Progress in Recent Research on Multiple Sclerosis

ALAN N. DAVISON

Miriam Marks Department of Neurochemistry, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG, U.K.

In recent years, there has been a marked increase in new information about multiple sclerosis. Undoubtedly this is related to advances made in scientific medicine, especially immunology, but there is also a greater awareness that something can, and must, be done to solve the problem of a crippling disease that affects 40000–50000 individuals in this country. Multiple sclerosis, or disseminated sclerosis, is a relatively common neurological disease, affecting 1 in 2000 of the population in England and about 1 in 800 in the Hebrides, Scotland. The age on onset is usually between 20 and 30 years, but it may occur up to the age of 50. The white peoples of North and Central Europe are generally susceptible to the disease as are races in other temperate areas. Data from epidemiological studies on migrants from high-risk areas (e.g. Europe) to low-risk areas (e.g. Israel or South Africa) have been interpreted as showing that the migrant carries with him, after the age of 15, the high risk of his country of origin. This suggests that an infective agent is acquired by multiple-sclerosis sufferers during childhood but alternative explanations should be considered. Orientals and many South Europeans and black Africans may be relatively unsusceptible to the disease (Acheson, 1977).

Multiple sclerosis is associated with various signs of damage to the insulating myelin sheath of the central nervous system and is characterized by the disseminated nature of the lesions in both time and space. As in rheumatoid arthritis, it is the resulting unpredictable relapses and remissions that make diagnosis and evaluation of treatment in clinical trials so difficult. Hence the development of improved laboratory methods for diagnosis and monitoring of the disease is of considerable value to the neurologist.

Neurophysiology

One considerable advance has been the use of neurophysiological techniques, such as the visual-evoked potential, which can help in quantifying the degree of central-nervous-system damage in a particular subject (Halliday & McDonald, 1977). Alternatively the auditory system may be examined by use of a standardized click stimulus. The evoked potential is more complex and depends on the structures in the brain stem as well as the forebrain. A high proportion of patients with clinical evidence of brain-stem demyelination have abnormal auditory-evoked responses and 50% of those without any apparent such disturbances also have abnormalities, in spite of the rarity of clinically apparent deafness (Robinson & Rudge, 1977). It is believed that, ultimately, a series of evoked-potential data in a single patient will enable hidden plaques to be detected in those patients with clinically probable, or possible, multiple sclerosis, and thus make the diagnosis definite.

Tissue antigens

There is some evidence, from familial studies, that an inherited factor is implicated in multiple sclerosis. It has recently been shown that subjects with certain tissue-antigen types (first identified as transplantation antigens, associated with cell rejection) have an increased incidence of certain diseases (including multiple sclerosis) that are thought to depend upon a disordered immune process. For example, patients with myasthenia
BIOCHEMICAL SOCIETY TRANSACTIONS

Gravis frequently possess the HLA-B8 type. The recent development of research on B-lymphocytes has shown that about 83% of multiple-sclerosis patients have one of the antigens (BT 101) compared with 33% in the normal population (Compston et al., 1976), so increasing their risk by 9.8 times. Susceptibility to multiple-sclerosis (associated with HLA antigen) may be due to an inherited defect in immunity, for the genes on chromosome 6 controlling the expression of antigens detected by lymphocytotoxic antibodies (designated A, B and C) are closely related to those of the immune response region (la gene). It is noteworthy that multiple-sclerosis patients in Jordan have a different antigen type (BT 102) (Kurdi et al., 1977), suggesting that they are exposed to a different virus affecting Arab compared with Northern European populations. It is more likely that the altered distribution relates to a different linkage disequilibrium with a specific la gene.

Antibodies in the cerebrospinal fluid

Although the total protein content of cerebrospinal fluid is 200 times less than that of serum, the concentration of immunoglobulin G is disproportionately lower (15% in serum, 3% in cerebrospinal fluid). During normal production of cerebrospinal fluid, plasma is selectively filtered so that only one molecule in 200 crosses the barrier of the choroid plexus (Thompson, 1977). Even this selection is in favour of smaller molecules [e.g. albumin (68000 daltons)] compared with immunoglobulin G (155000 daltons).

Since serum proteins are able to leak into spinal fluid owing to damage to capillaries (e.g. plaque near the ventricles), one must correct for any such passive transfer by relating immunoglobulin G values to another high-molecular-weight protein, e.g. a,-macroglobulin. Having allowed for this, multiple sclerosis is clearly still a disease in which the amount of immunoglobulin G in cerebrospinal fluid is much higher than amounts that can be explained by simple 'transfer' of the substance from serum.

The normal distribution of immunoglobulin G proteins in the serum is diffuse and homogeneous, and their isoelectric points vary greatly, i.e. they originate from many different lymphocyte clones. In multiple sclerosis and other diseases of the nervous system with a known 'infectious aetiology', a restricted heterogeneity of the immunoglobulin G is seen, i.e. there are a few 'bands' that are relatively diffuse in themselves and that probably represent the immunodominant antigens, each of which has stimulated several clones of lymphocytes to produce antibody.

About 75% of multiple-sclerosis patients have an increased concentration of immunoglobulin G in the cerebrospinal fluid and 90% of multiple-sclerosis patients oligoclonal bands can be detected on electrophoresis (E. J. Thompson, personal communication). The detection of oligoclonal bands in the cerebrospinal fluid is of considerable value in confirming the diagnosis of suspected multiple sclerosis and other conditions. The specific bands in the immunoglobulin G region indicate that there is probably an active inflammatory process occurring, for similar changes are seen in well-defined encephalitis (Thompson, 1977), and there are major changes in the oligoclonal pattern with an exacerbation (E. J. Thompson, personal communication). There is some evidence of an autoimmune reaction, namely, by using complement fixation it has been found that part of the increased antibody is directed against the brain itself (Ryberg, 1976). A small proportion reacts with various myxoviruses (particularly measles). Levy & Schoen (1976) have claimed, by using isotachophoresis of spinal fluid from multiple-sclerosis patients, that nucleotide-rich molecules co-migrate with immunoglobulin G on 5% polyacrylamide-gel electrophoresis.

Pathology

Multiple-disseminated lesions occur only in the central nervous system. They are frequently found in sites of predilection, such as close to the ventricles and in the optic tract (Adams, 1977). Older sites of damage became fibrotic so that fibrous plaques may be seen as scar tissue (sclerosis). Examination by optical microscope of plaques reveals primary demyelination with naked axons and accumulation of sudanophilic material (cholesteryl ester). One of the earliest changes preceding demyelination is a perivascular
Perivascular cuffing in brain of multiple-sclerosis patient (magnification × 850)

Plasma cells (p) and lymphocytes (l) are infiltrating from a blood vessel. (Photograph by courtesy of Dr. B. J. L. MacGregor.)
cuffing of mononuclear cells resembling that seen in experimental allergic encephalomyelitis and some virus-induced demyelinating diseases in animals (Plate 1). There are well documented examples (Adams, 1975) of perivascular cuffing without accompanying demyelination, suggesting that the inflammatory perivenule reaction precedes the attack on the myelin sheath. In the acute stage of multiple sclerosis the inflammatory reaction is accompanied by lymphocytes with a few lipid-filled macrophages and polymorphonuclear leukocytes are only occasionally seen. Later macrophages and plasma cells (activated B-lymphocytes) are observed within plaques. Some of the scavenging cells may be of endogenous origin [microglia, or even activated astrocytes (Raine et al., 1973)], but most are probably blood-borne macrophages.

Away from the lesion in areas of apparently normal white matter, it has been reported that there is astrocytic hypertrophy (Andrews, 1972; Arstila et al., 1973) in the absence of a cellular reaction. In apparently normal areas of white matter from multiple-sclerosis patients, Cuzner et al. (1976) found increased $\beta$-glucuronidase, arylsulphatase and acetylcholinesterase activity, probably reflecting lysosomal enzyme changes in affected glial cells. This is comparable with similar alterations seen in the early stages of scrapie, the slow virus disease of sheep (Kimberlin, 1973; Millson & Bountiff, 1973). Thus, it may be that in multiple sclerosis, an infective agent (e.g. paramyxovirus) acquired in the brain during childhood initiates damage to glial cells, releasing viral or glial antigens. However, despite repeated attempts, there has been no clear demonstration of such an infective agent either in tissue-culture experiments or clinically, after intracerebral inoculation.

Chemical changes in the plaques

Analysis of the demyelinated plaque tissue shows replacement of typical myelin proteins and lipids by glial fibrillar protein and choleseryl ester. At the active rim of the plaque and in newly demyelinating white matter, there is a selective loss of the myelin basic protein associated with increased acid catheptic and phospholipase A activities (for a review, see Davison & Cuzner, 1977). This observation has stimulated work both on the possible mechanism of myelin dissolution and on the likely involvement of encephalitogenic peptides in the pathogenesis. It seems likely that selective loss of the basic protein is effected via the cytoplasm of the formative cell, for there is good evidence that the basic protein is localized on the cytoplasmic surface of the myelin sheath and is therefore not exposed to easy extracellular-surface attack. Basic protein or derived antigenically active peptides have been detected in the cerebrospinal fluid of multiple-sclerosis patients with an exacerbation of the disease (Cohen et al., 1976). In addition Allen et al. (1976) have shown that, only up to 3 weeks after a relapse, there is a decrease in macrophage inhibition to a myelin-basic-protein peptide fragment that still retains encephalitogenic activity. It does seem that there is a defect in the immunological defence mechanism of the multiple-sclerosis patient, possibly related to an inherited factor. For example, a defective response of lymphocytes to measles has been demonstrated (Zabriskie, 1975) and the proportion of T-lymphocytes is decreased in an exacerbation.

Myelinotoxic factors in the serum of multiple-sclerosis patients

It has been shown that the serum from some multiple-sclerosis patients can demyelinate cerebellar explants grown in tissue culture (Bornstein, 1963). The factor present in serum from animals with experimental acute encephalopathy, multiple-sclerosis patients and some controls appears to act on oligodendrocytes and is probably antibody (Berg & Bergstrand, 1974). Thus Wolfgram & Duquette (1976) found that the myelinotoxic factor could be removed from serum of multiple-sclerosis patients by preincubation with a white-matter fraction that contains oligodendrocytes, but not with myelin. By using myelin-protein synthesis by rat white-matter slices as a measure of glial-cell viability (M. I. Sabri & A. N. Davison, unpublished work), evidence has also been obtained for a gliotoxic factor in the sera of patients with a relapse. Time-lapse studies in tissue culture by Yonezawa et al. (1976) suggest that the serum factor
initiates a cellular demyelinating action, for after addition of serum from animals with experimental acute encephalopathy macrophages can be seen removing myelin from nerve fibres. An alternative and less complex method for assaying oligodendrocyte viability is to measure myelin-protein synthesis by slices of white matter (Sabri & Davison, 1977). Serum from rabbits with experimental acute encephalopathy and serum from multiple-sclerosis patients with a relapse inhibits incorporation of amino acid (Pellkopër & Jatzkewitz, 1976; Sabri & Davison, 1978). Preliminary experiments suggest that this effect may be ascribed to complement-dependent antibody (M. I. Sabri & A. N. Davison, unpublished work). Antibody-dependent target-cell cytotoxicity in vitro can be mediated by different cell types, especially neutrophils (Rouse et al., 1977).

Antibody and cellular reactions in the brain of multiple-sclerosis patients

The presence of antibody (immunoglobulin G) in the brain of multiple-sclerosis patients has been known for some time (Tourtellotte, 1975) and appears to be derived from plasma cells in the brain, particularly round the ventricles (Fig. 1). At least some of the antibody is thought to be bound as immune complex localized on perivascular astrocytic membranes (Dubois-Dalcq et al., 1975). When immune complexes are deposited in tissues they produce an acute, sometimes severe, inflammatory reaction. Chemotactic factors derived from the complement system can attract lymphocytes and polymorphs that ingest the immune complexes, releasing chemical mediators that induce severe tissue and vascular injury, including haemorrhage and thrombosis (Movat, 1976). It is therefore of interest that increase in total neutral proteinase activity of polymorphonuclear leucocytes has been found to coincide with an exacerbation of the disease (Cuzner et al., 1975). There is a concomitant loss of acid phosphatase and neutral proteinase activities in the lysosomal granules (Tchorzewski et al., 1976), but no changes occur in the lymphocyte hydrolases. Slightly increased concentrations of lysozyme have been observed in the cerebrospinal fluid of multiple-sclerosis patients (Hansen et al., 1977). Since lysozyme is secreted by leucocytes, especially polymorphs and macrophages, this effect is consistent with an inflammatory reaction in the disease.

Conclusion

It appears that many multiple-sclerosis sufferers possess a characteristic tissue antigen type and that this is possibly associated with the nature of the immune response of the
individual. Defects in the delayed hypersensitivity reaction and biochemical changes in polymorphonuclear leucocytes during an attack of the disease again point to an important immunological component in the pathogenesis of multiple sclerosis. Although the question of the primary infective agent remains open, permanent damage to the central nervous system might be prevented if the secondary inflammatory reaction could be repressed by long-term treatment with selective anti-inflammatory drugs. Nevertheless it is unlikely that such treatment would be effective in chronic cases where degenerative change already predominates.

Kimberlin, R. H. (1973) Biochem. Soc. Trans. 1, 1058–1061
Robinson, K. & Rudge, P. (1977) Brain 100, 19–40
Sabri, M. I. & Davison, A. N. (1978) in Myelination and Demyelination Recent Chemical Advances, in the press