five of eight patients.

The lymphocyte stimulation to MBP in ME is a most important observation. Johnson et al. also found lymphocyte stimulation to MBP in an individual with complications from rabies immunization, a disease considered the human counterpart of EAE.[34] Others have reported an increased lymphocyte response to MBP in PIE without exanthematous disease,[53] as well as in EAE. The general feeling of Johnson's group was that ME was caused by a viral-induced immune disturbance lending to reactivity against MBP; virus presence in the CNS was neither apparent nor necessary. More sensitive tests, such as in-situ hybridization, must be employed to substantiate their thesis that measles virus is absent in the CNS in ME.

Why should immune cells home to the CNS in ME? It is of course possible that virus initially invades the CNS, causes some inflammation, and then is cleared; recent in-situ hybridization studies suggest measles virus genome persists for prolonged periods of time.[26a] As has been demonstrated in other systems, continuing inflammation may lead to the homing of additional lymphocytes in the area, even if they are not specifically sensitized to antigens exposed in the area;[3, 58, 61] that is, lympho-

cytes directed against MBP (because of MBP spillover in the CSF) may home to inflamed but intact CNS areas and cause demyelination. Alternatively, measles virus may not initially reach the CNS. The high frequency of electroencephalographic changes and CSF pleocytosis seen in uncomplicated measles may represent lymphocyte, not viral, invasion, as Gendelman et al. suggest.[21]

Why would lymphocytes directed against MBP home to uninfected, noninflamed CNS areas? It is important to recognize that the lack of mitogenic response in measles may be a reflection of changed cell surface properties of lymphocytes. Perhaps these lymphocytes with changed cell surface properties are able to invade the CNS. Once cells enter the CNS and establish an inflamed focus, cells directed against a variety of antigenic specificities, including MBP, could home to inflamed areas nonspecifically and could produce demyelination. Of interest is the finding that lymphocytes alter their usual distribution in the body after infection with Newcastle disease virus.[105] It has been reported that CDV reaches the CNS by means of infected lymphocytes.[89a] It is also of interest that the viruses most often associated with PIE are enveloped viruses that presumably produce changes in lymphocyte cell-surface properties during virus budding.

Viruses and MS

What is the evidence that viruses actually play a part in MS? Generally the relationship of viruses to MS is based on information from four main sources: epidemiologic studies, ultrastructure, virus isolations, and IgG studies. Epidemiologic studies have suggested that an environmental factor is important in the acquisition of MS.[60] Initial enthusiasm over electron microscopic visualization of paramyxovirus-like nucleocapsids in MS CNS has waned with the recognition that these inclusions are nonspecific and probably nonviral.[60] There have been many virus isolations in MS over the years, and perhaps it is sufficient at this time to emphasize that none have been confirmed. The future unquestionably will involve the use of new technologies and molecular studies[26a] to look for the etiologic agent of MS.

Serologic studies have demonstrated a frequent elevation of intrathecal measles virus antibody synthesis of patients with MS compared with controls. However, Norrby et al.[69] showed that MS CSF anti-viral antibody activities are variable and at times multiple; 48 per cent of patients with MS have intrathecal production of antibody to one virus, 16 per cent to two viruses, and 7 per cent to three or more viruses. The multiplicity of the antibody targets indicate that at least some of the antibody is "nonsense," that is, unrelated to MS pathogenesis (see the article by Oger et al. in this issue).[58a] Perhaps measles virus antibody is most frequently increased because it commonly infects the nervous system, leaving residual lymphoid cell clones that can produce IgG when "nonspecifically" stimulated in a "heteroclitic" response. This thesis gathers support from observations of Libikova et al.[47] that indicate that Czechoslovakian patients with MS most commonly do not have intrathecal synthesis to measles virus but to an orbivirus, a common viral pathogen in this European area. It should be mentioned that the characteristic MS CSF oligoclonal IgG bands can be seen in nonviral autoimmune processes as well as in infectious ones.[103] In

summary, the serologic studies show B cell hyperactivity but fail to implicate specifically a viral etiology.

We know that viruses can produce demyelinating diseases in animals and humans, but we have no firm evidence at present of their involvement in MS. As Charcot wrote:

"In order to conclude this study, gentleman, it remains for me to discuss . . . the etiology . . . of multilocular sclerosis of the nervous centres. Unfortunately, the facts and documents which refer to these different subjects are few in number, and as yet mostly imperfect. . . ."[9]

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