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Altered immune function in demyelinative disease

from Dale McFarlin and Byron Waksman

Experimentally induced demyelination has an autoimmune origin in some mouse models and is somehow virally induced in others. In June, invited participants in a Kroc Foundation workshop discussed the damaging mechanisms involved and their relevance to an understanding of multiple sclerosis and human encephalomyelitis.

Human demyelinating diseases include post-vaccinal encephalomyelitis (PVE), post-infectious encephalomyelitis (PIE) and various forms of multiple sclerosis (MS). PVE was first described after the introduction of rabies virus vaccine and was almost

certainly caused by autoimmune reaction to the spinal cord used in the vaccine preparation. PIE can occur after immunization with vaccinia virus or infection with varicella, rubella or rubeola. Because live virus vaccination against rubeola has strikingly reduced

the incidence of measles epidemics, PIE has become rare in the U.S.A. but in the many regions which still have measles epidemics around 1 in 1000 cases is complicated by PIE. Consequently, PIE after measles infection may be the world's most common demyelinating disorder. Approximately 1 case in 4 is fatal. Pathologically it is characterized by primary demyelination (breakdown of myelin with preservation of axons) and perivascular infiltration of mononuclear cells. However, measles antigen cannot be detected in the nervous system immunocytochemically and the production of antimeasles antibody in the CNS is not increased. Recent investigations of measles epidemics in Lima suggest that the condition is related to an immune reaction against components of the nervous system. Blood lymphocytes from most patients show a proliferative response to myelin basic protein (MBP) — a somewhat specific response which is much less frequent in other neurological diseases such as uncomplicated rubeola infection and rubeola pneumonia.

The most common human demyelinating disorder in North America and Europe is MS, manifested by inflammation and primary demyelination at multiple sites and times within the nervous system. Although its cause and pathogenesis are unknown, both genetic and environmental factors appear to contribute. The disease is common in Caucasians but is virtually absent in Bantu, Inuit, and certain Mongolian populations. In Northern Europe and North America it is linked to HLA-DRw2 but to other DR-specified antigens in other geographic regions. Epidemiological observations link MS to an exogenous environmental agent. First, there are areas of high or low risk, with a well established north/south gradient in North America and

The protein-phospholipid material myelin swathes axons in a multi-layered dense spiral, akin to insulating material around wires. In the central nervous system (things differ in the periphery) myelin layers are formed by the compacted membranes of extensions of oligodendrocytes, one of the two types of satellite cell that surround most of the brain's neurons. The integrity of the myelin sheath therefore depends on the maintenance of normal oligodendrocyte function. Demyelination need not be accompanied by damage to the axon itself but it considerably slows nerve conduction and so can result in loss of coordination, paralysis and death.

In the diagram the axon is shaded in grey and the oligodendrocyte cytoplasm in black.

continued on p. 323

Demyelinative diseases – from p. 321

Northern Europe. Second, migrants between areas assume the risk of the region to which they migrate if they go before approximately aged 15; if they go later they take the risk of the region of origin with them. Third, epidemics of MS have recently been described. Such findings suggest that a viral agent produces a chronic or latent infection which causes MS but extensive efforts to isolate a MS-specific agent have failed. It has been suggested, on the basis of recent findings, that any of the more common viral infections may non-specifically initiate the MS process in susceptible individuals and trigger later exacerbations of the disease.

How an infectious agent could produce demyelination in MS or PIE is unknown. At least three mechanisms are possible: direct infection of the oligodendroglia (the myelin-producing cells) in brain and spinal cord; an immunopathological reaction and myelin damage produced by an immune response against viral antigen; induction of autoimmunity to myelin antigens. There is immune aberration in MS. This includes a pathology with many features in common with PVE, PIE and experimentally induced allergic encephalomyelitis (EAE); increased immunoglobulin production within the central nervous system; serum factors, some immunoglobulin, that produce demyelination *in vitro*; abnormalities of peripheral blood leukocytes (including reduced T_H cells, reduced suppressor activity induced by Con A, reduced peripheral blood lymphocytes bearing either OKT5 or OKT8 phenotypes, and decreased natural killer activity and interferon production). However, attempts to show cellular immune reactivity against a component of myelin have universally failed.

Animal models

Recent developments in the understanding of immunoregulatory circuits, idiosyncratic responses and genetic control of the immune response to H-Y antigens have provided important background information to the study of demyelination in mice. Many of the disease models have been produced in the SJL strain which has some unique immunological features. SJL mice are deficient in T-cell-mediated suppression, manifest spontaneous autoimmune pheno-

mena, and are resistant to tolerance induction, even early in life. This strain also has a high incidence of spontaneous recurring reticulum cell sarcomas which have NK activity and produce interferon. The $I-A^+$ tumor cells stimulate proliferative reactions by the normal immune cells. These alterations in immune function may contribute to the occurrence of CNS diseases.

EAE is produced easily in many species. The encephalitogenic determinants of MBP have been defined in rats and guinea pigs and susceptibility to the disease is clearly linked to genes of the major histocompatibility complex (MHC). Murine EAE traditionally has been more difficult to induce but in recent years three different forms of the disease have been studied.

Acute experimental allergic encephalomyelitis (AEAE)

AEAE is produced by the injection of mouse spinal cord homogenate in Freund's adjuvant followed usually on days 0 and 2 by two doses of *B. pertussis* i.v. The strain and dose of pertussis are important variables. AEAE can also be induced with MBP and the encephalitogenic determinants are apparently not the same for all strains, (residues 89–170 in SJL mice but residues 1–37 in PL/J mice). AEAE can be produced in a limited number of mouse strains and has been studied most extensively in SJL mice, where it is under the control of multiple genes. At least two have been identified, one linked to *H-2* and involved in the response to components of cord homogenate, the other not linked to *H-2* and apparently controlling vascular sensitivity to the histamine sensitizing factor (HSF) produced by some strains of *B. pertussis*. Presumably this affects the passage of immune cells from the microvasculature to the CNS. The most convincing evidence of the interaction of these two genes comes from the study of F1 progeny of two relatively resistant parents. For example, F1 progeny of B10.S (*H-2*-determined susceptibility, HSF resistant) and DDD (*H-2*-determined resistance and HSF susceptible) were highly susceptible to EAE. Studies of EAE in recombinants between (BALB/c × SJA) F1 and each parental strain indicate that other genes may also contribute. Throughout the conference other examples of polygenic immune influences became apparent. These include the response

to the H-Y antigen and the immune abnormalities leading to the development of immune complex disease in mice.

Resistance to AEAE in some but not all mouse strains is due to T_S cells which seem to be operative during induction. For example, treatment of resistant BALB/c mice with cyclophosphamide (20 mg kg^{-1}) two days prior to immunization renders them susceptible. Resistance can be re-established by the transfer of splenic T cells. However, other resistant strains, such as C57 BL/6 and DBA/2, are unaffected by cyclophosphamide pretreatment and resistance is attributable to other mechanisms. Efforts are in progress to obtain T_S factors which will inhibit the induction of AEAE. An extract of splenic T cells, obtained from resistant BALB/c mice by adherence to MBP-coated plastic dishes, contains an antigen-binding factor which suppresses the disease as well as delayed hypersensitivity reactions to MBP in other strains. AEAE was also inhibited by treatment during induction with anti-I-A^s but not anti-I-J^s sera. This treatment may modify I-A^s effector lymphocytes, antigen-presenting cells or both. Pharmacological agents such as cyproheptadine and methysergide which block receptors for histamine and 5-hydroxytryptamine, also inhibit AEAE.

Although AEAE is valuable for the investigation of certain immunological problems, it was pointed out that its pathological characteristics are somewhat different from those in MS. The overall picture is mid-way between that seen in other species sensitized with whole nervous system tissue in complete Freund's adjuvant (CFA) and the hyperacute EAE produced in Lewis rats by the addition of *B. pertussis*. In AEAE there is a prominent polymorphonuclear response associated with extravasation of fibrin and red cells. Although primary demyelination is an early transient feature, nerve fiber depletion resulting in Wallerian degeneration and gliosis are prominent. Axonal destruction may occur, in part, because the spinal cord preparation used to induce disease contains neuronal as well as myelin antigens.

Chronic relapsing experimental allergic encephalomyelitis (REAE)

This disorder has been produced only in SJL mice, by two regimens:

immunization with an emulsion of spinal cord, CFA and *B. pertussis*; or 1 mg cord in CFA containing 30 µg *Mycobacterium tuberculosis* H37RA given on two occasions 8 days apart without pertussis. Clinically, REAE is characterized by multiple episodes of neurological dysfunction. These can be correlated pathologically with lesions of different ages. With each episode there is edema and influx of perivascular leukocytes including PMNs, lymphocytes and macrophages, associated with primary demyelination followed by gliosis and partial remyelination. Schwann cells infiltrate the CNS and participate in the remyelination. Reduction in suppressor-cell activity by treatment with cyclophosphamide results in both an increase in relapses and intensification of the lesions. Triphasic EAE occurs in SJL × PLF1 mice after challenge with MBP but its pathology is not known. The possibility that other myelin antigens, such as proteolipid, various glycoproteins, or combinations of proteins with simple lipids, may play a role in induction of REAE also, has not been investigated.

Adoptively transferred experimental allergic encephalomyelitis (TEAE)

TEAE has been studied in rats, mice, and guinea pigs. To produce it in SJL mice, lymph node cells are removed from animals 10 days after sensitization and cultured with MBP for 4 days before adoptive transfer into syngeneic recipients. Neurological disease appears in nearly all the animals given 3×10^7 cells or more. The disease is monophasic and mononuclear cells infiltrate along veins within the CNS but other pathological details and specifically the amount of myelin destruction have not been established.

Negative selection experiments have shown that $\text{Ly}1^+2^-$ T cells transfer the TEAE while $\text{Ly}1^-2^+$ T cells do not. What then do $\text{Ly}1^+2^-$ T cells do? Antibody against MBP has not been detectable in the recipients and in a single experiment the transfer of cells into SJL nude mice resulted in disease. $\text{Ly}1^+2^-$ cells may therefore directly produce the disease. Other observations support this idea. In a single experiment AEAE could not be induced in SJL hairless mice, which have a defect in the maturation of $\text{Ly}1^+$ T cells and immunostaining techniques have demonstrated that most of the cells in the perivascular

lesions of AEAE bear the $\text{Ly}1^+$ marker. Similar studies of TEAE in rats have shown that antigen-presenting cells plus IL2 are involved in the stimulation of the cells responsible for the transfer. These bear the markers which belong to the helper/inducer subset of T cells. Finally, a T-cell clone which can transfer EAE in Lewis rats has been developed and found to have markers for the helper/inducer subset of T cells.

This combination of experiments has identified important questions. Contemporary immunological dogma indicates that the helper/inducer subset of T cells recognizes antigen in association with Ia molecules, encoded by immune response genes. In the various forms of EAE, what do the effector cells recognize, where does this recognition occur, and how does it lead to tissue damage?

Viral infection of the CNS

Demyelination results from CNS infection by a number of viruses. Among the most widely studied are Theiler's virus (TV), a picornavirus, and mouse hepatitis virus (MHV), a coronavirus. Inoculation of mice with TV produces an acute poliomyelitis associated with infection of anterior horn cells. Antibody appears after seven days and persists throughout life. A chronic disease ensues, with plaques of inflammation and primary demyelination in CNS white matter. Its development is somewhat strain dependent, being most frequent in SJL mice but also appearing in C57/BL6 and C57/BL10 animals. Some strains of TV produce the chronic disease without any preceding acute lesion. Virus can be recovered throughout the duration of the disease but it is not known if oligodendroglia are persistently infected; TV antigen is present in large cells within the CNS which appear to belong to the macrophage series. Immunosuppressive treatment can prevent the chronic demyelination, which strongly suggests that the lesion has an immunological basis.

The type -4 or JHM strain of MHV causes primary demyelination as well as encephalomyelitis by direct infection of oligodendroglia and neurons, respectively. Strain susceptibility to JHM varies and is controlled by a dominant autosomal recessive gene not linked to *H-2*. Temperature-sensitive mutants of

MHV-4 have been generated which produce either demyelination or encephalitis and differ in target-cell tropism. Inflammation accompanied by primary demyelination has been observed after infection with a number of other viruses including canine distemper virus, human herpes simplex virus, Semliki forest virus, Ross river virus, and vesicular stomatitis virus.

These findings raise important questions about the pathogenic mechanisms which result in myelin loss in any individual model. The most direct mechanism is a persistent infection of oligodendrocytes. This would produce lysis or at least some dysfunction of these myelin-producing cells and is the likely cause of abnormalities produced by certain mutants of MHV. At least two other mechanisms are possible. First, viral infection could induce an autoimmune reaction directed at myelin components: in Reo virus infection in SJL mice, virus invades the pancreas, pituitary and gastric glands, and this is associated with the production of autoantibodies against insulin, growth hormone and other cellular antigens present in these tissues. Comparable experiments seeking autoreactivity against myelin components in mice with persistent virus infections of the CNS seems likely to be done soon. While autoreactivity in the serum was low and transient in the Reo virus experiments, the range of specificities in the autoantibody response could be assessed by production of hybridomas making monoclonal antibodies from spleens of the infected animals. It should be kept in mind that the effector mechanisms responsible for myelin loss may be cellular rather than humoral. Hence, in designing such experiments cell-mediated immunity against myelin antigens should also be sought. An obvious extension of the Reo virus experiments would be to attempt the production of T-cell clones from virus-infected animals, for example from the CNS. It is reported that typical EAE appears in recipients of lymphoid cells from TV-infected donors.

A final possible mechanism, which was much discussed, was demyelination as a bystander consequence of an immune response by $\text{Ly}1^+2^-$ T lymphocytes to viral or neural antigens presented on Ia⁺ cells within the nervous system. Demyelination could thus be the final effect of a

number of concurrent or alternative immune responses. The antigen-presenting cells might be oligodendrocytes, microglia, monocytes invading the tissue, or vascular endothelium.

One example of a single immunopathological state produced by multiple mechanisms is mouse immune complex disease, which shares many features with human SLE. The disease is now known in at least three mouse strains, NZB $\times$ NZW, MRL

and BXSB, and in each different genetic factors are involved, affecting various elements of the T-cell regulatory network or the effector B cells themselves.

Murine models of demyelination and other disorders can now be analysed in terms of contemporary concepts of immune regulation. Bold new methods for modifying the molecular events involved in regulation of the specific immune response are available and likely to be applied

directly to the reduction of tissue change in the various animal models of MS and even possibly in human disorders.

Dale E. McFarlin is Chief, Neuroimmunology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, U.S.A., and Byron H. Waksman is Director, Research Programs, National Multiple Sclerosis Society, New York, New York, U.S.A.

The workshop participants were:

Ruth Arnon, Rehovot, Israel; Harvey Cantor, Boston, Massachusetts; Peter C. Doherty, Philadelphia, Pennsylvania; Robert B. Fritz, Atlanta, Georgia; Edmond A. Goidl, New York, New York; Mark I. Greene, Boston, Massachusetts; Martin V. Haspel, Bethesda, Maryland; Richard T. Johnson, Bethesda, Maryland; Robert L. Kroc, Santa Ynez Valley, California; D. Scott Linthicum, Los Angeles, California; Howard L. Lipton, Chicago, Illinois; Leonard D. Schultz, Bar Harbor, Maine; Elizabeth Simpson, Harrow, Middlesex, U.K.; Alfred Steinberg, Bethesda, Maryland; Lawrence Steinman, Stanford, California; B. David Stollar, Boston, Massachusetts; Robert H. Swanborg, Detroit, Michigan; Argyrios Theofilopoulos, La Jolla, California; Byron H. Waksman, New York, New York; G. Donald Whedon, Bethesda, Maryland; John B. Zabriskie, New York, New York.