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# Aetiology and pathogenesis of Multiple sclerosis

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In the first part of our special feature, the authors outline the genetic and viral factors involved in the aetiology of multiple sclerosis. Also discussed is the use of an animal model that provides an insight into the pathogenesis of the disease

## IMAGE REMOVED

Coloured magnetic resonance image (MRI) scan of a vertical section through the brain of a patient with multiple sclerosis. The two cerebral hemispheres, containing the fluid-filled ventricles (white), occupy the top part of the brain. Below these is the cerebellum (yellow). Lesions caused by MS appear red in the cerebral hemispheres (upper right and centre left of brain) and white in the cerebellum (lower centre)

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS).<sup>1</sup> It was in the 1830s when Cruveilhier and Carswell first presented reports and provided illustrations of pathological specimens that later proved to be consistent with MS.

In 1868, Charcot produced his lectures on "Sclerose en plaques", providing the first detailed clinico-pathological description of the new disease, now termed multiple, or disseminated, sclerosis.

By consensus, there are four main clinical categories of MS.<sup>2</sup> The commonest is relapsing remitting MS (RR-MS), which affects 80-85 per cent of patients. These patients develop disease relapses, often without a specific trigger but possibly associated with infections. Disease relapses can last between 24 hours and several months, and patients may, or may not, completely recover. Patients are clinically stable between relapses, although they can have residual symptoms and disability.

After several years, the majority (70 per cent) of these patients will develop secondary progressive disease (SP-MS), whereby they experience a progressive neurological deterioration. They may still suffer from superimposed relapses. A subcategory of RR-MS patients (between 15 and 20 per cent) have benign MS. These patients have rare and mild relapses and a long course of disease, with minimal or no disability.

If patients have a steady neurological decline from the outset, without relapses, they are described as having primary progressive MS (PP-MS). This comprises approximately 15-20 per cent of sufferers. A fourth, rare category of patients is progressive relapsing MS (PR-MS), which is considered a variant of PP-MS, with similar prognosis. In patients with PR-MS, there is a gradual neurological decline from the beginning. It is similar to PP-MS, but has superimposed, acute relapses.

## EPIDEMIOLOGY

The prevalence of MS in the United Kingdom is approximately 120 per 100,000, with an incidence of seven cases per 100,000 per year.

The usual age of onset is within the third and fourth decades, although the disease

can begin in childhood and also above the age of 60 years. Overall, MS occurs more frequently in women than in men, and the ratio is approximately 2:1. This female predominance is less defined in patients with PP-MS, which typically develops at a later age.<sup>3</sup>

There is a variation in the worldwide distribution of MS, with the highest prevalence in northern and central Europe, North America, Canada and south-eastern Australia. Clusters, or so-called epidemics, occur. For example, in the Faroe Islands between the 1940s and 70s, there was a significantly higher than expected incidence of new MS cases.

There are also racial differences, with a low prevalence in black Africans and Asians, and a higher frequency in Caucasians, especially of northern European descent.<sup>1,3</sup>

## AETIOLOGY

The cause of MS is uncertain, but there is evidence of both genetic and environmental influences. Support for a genetic component is significant. Twin studies have shown an increased risk of 30 per cent in monozygotic, and around 5 per cent in dizygotic, twins. First degree relatives have

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## IMAGE REMOVED

Coloured magnetic resonance imaging (MRI) scan of damage (pink, arrowed) to the spinal cord due to multiple sclerosis (MS). Healthy nerve tissue is yellow. The damaged areas (plaques) are where the myelin covering of the nerves in the spinal cord has been destroyed.

a 2 to 3 per cent increased risk, which, although small, is considerably higher than in the general population. The risk decreases as the closeness of the relative decreases; children of a sufferer are at a higher risk than nephews or nieces. Adopted relatives have the same risk of developing MS as the general population.<sup>4</sup>

Devic's syndrome, or neuromyelitis optica, is a demyelinating disease in which only the optic nerves and spinal cord are clinically affected. Some neurologists feel it is a variant of MS, and certain studies have shown that this disease can run through families. However, there is no evidence to suggest further that the early clinical expression of MS is hereditary.<sup>5</sup>

## GENETIC INVOLVEMENT

No specific gene has been identified and MS is likely to have a polygenic mode of inheritance. Nevertheless, there is one well documented association between MS and the major histocompatibility complex (MHC), in particular, the human leukocyte antigen (HLA)-DR2.

The MHC is a group of proteins which are expressed on the surface of cells and enable self and non-self cells to be distinguished. The human MHC is named HLA (human leukocyte antigen). There are two types of HLA protein — class I and class II — and each one is coded for by three separate genes. These genes are HLA-A, -B and -C for class I, and HLA-DP, -DQ and -DR for class II. Each person has two copies of each gene, one maternal and one paternal. There are multiple forms of each gene available within the population and these are numbered, for example, HLA-B27.

There is evidence for a high proportion of HLA-DR2 in MS sufferers and, in areas with a high frequency of HLA-DR2 in the local population, there is, accordingly, a high prevalence of MS. However, this genotype is not associated with the clinical expression or course of the disease. Polymorphisms in the gene for tumour necrosis factor, an important proinflammatory cytokine, are also associated with susceptibility to MS.<sup>5,6</sup> Tumour necrosis factor is part of the MHC.

Other genes have been investigated that may be associated with the severity of MS. One example is the apolipoprotein E (APOE)-4 allele, which has been shown to

be associated with axonal degeneration in conditions such as Alzheimer's disease. Axonal loss is an important pathological feature of MS and evidence exists for its contribution towards irreversible clinical disability. Consequently, studies have also shown that the APOE-4 allele is associated with a faster progression of disability in MS sufferers.<sup>7</sup>

The fact that 70 per cent of monozygotic twins are discordant for MS strongly suggests that environmental factors also play a significant role in the development of the disease. Ever since Charcot's initial detailed clinico-pathological description of MS as an entity, investigations for an environmental cause have concentrated on transmissible agents. Support for this comes in the form of the epidemics of the disease described earlier. Some viruses are also known to cause diseases that have similar clinical features to MS. One example is human T-cell lymphotropic virus (HTLV)-I, which can lead to a spastic paraparesis and, sometimes, optic nerve involvement. Human immuno-deficiency virus can also, among other neurological complications, cause a myelopathy.

One of the characteristics of MS is the presence of oligoclonal IgG bands, in around 90 per cent of patients, on cerebrospinal fluid (CSF) examination. These bands reflect the intrathecal (within the CNS) synthesis of antibodies. Although sensitive for MS, and thus helpful in supporting the diagnosis, oligoclonal bands are not specific for the disease.<sup>1</sup> One of the conditions in which they are also prevalent is subacute sclerosing panencephalitis (SSPE), a condition that is caused by the measles virus as a late complication. The antigenic specificity of the antibody in oligoclonal bands is not known in MS. In SSPE, however, it is known to be a protein component of the measles virus. Raised CSF IgG production is also associated with other neurological inflammatory or infective disorders.

## VIRAL PATHOGENS

Viral pathogens investigated and considered to be involved in MS include human herpes virus-6 (HHV-6). Some studies have shown the presence of HHV-6 DNA (detected by polymerase chain reaction) in the brain tissue or CSF of patients with MS. Subsequent investigations, on the other hand, have not supported these results.<sup>8,9</sup>

Epstein-Barr virus (EBV) has also been implicated in MS. Nearly 100 per cent of patients with MS have circulating antibodies against EBV, compared with approximately 60 per cent in the general population. In addition, a history of symptomatic infectious mononucleosis (caused by EBV), especially at an older age, is

associated with a higher risk of MS.<sup>10</sup>

Another possible association is with *Chlamydia pneumoniae* which, in one study, was found to have a high prevalence (64 per cent) in the CSF of MS patients. This was, however, not corroborated by further investigations.<sup>11</sup>

It is unlikely that a specific infectious agent directly induces MS, and searches for such agents over the decades have been fruitless. More likely, it is the immunological consequences of an exposure to a potentially more common virus that triggers the disease in prone people. The mechanisms of this phenomenon may be multiple, and may involve molecular mimicry, bystander activation (activation of self-reactive T cells, eg, myelin-reactive T cells, in an inflammatory environment created by an infection in close proximity to these T cells), or superantigen activation.<sup>12</sup>

Environmental factors, other than infectious agents, for which there is some evidence of an association with MS, include low sunlight exposure, toxins, diet factors and trauma.

Despite the unknown aetiology, the working consensus is that environmental factors, possibly combined with chance, trigger an autoimmune reaction against CNS myelin in genetically susceptible individuals.

## PATHOGENESIS

The pathogenesis of MS is unclear, but a large body of evidence supports an autoimmune mechanism. Features that support this mechanism include the higher frequency in females (known to be more affected by autoimmune disease), the predominantly relapsing-remitting nature of the disease, evidence of an association with HLA and oligoclonal IgG bands in the CSF, and the benefit derived from some forms of immunomodulation and immunosuppression. The effect of pregnancy on MS is also a characteristic shared with other known autoimmune diseases. In pregnant patients with MS, the relapse rate is reduced during the second and third trimesters, but the rate increases for approximately three to six post-parturition.<sup>13</sup>

There are several key pathological features of MS, and these are inflammation (predominantly caused by T cells, macrophages, and some plasma cells), demyelination, axonal loss, and gliosis (overgrowth of nervous tissue). Axonal loss leads to indurated areas called plaques.

Plaques are the pathological hallmark of MS, but the pathogenic mechanism by which they develop is unknown. The exact process varies from person to person because, although the pathological features are consistent in different lesions within an individual, there is a significant

degree of heterogeneity between lesions within different patients.<sup>14</sup>

Plaques are generally located around blood vessels and extend along the line the vessel takes. This is described as Dawson's fingers. Plaques have a propensity for certain areas, such as the optic nerves, periventricular white matter, cerebellum, brain stem and spinal cord.

Plaques are characterised by loss of myelin or oligodendrocytes, or both. The myelin sheath is a lipid and protein cell membrane, derived from the oligodendrocyte, that wraps several times around an axon. The myelin sheath is segmented along its course and the areas between the sections are called nodes of Ranvier. These allow for saltatory conduction of nerve action potentials, whereby the impulses jump from node to node. This is much more rapid than conduction along an unmyelinated or demyelinated axon. The important protein constituents of myelin that are potential targets of an autoimmune attack include myelin basic protein (MBP), proteolipid protein (PLP), myelin-oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG).

In the acute or active plaque, there is an inflammatory infiltrate consisting predominantly of T lymphocytes and macrophages, as well as some B lymphocytes. Chronic, inactive plaques are largely hypocellular. Disruption of the blood-brain barrier also occurs in the acute phase and may be the first evident feature to develop on magnetic resonance imaging (MRI). In the MRI sequence called T1-weighted, disruption of the blood-brain barrier can be seen as an increase in the MRI signal intensity (enhancement) after administration of the paramagnetic contrast agent, gadolinium.

In the MRI sequence called T2-weighted, the presence of hyperintense, ovoid or round lesions on the images is a radiological hallmark and an important feature for the diagnosis of MS. These lesions occur in the white matter, with predominantly periventricular localisation, and also in the corpus callosum, brain stem, cerebellum and spinal cord.

Axonal loss is an increasingly recognised feature of MS plaques although, traditionally, it was believed that MS is a demyelinating disorder with relative preservation of axons. On MRI, low signal areas on T1-weighted imaging, as well as cerebral atrophy, are considered to be evidence of axonal degeneration, which correlates with irreversible clinical disability. Gliosis and scarring can be a feature, particularly of chronic plaques.

#### ANIMAL MODEL

The animal model, experimental allergic (autoimmune) encephalomyelitis

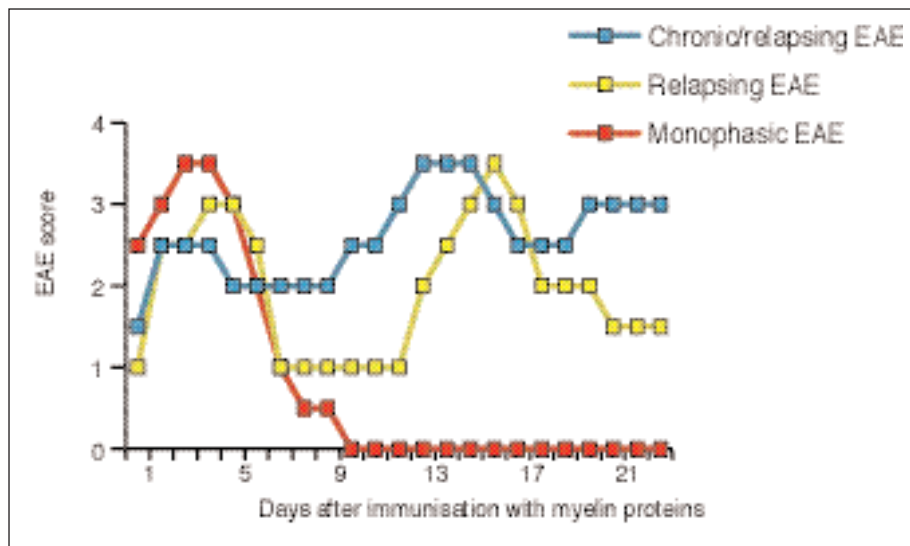


Figure 1. Examples of monophasic, relapsing, and chronic/relapsing EAE. Scoring of clinical signs of EAE are on a scale of 0 (normal) to 5 (moribund or dead resulting from EAE)

(EAE), is an autoimmune CNS demyelinating disease that has made a major contribution to the understanding of the pathogenesis of MS. EAE can be induced in a variety of species, including mice, guinea pigs and primates. It is a good model of MS and of organ specific autoimmune disease in general. Monophasic, relapsing and chronic progressive forms of EAE have been developed (see Figure 1). The monophasic form is a closer human counterpart of acute disseminated encephalomyelitis than of MS itself.

MS is induced by active immunisation with myelin proteins, such as PLP, MBP or MOG, or their encephalitogenic peptide fragments, in the presence of adjuvants. Alternatively, it is induced by passive (adoptive) transfer of T lymphocytes from immunised animals that are specific and auto-reactive for various neuroantigens. The adoptive transfer model suggests that EAE is T cell mediated, and that T cells are clearly important in MS.<sup>15</sup> Highly active T cells that recognise myelin antigen have been found in the blood and CSF of patients with MS, and this correlates with disease activity.<sup>1</sup>

There are two subsets of T lymphocytes, categorised according to which surface proteins they express and which class of MHC protein they recognise. Helper T lymphocytes (T<sup>H</sup> cells) are CD4 positive and recognise MHC class II protein. Cytotoxic T lymphocytes (T<sup>C</sup> cells) are CD8 positive and recognise MHC class I protein. There are also two sub-types of T<sup>H</sup> cells. These are termed T<sup>H</sup>1 and T<sup>H</sup>2 cells and differ in the cytokines they secrete and, therefore, the functions they perform.

For the greatest part, T<sup>H</sup>1 cells mediate EAE. The cytokines they release include interferon-gamma (IFN-g), tumour necrosis factor beta (TNF-b) and interleukin-2 (IL-2). IFN-g and TNF-b both activate

macrophages. Macrophages can strip myelin, destroy oligodendrocytes, and can produce further proinflammatory cytokines, such as TNF and IL-12. TNF may be directly cytotoxic to oligodendrocytes.

EAE is, therefore, largely a T<sup>H</sup>1 lymphocyte mediated disorder. Similarly, myelin-reactive T cells in the blood of an MS patient are predominantly T<sup>H</sup>1 lymphocytes.<sup>15</sup> However, the T<sup>H</sup>1/T<sup>H</sup>2 ratio may not be so clear-cut. In some circumstances, a shift to T<sup>H</sup>2 has been found to worsen the disease and EAE-like CNS inflammation can be mediated by T<sup>H</sup>2 lymphocytes.<sup>16</sup>

In MS, currently available immunomodulatory treatments (interferon beta or glatiramer acetate) act, in part, by suppressing T<sup>H</sup>1 lymphocytes while increasing T<sup>H</sup>2 lymphocytes.

CD8 positive T<sup>C</sup> cells have also been implicated in EAE and MS. T<sup>C</sup> lymphocytes specific for myelin and MBP have induced EAE.<sup>17</sup> These cells have also been found in significant numbers in MS lesions. They can lead to pathology in various ways. They can cause apoptosis (programmed cell death) by expressing Fas ligand and binding to Fas antigen on oligodendrocytes. T<sup>C</sup> cells can also release enzymes, such as perforin and granzymes, which can destroy targeted cells.

B lymphocytes are also suspected of having a role. Once activated, B cells produce antibodies or immunoglobulins. It was previously mentioned that CSF examination commonly demonstrated raised IgG synthesis in MS. Auto-antibodies to MOG, MBP, and other myelin proteins, have also been found in the serum or CSF of patients with MS. In the animal model, it has been recognised that administration of anti-MOG antibodies can exacerbate EAE.<sup>18,19</sup>

These processes could all lead to

demyelination. Denuded axons could be prone to axonal degeneration by the absence of trophic support, and are at increased vulnerability to an immune attack. Another contributing mechanism of axonal loss is destruction via toxic mediators, such as free radical nitric oxide, released by the inflammatory process.

In the inflammatory process, T cells in MS patients are activated and express adhesion molecules, allowing them to cross the blood-brain barrier. Proinflammatory cytokines enhance the expression of these adhesion molecules. Some proinflammatory cytokines, such as IL-12, also direct the differentiation of T cells into T<sup>H</sup>1 cells. Chemokines chemoattract inflammatory cells to the CNS. Matrix metalloproteinases have a dual role in directly mediating myelin destruction and facilitating blood-brain barrier permeability.

A plausible series of events in MS pathogenesis can occur, as in the following example. In a genetically predisposed individual, an environmental trigger (such as a viral infection) could lead to the development of antibodies, or autoreactive T cells, against myelin-based antigen. These antibodies cross from the systemic circulation to the CNS via the blood-brain barrier, which may be disrupted and, therefore, becomes more permeable. The circulating autoantibodies could induce demyelination. Presentation of the myelin-based antigen to T lymphocytes (combined with the appropriate MHC class protein) could also activate T<sup>H</sup> cells. This process releases cytokines that activate macrophages which, in turn, mediate neural injury. T<sup>C</sup> cells may also be triggered. The damaged CNS may expose new antigens, or new portions of an antigen, to the immune system, with continuation of inflammation. This is called epitope spreading and is demonstrated as a possibly important mechanism, both in EAE and in MS.<sup>20</sup>

Blocking this complex pathological process at any of these steps may be beneficial. Immune interventions directed at these processes have met variable, but encouraging, success in EAE. These include adhesion molecule blockade, cytokine suppression, nitric oxide suppression, enhancement of anti-inflammatory cytokines, and elimination of T cell subsets or macrophages. In addition, other approaches have been aimed at restoring neural function and integrity, or stimulating regeneration/remyelination. In addition, more non-specific immunomodulatory treatments may inhibit other pathological pathways simultaneously. Some of these interventions are beginning to be considered as possible ways forward for treating MS.

## CONCLUSION

The aetiology and pathogenesis of MS is unknown, but probably involves a combination of genetic and environmental factors and, possibly, chance. This combination triggers an immune process leading to inflammation, demyelination and axonal loss. The mechanism by which this occurs can vary between individuals, as evidenced by the heterogeneity in pathology, investigational results and treatment response.

EAE provides a useful experimental model, although it raises questions as well as providing answers. Further research is required to determine the cause of MS and to identify pathogenic mechanisms and, thus, therapeutic targets. This will then, it is hoped, lead to definitive treatment and cure.

*Credit for Learning begins on p29*

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