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Chapter 19

VIRAL ETIOLOGY OF MULTIPLE SCLEROSIS: A CRITIQUE OF THE EVIDENCE

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I. INTRODUCTION

Multiple sclerosis (MS) must currently be ranked among the major enigmas in medicine. On the one hand, it has been the subject of a vast effort by a host of investigators, and on the other, its etiology remains elusive. During the last 20 years, when research on MS has employed modern methods, the proposal that this mysterious disease is caused by one or several viruses has been a leading hypothesis. Yet, if so, the putative agent has defied discovery. Futile efforts to identify a conventional virus suggest that, if the hypothesis is still viable, an unconventional subviral agent might be implicated. For this reason, it is of interest to review briefly the current evidence for a viral etiology of MS, in a monograph devoted to subviral pathogens.

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II. THE VIRAL HYPOTHESIS: BACKGROUND AND HISTORY

The idea that a virus might cause MS springs from several quite different sources, each of which provides suggestive clues. First, studies of identical twins where one has MS show that the second twin develops the disease in only about 30 percent of instances (Spielman and Nathanson, 1982). This low concordance suggests that an exogenous event must be imposed on even a proven susceptible genotype in order to cause disease. Migrant studies, described in greater detail below, also indicate that the risk of MS is influenced by environmental factors, since movement of a population from a low to high risk region alters the subsequent incidence of the disease (Nathanson and Miller, 1978).

The discovery and elucidation of persistent viral infections has provided a separate line of evidience suggesting that, even in humans, chronic neurological disease may be caused by viruses (Johnson and Herndon, 1974; Johnson, 1975). Three quite distinct diseases have been well documented in the past 15 years. Creutzfeldt-Jakob disease is a spongiform encephalopathy,one of the group of agents related to scrapie, the exact nature of which remains elusive (see Prusiner, this volume). Progressive multifocal leukoencephalopathy is a progressive disease with focal demyelination which is caused by JC virus,a human member of the polyomavirus group. Finally, subacute sclerosing panencephalitis is a fatal chronic destructive encephalitis caused by measles or, rarely, by rubella virus.

A third clue is provided by studies of the spinal fluid in patients with MS (Thompson, 1977). In most cases the level of immunoglobulins is elevated, with evidence of intrathecal antibody synthesis of oligoclonal antibodies. This suggests that a local immune response is occurring within the central nervous system (CNS) and that this response may be indirect evidence of a foreign microbial agent, conceivably a virus (Paterson and Whitacre, 1980). Furthermore, when CSF samples were tested for anti-viral antibody activity, a high proportion of patients with MS were found to have antibody against one or more of a number of human viruses (Norrby, 1978). Based on the serum to CSF ratio, these antiviral antibodies were synthesized within the CNS.

In sum, several independent lines of evidence have bolstered the hypothesis that a viral agent might trigger the development of MS. In the following sections, we will review and critique these different sets of data.

III. EPIDEMIOLOGY OF MS: DOES IT SUPPORT THE VIRAL HYPOTHESIS?

Prevalence of MS in Different Populations. The prevalence of MS is well-defined in only limited areas of the world, particularly in some of the developed countries (Acheson, 1977). Studies in North America, Europe, and Australia indicate that risk increases with distance from the equator. For instance, the rates are higher in northern than southern Europe. In the northern part of the United States and in Canada, the rate is about twice that in the southern United States (Beebe *et al.* 1967).

Studies in several countries demonstrate that, following migration between areas with different incidence, the migrating population, or its offspring, take on the risk of the new area of residence. The evidence is particularly impressive among veterans in the United States, where movement in either direction (North to South or the reverse), between birth and induction into service, alters the risk(Dean and Kurtzke, 1971; Kurtzke *et al.*, 1979). Likewise, migration from Great Britain to South Africa reduces incidence while migration from North Africa to Israel increases incidence (Alter *et al.*, 1966; 1977).

Although the effect of migration upon the risk of developing MS is generally accepted, it is less clear what this observation means. If the migration effect is assumed to reflect differences in the age of exposure to an infectious agent which causes MS (the usual interpretation), several predictions may be made regarding age distribution, soci - oeconomic and rural-urban differences in rates, and secular trends in incidence and age distribution. Many of these predictions fail to be confirmed by the epidemiological data (Nathanson and Miller 1978). Therefore, interpretation of the epidemiologic evidence must be viewed with scepticism.

IV. ANIMAL MODELS

A. Persistent infection of the CNS. One of the strongest arguments for the search for viruses in MS is that a number of diseases of animals that are well documented to have a viral etiology, resemble MS both pathologically and clinically (Doherty and Simpson, 1982). Three diseases have been studied thoroughly: Theiler's Murine Encephalomyelitis Virus (TMEV) and Mouse Hepatitis Virus (MHV) in mice and rats, and visna of sheep. The hallmarks of these diseases are the presence of symptoms of white matter disease and the pathological evidence of demyelination. Exacerbations and remissions are seen both naturally (visna) and by manipula-

tion of the model (TMEV). Although none of the models is perfect, in combination they suggest that a disease such as MS could arise from a viral infection.

B. Theiler's Murine Encephalomyelitis. The group of viruses responsible for TMEV are picornaviruses originally isolated by Theiler in the 1930's (Theiler, 1937, 1940). Although they were a known cause of both asymptomatic enteric infection and of CNS disease, their true significance as a model for demyelinating disease was not recognized until Lipton began a systematic study of their pathogenesis in the 1970's (Lipton, 1975). TMEV is an endemic cause of asymptomatic disease in mouse colonies. In occasional animals, it spreads to the nervous system, where it results in a flaccid paralysis originally described as "mouse poliomyelitis". Experimentally, after intracerebral inoculation TMEV causes a biphasic illness, initially involving the gray matter and subsequently (in survivors) the white matter (Lipton, 1975).

The TMEV group can be subdivided according the predominance of gray or white matter disease after intracerebral inoculation. Recently, it has become clear that these biological differences reflect true genetic heterogeneity. The more virulent viruses (GDVll, FA) share large plaque size, polypeptide mobility, and Tl oligonucleotide pattern (Lipton, 1980; Lipton and Friedmann, 1980; Lorch *et al.*, 1981). These viruses tend to cause a severe neuronal infection and a high mortality. From the standpoint of MS research, those viruses that cause a more persistent disease (DA, WW, TO4 and Yale) are more interesting, and the remainder of the discussion will be confined to them.

The demyelinative myelopathy associated with persistent TMEV infections has been studied extensively by light and electron microscopy. After an inital infection which involves both neuronal and glial cells (Penney, 1979) the virus persists in the CNS in spite of the presence of neutralizing antibody (Lipton and dal Canto, 1979). The amount of infective virus present during this late phase is small, and there is little detectable antigen seen by fluorescence studies or electron microscopy (Lipton, 1975; dal Canto, 1975). In situ hybridization has demonstrated the presence of viral RNA in glial cells during this late demyelinative stage (Brahic *et al.*, 1981).

An autoimmune component has been postulated for the late white matter disease seen in TMEV. Immunosupression ameliorates the CNS lesions (Lipton *et al.*, 1977) and the SJL/j mouse, known to be particularly susceptible to autoimmune phenomena, develops more intense demyelinative lesions (dal Canto, 1979).

Brahic (unpublished,1983) has suggested a credible hypothesis for the series of events that result in chronic TMEV infection and demyelination. A virulent panencephalitis kills neurons and infects glial cells, which do not support replication, at least not productively. In the animals that survive the initial disease, and where the virus belongs to the less virulent group, a low level of virus production persists. This low level replication is sufficient to either destroy a number of oliodendrocytes, resulting in demyelination, or to trigger a cellular immune response, with the same final result. In either case, the initial immune response, though adequate for clearance of the majority of virions, allows the persistence of low levels of TMEV.

C. Mouse Hepatitis Virus. The MHV group, which infects both mice and rats, was initially isolated in 1949 (Cheever *et al.*, 1949). The first isolate, JHM virus, was a neurotropic strain. Since then, these viruses have been classified as either hepatotropic (MHV-3 for example) or neurotropic (JHM and others). They are part of the family Coronaviridae, which are positive stranded RNA viruses so named because of their electron microscopic appearance. The family includes a number of human isolates, responsible for a significant proportion of upper respiratory infections in man.

The CNS pathology was initially described as demyelinative (Bailey *et al.*, 1949). It is now known that the neurotropic strains of MHV infect both neurons and oligodendrocytes. The survivors from the initial panencephalitis may go on and develop a demyelinating syndrome, with chronic persistence of the virus in oligodendrocytes (Herndon *et al.*, 1975; Weiner *et al.*, 1973).

Three recent developments have increased interest in MHV; (i) the isolation of a human coronavirus from MS brain; (ii) the isolation of mutants that are highly neurotropic yet have a low mortality associated with them; and (iii) the development of an autoimmune-type illness after adoptive immunization of rats with lymphocytes from MHV infected animals.

The isolation of a coronavirus from MS brain (Burks *et al.*, 1980) has not been duplicated, and is discussed below. Over the past several years, Oldstone and associates have isolated a number of temperature sensitive (ts) mutants of JHM, the prototypic MHV neurotropic strain. One of these, ts8, shows a low mortality when injected intracerebrally into mice, yet induces a persistent infection with recurrent demyelination (Knobler *et al.* 1982). This mutant also demonstrates high infectivity for oligodendrocytes, rather than neurons. The CNS of these animals shows demyelinated and

remyelinated axons, suggesting potential similarities with MS. Since the disease can be induced in inbred strains of mice, a variety of genetic parameters can now be studied with this system.

In addition to the finding that ts mutants of MHV can cause an exclusively demyelinating disease, recent work has demonstrated that auto-immune mechanisms may play a role in the demyelination caused by these viruses. Ter Meulen and collegues (Watanabe, 1983) have now been able to infect rats with JHM strain, restimulate the lymphocytes from these diseased rats with myelin basic protein *in vitro*, and cause a demyelinative disease in healthy rats adoptively immunized with these lymphocytes. These findings are an important confirmation of the long-standing hypothesis that viruses may be able to trigger an immune response to self antigens. At this point it is not clear how this sensitization takes place, but the more likely possibilities are that: (i) injury to oligodendrocytes exposes antigens not usually available to the immune system; (ii) there are antigens on the JHM virus which cross react with cellular antigens; or (iii) in the process of infecting cells the virus acquired cellular antigens which are then presented to the immune system. Although there is as yet no evidence for the first of these possibilities, cross reacting antigens have been established for other viruses (Fujinami *et al.*, 1983). Whichever of these mechanisms is shown to be responsible for this unusual response to MHV infection, this promises to be an exciting area for research related to MS.

D. Visna. Visna is a disease of sheep (Palsson, 1976) caused by a naturally occurring retrovirus (also called progressive pneumonia virus, or maedi virus). In the field, the agent is spread as a respiratory infection, between ewe and lamb or between adults, and usually causes a progressive interstitial pneumonitis; some infected animals also develop the CNS phase, known as visna.

Under experimental conditions,when a large inoculum of a neurotropic strain is injected intracerebrally into genetically susceptible Icelandic sheep, a high proportion develop a chronic subacute encephalitis (Nathanson *et al.*, 1983; 1984). At irregular intervals of months to years, additional focal demyelinating lesions arise in brain and spinal cord, with consequent progressive paralysis which is eventually fatal. All infected sheep undergo lifelong virus persistence, in lymphoid tissues, lung, or CNS. However, the genome apparently persists as a latent provirus and most infected cells produce little or no infectious virus and are not killed by the infection. Virus isolation often requires co-cultivation

with permissive sheep fibroblasts. During the course of infection, sheep develop serum antibody against the envelope glycoprotein and against the major internal protein. A transient cellular response can often be demonstrated by an *in vitro* lymphocyte proliferation in the presence of viral antigens.

It appears that the slowness of the infection is due to a host cell restriction on the expression of the viral genome (Brahic *et al.*, 1981); in cultures, sheep fibroblasts are highly permissive while selected other cell lines are severely restrictive (Nathanson *et al.*, 1984). A second factor limiting infection *in vivo* is the high titer of neutralizing antibody in serum and CSF. The same mechanisms which slow the infection also account for its persistence, particularly the maintenance of the viral genome as a latent provirus. In addition, in some sheep the occurrence of antigenic variants of the virus probably contributes to persistence (Narayan *et al.*, 1978; Lutley *et al.*, 1983; Thormar *et al.*, 1983).

The mechanism of the development of the CNS lesions is less well understood. The early subacute inflammatory response is prevented by immunosuppressive intervention and probably respresents a virus-specific immune response (Nathanson *et al.*, 1976; 1981). The late focal demyelinating lesions may be due to the bystander effect of a classical delayed type hypersensitivity but this is only speculative.

E. Virus-induced demyelination. One salient observation which arises from the animal models described above is that primary demyelination, of a focal nature, can be caused by certain persistent viral infections. The pathologic hallmark of MS is the plaque, also a focal demyelinating lesion. This connection establishes the plausibility that a virus could be instrumental in the pathogenesis of MS. For this reason, the mechanisms of virus-induced demyelination are of interest.

Two different mechanisms appear to operate in different models (see above). Coronaviruses apparently demyelinate by initiating a lytic infection of oligodendrocytes (Weiner, 1973) although auto-immune mechanisms may also come into play (Watanabe *et al.*, 1983). With Theiler's virus, there is evidence that the antiviral immune response may be important, since immunosuppression will prevent demyelination under certain conditions (Lipton and dal Canto, 1977). On the other hand, virus apparently persists in oligodendroglia although no infectious virions are synthesized. Possibly direct virus lysis may also operate in this model. In visna, it is likely that demyelination is an indirect process, since there are very few infected cells in the CNS. Possibly, an immunological process could provide an amplifying mechanism to explain

the very severe lesions which can occur (Nathanson *et al.*, 1976; 1981). However, this model does not lend itself to the precise testing of such a hypothesis.

A final point of importance is that, in all of the animal models, it may be very difficult to detect virus during the chronic phase of demyelination (Martin and Nathanson, 1979). This is particularly true of mouse hepatitis and visna infections. Thus, it is plausible that a putative but unidentified viral agent might go undetected in MS tissue, in the absence of agent-specific probes.

V. AUTO-IMMUNE DISEASE INITIATED BY VIRUSES

The hypothesis that a chronic viral infection of the CNS might initiate an autoimmune demyelinating process has long been suggested as a mechanism in MS (McFarlin and Waksman, 1982). Such a hypothesis could explain two key observations: (i) the great difficulty in detecting a viral agent on CNS examination of patients with longlasting progressive MS, and (ii) the resemblance of the pathology of MS to relapsing forms of experimental allergic encephalitis (EAE). In the past, there was little evidence to support such a concept, appealing as it was. Recently, however, two sets of observations have given the concept of virus-induced auto-immunity enhanced credibility.

A. EAE following mouse hepatitis virus (MHV) infection. ter Meulen and his colleaques (Watanabe *et al.*, 1983) have recently reported studies in Lewis rats (highly susceptible to EAE) infected with MHV. Several months after infection, these animals develop partial paralysis, and show histologic evidence of chronic demyelination; some lesions resemble those of EAE while others appear more like the demyelination produced by MHV in mice. When spleen and lymph node cells from such rats are transferred to Lewis recipients a classical EAE occurs, about one week after transfer. That the effect is due to cells not virus is indicated by the short incubation period and by the limitation of the effect to syngeneic recipients, while MHV-susceptible allogeneic recipients do not develop disease. This appears to be the first bona fide model of virus-induced EAE.

B. Virus-induced autoimmunity. The advent of hybridoma technology has permitted the isolation of clonal B cell populations from virus-infected mice. Mice infected with many viruses yield clones synthesizing anti-viral antibodies. Recently, it has been shown that, in some viral infections, the animals also raise monoclonal antibodies against a variety of self antigens (Haspel *et al.*, 1983). Appropriate controls indicate that the viral infection is required and

that this response is not due to self antigens contaminating the inoculum.

C. Molecular mimicry. One possible mechanism for virus-induced auto-immunity is the sharing of epitopes between proteins and self proteins. Although long postulated, direct evidence has been lacking. However, several recent reports indicate that occasional antibody clones do indeed recognize shared epitopes on virus and self (Fujinami *et al.*, 1983; Dales *et al.*, 1983).

Taken together, these recent observations from several laboratories suggest that credence must be given to the proposal that virus infection could initiate auto-immune demyelination, and that immunologic mechanisms exist which could account for such an occurrence.

VI. SEARCH FOR VIRUSES IN MS

A. Attempts at isolation. Conventional efforts at defining a transmissible agent in MS tissue have been directed at both visualization and isolation (Johnson, 1975; Johnson and Herndon, 1974; Fraser, 1977; Sever and Madden, 1980; Melnick, 1982; Cook and Dowling, 1980). Most of the efforts, by the nature of the disease, have utilized brain tissue obtained at autopsy. Processing of such tissue prior to the onset of autolysis is difficult. Many patients with MS die in nursing homes and other chronic care institutions where initial etiology of the neurologic disorder may not be well known and where there is no interest in research. Consequently, experimental tissue is scarce, and most investigators have not limited their efforts to patients in whom the disease is known to be active, as might be judged by the continuing appearance of new symptoms and signs. Chronic "burned out" cases may have little inflammation and/or active myelin destruction and could conceivably no longer carry a putative etiologic agent.

B. Visualization. There have been several reports of visualization of extraneous virus-like particles in either MS tissue obtained at autopsy or explants from such tissues. The descriptions have ranged from "herringbone" to paramyxovirus-like particles, and they have been localized to nucleus (Prineas, 1972) and endoplasmic reticulum (Tanaka *et al.*, 1975) of astrocytes and of infiltrating mononuclear cells. Because of the persistent association of elevated measles antibody titers with MS, discussed above, paramyxovirus-like particles have been of particular interest. Unfortunately, the findings on electron microscopy have not been reproducible.

C. Isolations. Isolations of viruses or viral-like

agents from MS tissue by routine explantation and inoculation methods, either in tissue culture or in a variety of animal species, have been reported consistently since the late 1940's (Margulis *et al.*, 1946; Dick *et al.*, 1958). In recent years the number of isolations has reached a frequency of about one each year, and the reported agents range from conventional organisms, like CMV and herpes (Wroblewska *et al.*, 1979; Gudnadottir, 1964) to ill defined factors like the MS- associated agent (Carp *et al.*, 1972; 1978) and measles antigen (Pertschuk *et al.*, 1976). These isolations have, to the present time, been disappointing, and the reported isolates have either not been confirmed in other laboratories or, in some cases, have been shown to be mycoplasma (Mitchell *et al.* 1978) or other passenger agents such as mouse hepatitis virus (Burks, 1979). Table 1 shows a list of the best known reports.

Table I
Viral Isolations from Multiple Sclerosis Tissue

Year	Virus	Reference
1946	Rabies	Margulis *et al.*
1972	MSAA*	Carp *et al.*
1972	Parainfluenza/(6/94)	ter Meulen *et al.*
1978	Bone marrow "agent"	Mitchell *et al.*
1979	Cytomegalovirus	Wroblewska *et al.*
1979	Coronavirus	Burks *et al.*
1982	Unidentified	Melnick *et al.*

*MSAA: MS associated agent.

Current efforts by one group (Gilden, Wroblewska, and colleagues) consist of explantation of tissue from diseased areas of white matter and growth of the brain either as primary cultures or in a variety of indicator cell lines. In parallel, some samples are inoculated into chimpanzees and mice. Two isolates, TMEV and CMV strains, have been properly identified as unrelated to MS(Wroblewska *et al*, 1979 Approximately 24 other brains have been negative (D.H. Gilden, personal communication). These extensive efforts suggest that if an agent is present in MS tissue obtained at autopsy, it does not grow in conventional tissue culture nor by the more common methods of explanation.

D. Use of Nucleic Acid Probes. The rapid expansion of techniques in molecular biology has added a powerful armamentarium for those interested in the search for putative infectious (replicating) agents in chronic systemic and neurologic diseases. A number of investigators have now begun searching MS tissue with a variety of nucleic acid probes, utilizing viruses which have in the past been associated with

the disease by either serological methods or isolations. A recent conference sponsored by the MS Society (Haase *et al.*, 1983) focused on the current and potential use of these probes. Investigators are now looking at MS tissue with probes designed to detect canine distemper virus (P.C. Dowling and collaborators), coronavirus (S. Weiss), and measles virus, (A. Haase). The work with the measles probes has progressed the furthest, and it shows that both MS and control tissue contain sequences that will hybridize with measles complementary DNA (Haase *et al.* 1981). The sequences in brain are sensitive to ribonuclease, suggesting that the nucleic acid in brain is viral RNA and not an integrated DNA copy. As controls for this work, visna and Theiler's murine encephalomyelitis virus probes were also hybridized with brain, and these were negative in both MS and normal tissue. Work in another laboratory has shown that canine distemper virus probes do not hybridize with the brain of MS cases.

The work with other viral probes is still at a preliminary stage. Nucleic acid probes are becoming increasingly sensitive and precise, and it is unlikely that this kind of work will be completed at a level that satisfies exacting minds in the near future. In addition, the positive findings with measles will color any results obtained with other viruses; additional complementary evidence will be needed to suggest that any agent has a role in this disease.

VII. SUMMARY AND CONCLUSIONS

The etiology and pathogenesis of multiple sclerosis is a major enigma which continues to defy solution in spite of a great deal of research employing techniques which range from epidemiology to molecular biology. Family and population studies strongly suggest that the disease is triggered by an exogenous environmental event. Viral and immunological investigations of animal models make it plausible that a viral infection can cause a chronic multifocal demyelinating process in the CNS, and recent observations suggest that this could involve an auto-immune process initiated by a viral infection. A direct viral cytolytic effect or an anti-viral immune response offer alternative mechanisms.

However, attempts directly to demonstrate a viral agent, viral genome, or viral proteins have been unsuccessful to date. This sugests that, in addition to conventional viruses, it is possible that a novel virus or virus-like agent might be involved. Clearly, the detailed investigation of viroids, virusoids, and other subviral agents will provide an essential foundation for the continuing search for the cause of MS.

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