2 Pathogenesis of Multiple Sclerosis: Relationship to Therapeutic Strategies

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Introduction

Among the chronic inflammatory disorders of humans, multiple sclerosis (MS) represents perhaps the most complex puzzle and a frustrating challenge to medical science. Having successfully teased three generations of immunologists and virologists, the underlying biology of MS remains only vaguely understood. The immunology of MS appears complex, in part because there have been so many observations, some conflicting, that do not result in a clear model of pathogenesis. Einstein noted that good science simplified one's understanding of the world, a criterion thus far not met in the MS arena. Certain fundamental questions must be answered before a coherent picture emerges. For example, what is the mechanism of chronic inflammation in MS? What triggers MS? What antigens (if any) are targeted? MS is generally considered to be an autoimmune disease, yet inflammation and selective destruction of central nervous system (CNS) elements may also occur in non-autoimmune conditions, diseases of known etiology including genetic disorders (adrenaleukodystrophy, metachromatic leukodystrophy) or chronic virus infections (HTLV-I or Theiler murine encephalomyelitis virus). The inflammatory changes that occur in MS may ultimately be shown to be secondary rather than primary, and only tentative assumptions of the nature of MS can reasonably be made at this time. This said, recent data from multiple converging sources lend support to the classical concept that MS is mediated by an aberrant immune response directed against one or several myelin proteins of the CNS (Tables 2.1 and 2.2). Such a response will develop only in a genetically susceptible individual, following some as yet undefined environmental exposure. The autoimmune model of the pathogenesis of MS has set the tone for immunotherapy in this disease, first by general immunosuppression using cytotoxic drugs and, more recently, by selectively targeting a specific component of the immune response (Oksenberg 1994).

A primary role for the immune system in the etiology of MS has been debated for more than a century, since the initial suggestion by Pierre Marie in 1884 that lesions of MS might represent a common complication to multiple infectious agents. Spirochaetes were claimed to be the cause of MS in 1917 and during the

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past 80 years more than 20 infectious agents have been reported, but not confirmed, to cause the disease. Beginning in the 1930s, work by Rivers and others defined the experimental autoimmune disease, experimental allergic encephalomyelitis (EAE), which bore some clinical and pathologic resemblance to human MS. In the cerebrospinal fluid (CSF) of patients, evidence for cellular inflammation and a selective increase in levels of locally synthesized immunoglobulin, was presented in the 1930s and 1940s, respectively. Searches for autoantibodies or other myelinotoxic factors date also from the 1930s. The demonstration in 1961 by Patterson that EAE could be adoptively transferred by specifically sensitized T cells inaugurated the era of T-cell immunology in MS research, an area that in many respects dominates the field to this day.

Table 2.1. MS as an autoimmune disease: evolution of thinking

Date	Development of knowledge
1835	First pathologic description of MS
1884	Relationship to infection is proposed
1932	Encephalitis accompanied by myelin destruction is experimentally produced in monkeys by multiple injections of rabbit cord tissue
1934	CSF inflammatory changes recognized in MS
1947	Elevated immunoglobulin levels in CSF
1960	T-cell mediation of experimental allergic encephalitis (EAE) demonstrated by adoptive transfer
1972	MS genetic susceptibility is associated with the major histocompatibility complex on chromosome 6
1977	The administration of soluble neuroantigens induce tolerance and regulate EAE
1980	IgG of restricted clonality with reactivity to myelin basic protein (MBP) eluted from MS brain
1982	The cellular composition of the inflammatory reaction in the MS plaque is described
1985	Molecular mimicry may operate in MS
1988	Suppression of EAE is achieved by oral administration of MBP
1988	Limited heterogeneity of lymphocytes mediating EAE allows T-cell antigen receptor (TCR)-specific immune intervention
1990	Identification of activated MBP-reactive cells in MS peripheral blood
1993	TCR rearrangements from MS brain lesions encode CDR3 regions identical to those found in T cells recognizing MBP
1993	Superantigens may be implicated in the initiation and/or recurrence of demyelination
1993	MBP-specific TCR transgenic mice develop spontaneous autoimmunity

Table 2.2. Putative autoantigens in MS

Myelin basic protein (MBP)
Proteolipid protein (PLP)
Myelin oligodendrocyte glycoprotein (MOG)
Myelin associated glycoprotein (MAG)
Heat shock proteins
β-arrestin and arrestin
Glial fibrillary acidic protein (GFAP)
Astrocyte-derived calcium-binding protein (S1000β)
Transaldolase

Our present view of the pathogenesis of MS has been markedly enhanced during the past several years by the identification of credible candidate autoantigens, the study of the role of major histocompatibility complex (MHC) gene products, the genetic analysis of the T-cell antigen receptor (TCR), and progress in the understanding of cytokine physiology. In addition, significant advances in the capacity to manipulate, control and understand EAE has led to a true revolution in knowledge of T-cell mediated demyelination. Indeed, sophisticated approaches for treatment of EAE by selective immune intervention are being attempted in MS at the present time (see Chapter 3) (Table 2.3). This review will focus on the immunopathology of the MS plaque, the mechanisms of plaque formation, the analysis of regulatory circuits required to maintain tolerance to CNS antigens, and the genetic basis of susceptibility to MS. We believe that the development of new and more effective therapy is likely to result from the use of new molecular tools to define the immune response in MS and to characterize its genetic basis.

Table 2.3. Experimental strategies for selective immunosuppressive therapy

Monoclonal antibodies to:
T-cell sub populations (CD4)[a]
T cell receptors[b]
Adhesion molecules[a]
Accessory molecules (CD40, CD80)[c]
MHC class II molecules[c]
Cytokine receptors[c]
Activation markers[c]
Macrophages[c]
Cytokines[c]
T-cell vaccination[a]
TCR peptide vaccination[a]
Immunomodulation (COP 1[a], Linomide[a])
Oral induced tolerance[a]
Inhalation induced tolerance[c]
Cytokine receptor analogs and antagonists[a]
Cytokines (IFN-β[a], TGF-β[b], IL-4[c], IL-10[c])
Antigen-induced programmed T-cell death (apoptosis)[c]
Antigen peptides TCR analogs and antagonists[c]
Blocking costimulation pathway (anergy)[c]
Blocking costimulation pathway (Th1/Th2 commitment)[c]
MHC class II–peptides complexes[b]
Anti-IgD peptide conjugates[c]
Superantigen modulation[c]
Metalloprotease inhibitors[c]
Blocking-signal transduction pathways[c]
cAMP-phosphodiesterase inhibitors[c]
Complement inhibitors[c]
Regulation of MHC gene expression[c]
Apoptosis-inducing antigens[c]

[a]Clinical trials
[b]Pre-clinical trial stage
[c]Experimental stage

Immunopathology of the MS Lesion

The pathologic hallmark of MS is the plaque, a well demarcated gray or pink lesion, characterized histologically by complete myelin loss, an absence of oligodendrocytes and relative sparing of axons. MS plaques are multiple, generally asymmetric, and tend to concentrate in deep white matter near the lateral ventricles, corpus callosum, floor of the fourth ventricle, deep periaqueductal region, optic nerves and tracts, corticomedullary junction and cervical spinal cord. The acute MS lesion is characterized by perivascular and parenchymal infiltration of mononuclear cells, both T cells and macrophages, and by myelin breakdown that appears to be mediated by the infiltrating cells. B cells and plasma cells are only rarely present. As lesions evolve, axons traversing the lesion show marked irregular beading; proliferation of astrocytes occurs, and lipid-laden macrophages containing myelin debris are prominent. Progressive fibrillary gliosis ensues and mononuclear cells gradually disappear. In some MS lesions, but not others, proliferation of oligodendrocytes appears to be present initially, but these cells are apparently destroyed as the gliosis progresses. Gliosis is more severe in MS than in most other neuropathologic conditions. In chronic MS lesions, complete, or nearly complete, demyelination, dense gliosis and loss of oligodendroglia are found. In some chronic active MS lesions, gradations in the histologic findings from the center to the lesion edge suggest that lesions expand by concentric outward growth. Axonal preservation is relative rather than absolute. In approximately 10% of lesions there is significant axonal destruction. Rarely, complete destruction of the neuropil and cavitation occur.

Controversy still surrounds the nature of the initial pathologic event in MS. The earliest detectable event in plaque development is an increase in permeability of the blood–brain barrier (BBB), associated with inflammation (McDonald 1994). Following the breach in the BBB, myelin appears to be the primary target of the pathologic immune reaction (Kermode et al. 1990; Raine 1994a). Lassman and colleagues found that oligodendrocytes were preserved in early lesions of relapsing MS but were destroyed in chronic lesions (Ozawa et al. 1994). Oligodendrocytes were also apparently destroyed in early aggressive cases. These findings suggested some variability of oligodendroglial destruction in different clinical forms of MS. Rodriguez et al. (1993) has proposed a different sequence of neuropathologic events. In a study of 11 stereotaxic brain lesion biopsy specimens, uniform widening of inner myelin lamellae (biphasic myelinopathy) and degeneration of inner glial loops ("dying-back" oligodendrogliopathy) were early pathologic abnormalities that preceded complete destruction of myelin sheaths. Because the oligodendrocytes were morphologically preserved in this early stage, the authors proposed that the initial event in MS is the functional interference with the myelinating capacity of these cells. Subsequently, degeneration of both the inner myelin lamellae and the inner oligodendroglial loop occur. As a consequence of this injury, novel or aberrant antigens may be exposed, triggering the infiltration of inflammatory cells (Rodriguez et al. 1993).

As the inflammation proceeds, oligodendrocytes at the periphery of the plaque, as well as astrocytes, proliferate under the influence of factors released

into the microenvironment. Such oligodendrocytes, which appear to continue to function as myelinating cells, may be derived from surviving or progenitor cells (Wu and Raine 1992). When inflammation decreases, the edema disappears and conduction is restored, possibly as a result of the expansion of sodium channels into the demyelinated axon (McDonald 1994). Remyelination is not essential to remission.

Upregulation of MHC molecules has been proposed as a marker of plaque activity. Class I MHC antigens have been identified in plaque tissue on endothelial cells, infiltrating lymphocytes and astroglia, while class II determinants are reported to be expressed on endothelial cells, macrophages, microglia and astroglia. On the other hand, a recent study by Bo and colleagues (1994) provides compelling evidence that the only cells in the active lesions expressing class II antigens are macrophages and microglia. In any case, the high expression of MHC class II molecules in MS brains suggests that the local microenvironment may be enriched in MHC-activating factors such as interferon (IFN) -γ, and that antigen is possibly presented to T cells (Traugott et al. 1983). It is important to note, however, that in many silent plaques devoid of T-cell infiltrates, class II MHC may be expressed at high levels on reactive microglia. Upregulation of MHC class II antigens is not unique to MS tissue, as it has also been detected in neurodegenerative diseases and following trauma.

As noted above, the inflammatory reaction in active plaques is dominated by T lymphocytes and macrophages, whereas B lymphocytes and plasma cells are rare. The percentage of plasma cells in the inflammatory infiltrates is significantly higher in late chronic MS compared with acute MS (Lassman et al. 1994). Early studies demonstrated that lymphocytic perivascular cuffs are prominent at the edge of active plaques and are occasionally seen in areas with no evidence of demyelination or macrophage infiltration. T cells in the parenchyma and in the perivascular cuffs consisted of CD8+ (suppressor/cytotoxic) cells and variable numbers of CD4+ (helper/inducer) cells (Hauser et al. 1986b). The selective accumulation and compartmentalization of T cells indicate a specific pattern in the homing of T cells to the lesion, and suggests an immune response to a discrete antigenic complex. Indeed, restricted populations of activated T cells reactive against myelin components are present in the peripheral blood (Allegretta et al. 1990, 1994) and are compartmentalized in the CNS of MS patients (Lee et al. 1991; Oksenberg et al. 1990a; Renno et al. 1994; Usuku et al. 1992).

T Cells and Macrophages

The vast majority of CD4/CD8 T cells in the MS brain bear the common form of the antigen cell receptor (i.e. the α/β heterodimer). The TCR is expressed on the surface of mature T lymphocytes, which subserves T-cell recognition by fragments of antigen associated with MHC molecules. The antigen-binding variable domains of the TCR have a β barrel structure in which a conserved framework of β strands support three hypervariable loops termed complementary determining regions (CDRs). The putative CDR1 and CDR2 loops are encoded within the

germline sequences of the variable (V) gene segments, while the CDR3 loops are encoded by the V, diversity (D) and joining (J) genes that rearrange in unique ways in individual developing T-cells, and include the use of non-germline N region nucleotide additions and/or deletions, generating dramatic increases in T-cell diversity (Usuku et al. 1992b). CDR3 regions play a critical role in peptide recognition. Modeling of the trimolecular interaction between the TCR, the MHC antigen-presenting molecules and bound antigenic peptide, suggests that the complementary-determining regions, CDR1 and CDR2 of the TCR, interact primarily with the alpha helical regions of the MHC, and provide the structural framework and topology for the interaction of a particular CDR3 region, (N)Jα and (N)Dβ(N)Jβ, with the peptide bound in the MHC cleft (Chothia et al. 1988; Jorgensen et al. 1992; Katayama et al. 1995). One of the authors (JRO) recently reported the study of TCRAV and TCRBV rearrangements using the polymerase chain reaction and sequence analysis in MS plaques (Oksenberg et al. 1993a). A limited number of TCR-V gene rearrangements were seen in 30 specimens from 16 MS brains. Of eight MS patients who had the MS-associated MHC type human leukocyte antigen (HLA)-DR2 (DRB1*1501, DQA1*0102, DQB1*0602 and either DPB1*0401 or 0402), seven had rearrangements of the TCRBV 5.2 gene in the lesions, compared with only two of seven MS brains from patients who were not DR2-positive. Genetic susceptibility to MS has been shown to be associated with the HLA-DR2 haplotype in caucasoid populations. It is conceivable that the bias in TCRBV gene expression resulted from activation of reactive T cells after encounters with peripheral or local exposed antigens presented by HLA-DR2-associated antigen-presenting molecules, and their subsequent trapping in the brain. In a related observation, Kotzin and colleagues (1991) found that 90% of T-cell lines that could be expanded from peripheral blood of DR2-positive patients and that reacted against the myelin component, myelin basic protein (MBP), expressed TCRBV 5.2 and/or TCRBV 6 genes. We then sequenced BV 5.2 positive cDNA clones from different anatomic regions of the brain of two DR2-positive MS patients. Instead of finding many different CDR3 sequences, as would be expected if no selection by antigen was operating, in the plaques of both patients five predominant amino acid CDR3 motifs were present: BV 5.2(Q)LR or BV 5.2LRGA, BV 5.2LGG, BV 5.2LVAG, BV 5.2LDG, and BV 5.2(Q)PT. None of these sequences was seen in BV 5.2 transcripts from control brain tissue or from the peripheral blood of individuals with the same HLA phenotype. After a search of more than 1500 CDR3 sequences that have been published, a few striking similarities emerged. One of the repeated BV 5.2 motifs found in MS brains contained the basic pattern LRGA at the V–D–J junction, which is identical to that found in a cytotoxic T-cell clone recognizing the MBP sequence 87–106, isolated from the peripheral blood of a DR2-positive MS patient. This CDR3 sequence is also seen in T-cell clones reactive to the MBP peptide 87–99, derived from the spinal cord of Lewis rats with EAE (MBP is the major autoantigen in most rodent models of EAE). Taken together, these results constitute the first evidence for an MBP-specific T-cell response in MS brain tissue. Although both the α and β TCR chains contribute to the peptide and MHC specificity of a T cell, identification of TCRB CDR 3 sequences identical to those found in bona fide T-cell lines known to be reactive against MBP 87–106

strongly suggests that MBP-specific T cells are present in the MS nervous system. The observation that some of the TCR sequences detected in the MS lesions are similar to those expressed by pathogenic encephalitogenic (i.e. disease-inducing) T-cell clones further suggests that these cells may be involved in the disease process. In a recent study, Hara and colleagues (1994) reported that the TCRBV gene sequences expressed by lymphocytes in spinal cord lesions taken from HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) autopsy cases, included restricted CDR3 motifs with a striking homology to those reported earlier in MS brains. Because no proviral DNA was amplified in any neuronal cells, including neurons and glial cells, demyelination of the spinal cord as a direct result of viral infection is unlikely. T cells expressing these restricted TCRBV CDR3 motifs may be somehow expanded or activated as a result of infection with HTLV-I. These results raise the possibility that tissue damage in HAM/TSP is mediated by autoreactive T cells, as in MS. An association between HTLV-I infection and MS was suggested in the mid-1980s, but the current weight of evidence argues strongly against a role for this human retrovirus in MS (Hauser et al. 1986a; Reddy et al. 1988; Oksenberg et al. 1990b).

T cells carrying the other form of the TCR, the γ/δ heterodimer, have been also identified in significant numbers in lesions of MS. Using immunocytochemistry, Selmaj and colleagues (1991) showed that γ/δ+ TCR T cells, while absent from control brain tissues, accumulated specifically at the margins of chronic active plaques, in which the acute phase reactant heat shock protein (hsp 65) was coexpressed on immature oligodendrocytes. In addition, clonal expansion of γ/δ T cells was detected in the brain, CSF and peripheral blood of MS patients with acute disease (Shimonkevitz et al. 1993; Wucherpfennig et al. 1992a). Bernard and colleagues reported that the majority of γ/δ cells in chronic plaques expressed the Vγ2 and Vδ2 chains. However, sequence analysis of such transcripts showed no evidence of clonal expansion (Hvas et al. 1993). Striking limited diversity of a Vδ2-Jδ3 TCR rearrangement was detected by Battistini and colleagues (1995) in chronic active lesions. Further studies are needed to ascertain whether γ/δ T cells are involved in the demyelinating process, whether they respond to heat shock and other stress-related proteins, or merely represent a non-specific recruitment of T cells into the CNS. It is noteworthy however, that peripheral blood γ/δ T cells can induce the lysis of fresh human oligodendrocytes in culture (Freedman et al. 1991). Detailed knowledge of the patterns of expression of TCR genes in MS might have implications for the treatment of this disease, given the success of preventing or reversing experimental demyelination with reagents that target specific V-region gene products (Acha-Orbea et al. 1988; Offner et al. 1991). It is important to remember however, that TCR studies on the inflamed brain represent just snapshots, which cover a short chapter in the history of the plaque.

The dynamics of the autoimmune T-cell infiltration into and out of the CNS parenchyma have proved difficult to investigate in humans. A more complete picture is emerging from studies in animal models. EAE can be induced in a variety of animal species, including non-human primates, by injection of myelin proteins or their peptide derivatives, as well as by adoptive transfer of CD4+ activated T cells specific for MBP or proteolipid protein (PLP) (Bernard et al.

1992). EAE is a prototypic experimental model for antigen-specific, T-cell-mediated autoimmunity. Based upon clinical, histologic and genetic similarities to the human disease, EAE is widely considered to be a relevant model for MS. The establishment of inflammatory EAE lesions and clinical disease is a multi-step event. It has been proposed that the first step requires that activated T cells cross the BBB. Activated lymphocytes that bear a memory phenotype (CD44+, Mel 14–), suggesting previous activation by antigen, and also express the adhesion molecule VLA-4 integrin ($\beta 1 \alpha 4$), become attached to appropriate receptors on endothelial cells at parajunctional areas, adjacent to the endothelial tight junctions, and then proceed to pass directly into the interstitial matrix. It is of considerable interest that this process occurs without regard to the antigen specificity of a T cell; thus MBP reactive cells cross the BBB with no greater efficiency than do activated cells that do not recognize a CNS antigen (Wekerle et al. 1994). Next, specific CD4+ T cells are reactivated in situ by fragments of myelin antigens presented in the framework of MHC class II molecules on the surface of antigen-presenting cells (macrophages, microglia and perhaps astrocytes). This leads to a second wave of inflammatory recruitment and clinical EAE. Proinflammatory cytokines such as TNF-α and IFN-γ are probably key mediators of the full-blown inflammatory response. Encephalitogenic myelin-specific T cells may not be capable of mediating EAE in the absence of this secondary leukocyte recruitment.

The prototypic EAE susceptible rat strain is Lewis. After injection of an encephalitogenic, MBP-reacting T-cell line into these animals, pathogenic T cells are detectable in the brain within a few hours (Karin et al. 1993). Until day 4, brain lesions are mostly populated by these cells. In contrast, by the time clinical paralysis occurs, the TCR genes rearranged in the CNS are quite diverse, indicating an heterogeneous (polyclonal) T-cell population (Bell et al. 1993; Karin et al. 1993). Following recovery from the acute attack, the TCR repertoire in the lesions is quite restricted again and similar to the early infiltrate. Similarly, in acute MS lesions, TCR gene transcripts are quite heterogeneous, whereas in chronic lesions they are more restricted (Oksenberg et al. 1993a; Wucherpfennig et al. 1992b). What is the likelihood of detecting TCR rearrangements associated with pathogenic T cells in a cellular infiltrate in inflamed brain? Brocke and colleagues recently characterized a murine (SJL/J) encephalitogenic T-cell clone specific for MBP 87–99. This particular clone contains TCRAV and TCRBV sequences homologous to those found in both human T-cell clones reactive to the same epitope and in the MS brain lesions (S. Brocke, personal communication). In addition to sequence homology, these pathogenic cells are of particular interest because they remain anergic in the EAE brain in animals that have recovered from clinical disease after selective immunotherapy with TCR agonist synthetic peptides. By studying chronic MS lesions, the critical signals among the TCRs rearranged in the cellular infiltrate could be deciphered from the noise. This may not have been feasible if more acute lesions had been examined (Oksenberg et al. 1993b).

Recently, the potent proinflammatory and chemoattractant functions of a new superfamily of chemokines were reported (Schall 1991; Schall et al. 1990). These structurally related chemokines share a conserved 4-cysteine motif and are

subdivided into two groups, namely C–X–C and C–C, depending on whether or not there is an intervening amino acid between the first two cysteines. The chemokines of the C–X–C class act mainly on neutrophils, whereas the chemokines in the C–C class appear to act on mononuclear cells. RANTES is a member of the C–C class and may have important biologic activities in inflammatory lesions, such as seen in MS. RANTES is a small glycoprotein secreted by activated T cells, monocytes and endothelial cells, and is a chemotactic factor for monocytes and activated CD4+ T cells of the memory phenotype (CD45RO), which is known to accumulate at the site of the MS lesion. Furthermore, RANTES is present in synovial lining cells of patients with rheumatoid arthritis and in delayed-type hypersensitivity reactions. Preliminary data indicate the high expression of mRNA for this cytokine at the edge of active MS plaques (J. Hvas, personal communication).

The waves of cell recruitment into the brain are accompanied by the expression of various cytokines, adhesion molecules, and their receptors (Raine and Canella 1992). In human disease, acute lesions are positive for the adhesion molecule ICAM-1 and its receptor LFA-1, and negative for VCAM-1/VLA-4, whereas chronic lesions are highly positive for both adhesion molecule complexes (Cannella et al. 1991; Raine 1994b). In a more recent study, Cannella and Raine (1995) confirmed the higher expression of VCAM-1 in chronic active plaques compared with acute lesions. VCAM was also present in microglial cells and blood vessels. Strong expression of VLA-4 was detected on cells in perivascular cuffs and in the parenchyma. ICAM-1/LFA-1 was uniformly higher at all stages of lesion formation. The lower expression of VLA-4 in the acute plaque is puzzling because this molecule appears to be required for the entry of CD4+ T cells into the CNS parenchyma (Baron et al. 1993). Furthermore, Yednock and colleagues (1992) showed that the in vivo administration of an antibody against human VLA-4 integrin $\alpha 4\beta 1$ not only prevented the accumulation of leukocytes in the CNS but also inhibited the development of EAE in Lewis rats. Romanic and Madri (1994) showed that T cells that have transmigrated through endothelial cells in vitro or in vivo exhibit a specific downregulation and decrease in $\alpha 4$ expression at the cell surface, providing an explanation to the lower expression of VLA-4 in the acute plaque. Following adhesion, T cells transmigrate through the endothelium and the subendothelial basal lamina into the matrix. Although macrophages are a rich source of enzymes that will disrupt the endothelium and allow traffic into the subendothelial basal lamina, T cells may have their own arsenal of proteases. Leppert and colleagues (1995) recently demonstrated that highly purified normal peripheral blood T lymphocytes express two matrix metalloproteinases, gelatinases A (72 kDa) and B (92 kDa). Both gelatinases are structurally related and share the proteolytic selectivity for basal lamina collagens. Gelatinase B is secreted constitutively, whereas gelatinase A is inducible in vitro on activation. The 72 kDa gelatinase A is also inducible in T cells upon adhesion to endothelial cells after binding to VCAM-1 (Romanic and Madri 1994). Inhibitors of gelatinases may offer new therapeutic avenues for MS and other inflammatory diseases.

T lymphocytes crossing the BBB would encounter perivascular macrophages constitutively expressing MHC class II. This encounter may be sufficient to

present myelin antigens to specific T cells (Perry 1994). The resident microglia, lying within the parenchyma, would then become activated as a result of locally released cytokines. Brain parenchymal non-bone-marrow derived cells, such as astrocytes and endothelial cells, may be also capable of functioning as antigen-presenting cells (Fontana et al. 1984; Myers et al. 1993). Conversely, astrocytes may also play a role in limiting the progression of inflammatory lesions by failing to provide appropriate antigen-presenting costimulatory signals to the responding lymphocyte (Weber et al. 1994). A cascade of events that will result in plaque formation and demyelination has been started. Macrophages act not only as antigen-presenting cells to T lymphocytes but also as scavengers that remove debris and serve as a source of growth regulatory molecules and cytokines. Interactions between T cells and macrophages can result in proliferation of each of these cell types through the mediation of such molecules as IL-2 and colony stimulating factors respectively. Furthermore, endothelial and T cells can provide colony stimulating factors to the macrophage to prevent apoptosis and cell death and maintain activation. In addition, activated macrophages can produce an extraordinary number of biologically active molecules with profound effects on lymphocytes and macrophages themselves, as well as on endothelial and CNS cells.

The recruited macrophage is likely to be an additional key mediator of vascular and myelin damage in MS. By depleting the macrophage populations from animals with EAE, either by intraperitoneal injection of silica (Brosnan et al. 1981) or by liposomes containing dichloromethylene diphosphonate (Huitinga et al. 1993), amelioration of disease was achieved. Liposomes, delivered before the onset of clinical signs, will prevent disease, demonstrating that recruited macrophages (liposomes do not actually enter the CNS) also contribute to the effector phase of the disease. Membrane proteins involved in macrophage adherence to the endothelium include the CD11b/CD18 integrin, also known as the type 3 complement receptor (CR3). Intravenous injection of antibodies directed against epitopes in the CR3 molecule suppressed clinical signs of EAE, confirming the role of CR3 in macrophage homing toward inflammatory CNS lesions (Huitinga et al. 1993). Numerous questions remain to be answered in studies of the macrophage in MS. It will be important to determine the time of arrival to the plaque in relation to lymphocytes, and the details of their cell cycle inside the brain. The critical role that the macrophage may play in lesion formation suggests that finding means of controlling macrophage participation at all levels of demyelination could be critical in altering lesion progression.

B Cells and the Humoral Reaction

In most MS patients, an elevated level of intrathecally synthesized immunoglobulins can be detected in the CNS. Although the specificity of these antibodies is mostly unknown, antimyelin specificities have been reported (Bernard et al. 1981; Newcombe et al. 1982; Olsson et al. 1990). The role, if any, of these putative autoantibodies in the pathogenesis of MS is unclear. The antibodies may serve to opsonize the myelin sheath and make it more available for phagocytosis by

macrophages. CNS immunoglobulins may also induce in vitro myelinolysis via activation of a Ca^{2+}-dependent myelin-associated protease acting on MBP (Kerlero de Rosbo and Bernard 1989). In the CSF, the presence of membrane attack complexes suggests a possible role for complement-mediated antibody damage in MS (Roddy et al. 1994). Evidence that antibodies may participate in CNS demyelination has been obtained in recent animal experiments. Little or no demyelination is usually observed in EAE induced in Lewis rats by injection of purified MBP or by passive transfer of MBP reactive lymphocytes (Bernard and Kerlero de Rosbo 1992). Extensive demyelination can be induced in these animals by intravenous injection of anti-MOG (myelin oligodendrocyte glycoprotein) monoclonal antibodies when the BBB is breached (Schluesener et al. 1987). It is important to note that polyclonal antibodies against MBP, PLP or myelin associated glycoprotein (MAG) have no such effect. Evidence for an anti-MOG role in the in vitro demyelination effect has also been reported (Kerlero de Rosbo et al. 1990). MOG is a member of the immunoglobulin supergene family and, interestingly, the gene encoding MOG maps within the MHC in human chromosome 6 (Pham-Dinh et al. 1993). It constitutes about 0.05% of CNS myelin proteins, and is located exclusively on oligodendrocyte surfaces and in the outermost lamellae of myelin sheaths, making it readily accessible to the immune attack (Brunner et al. 1989). As suggested by Bernard (Bernard and Kerlero de Rosbo 1992), these studies indicate that antibody-mediated demyelination may not necessarily involve recognition of quantitatively major myelin proteins as previously assumed, but rather of strategically located antigens. In addition, B cells probably participate in the process of antigen presentation to T cells (Parker 1993). The recent availability of B-cell knockout mice (i.e. genetically altered mice lacking immunoglobulin molecules) (Loffert et al. 1994), may allow a better definition of the role of B lymphocytes in autoimmune demyelination.

The Role of Cytokines

During the process of lesion formation, cytokines, growth factors and other small molecules, such as nitric oxide, induce and regulate numerous critical cell functions, including cell recruitment and migration, cell proliferation and cell death. Elucidation of their roles in MS may provide opportunities to use them as potential starting points for therapeutic intervention.

A variety of cytokines regulate the activation, differentiation, and proliferation of T lymphocytes. Under their influence, cells differentiate into two major pathways (Table 2.4). T(helper)h1 cells produce IL-2, IL-3 TNF-β and IFN-γ, and participate in inflammatory responses. Th2 cells produce IL-3, IL-4, IL-5 and IL-10. A third subset of T cells, Th0, with a pattern of cytokine production overlapping both Th1 and Th2, was identified, and may represent a precursor population. In contrast to Th1 cells, Th2 cells depend on IL-4 rather than IL-2 for their autocrine growth, and can proliferate to anti-CD3 antibodies in the absence of accessory cells.

One of the first cytokines to be recognized in MS lesions was IL-2 and its

Table 2.4. CD4+ Th1/Th2 dichotomy

Th1-type response	Th2-type response
Mediators	
IFN-γ	IL-3
IL-2	IL-4
IL-3	IL-5
GM-CSF	IL-6
TNF-β	IL-10
	IL-13
	GM-CSF
Functions	
Inhibition of Th2 (IFN-γ)	Inhibition of Th1 (IL-10)
B cell differentiation (IFN-γ, IL-2) (IgG2a+, IgG2b– and IgG3–)	B cell differentiation (IL-4, IL-5) (IgG1+, IgG4+ and IgGE+)
Promotion of cell-mediated immunity and DTH responses	Promotion of humoral immunity
	Promotion of tolerance
Macrophage Activation (IFN-γ)	Mast cells and eosinophils: differentiation (IL-3, IL-4, IL-5)

At least two different Th cell types arise from a common precursor (Th0 or ThP). IL-2 is the autocrine growth factor for Th1, and IL-4 acts preferentially on Th2 cells. In addition, Th cells are engaged in mutual antagonism. IFN-γ from Th1 and IL-10 from Th2 cells, inhibit the other subpopulations. Preferential activation of one T cell type may explain why the immune response may be predominantly "cellular" in some circumstances, and "humoral" in others.

receptor (Hofman et al. 1986). Since then, a large number of pro-inflammatory (IL-1, IL-6, RANTES, MIP-1α, TNF-α, TNF-β, IFN-γ) and regulatory (IL-10, IL-4, TGF-β) cytokines have been detected in the brain, peripheral blood and CSF of MS patients (Raine 1994a, b). It is probable that, acting in both paracrine and autocrine ways, they constitute a functional network that regulates the cellular interactions that operate in MS.

One of us (SLH) identified the cytokines IL-1 β, TNF-α, and IL-6 by specific radioimmunoassays in the CSF of patients with MS and other neurologic diseases (OND) (Hauser et al. 1990). There was a high incidence of detectable IL-1β in patients with active MS compared with inactive MS or OND patients. TNF-α was also more frequently present in active MS than in OND CSF. By contrast, most MS CSF samples did not contain detectable IL-6. There was no correlation between the degree of CSF pleocytosis and the level of individual cytokines, suggesting that cytokine accumulation may be derived from CNS, and not CSF, cells. Elevated levels of TNF-α in the CSF have been associated in one study with disease progression (Sharief and Hentges 1991), but the reproducibility of the assay system used may have been suboptimal. As IL-1β and TNF-α experimentally induce astrogliosis, demyelination, temperature elevation, lassitude and sleep, these results raise the possibility that these cytokines may contribute to a variety of clinical manifestations in MS.

The role of TNF-α in EAE and MS has been studied extensively. TNF-α, by upregulaton of cytokine production, such as of RANTES for example, enhances lymphocyte–endothelial cell adhesion and is an efficient mediator of cell recruitment in inflammatory infiltrates (Issekutz and Issekutz 1993). Administration of TNF-α augments EAE (Kuroda and Shimamoto 1991), whereas antibodies to TNF-α or TNF-α receptor, and inhibitors of TNF-α synthesis, abrogate the disease (Ruddle et al. 1990). More recently, it has been demonstrated that the

modulatory effect of soluble peptide variants of an MBP epitope is through downregulation of TNF-α production (Karin et al. 1994). Finally, injection of TNF-α into the vitreous, a fluid compartment of the CNS, instigates oligodendrocyte disruption and demyelination (Butt and Jenkins 1994). TNF-α is initially expressed as a precursor with a 233 transmembrane amino acid anchor. This precursor is proteolytically processed to yield a mature 157 amino acid cytokine. The sequence in the putative cleavage site reveals homologies with peptide sequences known to be cleaved by metalloproteinase-like enzymes. Two recent papers report that the in vitro and in vivo release of TNF-α is specifically prevented by metalloproteinase inhibitors (Gearing et al. 1994; McGeehan, et al. 1994). Thus, metalloproteinases may act not only as mediators of cell extravasation, but may also increase the inflammatory and homing reactions through TNF processing. From the clinical point of view, synthetic compounds able to block both TNF-α production and matrix degradation may be effective in controlling MS (Gearing et al. 1994; Genain et al. 1995; McGeehan et al. 1994).

Self-Antigens in Multiple Sclerosis

In this paper, we are considering the concept that heightened self-reactivity is in some manner operational in the MS disease process. A critical prerequisite to understanding the molecular basis of an autoimmune disease is knowledge of the autoantigen or autoantigens. Antigen identification will help to define the pathogenesis of the diseases, and may provide new opportunities for novel diagnostic and therapeutic approaches (Table 2.3). The characterization of the autoantigen, in terms of molecular structure and nucleotide or amino acid sequences, can facilitate epitope mapping and accurate definition of antibody binding sites. As already discussed, EAE can be induced in susceptible animals following active immunization with purified MBP or PLP and their derived peptides in a suitable adjuvant, as well as transfer with MBP- or PLP-specific T cells. Because MBP and PLP represent the two predominant myelin proteins (about 30% and 50% of myelin proteins by weight respectively), they have received the most attention as potential autoantigens in MS (Table 2.5) (Friez 1989). Using protein chemistry, electron microscopy and mass spectrometry, Moscarello and colleagues concluded that MBP in MS patients is arrested at the level of the first growth spurt, within the first 6 years of life, and is therefore developmentally immature (Moscarello et al. 1994). The authors postulated that a structural change in MBP is primary and not secondary to the disease process, and that immature myelin is more susceptible to degradation, providing the initial antigenic material to the immune system. This provocative observation has not been confirmed, and it is possible that the observed changes were secondary to MS rather than the inciting event. Nonetheless, the major immune response detected in the laboratory in MS patients is directed against MBP (Olsson et al. 1990; Steinman 1994). MBP reactive T cells appear to concentrate in the CSF, relative to their frequency in peripheral blood (Soderstrom et al. 1993). In addition, Warren and colleagues recently reported that 111 of 116 chronic progressive MS patients had anti-MBP antibody in the CSF (Warren et al. 1994). Most patients who had no anti-MBP antibody in the CSF did

Table 2.5. Incriminating MBP in the pathogenesis of MS

MBP makes up about 30% of central myelin proteins
EAE is inducible in susceptible animals by active immunization with MBP, fragments of MBP, or synthetic peptides from MBP epitopes, when injected in suitable adjuvants
EAE is inducible in susceptible animals by passive transfer of MBP-reactive T-helper cells
Brain derived endothelial cells from guinea pigs are able to present MBP, but not purified protein derivative or ovalbumin, to previously sensitized T cells
It is possible to prevent or even reverse EAE by neutralizing the immune response against MBP
MBP induced lymphoproliferation among MS patients is only slightly higher than in controls, but activated T-cell clones with specificity for MBP are observed in MS patients and not in controls
Extensive molecular homology has been detected between MBP stretches and pathogens such as adenovirus type 2
Antibodies to MBP are regularly found in the CSF of patients with acute optic neuritis and active MS, as well as in CNS tissue of MS patients
Direct cloning and sequencing of TCR rearrangements from MS brain lesions indicated that some of these rearrangements are encoding CDR3 regions identical to those found in T cells recognizing MBP
Immature MBP isoforms may have a higher distribution among MS patients
Linkage was reported between allelic markers located at 5′ of the MBP gene on chromosome 18, and susceptibility to MS in a Finnish population of familial MS patients

have antibodies to PLP. The anti-MBP IgG affinity purified from CNS lesions reacted with the MBP peptide p75–106, a putative dominant T-cell recognition site. Several studies have shown that human MBP-specific T cells predominantly recognize peptides located in the center and in the C-terminal part of the MBP molecule, around residues 84–102 and 143–168 (Kotzin et al. 1991; Martin et al. 1991; Ota, Matsui et al. 1990; Pette et al. 1990; Valli et al. 1993; Wucherpfennig et al. 1994a, b). Although a number of MHC class II determinants can serve as antigen-presenting molecules, the immunodominant epitope 84–102 binds with high affinity to HLA-DR2 molecules (Wucherpfennig et al. 1994a). As detailed above, the analysis of TCR gene rearrangements in the MS brain has also indicated that one of the major immune responses in the lesions of HLA-DR2 patients, is directed to the MBP epitopes 84–102 or 87–106 (Oksenberg et al. 1993b). The importance of MBP reactive cells in autoimmunity was further demonstrated in recent work, where MBP-reactive T-cell clones isolated from the peripheral blood of healthy, unimmunized *Callithrix jacchus* marmosets, efficiently transferred CNS inflammatory disease (Genain et al. 1994). This primate species is characterized by a natural chimerism of bone marrow elements between siblings, that allows the adoptive transfer of cells between individuals across histocompatibility barriers.

EAE mediated by transfer of MBP or PLP reactive T cells is characterized by paralysis and perivascular CNS inflammation, yet, in most models, little demyelination is observed. In contrast, sensitization with CNS tissue homogenates may result in extensive demyelination. This suggests that antigens other than MBP and PLP are involved in demyelination. Highly purified myelin antigens were used by Bernard and colleagues to assess cell-mediated immune responses in MS patients. The greatest incidence of proliferative response by MS peripheral blood lymphocytes was to MOG, as 12 of 24 patients reacted, and of these, eight

reacted exclusively to MOG. In contrast, only one control individual of 16 tested reacted positively to this antigen. The incidence of responses to MBP, PLP and MAG did not differ significantly between MS patients and control individuals (Kerlero de Rosbo et al. 1993). Furthermore, they induced demyelinating relapsing EAE disease in rats after a single injection of purified MOG (Johns et al. 1995). As discussed above, reactivity against minor components of myelin, as well as to other antigens including heat shock proteins, β-arrestin and arrestin, glial fibrillary acidic protein and astrocyte-derived calcium-binding protein (S1000β) may play equally important roles in this disease (Selmaj et al. 1991; Ohguro et al. 1993; Kojima et al. 1994; Wekerle et al. 1994) (Table 2.2). Both normal and disease T-cell repertoires against autoantigens may share similar or even identical specificities. Peripheral regulatory mechanisms are then necessary to keep such cells under control in order to prevent their activation and the development of spontaneous autoimmune responses.

Maintenance of Peripheral Self-Tolerance

During T-cell ontogeny in the thymus, lymphoid stem cells undergo maturation and differentiation in consecutive waves of thymic selection (Benoist and Mathis 1992; Marrack and Kappler 1988). Thymocytes that will be useful to the host undergo positive selection, whereas thymocytes with autoreactive potential undergo negative selection by clonal deletion or inactivation. The "affinity/avidity" model may explain the balance between negative and positive selection (Nikolic-Zugic 1994). This hypothesis suggests that only cells bearing receptors with an intermediate affinity/avidity toward self-peptides–MHC complexes would survive selection. Those with lower avidity would fail to be positively selected, whereas the ones with high affinity/avidity would be negatively selected. Thymic regulation is extended to the periphery in the form of clonal anergy, clonal ignorance and exhaustion, idiotype interactions, and suppression (Murphy et al. 1989; Schonrich et al. 1991). It is not completely clear why some of these cells escape this surveillance and later in life participate in autoimmune pathogenic processes, but the multiple events that are required to induce self-reactivity may include tissue damage by trauma or infection and genetically determined susceptibility. Several experimental systems have been used to understand the mechanisms involved in maintenance of peripheral tolerance to myelin antigens. Among others, such studies include myelin immunization (Levine et al. 1968), T-cell vaccination (Ben-Nun et al. 1981), CD8+ T-cell depletion (Jiang et al. 1992), apoptosis induction (Critchfield et al. 1994), CD8 knockout (Koh, et al. 1992) and TCR transgenic mice (Goverman et al. 1993).

A topic of intense recent interest is the possible association of apoptosis with autoimmunity (Carson and Ribeiro 1993; Tan 1994). Apoptosis, or programmed cell death, is a form of cell death characterized by cell shrinkage, nuclear condensation and surface blobbing. In tissues, apoptosis usually affects scattered single cells rather than clusters, and fragments of apoptotic cells are phagocytosed and digested by resident cells. In this way, potentially immunogenic cellular components are exposed, a process that might explain why antibodies in

autoimmune diseases, as for example in systemic lupus erythematosus, are directed at multiple antigens. In addition, it appears that apoptosis is one mechanism by which clonal thymic deletion takes place after immature lymphocytes bind autoantigens (Goldstein et al. 1991; Tan 1994). A defect in the deletion of these lymphocytes could predispose to autoimmunity. The MLR/Mp-lpr/lpr mouse, which develops a disease analogous to human lupus, has a molecular abnormality in the APO-1 or *Fas* gene that mediates apoptosis (Watanabe-Fukunaga et al. 1992). Because *lpr* mice do not express the *Fas* receptor, they do not efficiently delete autoaggressive T lymphocytes. It is not clear if apoptotic mechanisms operate in MS.

Oral administration of MBP suppresses EAE by inducing peripheral tolerance. Tolerance can be adoptively transferred by CD8+ T cells, which are generated following oral administration of antigens and release TGF-β after being triggered by the specific antigen. TGF-β suppresses immune responses in the microenvironment, creating a form of bystander suppression that will control the disease (Miller et al. 1992). An alternative mechanism based on the induction of anergy (unresponsiveness) to the autoaggressive T cells by the tolerogen was also proposed (Whitacre et al. 1991). It is possible that a low antigen dose induces suppression whereas a high antigen dose induces clonal anergy (Friedman and Weiner 1994). In a recent study, T-cell clones were isolated from the mesenteric lymph nodes of animals that had been orally tolerized to MBP and had received an intraperitoneal injection of MBP as adjuvant. These mucosal clones were CD4+ and shared TCR usage, MHC restriction, and epitope specificity with encephalitogenic T-cell clones. However, they suppressed EAE that was induced with either MBP or PLP by producing a Th2-like cytokine profile composed of TGF-β, IL-4 and IL-10 (Chen et al. 1994).

It is widely accepted that the lack of second signals or costimulation provided by accessory cells causes mature T lymphocytes to enter a state of tolerance or anergy, in which they fail to proliferate or produce lymphokines, and are refractory to subsequent stimulation by antigen. The B7 costimulatory pathway involves at least two molecules, B7-1 and B7-2, which interact with their count receptors, CD28 and CTL-4, on T cells. Blocking B7-1 interactions during T-cell activation induces functional inactivation in Th1 cells. Consequently, IL-2 and IFN-γ, but not IL-4, production is inhibited, leading to a state of hyporesponsiveness or anergy. Thus, it may be possible to anergize selectively the Th1 cells and enhance the modulatory Th2 response by presentation of antigen without costimulation. For example, Finck and colleagues treated lupus-prone NZB/NZW mice with soluble CTLA4Ig. This protocol resulted in the blocking of autoantibody production and prolonged life, even when treatment was delayed until the most advanced stage of clinical illness (Finck et al. 1994). Conversely, the injection of anti-B7-2 antibody substantially increased disease severity in the EAE model, whereas administration of anti-B7-1 antibody significantly reduced the incidence of EAE, possibly through the generation of Th2 clones (Kuchroo et al. 1995). Since cotreatment with anti-IL 4 antibody prevented disease amelioration, costimulatory molecules may directly affect cytokine secretion.

Infection in Multiple Sclerosis

Infectious agents have been postulated as causes of MS for over a century. Most work has focused on viruses known to be able to induce demyelination in humans and experimental animals (Johnson 1994). The possible role of a virus in MS is supported by data suggesting that some as yet undetermined childhood exposure somehow influences susceptibility to MS. This data is derived from migration studies and from study of apparent point epidemics of MS. Viral infections may also induce exacerbations. Some MS patients have abnormal immune responses to certain viruses. Higher antibody titers against measles, herpex simples, varicella, rubella, Epstein–Barr, influenza C and some para-influenza strains have been detected in the serum and CSF samples of MS patients compared with controls. The occurrence of viral infections was studied in a group of patients participating in a recent IFN-β clinical trial (Panitch 1994). A strong correlation was found between MS attacks and viral upper respiratory infections. In addition, a number of viruses have been recovered from MS patients' fluids and tissues (Johnson 1994). However, despite data obtained from epidemiologic, serologic and animal studies, no virus has been consistently isolated, or viral material uniquely identified, from MS patients. Nonviral micro-organisms or their toxins implicated in demyelination include *Acanthamoeba, Borrelia, Brucella, Campylobacter, Hartmannella,* mycobacterium, trypano-somes, diphtheria toxin and tetanus toxoid (Birnbaum et al. 1993; Brocke et al. 1994). A model even proposing that MS is mediated by prions exists in the literature (Wojtowicz 1993).

The role, if any, of pathogens in MS remains unknown. Mechanisms that may explain a pathogen–MS interaction include polyclonal activation of T and B cells, infection and destruction of regulatory cells, exposure of sequestered or modified antigens, and "molecular mimicry". Molecular mimicry refers to the initiation of an autoimmune response because of sequence or structural homol-ogies between a self-protein and a protein in a viral or bacterial pathogen (Wucherpfennig and Strominger 1995). For example, EAE may be induced by immune sensitization with a peptide sequence from a pathogen with homology to MBP (Fujinami and Oldstone 1985). MBP shares extensive homologies at the amino acid level with measles, influenza and adenovirus. Residues 91–101 of MBP, for example, share stretches of four to six amino acids with adenovirus (Jahnke et al. 1985). Homology may be necessary at only a few amino acids for efficient T-cell recognition to occur. Conservation of the native amino acid sequence at four of ten amino acids of the MBP Ac1–10 epitope is sufficient to induce EAE (Gautam et al. 1992). Tolerance to MBP can be also broken by viral infection of the brain, another "innocent bystander" model. For example, anti-MBP responses are seen in measles encephalitis in humans (Johnson et al. 1984) and HTLV-I infection (Hara et al. 1994). In rats, infection of the brain with the neurotropic coronavirus results in a breakdown in tolerance to MBP, and activation of MBP-reactive T cells capable of transferring EAE (Watanabe et al. 1983). Thus, a neurotrophic virus may infect the nervous system and, by doing so, stimulate an immune response not only to the virus but also to normal nervous system proteins.

An alternative mechanism has recently been proposed, which implicates "exogenous superantigens" in the etiology of autoimmune diseases (Paliard et al. 1991). The term superantigen describes antigens that, at very low concentrations (in the picomolar range), can stimulate subsets of T lymphocytes (Chatila and Geha 1992; Herman et al. 1991; White et al. 1989). Superantigens bind with high affinity to class II MHC molecules outside the antigen binding grove. They interact with the Vβ chain of the TCR in the region of the β-pleated sheet, away from the CDR3 region, the putative antigen binding site. In a non-MHC restricted manner, with no need for antigen processing, the class II superantigen complexes trigger the proliferation of T cells expressing particular TCR-Vβ chains. This stimulation is independent of accessory molecules, and induces the release of cytokines such as IL-2, IFN-γ and TNF-α. A notable feature of superantigenic stimulation is that responding T cells initially mount a vigorous response, but then disappear or display anergy. Two groups of superantigens have been defined: endogenous superantigens (Acha-Orbea and Palmer 1991; Choi et al. 1991), retroviral sequences encoded within the genome, which have been identified in the mouse, but not yet in humans, and exogenous superantigens. This second group includes the toxins of many common bacteria, and possibly components of certain viruses, for example, the nucleocapsid of rabies (Lafon et al. 1992; Misfeldt 1990).

Superantigens are associated with numerous human diseases, such as food poisoning, toxic shock syndrome, scalded skin syndrome, and others (Misfeldt 1990; Zumla 1992). The involvement of exogenous superantigens as etiologic agents in several autoimmune diseases is currently the subject of active investigation (Abe et al. 1993; Conrad et al. 1994; Paliard et al. 1991). Many autoimmune diseases are exacerbated, and perhaps even preceded, by infections. Theoretically, superantigens may reverse the state of anergy on CD4+ T cells tolerant to self-antigens, activating pathogenic pathways. In a recent study, Brocke and colleagues (1993) showed that relapses and exacerbations of EAE could be induced with the superantigen Staphylococcus enterotoxin B (SEB), and, to a lesser extent, with SEA. At least part of the observed effect is due to specific reactivation of autoreactive cells in the periphery and to the production of TNF-α, because administration of anti-TNF antibodies can delay the onset of disease induced by SEB. Interestingly, SEB can prevent disease when given at least 14 days before onset of disease. Burns and colleagues (1992) examined the ability of staphylococcal toxins to stimulate human T cells specific for MBP or PLP, which are putative autoantigens in MS. All myelin antigen-specific T cells responded in proliferation studies to at least one of the nine superantigenic toxins used in this study. In some experiments, the superantigenic toxins were up to 7×10^5-fold more potent in proliferation assays than were the myelin antigens to which the T cells were initially sensitized. The authors suggest that massive superantigen stimulation during infection may be associated with the activation of myelin-specific T cells and disease exacerbation. These results may have important therapeutic implications. MacNeil and colleagues (1992) demonstrated that it is possible to inhibit superantigen recognition with peptides that resemble the site of superantigen interaction on the TCR-β chain.

Activation of T cells by superantigens requires the presence of MHC class II

molecules, the cross linking of toxin, or other costimulation. In the absence of these conditions, superantigens such as SEB appear to cause anergy. Trace levels of SEB suppress the pathogenic effect of encephalitogenic BV8-expressing T-cell clones (Ben-Nun and Yossefi 1992). It was recently observed that SEB can form biologically active complexes with soluble TCR molecules in both the absence and presence of class II MHC molecules (Seth et al. 1994). This finding may provide an explanation for the anergic effect of superantigens in vivo in the absence of costimulants or cross linking, and may lead to new approaches for manipulation of the immune response. It is likely that these strategies and methodologies will yield crucial information about environmental factors involved in MS pathogenesis.

Genes Conferring Susceptibility to Multiple Sclerosis

MS is not usually considered a genetic disease in the classic sense. However, the racial and familial clustering of MS, the higher disease incidence in women (roughly 2:1, compared with males), and the high concordance rate in monozygotic twins (25%–30%) compared with dizygotic twins (2%–5%) and non-twin siblings (2%–5%) (Ebers et al. 1986), all suggest genetic influences on susceptibility to MS. A simple model of inheritance for all MS is unlikely and cannot account for the non-linear decrease in disease risk with increasing genetic distance from the MS proband. Although, in some families, a pattern suggestive of autosomal dominant inheritance (parent to child or grandparent to parent to child) is found, more often siblings or first cousins are coaffected, suggesting recessive or oligogenic inheritance. Indeed, concordance estimates in twins and relatives of MS patients differ from predictions based on single-gene inheritance, and suggests a multigenic etiology (Ebers et al. 1986). Although it is likely that genetic heterogeneity exists in MS, a simple mode of inheritance or a single underlying susceptibility gene cannot be ruled out in a subset of pedigrees. Using published data from twin and multiply affected family studies, different genetic models of MS susceptibility were evaluated by Phillips (1993) using mathematical techniques developed by Risch (1990). His analysis favored a model involving multiple interacting susceptibility loci, each with a relatively small contribution to the overall risk. His analysis also indicates that perhaps as many as 15 mutually influenced (epistatic) susceptibility loci are involved in familial MS transmission.

By analyzing backcross experiments in mice and rat strains susceptible to EAE or demyelination caused by Theiler's murine encephalitis virus (TMEV), Blankenhorn and Stranford (1992) concluded that at least six chromosome markers in EAE, and four in TMEV, are associated with susceptibility to immune demyelination. In addition to the MHC and the TCR gene complexes, T-cell suppressor activity and the response to pertussis-induced histamine sensitization, were included as candidate, albeit unconfirmed, genes. The *Bordetella pertussis*-induced histamine sensitization (*Bphs*) locus was mapped telomeric to the TCR-β-chain gene, on the murine chromosome 6 (Sudweeks et al. 1993). It is important to note that pertussis toxin is used as an adjuvant in the induction of

EAE in mice. This region also contains a number of other loci with immunologic relevance.

Early attempts to identify the "MS gene(s)" focused on those polymorphic genes whose products participate in the immune response, such as the genes comprising the MHC on chromosome 6, TCR genes on chromosomes 7 and 14, and immunoglobulin genes. Although suggestive correlations between certain alleles at these loci and the presence of MS have been described, conflicting results are not unusual and critical questions remain unanswered (Oksenberg et al. 1993a).

The Major Histocompatibility Complex

In humans, the MHC, also known as the human leukocyte antigen (HLA) region, consists of linked gene clusters located in the short arm of chromosome 6 at 6p21.3, spanning over 3 million base pairs (Simpson 1988; Trowsdale 1988). An individual's ability to respond to an antigen, whether it be foreign or self, is in part determined by the amino acid sequences of these highly polymorphic molecules (Pullen et al. 1989; Unanue 1992). Not surprisingly, susceptibility to a number of diseases, most of them autoimmune, has been associated with particular class II alleles (Nepom and Erlich 1991; Todd et al. 1988). The association between an MHC determinant and MS was first described in 1972 (Bertrams et al. 1972; Maito et al. 1972). An extensive literature supports this association (Oksenberg and Steinman 1990). The majority of studies have focused on Caucasians of northern European descent, where predisposition to MS has been associated with the HLA-A3, B7, DR2, Dw2 extended haplotype. The class II (HLA-D) region provides the strongest association with MS, and molecular analyses have identified the susceptible DR2 haplotype as HLA-DR2, DRB1*1501, DQA1*0102, DQB1*0602 (Allen et al. 1994; Olerup et al. 1989; Spurkland et al. 1991; Vartdal et al. 1989). Attempts to localize further the susceptibility genes within the DR-DQ region have not provided consensus. The strong linkage disequilibrium across the DR-DQ region and the fact that DRB1*1501 and DQB1*0602 are found exclusively on this DR2 haplotype in northern Europeans (Begovich et al. 1992), has prevented a clear resolution of the relative contribution of each gene. Other as yet undefined genes within this region or specific combinations of particular alleles at the DR and DQ loci may actually confer susceptibility.

Although it is clear from data on multiple ethnic groups that the DR and DQ subregions of the MHC play a role in susceptibility to MS, there is no consensus on the actual gene or genes involved. Because these studies have been performed on various ethnic groups, the apparent contradictions might be reconciled, or at least explained from the geographic and ethnic variation among the different studies. It is possible that different genetic backgrounds may provide different susceptibility patterns for unrelated environmental factors. In addition to the class I and class II genes, the human MHC contains at least 70 additional genes. Other genes mapped in the MHC region include complement proteins C2, factor B and C4, and genes for the steroid 21-hydroxylase, collectively known as class

III genes, as well as genes for tumor necrosis factor (TNF-α and β), genes involved in antigen processing (LMP 1 and LMP 2) and transport (TAP 1 and TAP 2), and possibly genes regulating nonimmune functions and development. The MHC determinants may merely represent markers for other susceptibility genes located in the same chromosomal area. Although there is no direct proof, susceptibility is more likely to be mediated by the class II genes themselves (DR, DQ or both) due to the known functions of these molecules in the normal immune response.

Formal linkage studies of the MHC region in MS sibling pairs have also yielded conflicting results, although pooled data indicate that a small MHC effect may be present. Cumulative data, representing 244 sibling pairs, indicate that 95 pairs (39%) share both haplotypes, 115 pairs (47%) share only one haplotype, and 34 pairs (14%) do not share an HLA haplotype in common. By random segregation of HLA genes, one would expect sharing of 25%, 50% and 25% of genes in common by sibling pairs. Thus a shift towards greater than expected HLA gene sharing by coaffected MS siblings indicates a genetic effect of this region. However, complete HLA discordance in 14% of affected sibling pairs indicates that coinheritance of an HLA gene does not always occur, and suggests that the genetic influence of the HLA region on MS is small (Phillips 1993).

The T-Cell Receptor Complex

T-cell receptor genes were expected to have an impact in the overall genetic susceptibility to MS. Linkage studies have shown a clear influence of the TCR-β-chain gene complex (or genes linked to it) in experimental murine demyelination (Blankenhorn and Stranford 1992). An early approach to identify variations in TCR genes that could affect the development of human disease was based on the detection of polymorphic markers using restriction fragment length polymorphism analysis. Several polymorphisms in human TCR genes have been described and in certain cases were found to correlate with the incidence of MS (Oksenberg and Steinman 1990; Steinman et al. 1992). In contrast, other studies have revealed no association between germline polymorphisms of the TCR and susceptibility to MS (Hillert and Olerlup 1992).

As for MHC genes, a useful approach to test for TCR influences on MS is to test families with multiply-affected siblings using a series of markers located at different regions of the TCR complex (Robinson and Kindt 1992). In a collaborative study, Seboun et al. analyzed the inheritance of TCR-β-chain genes in families, with 40 sibling pairs with relapsing–remitting MS, using both human and murine probes (Seboun et al. 1989). The mean proportion of TCR-β haplotypes identical by descent inherited by MS sibling pairs was significantly increased compared with expected values, whereas the distribution of haplotype sharing was random when MS patients were compared with their unaffected siblings. Additional support for an effect of TCR-β inheritance in MS sibling pairs was presented in preliminary form for an English data set. The association between MS and the sharing of TCR-β haplotypes in families is not absolute (Lynch et al. 1991) and its actual contribution to susceptibility largely remains to be defined.

Other Candidate Markers

A multiallelic tetranucleotide repeat polymorphism identified 5′ to the MBP gene on chromosome 18 may be associated with MS (Boylan et al. 1990a, b). More recently, this genetic marker was studied in a homogeneous pool of 21 Finnish MS families (Tienari et al. 1992). A significant association was found between MS patients and the MBP marker, mostly attributable to the higher frequency of an 1.27 kb allele among patients in comparison to controls. The same Finnish group have since reported a highly significant two-locus linkage in MS, when MBP was analyzed in conjunction with the HLA locus (Tienari et al. 1994). Subsequent reports in other populations have failed to confirm linkage or association between MS and this genomic segment (Graham et al. 1993; Rose et al. 1993; Wood et al. 1994). Most of these studies, however, used a short stretch of the repeat and potentially informative polymorphisms may therefore have been missed.

In the past 5 years, genes that participate in antigen processing have been mapped to the MHC class II region (Monaco 1992; Trowsdale 1993). Two of these genes, which map between HLA-DNA and HLA-DOB genes, belong to the ATP-binding cassette transporter superfamily. They have been named TAP1 and TAP2, for transporters associated with antigen processing, and it was proposed that their products form a complex that transports peptides into the lumen of the endoplasmic reticulum for interaction with MHC class I molecules. Two additional genes, LMP2 and LMP7, which map close to TAP1 and TAP2, encode products that are related to subunits of a large cytoplasmic complex called the proteasome, thought to be involved in degradation of proteins in the cytoplasm. Limited polymorphism has been detected in both sets of genes. Powis and colleagues (1992) demonstrated that, in the rat system, allelic variation within TAP2 determines the peptides assembled in MHC class I RT1.Aa molecules, and their subsequent recognition by allogeneic T cells. Due to their location in the MHC class II cluster and their possible involvement in antigen processing of endogenous class I restricted proteins, it is tempting to postulate that polymorphism in the TAP and LMP genes may be involved in antigen peptide selection that might lead to autoimmunity. However, no association was detected between LMP or TAP polymorphisms and MS susceptibility (Liblau et al. 1993; Szafer et al. 1994). Further studies will be required to discover the extent of polymorphism in these genes, and to test whether different alleles contribute to autoimmune susceptibility.

A recent study employing transgenic methodology may shed light on some functional aspects of genetically determined susceptibility to autoimmunity (Scott et al. 1994). In this experimental model, transgenic expression of an influenza hemagglutinin (HA) on islet β cells, was combined with a diabetogenic BV8.3 TCR transgene specific for a class II-restricted HA peptide. Double transgenic mice displayed either resistance or susceptibility to spontaneous autoimmune diabetes, depending on genetic contributions from either of two common inbred strains, BALB/c or B10.D2. Functional studies of autoreactive CD4+ T cells from resistant mice showed, contrary to expectations, no clonal anergy, clonal deletion or receptor desensitization; rather, there was a non-

MHC-encoded predisposition toward differentiation to a non-pathogenic effector phenotype (regulatory Th2 cells versus proinflammatory Th1 cells). T cells from resistant double negative transgenic mice also showed evidence of prior activation by antigen, suggesting that disease may be under genetically controlled active regulation by autoreactive Th2 cells.

With the advent of genomic screening, a comprehensive and ambitious approach is now possible for the genetic dissection of complex traits such as MS susceptibility (Lander and Schork 1994). The goal in genomic screening is to scan the entire human genome for chromosomal regions that may harbor susceptibility genes. Regions linked to the disease can then be studied by positional cloning methods. This strategy takes advantage of two rapidly developing sets of tools: a large number of highly polymorphic microsatellite markers, and modern statistical techniques (Dawson et al. 1990; Pericak-Vance et al. 1991). Microsatellites are tandem DNA repeats (2–4 bases) characterized by the high degree of polymorphism in the number of repeated units. These markers were first identified about 5 years ago, and have since become the polymorphism markers of choice for genetic studies (Weber 1990; Weber and May 1989). Recent work has now generated microsatellite maps of every chromosome, and more refined maps are in progress as part of the human genome initiative. Genomic screening with anonymous markers followed by detailed analysis of candidate regions using positional cloning methods, represent a powerful tool to identify susceptibility genes. By collecting appropriate pedigrees, it will be possible to perform an efficient screen of the entire human genome to identify the genetic components of MS. Their characterization will help to define the basic etiology of the disease, improve diagnostic ability and risk assessment, and influence therapy.

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