Human disease

Mechanisms in multiple sclerosis

from Byron Waksman

THE splendor of the great stairs and ceilings of the Würzburg Residenz, created by Balthazar Neumann and Tiepolo, provided a fitting backdrop for a recent discussion* of the baroque complexities of contemporary immunology in its application to multiple sclerosis (MS), a crippling disease of young adults.

Susceptibility to MS seems to be under multigenic control, with links to *HLA-D* locus genes *DW2* (*DR2*) on chromosome 6 (C. Jersild) and possibly to IgG allotype genes (*Gm*-1, 17;21) on chromosome 14 (J. Pandey *et al.*). A role for genes governing the T-cell receptor subunits and/or vasoactive amine sensitivity, suggested by the results of animal studies (see below), remains conjectural, as is the significance of increased frequency of spontaneous and induced chromosomal translocation in MS cells (G.R. Sutherland).

The evidence that MS in susceptible individuals is initiated by viral infection, at least in some cases, is increasingly persuasive. For the epidemic in the Faeroe Islands that followed the arrival of British troops in 1940, it seems that approximately 2 years of exposure to the putative infectious agent was necessary and that the period from infection to onset of clinical disease was about 6 years (J. Kurtzke). Susceptibility was low before puberty. In informal discussion, it was mentioned that MS elsewhere is associated with infections (measles, mumps, rubella, Epstein–Barr virus) caught significantly later in age than in HLA-matched controls (A. Compston, O. Andersen). In a well-studied cohort of patients, over 25 per cent of exacerbations were associated with such viral infections; from two weeks before to five weeks after infection, the exacerbation rate was increased more than threefold (W. Sibley).

Viruses such as measles, rubella, and varicella have been shown to sensitize T lymphocytes to myelin basic protein (MBP) in some subjects; this sensitization is very frequently associated with 'post-infectious' encephalomyelitis (R.T. Johnson). In a study of such encephalomyelitis associated with rubella, multiple T-cell clones obtained from cerebrospinal fluid showed equal reactivity with the virus and with MBP (P. Marquardt). Three independent computer searches have indicated the possible chemical basis for this cross-sensitization by demonstrating the presence of homologous peptide sequences in encephalitogenic regions of MBP and in antigens of measles, influenza, canine distemper, Epstein–Barr virus and several papova and adenoviruses (R. Fujinami, U. Jahnke *et al.*, G. Stoner).

A key point of current research is to determine the exact location where specifically-immune T Lymphocytes, circulating in the blood, first recognize myelin (or other) antigen, 'presented' in conjunction with major histocompatibility antigen (Ia). In agreement with the findings in animal studies, Ia has now been demonstrated on the endothelium of blood vessels within MS lesions and on activated astrocytes near the edges of lesions, as well as on the population of infiltrating macrophages (U. Traugott). MBP has also been demonstrated at these locations. Thus presentation sufficient to trigger T cells in the blood, or such T cells as enter the perivascular space, may occur at the luminal surface of the endothelium or the foot processes of the astrocytes. In older lesions, presentation may take place on the cell bodies of astrocytes, or other glia, and on the surface of macrophages.

The early MS lesions, triggered in the white matter of the central nervous system by these reacting T cells, show vascular injury with leakage of fluid, invasion of the tissue by a mixed population of lymphocytes and macrophages, and myelin destruction (C. Raine, H.L. Weiner). T lymphocytes, mostly of the T8$^+$ class but with some T4$^+$, predominate. Haematogenous macrophages play the principal role in myelin destruction at the advancing edge of the lesion (J.W. Prineas) by a mechanism that seems to involve receptor-mediated endocytosis. That the ligand for the receptor may be antibody is suggested by immunoglobulin 'capping' on many of the actively phagocytic macrophages. But extracellular lysis of myelin is also seen: oligodendrocytes at the edge of the lesion look healthy, while those in contact with lymphocytes or macrophages show mor-

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phological evidence of cell damage.

T cells found in the cerebrospinal fluid may reflect events in the central nervous system. Both $T8^+$ and $T4^+$ types are present and many carry markers characteristic of long-term cell lines (Weiner). There is one report of cells specific for MBP in very acute cases of MS (R. Lisak and B. Zweiman), but most studies of specificity suggest that the T cells are polyclonal and that many are MHC-autoreactive (A. Salmi, G. Birnbaum). The cerebrospinal fluid also contains raised levels of γ-interferon, even during clinical remission (R. Hirsch). The B cells and plasma cells of the lesions and the cerebrospinal fluid show a polyclonality that is comparable to that of the T cells and affects all the major immunoglobulin classes (K.P. Johnson, H. Link). The much debated 'disappearance' of $T8^+$ cells from the peripheral blood early in acute attacks (J. Antel and B.G.W. Arnason) has been attributed either to their actual migration into the target tissue (Weiner) or to modulation of their phenotypic markers by, for example, prostaglandins released from activated monocytes in the circulation (P. Dore-Duffy, J. Merrill). A striking new finding is the absence, in most patients, of cytotoxic T-cell precursors specific for measles virus (H. McFarland). There is no comparable deficiency for influenza virus.

For many of these crucial new findings, the study of animal models with conditions that resemble MS has led the way. Experimental allergic, or autoimmune, encephalomyelitis (EAE), induced by immunization with MBP or proteolipid protein, may take a chronic relapsing or progressive form, particularly if immunization is at the time of weaning (equivalent to puberty) (Raine, D. McFarlin, F. Lublin, T. Tabira). Certain temperature-sensitive mutants of the JHM virus produce a subacute or chronic inflammatory demyelinating disease in rats that is associated with sensitization against MBP; lymphocytes from these animals produce typical EAE in normal recipients (H. Wege, R. Watanabe). Theiler's murine encephalitis virus (TMEV) produces similar disease in SJL mice and certain other strains (H. Lipton); although there is an immune response against myelin, the continuing reaction may be directed at persistent viral antigen (Lipton, P.L. Lampert).

The use of recombinant inbred mouse strains has shown there to be multigenic control of susceptibility to the MS-like disease, as well as to its intensity and character, in both EAE and TMEV models. For EAE, both high-responder and low-responder strains have been found (Lublin). The high responders, most frequently associated with $H\text{-}2^s$, readily develop chronic relapsing or progressive disease. Earlier studies in acute EAE had mapped the responsible *H–2* gene to *I–A* (R. Fritz). Genes controlling vascular sensitivity to vasoactive amines are also important (S. Linthicum). For the TMEV system, an important genetic control, related to the intensity of the T-cell mediated response against viral antigen, maps to *H–2D*; another seems to be related to the gene encoding the β-chain of the T-cell receptor.

Lines or clones of T cells specific for MBP produce acute or chronic EAE when activated (with mitogen or specific antigen) *in vitro* and transferred to normal syngeneic recipients (I.R. Cohen, H. Wekerle). The delay of 72 – 96 hours before the first inflammatory cells appear in the central nervous system (R. Meyermann) can be accounted for by the time required for the transferred cells to produce heparanase, which permits them to penetrate the vessel wall and enter the tissue (Cohen), and to produce γ-interferon, which activates vascular endothelial cells and astrocytes. The delay includes time for both cell types to synthesize and express Ia, which can be demonstrated on macrophages infiltrating lesions (W. Fierz, Sobel).

An important new finding is that purified endothelium from brain vessels can be stimulated *in vitro* by activated T-cell supernatants or purified γ-interferon to express Ia and present antigens such as MBP to specifically sensitized T-cells (McFarlin). Similar data for purified astrocytes have been reported (B. Fontana). The importance of presenting myelin antigen in association with Ia is clearly established in experiments with cell lines transferred from, or to, either of two rat strains and their F1 hybrid (C. Linington). JHM virus particles, however, are capable of directly activating astrocytes without mediation by γ-interferon (P. Massa). Manoeuvres which stimulate systemic γ-interferon and/or reduce suppressor T-cell activity trigger relapse, even in normally resistant animals, such as Lewis rats that have recovered from acute EAE. An important confirmatory finding is that MBP-specific suppressor cells are present in the spleens of sensitized rats during periods of remission but not during exacerbation (W. Lyman and C. Brosnan).

A hypothetical sequence of events in MS, which merges findings reported at the symposium with reports in the recent literature, is presented in the table. Several points of attack for potential therapies are suggested by the scheme. It would be worth testing the value of monoclonal antibodies directed at phenotypic markers or characteristic activation antigens of appropriate T-cell subsets; at the T-cell receptor or its subunits, particularly idiotypes related to specific myelin antigens; or at Ia (Weiner). Hybrid or toxin-linked antibodies may be advantagous. Other leads come from studies of EAE. Vaccination with MBP-specific lymphocytes, inactivated with X rays or mitomycin C, induces resistance (idiotype tolerance?) to actively induced EAE (Wekerle); lymphocytes inactivated by hydrostatic pressure (which changes the physical state of membrane glycoprotein) are particularly effective, inducing a resistance that is transferable with T cells (Cohen); prolonged treatment with MBP plus galactocerebroside not only turns off chronic EAE but promotes remyelination, perhaps due to oligodendrocyte stimulation by antibody to galactocerebroside (Rodriguez).

All in all, participants at the meeting were imbued with a sense of optimism that both the complex scientific problems related to MS and the practical issues of its prevention might be nearing solutions. □

Hypothetical sequence of pathogenetic events in multiple sclerosis

Acute phase

Systemic virus infection (measles, varicella, influenza)

Primary (cross-reactive) immunization to MBP

Circulating T lymphocytes specific for MBP

Systemic interferon (α, β, γ) response

Activation of vascular endothelium (and astrocytic foot process?) by viral particles and/or by γ-interferon

Expression of Ia in vascular endothelium body-wide and in CNS

Acute phase events in CNS vessels

Expression of Ia and presentation of MBP

Dual recognition by MBP-specific T cells

Activation of T cells leading to nonspecific secondary inflammation

Leakage of fluid producing oedema

Invasion of tissue by monocytes (macrophages), T cells and B cells

CNS tissue damage by oedema and invading cells

Demyelination by activated macrophages

Damage enhanced by local antibody formation, complement from circulation, free oxygen radicals (?)

Arrest of conduction, due to fluid pressure, loss of myelin, and perhaps T-cell lymphokines and free MBP

Chronic phase

Activated T cells produce IL-2 and γ-interferon locally

Activation of astrocytes by γ-interferon

Expression of Ia, release of IL-1 and prostaglandins by astrocytes invading macrophages

Proliferation, synthesis of GFAP, gliosis

Activation of Ia-reactive T cells in response to Ia, IL-1 and IL-2 in lesions

Persistent foci of Ia-reactive T cells

Continuing nonspecific secondary inflammation

Systemic virus infection-reactivation of process

Astrocyte activation by systemic γ-interferon

Renewed activity of local Ia-reactive cells

Enlargement of existing lesions

New lesion formation, as in acute phase

MBP, myelin basic protein or other myelin antigens; Ia, major histocompatibility complex antigen (Class II); IL-1 and IL-2, interleukins-1 and 2; GFAP, glial fibrillary acidic protein; CNS, central nervous system.

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Multiple sclerosis and viruses

Evidence that human T-cell lymphotropic viruses are associated with multiple sclerosis may lead to identification of viruses that cause the disease — or to an addition to the list of failed candidates.

It is not every day that the Faroe Islands are mentioned in the scientific literature, but no contemporary review on the causes of multiple sclerosis is complete without a mention of them. While the disease was unknown in the Faroes before 1940, a mini-epidemic was recorded in the years after British troops were garrisoned there in 1940. There seems little doubt that an infectious agent was involved; it is now conventional to suppose that multiple sclerosis can be initiated by viral infection in genetically susceptible individuals. But which virus or viruses are the culprits?

Frustration has marked the hunt. By some counts, twelve separate viruses have at one time or another been implicated as initiators of multiple sclerosis. But in no case has the case been made to stick. So the first evidence of the frequent exposure of multiple sclerosis patients to a new virus type is bound to excite an equal mixture of interest and scepticism. Publication of the data, moreover, is bound to quicken the pace of the further research that will show whether the new type of virus is genuinely an initiator of multiple sclerosis, or whether it will join its dozen or so predecessors on a list of suspects. It is in this spirit that we publish, on page 154, the tantalizing but inconclusive evidence from Hilary Koprowski, Robert Gallo and their colleagues of an association between multiple sclerosis and the human T-cell lymphotropic viruses (HTLVs).

This provisional group of viruses comprises three members. HTLV-I was the first to be discovered, in 1980, and is the cause of adult T-cell leukaemia discovered in Japan. HTLV-II is a variant of HTLV-I; and HTLV-III (also known as lymphadenopathy-associated virus, LAV) is the cause of acquired immune deficiency syndrome (AIDS). Although HTLV-III lives up to this name in some ways, notably a propensity to infect human T lymphocytes, its nucleic acid sequence is not related to that of HTLV-I or HTLV-II. Another view, now strengthening, is that HTLV-III is more related to the lentiviruses than to the other HTLVs. Evidence comes from both structural comparisons and the fact that the HTLV-III can be found in brain tissues of certain AIDS patients (Shaw, G.M. *et al. Science* **227**, 117; 1985). Lentiviruses are animal viruses that cause progressive demyelinating diseases of the brain and spinal cord. Indeed, the disease caused in sheep by visna virus, the most studied of the lentiviruses, is often said to be the best naturally occurring model for multiple sclerosis.

Inevitably, therefore, attention has been turned to the possibility that HTLV-III or a related virus is the cause of multiple sclerosis. Ironically, the evidence of Koprowski *et al.* is if anything more suggestive of an association between multiple sclerosis and HTLV-I or HTLV-II than with HTLV-III. Indeed there is no uniform response among the patients studied to tests for any of the three HTLV types; the authors say this conceivably indicates the presence of one or more entirely new HTLV-like viruses, possibly including a virus that combines some of the features of all three known HTLVs.

Leaving aside the identity of the viruses responsible for the signals detected in the tests, what and how strong are the signals, and to what extent can they be interpreted as evidence that the viruses in any way cause the disease? The signals are of two distinct types: the presence in the blood and cerebrospinal fluid of antibodies that cross-react with HTLV proteins, and the presence in cells cultured from cerebrospinal fluid of HTLV RNA. The former indicates that the viruses have been present, the latter that they are still present and active.

There are three problems in interpreting the antibody data. First, although it is clearly shown that the concentration of antibodies that cross-react with some HTLV proteins is greater in most serum and cerebrospinal fluid samples from multiple sclerosis patients than in various controls, such antibodies are not invariably present. Second, no consistent picture emerges as to which HTLV the antibodies are most closely related, although HTLV-I is much more frequently and usually more strongly implicated than HTLV-III. Third, and most important, it is already known that elevated concentrations of antibodies to measles and Epstein–Barr virus are frequently found in the cerebrospinal fluid of multiple sclerosis patients, throwing doubt on the significance of raised antibodies to other viruses.

For the data that indicate the presence of RNA that is related to HTLV RNA in cultured cerebrospinal fluid cells, at least there is no consistency of HTLV type. In the 4 out of 8 cases where RNA has been detected, it is related, though not very closely, to RNA of HTLV-I but not HTLV-III. When present, the RNA is detected in less than one cell in ten thousand, which may at first seem too low to be of significance, but this rate is similar to the frequency of HTLV-III RNA in T cells of AIDS patients. Moreover, it is a general observation in chronic virus infections, including visna and measles, that only 0.1 to 1.0 per cent of cells in any tissue contain viral genetic information. Much more of a problem is that the controls are so far limited to two healthy subjects, neither of whom had HTLV-related RNA in the cells of their cerebrospinal fluid.

When Ashley Haase and his colleagues first reported measles virus RNA in several multiple sclerosis brains (*Science* **212**, 672; 1981), the data, together with those on elevated antibodies to measles virus in multiple sclerosis, made the measles virus a strong candidate as the initiator of the disease. But further investigations revealed that measles virus RNA can be detected almost as frequently in the brains of patients with other neurological and non-neurological diseases as in those with multiple sclerosis, weakening the case for the involvement of measles virus in multiple sclerosis, though not excluding it.

Clearly, more data, and particularly more controls, will be needed before the case for an HTLV-type virus as the initiator of multiple sclerosis can be realistically assessed: the National Multiple Sclerosis Society in New York has already organized several laboratories to begin participating in studies of the putative association with HTLV. There will also be intense efforts to try and isolate the virus or viruses that are the source of the HTLV-I-like RNA. Until that can be achieved, there can be little progress in understanding the variability of the HTLV-related antibodies in multiple sclerosis.

Even if a virus can be proved to be implicated in multiple sclerosis, it is likely to be merely the initiator of a chain of immunological events leading to demyelination of nerve fibres rather than a direct cause. It seems probable that the initial viral infection somehow triggers an autoimmune reaction against components of the myelin sheath of nerves. "The baroque complexities of contemporary immunology in its application to multiple sclerosis", as Byron Waksman puts it in summarizing current research on page 104, will require considerable unravelling before it is clear how the disease progresses from the triggering event.

Peter Newmark