The pathogenesis of multiple sclerosis

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A complete understanding of the way in which the nervous system is damaged in multiple sclerosis (MS) must take into account a number of observations. They fall into three classes: first, pathological—myelin is destroyed with relative preservation of axons and a vigorous astrocytic response; second, epidemiological—the disease is commoner in high latitudes than in low and affects members of some races more often than others; and third, immunological—there is a strong association of multiple sclerosis with the HLA system and there are elevated titres of antiviral antibodies in blood and cerebrospinal fluid.

At present, we have no wholly consistent framework within which all these observations can be accommodated. After reviewing the aetiological factors implicated in MS and the nature of its immunological disorder, I shall enquire how the facts might fit together.

Aetiological factors in multiple sclerosis

Our present ideas about the aetiology of MS stem largely from the observation that it is more common in some parts of the world than others. Analysis of the data indicates that the peculiar geographical distribution of the disease results from a complex interaction of environmental and genetic factors. Much of the evidence is well known and so I shall concentrate on some recent observations that help to clarify the nature of the factors involved.

Environmental factors

The study of migrating populations, especially from high risk areas of Northern Europe to lower risk areas in lower latitudes has provided good evidence that the risk of developing MS is strongly influenced by where one spends one's pre-adolescent years—if in a region of high prevalence like the UK, the risk remains high if one migrates in adult life to a kinder climate with a lower risk; but it is reduced if one migrates there in childhood [1, 2]. The same principle *mutatis mutandis* is probably true in reverse [3].

The importance of the pre-adolescent years is emphasised by a study of a remarkable outbreak of MS in the Faroe Islands which are situated in the North Atlantic between Iceland, Norway and Scotland—all areas of high prevalence [4–6]. The islands' population is now about 45,000.

No cases of MS were seen there prior to the Second World War [4], but between 1943 and 1973 there were 32 [5]. The calculated mean incubation period was six years. The cumulative risk of MS for those born by 1940 was 110 per 100,000, about that expected for other high prevalence regions. When the time to onset from 1943 (or puberty, whichever came later) was plotted the distribution was log-normal, the hallmark of a point-source epidemic of infective nature.

The major change which occurred about this time was the advent of British troops in 1940. There was a close correspondence between the location of army camps and the residence of the patients who developed MS [5], which lends support to the idea of an infection having been transmitted from the troops to the previously unexposed Islanders. There are no clear leads as to its nature.

Evidence from other sources as to infection in MS is no more satisfactory. The increased levels of antibodies against viruses which have been repeatedly reported indicate that viruses *have* been present, but the evidence that they still are is wanting. There is no confirmed example of isolation of the same organism or fragments of it, from different brains in different laboratories, and no confirmed example of transmission of a central demyelinating disease from man to animals [7]. Finally, the virus-like particles seen from time to time at postmortem can, with one possible exception, be accounted for in other ways [8].

Although the epidemiological evidence strongly suggests that an infective agent is involved in the aetiology of MS, the evidence for a causal connection between a specific organism—viral or bacterial—and the initiation of the disease process is lacking. A connection between relapse and common presumed viral infection is however much more convincing. Sibley and his colleagues [9] in a carefully conducted prospective case-controlled study demonstrated a highly significant ($p < 0.001$) relationship between the occurrence of a relapse and a recent viral (but not bacterial) infection.

Genetic factors

A genetic factor in the aetiology of MS has long been suspected from the occasional familial clustering of cases and the racial predilection for the disease—it is an order of magnitude less frequent in Orientals than Caucasians,

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even when, as in Seattle, they share the same environment [10]. Convincing evidence that this effect *is* genetic rather than environmental in origin has only recently been forthcoming.

Ebers and his colleagues [11], in a Canadian population, compared the concordance rate for MS in monozygotic and dizygotic twins—a crucial test. Their study had the advantage of a demonstrably minimised ascertainment bias: the frequency of twinning was 13 per thousand which is as expected for their Canadian population. They found that the concordance rate in non-identical twins (2.3 per cent) was similar to that in siblings (1.9 per cent)—but in identical twins it was 25.9 per cent. This observation provides very strong evidence that a genetic factor is operating.

The second line of evidence comes from the study of genetic markers. It is now well established that there is an association between certain alleles of the HLA system and MS [12, 13]. The strongest is with HLA-DR2. Our London data are representative: HLA-DR2 was present in about 20 per cent of controls and 55 per cent of patients with MS [14]. A similar association is found in people of Northern European stock wherever they live.

The strength of the association decreases as one goes south in Europe towards the Mediterranean, and increases towards the north, even within the UK. So does the frequency of DR2 in the control population. This is well shown by Swingler and Compston's [15] recent study. They plotted the prevalence of MS in the UK against the frequency of HLA-DR2 in control populations matched as closely as possible with the sites of the prevalence studies. Although they are cautious in their interpretation, it is clear that MS is more common where DR2 is higher in the healthy population, this being most strikingly shown in Aberdeen [16] and the Orkneys and Shetlands [17].

This study raises an interesting point in relation to the geographical prevalence of MS. The forebears of people in the high risk areas of the New World came from high risk areas of Northern Europe. Understandably, migrants tended to settle in regions which resembled their homeland, where their skills would be useful in earning a living.

In New Zealand, for example, immigrant Scots settled in the south, while the English went further north. To this day, the proportion of Macs in the New Zealand telephone directories increases as one goes south [18] much as the frequency of HLA-DR2 increases as one goes north in the UK. And the prevalence of MS in Otago in 1981 was nearly three times that found close to Auckland.

Ebers [19] showed that the ethnic origin of the populations in high prevalence regions of the USA was from Finland and Scandinavia. The Fenno-Scandinavian population has strong racial ties with the North-East of Scotland where the highest known prevalences are found.

Thus, a significant determinant of the geographical distribution of MS is genetic, reflecting the natural predilection of human beings for surroundings which give them a sense of familiarity.

DR2 of itself does not confer susceptibility to MS. First, the association is not universal.It is not seen in the Japanese [20] or in the Gulf Arabs [21]. It is in some Mediterranean Arabs [29], though in others, as we found in Jordan [22], the association is with DR4. Secondly, in Hungarian gypsies, the population with the highest frequency (56.5 per cent) of DR2, MS is rare [23, 24].

The frequency with which some association is found suggests that it is likely to be relevant, and the simplest explanation would be that DR2 is acting as a marker for another gene (or genes) which is conferring susceptibility, and/or that additional genetic factors are involved. There is some evidence that this is so. We have recently found in Aberdeen, where the prevalence of MS is 178 per 100,000, an association with an allele of the newly identified DQ locus—DQw1 [16]. The same association has been found in the Pyrenees [25], and by comparing the sera in our London study of 10 years ago [26] with the DQ sera, it turns out that the same association (though originally given a different local laboratory name, BT101) is found there.

We also found evidence for an association with the 14th chromosome: the risk of developing MS in Aberdeen more than doubled for those who were DQw1 if they also possessed a particular Gm allotype (3;5,10,11,13,14) [27]. There are now three reports of an association of MS with Gm allotypes, though not all with the same one [28, 29].

Although the details of hereditary factors in the aetiology of MS have still to be worked out, there is clear evidence that genetic factors are operating and that they are probably concerned with the genetic control of the immune response.

The mechanism of tissue damage

The HLA association with MS raises the possibility that an immune mechanism is involved, since all the other diseases showing a significant association with DR2 have an autoimmune basis, with the possible exception of narcolepsy. The evidence for an immunological disturbance in MS comes from four main sources.

Cerebrospinal fluid. Oligoclonal bands and an increase in production of IgG by the brain are found in about 90 per cent of patients [30]. The level of C9, the terminal membrane attack component of complement, is lowered [31].

Antibody titres. There are raised titres of antibodies in blood and CSF against a variety of common viruses (see review by Johnson [7]). In one study nearly a quarter of patients had evidence of increased CNS synthesis of antibodies against two or more of these viruses [32]. In the absence of evidence of persisting viral infection these observations suggest that there is a pathological overproduction of humoral antibodies.

Peripheral blood lymphocytes. The ratio of T helper to T suppressor cells in peripheral blood is disturbed in active MS, most noticeably in the patients with steadily progressive disease [33, 34].

The brain. One of the reasons why progress in understanding the pathogenesis of MS has been slow is the impossibility of examining tissues at selected stages of the disease. Attempts have been made to correlate the patho-

logical findings at postmortem with the clinical state of the patient just before death. But the case notes are often poor at this stage and only rather crude distinctions—such as between an old lesion in which there is no evidence of myelin debris and a more recent lesion in which there is—are possible.

The plaques of MS are orientated around venules [35] which are frequently cuffed with cells. It is sometimes possible to trace a cuffed venule in continuity from a demyelinating region containing myelin debris into adjacent white matter of normal appearance. The cuffs are composed mostly of T cells but B cells and plasma cells are also present [36, 37]. T cells are also present in the lesion itself and although there is some disagreement about the proportions of different subsets, the balance of evidence is that suppressor cells predominate [38].

Other immunologically competent cells are also present as indicated by strongly positive staining for the immune-associated or IA protein. Some of this staining is undoubtedly on macrophages but Traugott and Raine [39] showed that it is also present on the endothelial cells of blood vessels in the lesion, and on astrocytes which are increased in number in the plaques and in the adjacent white matter of normal appearance [40, 41]. The recent work of Fontana and his colleagues [42] has shown that the astrocyte, at least in culture, is capable of acting as an antigen presenting cell; the possibility thus arises that both the astrocytes and the endothelial cells play a part in the active advance of the lesion.

The role of the T cells in the lesion is not yet clear. There are three possibilities. First, they may be an integral part of the process leading to myelin breakdown. There is no direct evidence that this is so, but the idea is attractive since demyelination resembling that seen in multiple sclerosis is mediated by T cells in experimental allergic encephalomyelitis. Secondly, the T suppressor cells at the edge of the lesion may represent a process limiting its spread: Esiri has argued rather convincingly that this is so [43].

Thirdly, it is possible that the lymphocytes and macrophages are unrelated to the pathogenesis of myelin breakdown and simply reflect a reaction to demyelination produced in some other way [8, 44]. Because of the difficulties of making precise correlations between the age of the lesion and its morphological appearance, it has not been possible to decide whether the immunopathological features are primary or secondary.

Our recent observations on the retina are relevant. Over the past 40 years there has been a number of reports of perivenous sheathing of the retinal venules in MS [45, 46], but there has been disagreement about the significance and even the validity of the observation. We therefore undertook a systematic study of the retina in 50 patients with isolated optic neuritis presenting to Moorfields Eye Hospital [47]. Perivenous sheathing was indeed visible in some patients and was associated with leakage of fluorescein at angiography. Approximately one quarter of the patients had evidence of vascular abnormality and/or cells in the vitreous at the time of an attack of acute optic neuritis.

Sheathing represents the visible expression of perivascular cuffing which is readily seen in the retina in MS [48], and there is a single example in the literature of a cuffed vessel which had been seen to be sheathed during life [35]. It is of particular interest that nearly two thirds of our patients with sheathing have already developed MS after a mean follow up of $3\frac{1}{2}$ years as compared with only one tenth of the remainder.

An early event in many immune reactions in the tissues is a vascular change: immunologically competent cells leave the circulation to reach the target tissue and are accompanied by exudation of fluid. In the retina and the brain in MS the normal vascular barriers break down, as shown by the abnormal permeability to fluorescein in the former and to iodine containing compounds [49] and gadolinium in the latter. The vascular changes in MS occur where there are certain anatomical peculiarities: continuous tight junctions between the endothelial cells [50, 51], and investment of the endothelium by glial processes derived from cells which express the glial fibrillary acidic protein, ie the astrocyte in the brain and the Mueller cell in the retina [52]. In some locations where these events occur (the retinae) there is neither myelin nor myelin forming cells; in others (in normal appearing white matter of the brain) the myelin is intact, and in yet others there is demyelination. That the vascular events occur in the absence of myelin breakdown products provides strong evidence that they are not a reaction to them. A more likely alternative is that they precede demyelination and lead to it as they do in experimental allergic encephalomyelitis [53].

The use of nuclear magnetic resonance

I have emphasised the difficulty of establishing the true sequence of pathological events in the development of an episode of MS because of the difficulty and ethical unacceptability of obtaining pieces of tissue at defined stages of the clinical evolution. The application of nuclear magnetic resonance (NMR) to the brain is beginning to change that.

Ian Young and his colleagues [54] at the Hammersmith Hospital showed just six years ago that NMR imaging is exquisitely sensitive in detecting abnormalities in the brain in MS. Through the generosity of the Multiple Sclerosis Society and with MRC support a group has been established at Queen Square for the investigation of MS using NMR.

Figure 1 shows a series of axial slices from a patient with MS. The irregular white areas are abnormal. When a complete set of images from this patient is processed by the computer techniques being developed in the Department of Medical Physics at University College, it is possible to gain a three-dimensional impression of the arrangement of much of the abnormal tissue (Fig. 2). The resulting image looks much like a cast of the ventricular system, but it is not: the computer has been set to discriminate between normal brain and CSF on the one hand and areas of abnormal signal intensity on the other. This distribution of lesions reflects that of the subependymal venous plexuses. There are usually additional discrete lesions remote from the periventricular region in

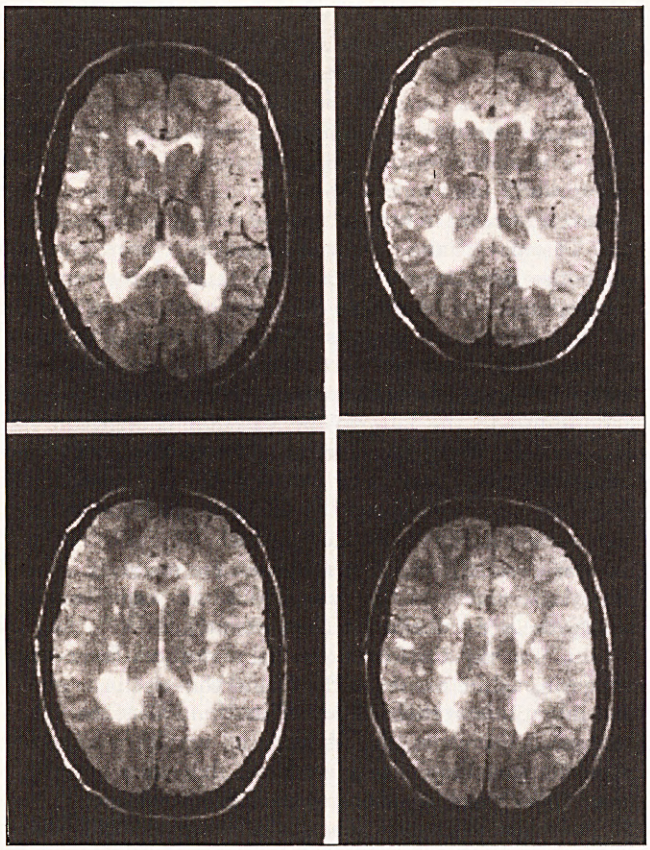

Fig. 1. *T_2-weighted axial NMR images of the brain in multiple sclerosis.*

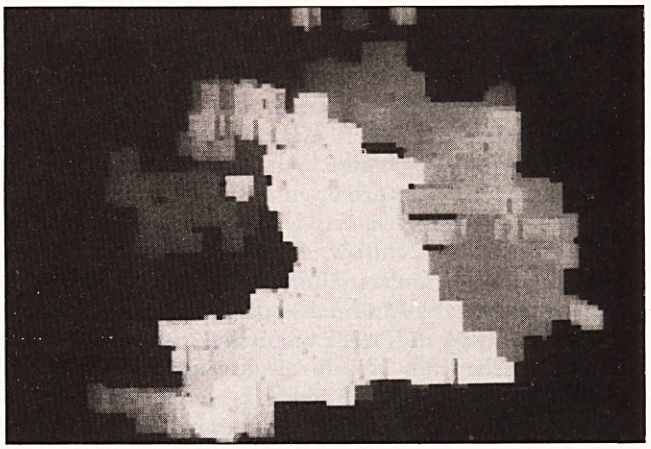

Fig. 2. *Quasi-3-dimensional reconstruction of the cerebral abnormalities in the brain of the patient shown in Fig. 1. (By permission of Mr J. S. Clifton, Mr S. R. Arridge, Ms S. R. Grindrod and Professor J. P. Moss of the Departments of Medical Physics and Orthodontics, University College, London)*

the cerebral and cerebellar hemispheres and the brain stem and spinal cord.

Ormerod and his colleagues [55] investigated the nature of the regions from which the abnormal signals were coming by scanning six formalin-fixed brains. Sections were subsequently cut in the same plane as the NMR sections and the abnormal images were found to correlate well with the areas of demyelination and gliosis, indicating that the plaques of MS can be identified by NMR imaging.

The principle of nuclear magnetic resonance suggests that it can give information on the different components of a lesion. The patient is placed in a powerful magnetic field. The free protons (mostly in water) behave like tiny bar magnets and align themselves with it. As a result the tissue becomes magnetised in the direction of the external field. To generate an NMR signal the protons must be at an angle to the external field, and this is achieved by applying a radio frequency pulse.

Each proton is spinning on its own axis, like a top. When it is deflected, the situation resembles that of a child's toy top tipped off the vertical — each proton then rotates about the direction of the field, as well as on its own axis. Rotating bar magnets induce electric currents in a suitably placed coil — which in NMR imaging is placed around the patient's head. The induced electric currents form the basis of the NMR signal. When the radio frequency pulse is turned off, the protons return to their original position in line with external field, and the current — that is the NMR signal — disappears.

Two processes are taking place. First, there is transverse relaxation which is the decline of the component of the tissue magnetisation in the transverse plane. Since the NMR signal is generated by this component of the tissue magnetisation, it is transverse relaxation which results in the decay of the signal. Its rate is characterised by T_2 and is strongly influenced by both the amount of water in the tissue, and the physical interaction between water and neighbouring macromolecules such as the components of membranes. Secondly, there is longitudinal relaxation, ie return of the direction of tissue magnetisation to that of the external field. The rate at which this occurs is characterised by T_1, the value of which is strongly influenced by the amount of water in the tissue.

Thus it should be possible to learn something of the tissue changes in disease by measuring T_1 and T_2, and, by comparing results from diseased brain with normal brain, to determine the amount of water in the tissue and the extent to which it is associated with protein.

To test this hypothesis Dr David Barnes has compared the NMR characteristics of two different types of experimental cerebral oedema, one rich in protein and the other poor [56, 57]. Protein-rich oedema was produced by applying a cold probe at the temperature of liquid nitrogen to the exposed cerebral cortex of the anaesthetised cat. This produced a superficial area of necrosis and extensive oedema of the subjacent white matter. Electron microscopy showed that the expanded extracellular space contained much granular material, characteristic of plasma protein. NMR imaging at 24 hours showed an abnormality corresponding with the distribution of oedema histologically (Fig. 3a).

Protein-free oedema was produced by intoxication with triethyl tin which gives rise to intramyelinic vacuoles

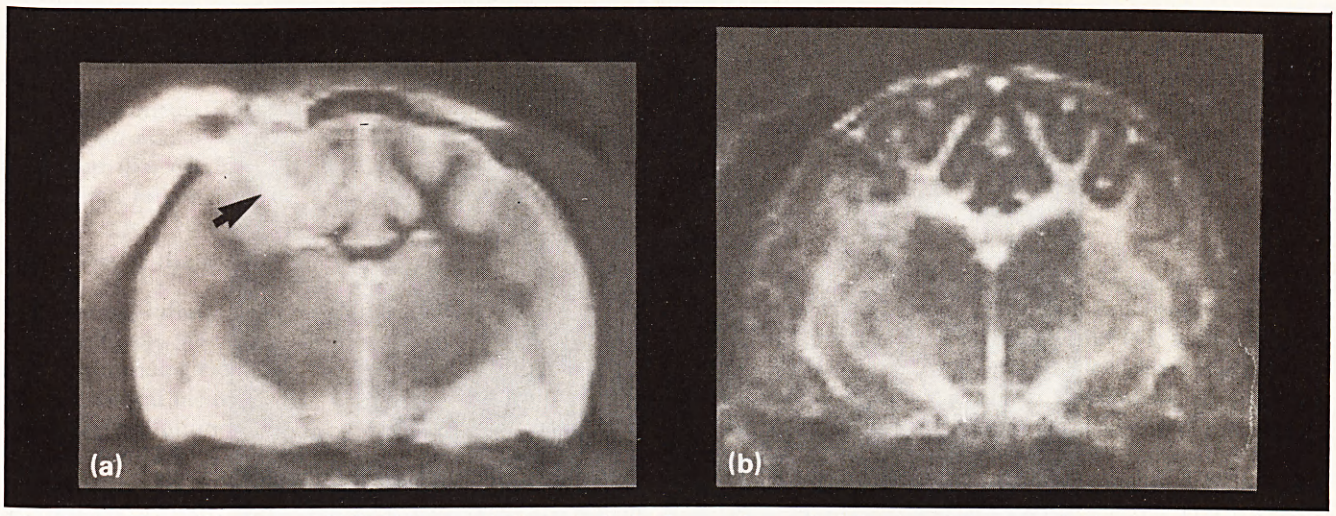

Fig. 3. (a) *T_1-weighted coronal NMR image of the cat brain 24 hours after cortical freezing. The arrow indicates oedematous white matter.* **(b)** *T_2-weighted coronal NMR image of the cat brain after daily injections of triethyl tin sulphate.*

throughout the white matter. Each is filled with fluid which is known from previous work to be protein free. NMR images showed abnormalities corresponding with the diffuse distribution of oedema shown histologically (Fig. 3b).

The two types of oedema are similar in appearance but can be distinguished by measurement of T_1 and T_2 (Table 1). First Barnes measured serially the changes in vasogenic oedema, in which protein is initially high and is absorbed by the endothelial cells and astrocytes over the next few days. Initially both T_1 and T_2 were increased approximately equally. As the protein was absorbed, the T_2 of the water protons increased relative to T_1 because their motion was now less restricted than it had been when they were associated with protein molecules. The ratio thus fell and approached that of triethyl tin oedema which is protein free.

Table 1. Two types of oedema distinguished by measurement of T_1 and T_2.

Experimental group	$\frac{\%\text{ increase } T_1}{\%\text{ increase } T_2}$
Vasogenic oedema 1 day (n = 16)	0.87 ± 0.14
Vasogenic oedema 3 days (n = 8)	0.68 ± 0.06
Cytotoxic oedema (n = 10)	0.50 ± 0.04

The oedema in the cold-induced lesion is absorbed during the first week and as this happens the NMR image of the oedematous white matter returns to normal. After about three months the image appearance of the previously oedematous white matter becomes abnormal again as astrocytic gliosis develops [58]. It is obvious that in the gliotic region there is a greater amount of cytoplasm per unit volume—and therefore of water on which the NMR characteristics depend—than in normal white matter. The NMR characteristics of gliosis are, however, different from those of oedema: whereas in oedema there

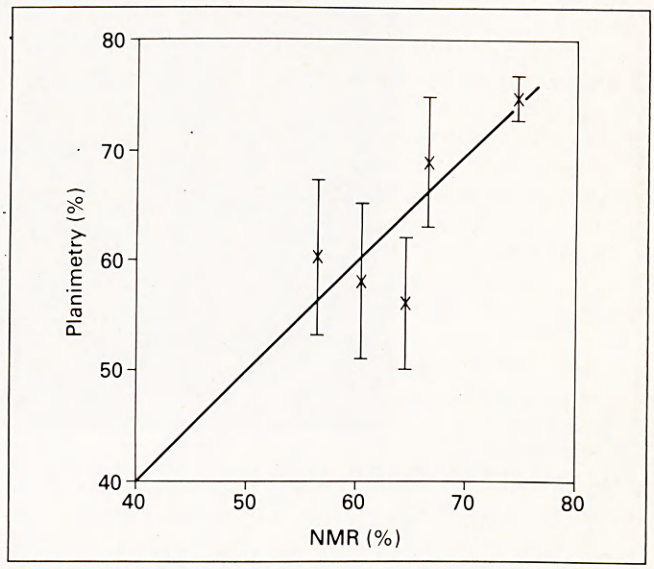

Fig. 4. *Relationship between the size of the extracellular space in experimental vasogenic oedema as determined by planimetry on electron micrographs and from magnetisation decay curves* in vivo. *The line is the line of identity.*

is a marked increase in T_2 due to an increase in the tissue's free water content, in gliosis T_2 remains relatively stable. This finding is probably due to extensive binding of intra-astrocytic water to densely packed glial fibrils and other tissue elements.

This sequence of changes in the experimental lesion suggested that it might be possible to distinguish acute and chronic lesions in MS. Ormerod and his colleagues [59] studying brainstem lesions showed that it probably is. Serial observations in individual patients with acute lesions showed a fall in both T_1 and T_2 with time. These observations are compatible with a reduction in the amount of water, ie with a resolution of extracellular

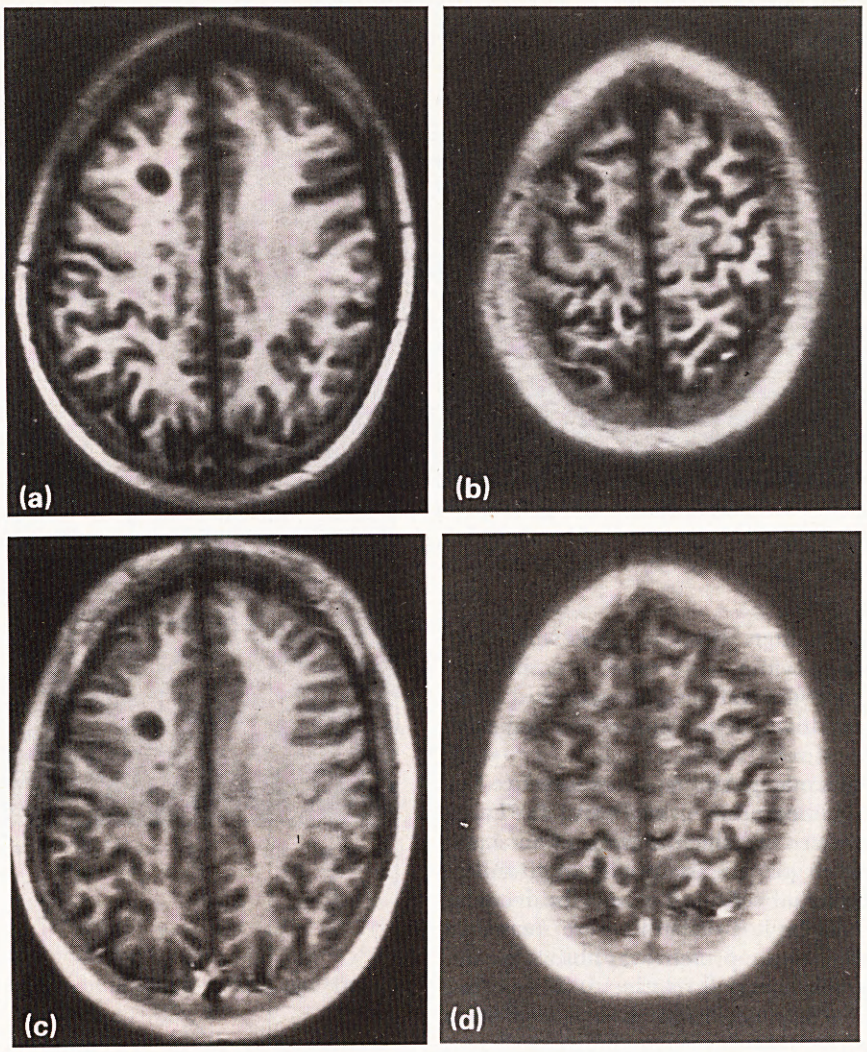

Fig. 5. *T_1-weighted NMR images from a patient with acute optic neuritis before* **(a,b)** *and after* **(c,d)** *injection of gadolinium-DTPA.*

oedema—and with an increase in the amount of water associated with macromolecules attributable to the replacement of myelin by cytoplasm-filled processes of astrocytes. We conclude that the abnormal NMR signals from the brain in the acute stages of development of a new lesion are primarily due to oedema.

The source of the abnormal signals in the chronic lesions is more complex. Tourtellotte and Parker [60] have shown that there is an increase in water in chronic cerebral plaques. This observation taken with the experimental findings suggests that gliosis plays a part but the relative importance of its contribution remains to be determined.

More detailed analysis of T_2 relaxation gives a measure of the relative sizes of the intracellular and extracellular spaces in acute experimental oedema. Figure 4 shows Barnes' plot of the relationship between the size of the oedema space in severe vasogenic oedema measured by planimetry on electron micrographs from five animals and that determined from magnetisation decay curves; the extracellular space is expressed as a percentage of the total tissue space. We are currently applying this technique to patients with MS and finding striking heterogeneity in the lesions in individual patients. Whether these differences between lesions represent differences in their biological activity or age is being investigated by examining the integrity of their blood-brain barrier using gadolinium as a marker.

The effects of gadolinium are well demonstrated by the inversion recovery sequence with which the lesions appear black instead of white. Figures 5a,b show cerebral scans from a patient with his first attack of acute unilateral optic neuritis who shows, as two thirds of such patients do, multiple lesions at presentation [61]. After the injection of gadolinium (Figs 5c,d) there is little if any change in the lesions shown in Fig. 5a, but the lesion high in the parietal region in Fig. 5b now appears white, indicating that the marker has leaked from the vessels into the tissues (Fig. 5d).

In summary we now have several complementary

NMR techniques for determining the way in which multiple sclerosis plaques change with time. The next step is to correlate these changes in tissue water and vascular permeability with the evolution of the changes in conduction and of the symptoms. This we are doing in optic neuritis, which has the advantage that the physiological and functional deficits are readily measurable.

Initiation of the disease process

I have argued that demyelination in the new lesion—or at least the newly active lesion—in established MS is mediated immunologically. But we do not know how an environmental factor might interact with the genetically determined immunological constitution of an individual to produce MS. Two different mechanisms might be relevant.

The first depends on the persistence of viral infection. Visna, a chronic lentiviral infection of sheep which results in central demyelination, provides the most attractive naturally occurring animal model for MS [7, 62]. It is strain specific and there is an incubation period of several years during which the animals appear healthy. The virus is difficult to recover. The infection persists and the periodic exacerbation of disease is due to sequential mutations in the regions of the viral genome that code for the production of envelope glycoproteins against which neutralising antibody is directed. As a result, the virus 'escapes' from time to time from antibody control and initiates new tissue damage. There are, however, some important differences between MS and Visna. Pathologically, Visna shows an intense meningitic reaction. It is readily transmissible, and MS is not. Viral DNA can be identified in infected cells and this has not so far been achieved in the human disease.

The second mechanism does not require the persistence of virus. Ter Meulen and his colleagues [63] infected mice with a corona virus which produced an acute encephalomyelitis, usually fatal. Some of the animals recovered and after an interval developed a second neurological illness but at a time when virus could not be recovered. Histologically, there was demyelination in the spinal cord. The disease could be transferred passively by lymphocytes that had been restimulated by exposure to myelin basic protein. Thus, a viral infection in a particular strain of mouse has led to the secondary development of autoimmune demyelination. As in the case of Visna, we must not press the analogy too far. We do not yet know whether this sequence of events can occur following infection by natural routes as opposed to intracerebral inoculation, and we do not know whether a spontaneously relapsing and remitting neurological disease can develop. At least we do now know of two possible mechanisms by which an infective agent could interact with a genetically influenced pattern of immune response to produce demyelination of the central nervous system.

Conclusion

All the necessary ingredients are present in MS for an immunologically mediated attack on central myelin. There is ophthalmoscopic, NMR and CT scan evidence of vascular damage. NMR provides clear evidence of oedema in acute lesions. At postmortem we see immune competent cells around venules and in the plaque itself, especially at its edge. There are cells expressing the IA protein that are capable of presenting antigens both in the plaque and in the nearby normal-appearing white matter. The evidence from the retina indicates that vascular changes histologically and functionally similar to those which occur in the brain in MS occur in the absence of myelin and oligodendrocyte antigens. These changes, being independent of myelin breakdown, are perhaps primary. When such changes occur in white matter, as in experimental allergic encephalomyelitis, immunologically mediated central demyelination can occur. Finally we know of two mechanisms by which a virus could induce such a process.

But there are gaps. We do not yet know the precise sequence of events in the development of a new lesion, how they relate to the production of symptoms and how myelin itself is broken down. We do not know how viral infection precipitates a relapse or what is the antigen exciting the immune response.

I have stressed that an important reason for the incompleteness of our understanding 150 years after the first good pathological descriptions of multiple sclerosis by Carswell [64] and Cruveilhier [65] is the inaccessibility of central nervous system tissue. The techniques of NMR are changing this and it is likely that in the not too distant future a more satisfying picture of the pathogenesis of MS will emerge.

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Erratum

Volume 21 No. 3 July 1987, p.192

Clinical assessment of Doppler cardiac ultrasound in valvular heart disease.

The Bernoulli equation published in this article was printed incorrectly. It should have read:

pressure gradient (mmHg) = 4 × velocity^2 (m/s)