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the MHC and T-cell-receptor peptides involved in triggering. These can be blocked with monoclonal antibodies (anti-MHC, anti-idiotype) with immunodominant but non-immunogenic peptides, or with non-catabolizable molecules which bind with high affinity to the MHC (DP, DQ) site or to the T-cell receptor. The feasibility of blocking with peptides has been solidly established in other systems within the last year. A more remote possibility would be the use of anti-sense DNA or RNA to eliminate synthesis of one or more of the key peptides involved in recognition.

Non-specific therapy has many possible targets, from manipulating the level of MHC expression in vascular endothelium, glial elements or immunocytes infiltrating the lesions to ablating specific T-lymphocyte subsets or blocking the diffusible products which play a role in inflammation. Many such therapies are currently under investigation, as are attempts to correct (or compensate for) the conduction deficit which results from fluid pressure, demyelination, and the action of the products of inflammation. New therapeutic concepts are needed to correct the immunoregulatory abnormality identified in MS or the striking abnormalities of hypothalamic pituitary-adrenal function.

A sense of optimism pervades the papers assembled here. Understanding at the cellular and molecular level must inevitably provide the tools which will prevent MS, stop it once it has begun or correct the physiopathologic abnormalities resulting from the disease process.

MULTIPLE SCLEROSIS AS A VIRAL DISEASE

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The cause of MS is still unknown. One of the oldest hypotheses is that viruses may be responsible for the demyelinating lesions which characterize this disease. Over the years several viruses have been isolated from MS patients, viral antibodies have been detected in their cerebrospinal fluid or serum and viral nucleic acids have been demonstrated in CNS samples. However, no single pathogen has been reproducibly linked to the disease. In spite of this, the viral hypothesis remains persuasive for two reasons. First, its epidemiology seems to link MS to an environmental agent, possibly a virus. Second, some animal viruses cause demyelinating diseases resembling MS. In many instances, these animal models are caused by persistent infections in which the virus is present at very low levels and therefore is difficult to detect.

In this chapter we will review the evidence in favour of a viral aetiology and discuss the types of viruses which could be responsible, paying special attention to measles virus and

retroviruses. We will describe some mechanisms by which viruses can cause primary demyelination. Finally, we will propose a new molecular approach for the search for viral genes in MS tissues.

Epidemiological evidence.

The geographic distribution of MS implicates an environmental factor in the disease. Northern Europe, the northern United States and southern Canada are high incidence areas [20]. In both France and the United States, a clear increase in the number of MS cases exists going from the south to the north of the country. In the United States, however, high incidence areas also coincide with the distribution of populations of Scandinavian origin and therefore could reflect the existence of susceptibility genes in these individuals. The risk of developing MS has been studied in populations who migrated from high to low incidence areas and vice versa [5, 21]. Although somewhat controversial, the results seem to indicate that the risk of having MS is acquired by living in high incidence areas before the age of puberty.

In addition, there have been epidemics of MS. The most extensively studied occurred in the Faroe Islands. No cases of MS were diagnosed there before 1940, while three epidemics, peaking in 1946, 1957 and 1969 occurred following occupation of the islands by large numbers of British troops during WW II. The data are consistent with the introduction of an infectious agent into the prepubertal Faroese population during the war [22].

What kinds of viruses might cause MS?

There are two ways of looking at viruses as possible causes of MS. The disease could be a rare consequence of common viral infections of childhood in genetically predisposed individuals, in which case there is no «MS virus» as such. Instead, a variety of well known agents — myxoviruses, paramyxoviruses, picornaviruses, *etc.* — could be responsible, under special circumstances, for a unique neurological condition with clinical onset in early adulthood. The observation that a variety of animal viruses cause primary demyelination supports this idea [25]. There is also a correlation between MS and a late occurrence of childhood viral infections, suggesting that common viruses could play a role in the disease [1].

On the other hand, MS could be due to a single, specific human virus which is widespread in high incidence areas, causes inapparent or minor viral illness in the majority of the population, but causes MS in a small number of genetically susceptible individuals. An example of this type of pathogenesis is the infection of mice by Theiler's virus, an endemic murine picornavirus. In most cases, Theiler's virus causes an inapparent enteric infection, but in a small fraction of outbred animals it reaches the central nervous system where it persists for life [31] and causes an inflammatory primary demyelinating disease [4]. The genetic background of the host plays a major role in the disease, since inbred strains of mice can be either susceptible or resistant [23].

However, in spite of seroepidemiological surveys, virus cultivation attempts and searches for viral nucleic acids and antigens in brain, it has not been possible to demonstrate the existence of a specific MS virus. Several viruses have been isolated from MS tissues, including herpes simplex, the scrapie agent, parainfluenza virus I, measles virus, simian virus 5, a coronavirus and others [18]. Unfortunately, in no case has a convincing relationship to MS been demonstrated. Rather than describe these many isolates, our discussion will focus on two viruses most frequently invoked, measles virus and human retroviruses.

Measles virus.

Measles virus can be neurotropic on occasion. In this case, it behaves as a slow virus and causes the fatal disease, subacute sclerosing panencephalitis

(SSPE), in which inflammatory cells in the white matter are associated with loss of myelin. The presence in the CNS of large amounts of measles virus RNA and nucleocapsid antigens is a hallmark of SSPE [10]. Measles virus RNA sequences were looked for in CNS tissues from MS patients using sensitive *in situ* hybridization techniques. Hybridization was observed in roughly 30 % of MS cases examined, but also in the same percentage of controls [9].

The immune response of MS patients to measles virus is abnormal. They have high levels of anti-measles antibodies. Their cytotoxic T-cell response against measles-virus-infected cells, which is performed by class-II-restricted CD4 T cells, is impaired. In contrast, their responses against influenza virus and mumps virus, the latter of which is also class-II-restricted, are unchanged [15]. Although this implies some connection between MS and measles virus, the significance is unclear.

Overall, the evidence that measles virus could cause MS is weak. One should note that measles virus has a worldwide distribution, as opposed to MS, and that this agent was present in the Faroese population before the MS «epidemics». Finally, if MS is due to the measles virus, one should eventually observe its disappearance in countries such as the United States where vaccination against this virus has been widespread for the past 15 years.

Retroviruses.

Since the discovery of HTLV-I, human retroviruses have become favorite candidates as agents of diseases of possible viral aetiology. In the case of MS, the retrovirus theory was reinforced by the finding that HIV is related to the lentivirus visna [29], the agent of a slow demyelinating disease of Icelandic sheep. More interestingly, a clear association between HTLV-I infection and a chronic human neurological disease was established in 1985 [8]. Tropical spastic paraparesis (TSP), also called HTLV-I-associated myelopathy (HAM) in Japan, is a rare consequence of infection by HTLV-I. Although different from MS, the two diseases present some similarities. Both are chronic, and histopathological examination of the few cases of TSP which have been autopsied showed inflammatory demyelinating lesions in the white matter of the spinal cord [28]. Not only did seroepidemiology link TSP-HAM to HTLV-I infection, but the virus was also isolated from T lymphocytes present in the CSF of TSP patients [13, 16]. Whether HTLV-I infects CNS cells besides lymphocytes is still an open question.

Koprowski and his collaborators described elevated anti-HTLV-I antibodies in CSF and sera, and HTLV-I-related sequences in CSF cells, of some MS patients [19]. These findings could not be reproduced in other laboratories including ours [11]. Furthermore, the geographical distributions of HTLV-I infection and MS cases do not overlap. Whereas HTLV-I is endemic in tropical areas and infects mostly blacks, MS is not found in these areas, and black populations, even living in high MS incidence areas, are resistant to the disease [5]. Therefore, the evidence that retroviruses may cause MS is still circumstantial.

Mechanisms of virus-induced demyelination.

Over the past years, the study of viral animal models of MS has uncovered several mechanisms by which viruses can cause demyelination. They fall into two categories: direct effects of viral infection on myelin synthesis and immune-mediated mechanisms.

Several viruses can infect oligodendrocytes (*e.g.*, JC virus in man and Theiler's virus in the mouse). The host cell can be killed by the infection resulting in demyelination. In other cases, such as Theiler's virus infection, viral replication in oligodendrocytes is restricted, little or no viral protein is expressed and the host cell survives [2, 3]. The consequence is a persistent infection of oligodendrocytes and chronic

disease. Viral genes in oligodendrocytes may alter the turnover of myelin without killing the cell. Specific loss of cell function as a result of non-lytic persistent viral infection has been demonstrated in several instances [27].

Virus-induced demyelinating lesions are often associated with mononuclear cell inflammatory infiltrates. This suggests that demyelination could be immune-mediated. In the case of Theiler's virus, inflammatory cells consist of activated macrophages, immunoglobulin-secreting B cells, and both $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells, some of which are directed at viral antigens (E. Cash *et al.*, in preparation). Although viral replication is severely restricted in the majority of infected cells, there is always a small fraction in which low amounts of capsid antigens can be detected [3]. If these antigens are displayed at the cell surface, there could be immune damage inflicted on a very small number of oligodendrocytes.

Demyelination may also arise from autoimmunity caused by chronic inflammation instigated by viral antigens. The expression of MHC antigens and other cellular proteins normally absent in the CNS is induced by persistent viral infection [24, 30]. In particular, the release of γ-interferon by inflammatory T cells induces the expression of class II antigens on astrocytes and vascular endothelial cells. These activated astrocytes can present antigens [6]. These events could lead to some autoimmunization [14]. In the case of Theiler's virus, some B lymphocytes associated with demyelinating lesions are specific for unidentified-virus-induced host antigens (E. Cash *et al.*, in preparation).

Finally, autoimmune demyelination could arise through «antigenic mimicry». Computer sequence comparisons have revealed the existence of short homologous peptides in viral and myelin proteins [17, 26]. Therefore antiviral antibodies or T cells could cross-react with myelin antigens. There is some evidence that this happens in nature. Immunization of rabbits with peptides of 8-10 amino acids from the hepatitis B virus polymerase induces antibodies and T lymphocytes which react with myelin basic protein and causes some CNS inflammation [7].

The role of virologists in MS research.

Which avenues can be followed by virologists trying to understand the cause of MS? If the disease is a rare consequence of childhood viral infections in genetically susceptible individuals, it will be important to determine which of these viruses may persist, in the form of nucleic acids, in CNS or possibly in cells of the immune system. Unfortunately, a systematic search for human viral nucleic acids in a large number of MS and control cases is an extremely burdensome task which has not yet been undertaken. Hopefully, new methods such as polymerase chain reactions may facilitate these studies.

The failure of repeated attempts to isolate a unique agent associated with MS does not mean that such a virus does not exist. It does suggest, however, that new methods may be required to achieve this task. In the past years, molecular biologists described techniques which may offer a powerful alternative to the conventional approaches to virus isolation. Subtractive cDNA hybridization has been used successfully to isolate tissue-specific mRNA. For example, the cloning of the T-cell receptor made use of the difference between the mRNA populations of T and B lymphocytes [12]. Because viral infections result in the expression of foreign genes in target tissues, it should be possible to isolate mRNA from unknown viruses by subtracting control tissue mRNA sequences from a cDNA library prepared with the mRNA extracted from the infected tissue. This approach has been tested successfully with Theiler's virus infection (J.F. Bureau and M. Brahic, in preparation).

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MS AS AUTOIMMUNE DISEASE: MYELIN ANTIGENS

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Ever since autoimmunity was considered to play a decisive role in the pathogenesis of MS, the search for the responsible antigens was one of the main domains in MS research, and still is. Nevertheless, in contrast to the situation in experimental models of the disease, where there has emerged a gradually increasing wealth of information about the nature of antigens involved, we still are virtually ignorant about antigens in MS. We are not even sure whether such entities as MS antigens do exist. On the other hand, the ever more detailed analysis of antigens in experimental allergic encephalomyelitis (EAE) has helped immensely not only to identify possible candidates for MS antigens, but, perhaps more importantly, to elucidate the pathogenic mechanisms of autoimmunity.

The identification of antigens in EAE followed a more-or-less straightforward logical path from crude brain and spinal cord homogenate to myelin, and from there to the purified myelin proteins, proteolipid protein (PLP) and myelin basic protein (MBP). PLP is the major structural protein of brain white matter accounting for more than 50 % of the central myelin membrane protein.