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Genetic and molecular aspects of demyelination

from Nicholas K. Gonatas, Mark I. Greene, and Byron H. Waksman

Three groups of investigators came together to learn from each other in a workshop: neurologists and neuropathologists interested in the pathogenesis of multiple sclerosis (MS) and other demyelinative diseases of man and similar autoimmune and virus-induced model diseases in animals; immunologists and virologists concerned with the genetic controls found to play a role in these diseases; and geneticists and molecular biologists investigating specific gene products and their role in recognition function and cell interactions in model immunological systems.*

Demyelinative diseases, MS in particular, are characterized by patchy inflammatory lesions, with accompanying tissue damage, randomly scattered throughout myelin-containing areas of the central nervous system (N. Gonatas, Philadelphia). In MS, as well as in the animal model, experimental autoimmune encephalomyelitis (EAE), the initial lesions are perivascular, and they grow by radial enlargement and confluence. The inflammation includes edema, formation of perivascular cuffs, and infiltration of the parenchyma with both T and B lymphocytes and large numbers of macrophages. The primary element of tissue damage is destruction of the myelin sheath around nerve fibers – this is the hallmark of this group of diseases. The demyelination involves both receptor-mediated endocytosis by macrophages (anti-myelin antibody appears to serve as ligand) and extracellular lysis of unknown mechanism (candidates are macrophage enzymes, complement, endogenous myelin proteases, free radicals).

Progress in understanding the immunogenetic and cellular immunological basis of EAE has guided or influenced similar studies in MS (Gonatas). Immunohistochemical studies have shown the presence of both myelin basic protein and galactocerebroside, well known immunogens of myelin, in the vessel wall and on the luminal surface of vascular endothelium in the CNS (U. Traugott, New York). The possible importance of MHC-restricted recognition events in lesion pathogenesis is suggested by the simultaneous presence of immune associated (Ia) antigens on vascular endothelium within the MS or EAE lesion and on activated astrocytes, especially near the periphery of enlarging lesions, as well as on the infiltrating macrophages and B cells. T lymphocytes of both phenotypes are found in the infiltrate. There is a suggestion that T4-bearing cells (helper/inducer phenotype) predominate early and T8-bearing cells (cytotoxic/suppressor phenotype) in older lesions. Several studies have shown T4 to be the predominant T-cell phenotype in the cerebrospinal fluid in acute exacerbations of MS. Immunohistochemical and radioimmunoassay evidence has also been found for release of interleukins 1 and 2 and γ-interferon (γ-IFN) within the lesions, and γ-IFN may also appear in the CSF.

The relative importance of cells and antibody in inducing demyelination may vary at different stages of the process and in different natural and experimental situations (H. Lassmann, Vienna). Earlier experiments focused attention on antibody to galactocerebroside, a tissue-specific myelin glycolipid, as a direct agent of complement-mediated destruction. More recent work incriminates antibody to a minor glycoprotein of myelin as ligand in an antibody-dependent cell-mediated form of demyelination. Indeed such demyelination is readily induced with monoclonal antibody in experimental situations.

Persistent virus infection of neural tissue may provide a continuing antigenic stimulus resulting in inflammation and demyelination, as shown in model studies of Theiler's murine encephalitis virus (H. Lipton, Chicago) and a number of other viruses (M. Oldstone, La Jolla). However, of twelve putative viruses related to MS over a 40 year period, none has withstood the test of further investigation. In acute attacks of demyelinative disease following infection with common childhood viruses, so-called postinfectious encephalomyelitis, virus has not been found to enter the nervous system. Accumulated epidemiologic evidence, e.g. from twin studies, suggests that MS may be initiated by infection with common respiratory or exanthematous viruses in late childhood, and a recently published study by W. Sibley *et al.*[1] appears to show that a major proportion of exacerbations are similarly triggered.

It seems reasonable to infer that genetic controls may operate at the level of the T-cell repertoire, T-cell receptors for myelin or viral determinants, MHC structure and its expression by antigen-presenting cells (B cells, vascular endothelium, glia), immune regulatory activity, 'factor' formation and release, and vascular permeability responses (Gonatas).

Genetic loci in susceptibility and resistance to demyelinative disease

Studies carried out over a decade ago established a relationship between MS susceptibility and the HLA haplotype A3B7DR2, encoded on chromosome 6 (the extended haplotype also includes C4-A4B2 and properdin BfS). In a large group of Canadian MS patients over half were $DR2^+$ compared with a figure of 28% in controls (G. Ebers, London, Canada). There is a suggestion that heterozygotes at this locus carrying both DR2 and DR3 may develop unusually severe disease. At the same time Lapps and Gypsies, over 50% of whom are $DR2^+$, have very little MS. Recent studies have located the genetic control more narrowly to DQ1 and the DW2 subgroup of DR2. Other reported linkages are with C3F (chromosome 19) and Gc–1F (chromosome 4). No studies have yet been reported of genes in MS affecting the T-cell receptor polypeptides or vasoamine sensitivity.

The multifactorial aspect of genetic control in MS susceptibility is emphasized in population, family, and twin studies (Ebers; D. Sadovnick, Vancouver). Among 5463 MS patients in the Canadian series (in-

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cluding patients in 400 multiplex families) 71 proved to have twins. Among 27 monozygotic twin pairs studied, the MS concordance rate was close to 40%, and there were MS-like lesions in a significant proportion of the remaining 'healthy' twins. In dizygotic twin pairs, the concordance rate was 2.5%, not significantly different from the 1.5% rate in a large series of sibling pairs. Interestingly both Ebers and D.E. McFarlin (Bethesda) have found several dizygotic concordant twin pairs which show no HLA genes in common. Relative risk rates were estimated, in family studies, as above 5% for siblings, parents, and aunts and uncles, and about 1% for children, compared with 0.1% in the population at large. Segregation analysis applied to extended HLA haplotypes and other genetic data, obtained in 611 individuals in 60 pedigrees, suggested the involvement of at least one MHC, and one non-MHC gene in MS susceptibility (Ebers).

The environmental factor, presumably viral infection, which may play a role in the initiation of the MS process was also studied in sibling pairs (Sadovnick). Data obtained in siblings born 4–8 years apart support a role both for genes governing susceptibility and for shared exposure to an environmental agent. In siblings separated by more than 8 years, the data favor genetic control of susceptibility but do not distinguish shared from random exposure to environmental agents. The latitude effect (increased MS prevalence at higher latitudes) best exemplified in Kurtzke's studies of US veterans, is often considered to support environmental influences in MS. However Ebers finds the data are equally well accounted for, state by state, by the percentage of the population of Scandinavian (Swedish, Norwegian) ancestry. This is true as well in Europe, if one takes account of Viking migrations to the British Isles, Normandy, and Sicily. Even the well known 'clusters' of MS in Finland are in areas with a Swedish population above 50%. The reported association of MS susceptibility with IgG Gml, 17; 21 (chromosome 14) apparently reflects the fact that this genetic marker parallels the Scandinavian ancestry in the tested populations.

Genetic studies in rats and mice also emphasize the multifactorial nature of genetic controls in acute and in chronic relapsing EAE, induced, for example, with myelin basic protein (MBP). In rats, MHC genes appear to control EAE susceptibility in parallel with delayed-type (T cell-mediated) hypersensitivity to MBP (D. Gasser, Philadelphia). Several resistance genes have been mapped to other chromosomes (Gasser; B. Källén, Lund) and these differ in different resistant rat strains. While disease is transferred with sensitized T cells obtained from either susceptible or resistant strains of rats, the resistance can be transferred only with bone marrow cells (F. Waxman, Chicago; C. Whitacre, Colombus).

In studies of Balb/c × SJL recombinant inbred mouse strains, as many as five genes are found to govern susceptibility to acute EAE: one MHC gene, shown earlier by R. Fritz *et al.*[2] to govern the ability to respond to encephalitogenic peptides of MBP, and one non-MHC gene, also two genes governing vascular sensitivity to vasoactive amines, and up to three 'histamine-sensitization responses' genes (these are related to the use of pertussis as an adjuvant) (D. Linthicum, Houston). In SWR × SJL recombinants, 'high responders' (mostly H–2^s, some H–2^q) tend to have earlier and more intense disease as well as more frequent relapses, while 'low responders' differ in all these respects (F. Lublin, Philadelphia). Transfers of bone marrow or immune cells between SJL (H–2^s, susceptible) and B10.S (H–2^s, resistant) showed a non-immunological element in resistance, possible at the level of CNS blood vessels. The number of vasoamine (pyrilamine, 5–hydroxytryptamine) receptors on cultured brain vessel endothelial cells is said to differ strikingly in different mouse strains (M. Hart, Iowa City). The highly responsive SJL mouse has a high number of mast cells in muscle and dura, also a marked relative increase in Ia throughout the body (C. Raine, New York). In another well known autoimmune disease model, experimental autoimmune myasthenia gravis, induced by immunizing mice with purified acetylcholine receptor of the myoneural junction, a comparison of the susceptible C57 Bl/6 strain with the resistant bm12 mutant showed that the difference resides in a simple mutation of the gene encoding the Ia β-chain (L. Steinman, Stanford). The use of brain transplants to the anterior chamber of the eye, which develop acute EAE lesions when the host is immunized with myelin antigens, should make it possible in various strain combinations to sort out the relative contributions of immune cells, vascular, and neural elements to genetically controlled susceptibility and resistance (Lublin).

A number of viruses produce demyelinating diseases in animals, among them visna in sheep, the caprine arthritis-encephalitis virus in goats, canine distemper virus in dogs, and JHM (MHV–4, a coronavirus) and Theiler's murine encephalomyelitis virus (TMEV) in mice. The JHM virus, at least in mice, acts by direct infection of oligodendrocytes (R. Knobler, Philadelphia). Virulence resides in the 'E2'–glycoprotein, required for viral entry into the cells and cell fusion. The SJL mouse, which is highly susceptible to immunologically-mediated demyelinative disease, is the most resistant strain to JHM. Its 'resistance gene' may encode a protease which attacks E2; it has been tentatively mapped to chromosome 7. TMEV, on the other hand, produces a chronic inflammatory, demyelinative lesion much like EAE, which appears to represent an immunological reaction to virus persisting in glial elements (Lipton). A study in recombinant inbred strains derived from the SJL × C57B1/10 cross, in which disease susceptibility is recessive, incriminated a single control, related to delayed-type hypersensitivity to the virus, in H–2D. In strains derived from SJL × Balb/c, in which susceptibility is dominant, 2–3 non-MHC genes were involved, one apparently related to the T-cell receptor β-chain. The findings do not parallel the findings with EAE.

The application of molecular techniques to genetic questions in MS and similar diseases is in its infancy, with an emphasis on human chromosomes 6, 7, and 14 (Steinman). In a study of myasthenia gravis, related to DR3 rather than to DR2 (chromosome 6), RFLP study of DR3 homozygous typing cells has permitted identification of a 13 kilobase (kb) DQ β-chain fragment which was present in 8 of 15 myasthenia patients but only in 1 of 38 normal people. The same approach is beginning to be applied in MS. Similarly, in the IgH region on chromosome 14, a polymorphic 5.9 kb band in the IgG_1 locus was present in 13 of 49 MS

patients' DNA (pooled data from France and USA), 24 of 41 myasthenics (Germany and USA), and 24 of 47 normals. DNA sequencing may provide still more detailed information in time.

An important finding, which has yet to find its place in relation to the genetic studies outlined above, is the increased frequency of spontaneous and induced sister chromatid exchanges in MS (C. Kidson, Brisbane). The inducing agent used in most studies is x-irradiation, but ultra violet (UV) irradiation, mitomycin-C, and various cross-linking agents have also been employed. Target cells commonly used include T and B lymphocytes and fibroblasts, but the same changes are seen in all cell types of the same individual. MS cells show increased sensitivity to x-irradiation and bleomycin but not to UV irradiation, mitomycin–C, or alkylating agents. Different MS pedigrees demonstrate different patterns of inheritance of this abnormality from dominance to no pattern. Cell fusion analysis, in which one fusion parent has been prelabelled with BUDr, and the hybrids (MS × control) are identified by karyotyping, also shows either dominant or recessive inheritance patterns, depending on the identity of the other fusion parent. The findings are consistent with defective DNA repair, replication, or recombination or with abnormality of a pleiotropic system regulating DNA manipulation. Karyotypic studies of cycling T cells within MS lesions or in MS cerebrospinal fluid appear not to have been carried out thus far.

Myelin antigens and the presentation problem

The best studied of the myelin antigens, myelin basic protein (MBP), occurs in three molecular forms in man and six in the mouse, encoded by a single gene on chromosome 18 in both species (R. Lazzarini, Bethesda). There is strong homology between different species' MBP. The oligodendrocyte apparently makes all the molecular forms in an ordered sequence, as shown by the use of monoclonal antibodies specific for the two inserted peptide sequences. Abnormalities in the MBP gene, as in the 'shiverer' and 'MLD' mutations, result in 'dysmyelinating' diseases of the mouse, and similar mutations occur in other species. Different MBP peptides are encephalitogenic in different species and even in different strains of a single species: peptide 68–88 in Lewis rats (A. Vandenbark, Portland, Oregon), peptide 89–169 in H–2^s (SJL, A.S.W.) and H–2^q (SWR, B10.T) mice, and peptide 1–37 or 1–20 in H–2^k (A, B10.A) or H–2^u (PL) mice (R. Fritz, Atlanta). The PLXSJL hybrid responds to peptide 1–37; this may depend on non-codominant expression of the two Ia molecules or of the relevant α or β-chains, as suggested by presentation experiments or by inhibition experiments with monoclonal antibodies. Of 17 (H–2^u × H–2^s)F_1 T-cell clones specific for MBP, almost all could respond to MBP peptide 1–9 only when presented in association with H–2^u or with both H–2 in the F_1 (S. Zamvil, Stamford).

The major protein of myelin is proteolipid protein (PLP), encoded on the X chromosome, as recently shown by H. Willard and J. Riordan (M.B. Lees, Boston). Gene defects result in X-linked abnormalities of myelin formation in Pelizaeus–Merzbacher disease (human) and jimpy disease (mouse). PLP also is encephalitogenic, producing a chronic EAE in rabbits, guinea pigs, and mice. The 30 kDa molecule consists of highly conserved sequences with alternating hydrophilic and hydrophobic regions. A 3D model has been developed defining transmembrane segments T1, T2, and T3, also cytoplasmic and extracellular segments. Some of these, notably BPS4 peptides 142–150 and 209–217, show regions of homology with MBP.

Molecular mimicry between viral antigens and tissue components is now well established as a mechanism of autoimmunization, both by computer searches of known peptide sequences and by actual synthesis of suspected peptides and effective cross-immunization (R. Fujinami, La Jolla). A. Notkins (Bethesda) and Oldstone, together with the group at the Wistar Institute, tested 600 monoclonal antibodies specific for various viral antigens and found that 3–4% reacted with normal tissue elements. Fujinami has identified a six amino acid peptide in hepatitis B virus polymerase identical with an MBP sequence known to be encephalitogenic in rabbits. A synthetic 8 residue peptide, incorporating this hexapeptide induced both antibody and T cells reactive with whole MBP and even produced mild EAE in rabbits. G. Stoner has reported homologous sequences in MBP and some of the papovaviruses of man, and E. Alvord and his colleagues have made similar observations with a number of additional viruses. H. Wege and R. Watanabe have reported intense MBP sensitization with EAE-like disease in rats infected with certain JHM virus mutants but the mechanism of cross-immunization was not established. PLP too shows regions of partial homology with a variety of antigens (from HTLV–III, measles, and EBV) and a region of close homology with an adenovirus antigen in the N-terminal peptide 1–19 (Lees). An unusual but possibly common mode of cross-immunization is the induction of immune responses against cell membrane components by using the idiotype of anti-viral antibody as a steric analogue of the cell membrane receptor for the virus (M. Greene, Philadelphia). The details have been elegantly worked out for the case of reovirus and the β-adrenergic receptor and the key peptide sequence identified.

Virus itself, if it persists in the CNS, may serve as the antigen eliciting a cell-mediated immune response with chronic inflammatory demyelinating disease, as in the case of Theiler's murine encephalomyelitis virus infection in mice (Lipton) and as reported for chronic JHM virus infection in rats by Wege. In both these cases, the responding T cell, which induces inflammation is of helper/inducer phenotype. In mice persistently infected from birth with lymphocytic choriomeningitis virus (LCMV), there is virus-specific tolerance at the T-cell level (Oldstone). When tolerance is abrogated by transfer to these mice of LCMV-specific cytotoxic T-cell clones, virus is cleared with the formation of lymphocyte 'cuffs' limited to the perivascular space but without typical T cell-mediated inflammation.

Cells and the antigen presentation problem in the central nervous system

It remains unclear whether the circulating sensitized T cell's initial encounter with myelin or viral antigen in the CNS takes place at the luminal surface of vascular endothelium, at the foot processes of astrocytes impinging on the perivascular space, or on glial cells in the parenchyma. MBP appears to be present at all three sites, by immunohisto-

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chemical criteria (Traugott). In adjuvant-sensitized guinea pigs, the appearance of Ia on CNS vessel endothelium before the inflammation begins, is readily demonstrated by high resolution electron microscopy (R. Sobel, Boston). It can be demonstrated in older animals even without sensitization. In hybrids of the EAE-susceptible guinea pig strain 13 with the insusceptible strain 2, 13 Ia is elevated and 2 remains low. In skin vessels, tuberculin sensitization and testing with PPD lead to increased Ia in both strain 2 and 13, while GL gives increased Ia only in strain 2 and GT only in strain 13. Ia is also seen in human brain vessels (Traugott), but appears to be entirely absent in vessels of the rat CNS, even after active or passive induction of EAE (Lasmann, H. Wekerle, Freiburg). Isolated brain vascular endothelial cells (free of glial contamination) cultured for as long as a month, do not express Ia. However in the presence of γ-IFN or of supernatants from specifically sensitized T cells incubated with MBP, they express Ia, increasing over 48–72 h, and acquire the ability to reconstitute the thymidine incorporation response of monocyte-depleted, sensitized T cells to MBP (R. McCarron, Bethesda). This reaction is blocked by anti-Ia of the correct haplotype but not by anti-macrophage serum. Endothelial cells isolated from mice with EAE express Ia and can present antigen. Ia is also expressed on vascular smooth muscle at a level that may exceed that of endothelium (Hart).

Astrocytes have several important properties relevant to antigen presentation (A. Fontana, Zurich). They express class I MHC antigen constitutively and class II antigen after exposure in culture to lipopolysaccharide, Con A supernatant, or γ-IFN. They acquire at the same time the ability to present MBP in an Ia-restricted manner to MBP-specific T-cell clones, with resulting T-cell 'clustering' and proliferation. They also can induce and trigger cytotoxic T cells against allospecific H–2. Finally, when stimulated they release IL–1, indistinguishable by a variety of biological criteria from that produced by macrophages, and IL–3, which stimulates macrophage proliferation in culture.

Macrophages are of great importance in both MS and EAE. C. Brosnan (New York) has established that EAE is largely or entirely inhibited by doses of silica that wipe out the macrophage population. Their ability, when activated, to produce IL–1, γ-IFN, and prostaglandins of the E series is well known. Contact of accessory cells such as macrophages with mitogen-stimulated helper/inducer T cells ($L3T4^+$) appears to be essential in order for the latter to express IL–2 receptor (Y. Ron, La Jolla; E. Shevach, Bethesda). This function, which is inhibited by anti-LFA–1 but not by anti-Ia, requires actual cell contact and is not related to a diffusible factor. Cytotoxic/suppressor ($Lyt\ 2^+$) T cells do not require such contact.

The IL–1, produced by activated macrophages and astrocytes, has manifold biological activities relevant to the full development of the inflammatory demyelinative lesion (C. Dinarello, Boston). Perhaps the most important are the proliferative stimulus to T and B lymphocytes responding to antigen, also the stimulation of astrocytes, the augmentation of IL–2 and other lymphokine production, the chemotactic stimulus to various inflammatory cells, and the stimulation of PGE_2 production by macrophages and astrocytes and prostacyclin by vascular endothelium. There appear to be three, or possibly four, distinct IL–1 molecules, not cross-reactive among themselves but cross-reactive with their respective congeners in other species.

The antigen presenting cell, whether macrophage or astrocyte, usually acts by unfolding and cleaving the antigen (J. Berzofsky, Bethesda). The resulting 11 or 12 residue peptides serve as 'epitopes' reactive with T-cell receptors. These have closely associated 'agretopes' reactive with the 'desetopes' of Ia or H–2 (R. Schwartz, Bethesda). MBP (and presumably PLP as well) has several immunodominant epitopes and agretopes, which interact best with different auxiliary sites: different I–A, I–A versus I–E, H–2K versus H–2D. The essential information for the α–β chain interaction which underlies agretope structure in I–A or I–E resides in the terminal 15 residues of the β-chain (R. Germain, Bethesda). Up-regulation by γ-interferon may permit expression of rare MHC molecules, with an enhanced presentation of unusual autoantigen epitopes and an enhanced probability of autologous MLR (C. Janeway, New Haven).

Virus and antigen presentation

Persistent virus infection, in either immunocytes or antigen-presenting cells may profoundly influence recognition events and the course of demyelinative disease. The lactic dehydrogenase virus (LDHV) replicates only in macrophages expressing class II MHC (I–A or I–E) antigen; this antigen actually serves as the viral receptor (C. Mims, London). Infection of mice with LDHV depletes Class II macrophages and severely compromises their ability to develop contact allergy or graft-versus-host reactions. In SJL mice infected two weeks before or simultaneously with immunization for EAE, the disease was virtually eliminated. (Persistent LDHV infection in C58 mice is associated with a unique age-dependent polioencephalitis, anterior horn cell death associated with mononuclear cell infiltration. Virus is present in astrocytes but not the dying neurons, and there is intense DTH against the virus. The mechanism of neuronal death remains conjectural).

Retroviruses may interact with both the MHC and minor H loci and thus affect immune responses (D. Meruelo, New York). Radiation leukemia virus of mice (Rad LV) infects the thymus primarily. In resistant mouse strains it enhances expression of H–2D; this in turn enhances later killer T-cell recognition of virus, associated with H–2D, and immune lysis of leukemia cells. In susceptible strains this effect is not observed. Instead, viral integration in the MHC changes methylation patterns and interferes with gene rearrangements. Insertion sites are found within the MHC, in K, D, and Tla; there is an additional site in H–38, and another as yet unmapped. Using probes for different endogenous retroviruses two were found to be integrated in or close to class I MHC genes, and 16 others in close association with minor H genes.

These observations are relevant to the recent claim by H. Koprowski and R. Gallo and their colleagues[3] of finding HTLV-I-like viral RNA in cultured cells from MS patients' CSF, and to studies carried on over many years of visna, a demyelinative disease of sheep. Visna is a retrovirus closely related to human HTLVIII. Its proviral DNA is integrated in the genome of cells in the bone marrow, and the genes are transcribed primarily or exclusively in circulating

monocytes, as reported by O. Narayan and J. Clements[4]. In activated macrophages there are production of complete virions and increased expression of macrophage MHC. The lesions appear due to an immunologically mediated inflammatory response to virus. Why they are localized in the CNS white matter (and the lungs) is unclear. Successive waves of disease are associated with mutations affecting the viral envelope (antigenic variation).

Effector and suppressor lymphocytes in demyelinative disease

A technological 'breakthrough' was provided by the development of MBP-specific T-cell clones capable of producing apparently typical EAE in normal recipient rats, and more recently mice and other species (A. Ben Nun and I. Cohen, Rehovot; Wekerle; Vandenbark; Steinman). Clones specific for other tissue specific antigens (P2, acetylcholine receptor, synovial proteoglycan) produce the corresponding autoimmune diseases (neuritis, myasthenia, arthritis) on transfer. All require *in-vitro* 'activation' with mitogen or specific antigen to become capable of initiating lesions. Activation may simply enable the cells to enter the CNS non-specifically (Wekerle); to produce a heparanase, which facilitates passage of the vascular barrier and entry (Cohen); or to elaborate γ-interferon, which stimulates MHC antigen expression by vascular endothelium (and astrocytic footprocesses) and thus MHC-restricted antigen recognition. Many clones, if inactivated by irradiation, mitomycin–C, or hydrostatic pressure, can prevent EAE, apparently by inducing a specific suppressor T-cell response (Cohen), which may however be nonspecific in its suppressor effect.

The MBP specific T-cell repertoire, its MHC restriction properties, antigen specificity, and antigen receptors have been analysed with a series of T-cell hybridomas prepared from rats immunized with a single octapeptide of MBP (E. Heber-Katz, Philadelphia). These showed at least five different patterns of *in-vitro* reactivity with guinea pig and rat MBP and encephalitogenic MBP peptide, and their reactions were inhibited in multiple patterns by a series of monoclonal anti-Ia, implying that the effector cells recognized a substantial range of different epitopes in the test antigens. They were also heterogeneous in Ia restriction and DNA genomic rearrangement patterns, as shown with a T-cell receptor β-chain probe.

It should be noted that MBP-specific T cells are found in the blood of patients with postinfectious encephalomyelitis as shown by R. Johnson and his collaborators (Baltimore), but attempts to find and/or clone similar cells from MS blood or CSF have largely failed. Curiously MBP-specific cells are present in significant numbers in normal blood, as shown by J. Burns, and MBP or PLP-specific cells in blood from controls with a variety of other neurological diseases (H. Weiner, Boston, Massachusetts). G. Birnbaum (Minneapolis) has reported that a high proportion of T-cell clones from MS CSF are MHC-autoreactive and also heteroclitic, i.e. they react better to one or more allogeneic MHC than autologous MHC. The CSF T cells in MS, most of which have been shown to be continuously cycling carry surface markers characteristic of long-term cell lines in culture (Tal and TS2/7) and lack markers ($T11_3$ and IL–2 receptor) characteristic of acute blast transformation (D. Hafler, Boston).

Birnbaum's observations may have a straightforward explanation. MHC-autoreactive T-cells are apparently present in the circulation at all times but held in check by a specific suppressor population (D. Wilson, La Jolla). These autoreactive cells may represent a small proportion of the total of cells specific for self plus X (where X represents exogenous antigens) generated during the life time of the individual. If autoreactive cells are brought into the CNS in the wake of a myelin or virus-specific T-cell reaction, they can react with Ia on the activated macrophages and astrocytes and perpetuate the inflammatory process. M. Bevan (La Jolla) has suggested that association of MHC molecules with other molecules in the surface membrane of antigen-presenting cells may strikingly increase the number of reactive T-cells which can recognize them.

A few of the isolated T-cell clones in Cohen and Wekerle's experiments have proved to act as MBP-specific suppressor-induced cells. As noted above, an MBP-specific suppressor response can be induced by immunization with inactivated specific effector cells. The specificity of the system (antigen, T-cell receptor idiotype, other?) and the phenotype and function of the cells involved (suppressor/inducer, suppressor, contrasuppressor) have not been determined. W. Lyman[5] has reported the presence of MBP-specific suppressor T cells (inhibiting Con A-stimulated blast transformation) during the remission phase of chronic guinea pig EAE; these cells were absent during exacerbations. Agents capable of triggering relapses of EAE during remission may simply act to eliminate suppressor cells (cyclophosphamide, antithymocyte serum) while others may stimulate expression of Ia (pertussis, systemic graft-versus-host reaction). The possibility was raised that some suppressor cells may inhibit specific differentiated function such as immunoglobulin secretion rather than cell proliferation (R. Lynch, Iowa City).

Immunological manipulation affecting cellular and molecular events in demyelinative processes

The model demyelinative diseases have lent themselves to manipulation by the use of monoclonal antibodies specific for phenotypic markers of the participating cells, as well as for any of the polymorphic or non-polymorphic surface molecules or diffusible factors involved in the essential cell interactions. This type of manipulation provides insight concerning basic disease mechanisms and opens the door for new forms of immunotherapy in man. The demonstration by S. Brostoff and L. Steinman[6,7] that EAE could be prevented or arrested after onset with antibody to T_h markers such as L3T4 has been followed by the first attempts to use similar antibodies (of mouse origin) in MS (Weiner; Hafler). Anti-T12, an IgM pan-T antibody, wipes out circulating $T12^+$ cells for 7 days, apparently mainly by cell surface modulation. Anti-T11, an IgG_{2b} against the sheep RBC receptor, produces a similar effect, accompanied by some immunosuppression. Both stimulate a brisk antibody response against mouse immunoglobulin. Labelled T cells, i.e. cells carrying mouse immunoglobulin, were found for several days in the circulation and entering the cerebrospinal fluid in considerable numbers.

Perhaps more promising is the use of anti-Ia antibody therapy which is effective in inhibiting EAE, auto-

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immune myasthenia, thyroiditis, and collagen arthritis in mice, spontaneous diabetes in the BB rat, and lupus in the NZB/W mouse (Steinman). It is possible to treat heterozygotic mice with monoclonal antibody specific for the Ia allele relevant to the antigen of interest. Thus EAE in SJL × Balb/c hybrids is inhibited by anti-Ia^s but not anti-Ia^d. The method works in monkeys and will soon be attempted in man, with the use of mouse antibodies engineered to have a human Fc.

A monoclonal antibody, made against a single T-cell hybridoma specific for MBP, was able to protect Lewis rats from EAE when injected once, at the time of or 5 days after immunization with guinea pig MBP (Heber-Katz). The hypothesis was offered that a single shared idiotypic determinant on rat MBP-specific T-cells may be involved in EAE. On the other hand this may reflect the non-specific suppressive effect of specifically triggered suppressor T-cells (Cohen).

D. Willenborg (Canberra) reported that μ-suppression, in rats treated from birth to 9 weeks with anti-IgM, effectively abolishes both the clinical and histological EAE response. A variety of serum and cell transfer experiments have failed to clarify the mechanism of this effect which appears to conflict with the evidence that T cells alone can produce EAE. Experiments with protein antigens such KLH show that unprimed T cells see the antigen on B cells (in lymph nodes), even when given in complete Freund's adjuvant (Shevach). In μ-suppressed mice, there is a deficit in priming, restored by resupplying B-cells (J. Sprent, La Jolla). B. Benaceraff (Boston) has reported major changes in IgH-restricted suppressor T-cell function associated with μ-suppression. This problem provided a suitable note of mystery on which to conclude the meeting.

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Interleukin 1 production for detection of bacterial polysaccharide in fetal calf sera and other solutions

Sir,

J. Oppenheim and his colleagues (*Immunol. Today,* 1986, 7, 45) reviewed the properties and function of interleukin 1 (IL–1). We have found that the measurement of IL–1 production by human monocytes and testing in the thymocyte costimulator assay is a sensitive means of detecting pyrogens in protein-containing solutions. Plastic-adherent blood monocytes were incubated with lipopolysaccharide (LPS) at 3×10^5/ml and supernatants were collected and frozen 24 h later. C3H/HeJ thymocytes were incubated in serial dilutions of supernatant with added phytohemagglutinin for three days and thymidine incorporation was determined during the final 6 hours of culture[1]. Titres of IL–1 in the supernatants were defined as the final dilution of supernatants which gave at least twice the background incorporation of thymidine. The medium used throughout was RPM1 1640 containing 10% functionally LPS-free fetal calf serum (FCS). Supernatants generated in absence of LPS contained no detectable IL–1.

Adherent cells (AC) from each of 16 randomly selected donors produced IL–1 in response to at least 0.1 ng/ml of our standard LPS, and in 3 cases to as little as 0.001 ng/ml. It seems, therefore, safe to say that pyrogen concentrations equivalent to 0.1 ng LPS/ml can be detected by practically all normal donors while some allow 10-fold or even 100-fold greater sensitivity. It would be useful to have a cell line which would produce IL–1 upon stimulation with LPS.

We screened 19 different batches of FCS from different suppliers and found that many contained an activity which would induce IL–1 production by monocytes. Some batches induced no measurable IL–1 production with cells from more than 30 different donors and we consider these, therefore, as functionally LPS-free. These sera were used as a supplement in the cell preparation and IL–1 production phase of the asay. When the pyrogen contents of batches of FCS were calculated and expressed as LPS equivalent some sera had activities, corresponding to as much as 10 μg LPS/ml. Thus even minor contamination with such a serum may have drastic effects whenever monocytes/macrophages are present in a given experimental system. Four batches of LPS-free serum came from three different sources, and most suppliers seem to have positive and negative batches.

Although several bacterial products, e.g. staphylococcal protein A, induce IL–1, the active principle in the FCS preparations seemed to be LPS. To exclude that we were dealing with a hormone-like IL–1-inducing agent of fetal origin, we established that the kinetics of indication of IL–1 by FCS were identical to those of LPS-induced IL–1 production. IL–1 inducing activity was sensitive to treatment with pH 2 and was essentially uninfluenced by pH 9, by heat (30 min., 90°C) or by the presence of protease inhibitors (100 U trasylol). As a control, a negative batch of FCS was supplemented by 10 ng LPS/ml and treated under the same conditions. It showed the same pattern. We also found that polymyxin–B, which binds LPS, inhibited IL–1-inducing activity. Finally, the presence of endotoxin in one batch of FCS was confirmed in a lethality test in mice made sensitive to LPS by D-galactosamine treatment[2].

Tedious *in-vivo* pyrogen tests in rabbits are still widely used to assess preparations designed for injection into patients. These may be replaceable by the more efficient and precise test we have described. None of 15 different adult human sera and 5 cord blood sera induced any IL–1 production but they supported IL–1 induction by exogenous LPS. We are convinced that IL–1 production and assay can reliably detect LPS in all blood products and LPS-anti-LPS complexes in sera of patients with