MINIREVIEW

Multiple Sclerosis: Etiological Mechanisms and Future Directions

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Multiple sclerosis (MS) is a complex human autoimmune-type disease with a predominantly unknown etiology. Immunologic destruction of myelin basic protein (MBP) throughout the nervous system is the major pathology of multiple sclerosis. This review will attempt to update new information about basic mechanisms and therapeutic management of the disease. The significance of the structure of MBP is discussed with respect to the contribution of such structures to the disease process. A number of MBP peptides that serve as the immunodominant antigens in MS patients have been identified. These peptides have been studied in animal models for their antigenic characteristics and ability to induce disease. Evidence for genetic contributions is reviewed with multigenerational twin studies providing the best evidence for susceptible haplotypes. The role of microorganisms/viruses and environmental agents are discussed as potential etiological factors but are now thought to be of minor importance to the primary causal development of the disease. Of major consideration are immunological mechanisms that contribute to the development of autoimmunity. In particular, antigen expression, cytokine and leukocyte interactions, and regulatory T-cells are discussed. Particular attention is given to regulatory T-cells (Treg), which help balance/modulate other T-cells such as Th1 and Th2 cells, and how such Treg regulate autoimmunity is addressed. The importance of the role of Tregs is exemplified by the demonstration that administration of oral antigens can induce specific Tregs that counteract experimental autoimmune encephalomyelitis in animal models. The significance of animal studies to human multiple sclerosis is discussed. A potential role for natural antibodies and innate immune mechanisms to help provide resistance to disease development is also reviewed. Finally, a variety of therapeutic agents that have been and continue to be utilized for multiple sclerosis is reviewed. Trials with oral antigens, such as glatirmer acetate (copolymer 1) especially in combination with interferon-β, have shown promise. Antibody therapy and bone marrow transplantation are also briefly discussed.** Exp Biol Med 229:12–20, 2004

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Multiple sclerosis (MS) is an autoimmune human disease without fully effective treatment and largely unknown pathogenesis (1). The disease affects about 350,000 people in the United States and ranks as a major cause of nervous-system disability in young adults between the ages of 15 and 45 years. It is usually a sporadic disease and is characterized as a variably progressive human disease of the nervous system in which patchy degenerative and inflammatory changes occur within the brain and spinal cord. The degenerative and inflammatory changes are associated with the formation of sclerotic plaques due, in part, to abnormal hardening and fibrosis of the neuronal myelin sheath (a type of protective coat surrounding neurons). The symptoms are diverse, ranging from tremor, nystagmus, paralysis, and disturbances in speech and vision; it occurs chiefly in early adult life with characteristic exacerbations and remissions. Extensive demyelination is seen in the neuronal lesions and it is thought to involve the participation of antibody (1). There are basically three myelin-type proteins with specific antigenic epitopes thought to be involved in MS disease progression. These include myelin

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basic protein (MBP), proteo-lipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). In rats and mice, extensive demyelination is seen following administration of antigens that induce T- and B-cell activation (2). At the present time, the exact etiological mechanism of MS in man is not clear. However, several animal models are available that provide insight regarding the development of the disease. This review will attempt to update new information that may help define etiological mechanisms and contribute to the potential management of the disease.

Blood Brain Barrier and Lymphocytes

Breakdown of the blood brain barrier (BBB) is a crucial event in MS pathogenesis. Studies using magnetic resonance imaging (MRI) have shown that the first detectable change in lesion formation or the extension of old lesions is the breakdown of the BBB. In this regard, BBB breakdown and an increase in permeability is necessary for the induction of disease in rats with experimental allergic encephalomyelitis (EAE; Ref. 3). In addition, activation of T-lymphocytes is an event that appears to be essential for development of the disease. An important role for activated T-cells in BBB breakdown has been demonstrated by functional studies in which MBP-activated T-cells and antibody to MOG were coadministered systemically to Lewis rats. Marked augmentation of demyelination was demonstrated in these animals compared with those given T-cells alone, but no pathological changes resulted in animals given only anti-MOG antibody into the central nervous system (CNS). Histological studies revealed serum leakage into the brain associated with the presence of inflammatory cells and an increase in vesicular transport through BBB endothelial cells (4).

It is interesting to note that the breakdown of the BBB has been shown to be achieved by T-cells activated to CNS antigens not associated with the myelin sheath of neurons (5). Within the lesionous plaques of MS, perivascular inflammatory cell infiltrates are commonly found and are presumed to play an important role in BBB breakdown; however, there is no evidence that these cells show specific reactivity to any particular CNS antigen such as MBP, MOG, or PLP. There is also accumulating evidence that tumor necrosis factor (TNF) is an important cytokine in the process of cell migration and plaque formation (5, 6). Therefore, some studies indicate that, under certain conditions, the BBB can be disrupted by activated T-cells of nonneural specificity presumably by the synthesis of proinflammatory cytokines, which contribute to the formation of large plaque-like regions of demyelination.

Structural Aspects of MBP

Myelin basic protein is one of the most important proteins of the myelin sheath. The nucleotide and corresponding protein sequences of most forms of MBP from numerous species are known, with the human and bovine forms sequenced first (7, 8). It was also determined that MBP is coded for by a single gene and the mRNA sequence is known. Kamholz *et al.* (9) have isolated cDNA clones encoding three separate forms of human MBP with molecular weights of 21.5, 18.5, and 17.2 kDa, which transcribe proteins containing 197, 186, and 171 amino acids, respectively. Human MBP has a number of sequence similarities with other protein families (10, 11). Properties such as charge density, posttranslational modification by addition of fatty acids, and overall hydrophobicity are similar in MBP and PLP from the CNS and in the pulmonary surfactant proteins SP-B and SP-C (12). Other sequence similarities with viral proteins have also been identified, which has provided an index of suspicions for the possible viral involvement in the etiology and pathogenesis of human MS (13, 14).

Details of the tertiary structure of MBP have been hindered because of the difficulty in forming pure crystals of MBP (15). Ridsdale *et al.* have presented a three-dimensional structure of MBP based on electron microscopical investigations of the tertiary structure (13). These studies concluded that many residues subject to posttranslational modification (phosphorylation, methylation, or conversion of arginines to citrullines) were located in exposed loop regions and therefore were accessible to modifying agents or enzymes. Furthermore, posttranslational modifications that lead to reduced surface charge could result in a weakened attachment to the myelin membrane. It is thought that such mechanisms could be operative in diseases such as human MS (13). In fact, studies on MBP from a patient with fulminating MS (Marburgs Disease) revealed that 18 out of 19 arginine residues on the MBP molecule had been citrullinated (16). These modifications will decrease the net positive charge of the protein, promote lipid aggregation, and alter the functional properties of MBP (16).

Mapping studies of the sequence of MBP has led to the identification of a number of peptides, which have been individually studied for their relative antigenicity and possible involvement in disease (17). These peptides have also been shown to induce EAE when injected into animals. Of considerable interest are the MBP peptides 82-100, 84-103, and 85-99, which have high relative affinity for major histocompatibility complex (MHC) molecules expressed by cell lineages of MS patients and are therefore considered as candidate auto antigens in MS. Thus, these peptides bind to HLA-DR2 molecules and are recognized by T-cells from MS patients with the HLA-DR2 haplotype (17–19). The residues Val-96, Pro-98, and Arg-99 and the COOH-terminal part of the MBP 85–99 peptide bound to appropriate MHC molecules were found to specifically interact with their cognate T-cell receptors expressed by T-cells from MS patients (17).

Genetic Relationship/Susceptibility

The role of genetics and environmental factors in the susceptibility of MS remains complex and poorly

understood. It appears that factors such as geographical location, ethnic background, and clustering in temperate climates all contribute indirectly to MS susceptibility. In particular, individuals with a north European heritage appear to be statistically more susceptible to MS than those from a more tropical environment and it is more common in women (20, 21). It has been estimated that approximately 1 in 1000 persons of northern European origin who reside in temperate climates are more likely to develop MS. Epidemiological data from multiple whole-genome screens in multiplex families indicated that, in all probability, MS is not a single-gene disorder and, in addition, environmental factors contribute to the syndrome (22). It has been suggested that interactions between different genes could contribute to an increase in susceptibility (23). Therefore, the contribution of genetic factors are complex.

Strong evidence for potential genetic contributions comes from studies on families with twins (24). The risk of MS was found to increase with the degree of shared genetic information within a family and that multiple genes were thought to interact to increase susceptibility. In a comparative analysis of monozygotic and dizygotic twins, the risk for dizygotic twins and siblings was smaller but still increased 20–40-fold compared with that of the general population. Data from concordance rate analysis of individual twins indicated that genetic background as well as exogenous or somatic events appear to be essential for disease development (25). Among many candidate genes that have been analyzed, the MHC genes have been shown to be important. Some of the strongest associations with MS were genes of the HLA-Class II complex, in particular HLA-DR15Dw2 and DQw6 (25, 26). Interest in the MHC region focused on the short arm of chromosome 6 at band 21 (6p21) and a locus on the short arm of chromosome 5 (5p) (22, 27). Results from studies on a multigenerational family (3–4 generations) that had 15 individuals with MS suggested the possibility of a single major locus that was responsible for MS susceptibility, with HLA-DRB1 acting as an important modifier (22, 27). Furthermore, limited studies on a Finnish population showed some protective effect of the haplotype DRB1*13-DQB1*0603 and a greater disease risk with DRB1*15-DQB1*0602 haplotype (28).

Data from the above-mentioned studies suggest that, while genetically inherited MHC genes clearly contribute to disease susceptibility and/or resistance, it is likely that a combination of known/unknown environmental factors may in addition contribute to the development of the disease in these genetically predisposed subjects.

Role of Microorganisms and Environmental Agents

Many studies have suggested that there is an association between episodes of MS exacerbation and concomitant viral or microbial infections. It is thus reasoned that other antigens, such as those that are constituents of infective organisms, including viruses, could be cross-reactive with the major autoantigens of MS and as such, these mimics should be considered as potential T-cell targets (29). This view is supported by a number of other studies that have shown that T-cells reactive to brain antigens other than those associated with myelin can cause a breakdown of the BBB. In this regard, autoantigens from several types of microorganisms may be involved. For example, the 70-KDa heat shock protein of *Mycobacterium tuberculosis* and *Mycobacterium leprae* have been considered to be potential autoantigens in MS. In addition, a 28-KDa protein surface molecule of mycobacteria has been shown to mediate neuronal Schwann cell (myelin forming cell) entry or invasion (30, 31).

Although T-cells specific for MBP have been derived from MS brain tissue, the evidence for MBP to serve as the sole autoantigen in MS is lacking. Moreover, because T-cells from MS patients reactive to MBP also react strongly to peptides from a number of common viruses, it is not clear whether the primary antigen source is MBP or another viral peptide.

The possible viral/microbial-associated etiology for MS is considered to be based on (i) epidemiological evidence of childhood exposure to infectious agents and increase in disease exacerbations with viral infection; (ii) geographic association of disease susceptibility with evidence of MS clustering; (iii) evidence that migration to and from high-risk areas influences the likelihood of developing MS; (iv) abnormal immune response to a variety of viruses; and (v) analogy with animal models and other human diseases in which viruses can cause diseases with long incubation periods, relapse, and demyelination.

Many of these studies involve the demonstration of increased antibody titers to a particular virus, whereas some describe isolation of virus from MS material. Nevertheless, caution must be taken in the interpretation of data from studies suggesting virus association with this disorder (29, 32).

Recently, the human herpesvirus (HHV-6 and HHV-8) have been reported to be present in active MS plaques (29). HHV shares some homology with cytomegalovirus (CMV), and HHV has been considered as a possible viable etiologic agent in MS for several reasons, including (i) primary infection with HHV usually occurs during the first few years of life and is consistent with epidemiological evidence for MS, suggesting childhood exposure to an etiologic agent; (ii) HHVs, in general, are highly neurotropic and HHV proteins have been shown to be preferentially expressed by oligodendrocytes within MS lesions; (iii) a fundamental property of HHV is its ability to reactivate, and the same factors that lead to reactivation (stress, infections) have also been associated with MS exacerbations; and (iv) HHV has been found to infect cells of both lymphoid and non-lymphoid origin. Therefore, it has been reasoned that a pleiotropic virus such as HHV that infects both the immune and the nervous systems could explain abnormalities in both (29, 32).

Several environmental factors other than a virus could trigger the onset of MS. It has been suggested that a specific stress response and multiple factors within the CNS could influence the permeability of the BBB and the onset of MS (20, 33). Such factors include heat shock proteins, adhesion molecules, α-β crystallin expression by oligodendrocytes, and the secretion of certain cytokines such as TNF and interferon (IFN) (33, 34). A possible disease initiating trigger mechanism for MS expression could involve molecular mimicry between CNS tissue antigens and virus or other infectious disease agent molecules (35). Indirect immune-mediated injury to normal tissue in the course of clearing an infectious agent may come about due to antigenic similarity between microbes and other tissues. For example, the molecular similarity between virus and myelin antigens may be permissive for immunological cross-reactivity between HHV-6 and myelin antigens. Thus, T-cells become activated by exposure to virus, cross the BBB, and, in certain predisposed persons, misidentify normal myelin antigens as virus, resulting in tissue injury (36). In addition, ubiquitous viruses, such as retroviruses, are also considered as the possible environmental triggers to initiate the expression of MS (37).

It is presently considered by many that a virus or another environmental influence may trigger the expression of MS, but it is unlikely that HHV-6 is the basic cause of MS (20, 36, 37). However, previous exposure to certain microorganisms and antigens early in life may be important as a potential mechanism of resistance to development of not only MS but also a number of other autoimmune diseases.

Autoimmune Mechanisms

The findings that infectious agents can in some cases induce autoimmune diseases in certain experimental settings supports the view of an infectious etiology in the pathogenesis of MS (38). In contrast, infectious agents can also suppress allergic and autoimmune disorders under some conditions. (38). Epidemiologic data notes a steady rise in the incidence of allergies and autoimmune diseases in developed countries over the past three decades, exemplified by the fact that the incidence of MS doubled from 1969 to 1986 in several northern European countries (39, 40). It was also noted that the risk of MS has increased among persons who spent their childhood in an environment with a high level of sanitation (38, 41). Analysis of data indicates that industrialized countries have an increase in the frequency of asthma, allergy, and autoimmune diseases. In contrast, there has been a decrease in the incidence of many infectious and parasitic diseases in developed countries as a result of antibiotics, vaccination, and improved hygiene and socioeconomic status. It is of interest to note, however, that children of farmers in Western industrialized countries were found to have a much reduced frequency of asthma, allergy, and autoimmune disease as compared with children in rural settings (42, 43). In this farmer type of environment, children are exposed to more bacterial endotoxins in the dust from farm animals and it is thought that this could be a factor in the disease resistance in such a population. These types of observations suggested the hygiene hypothesis to help explain the onset of allergic and autoimmune diseases (42).

T-cells recognizing MOG have been detected in both MS patients as well as healthy people (44). However, MS-derived autoantibodies were found to be predominately directed at the 1–60 amino acid region of MOG, while naturally occurring anti-MOG antibodies derived from healthy people reacted selectively to the 154–218 amino acid region of the molecule. An important discrepancy between myelin-reactive T-cells in MS and those in normals is the activation state of T-cells. MBP reactive T-cells undergo *in vivo* activation and clonal expansion in patients with MS but not in healthy normals (44, 45).

The development of most autoimmune diseases is thought to somehow involve an imbalance between the frequency of prototype T helper 1 (Th1), T helper Type 2 (Th2) T cells, cytokines, and regulatory T-cells (46). Some evidence suggests that demyelination is associated with a predominant Th1 and allergic responses. The reciprocal down regulation of Th1 and Th2 cells by cytokines such as IFN-γ and IL-4 indicates to some investigators that the regulation of a polarized Th1 as compared with Th2 prototype immune response following exposure to an infectious disease agent can lead to protection against allergy and autoimmunity (46). Furthermore, induction of Th2 regulatory cells can exert bystander suppression for a number of autoantigens present in tissues. This may be important in order to control antigens that promote MS. Self-reactive CD4 T-lymphocytes are important effectors in many autoimmune diseases, including MS; however, the presence of self-specific CD4 T-cells does not ensure autoimmune disease development (47–50). In this respect, the frequency of such cells in people with disease does not differ significantly from normals (51). Experimental animal studies on rats and mice involving cotransfer and adoptive transfer of lymphocyte populations have shown that, in the presence of nontolerant self-reactive T-cells, a second small population of CD4 T-cells was responsible for prevention of the disease. These cells are referred to as types of regulatory T-cells (Tregs) and are a small but very effective population compared with other lymphocyte populations (46). The protective effect of regulatory T-cells has been demonstrated in several experimental animal models, including a spontaneous demyelinating disease or autoimmune encephalomyelitis, and EAE, which are similar in disease manifestations to that seen in MS patients (46). Immunological research has focused on the characterization of $CD4^+$ regulatory T-cells (Treg cells) and has so far identified these cells as CD4 T-cells that express the α chain of the IL-2 receptor (CD25) that synthesize TGF-β and IL-10 (52–54). Experimental data indicate that certain infectious agents stimulate the production of Treg cells, which have multiple protective effects, and that some of these Treg cells provide protection

against induction of autoimmune-like responses. Potential mediators of this regulatory and protective process involve production of IL-10 and TNF-β by the cells and the subsequent modulation of Th1 and Th2 responses (55, 56).

Antigen expression has important roles in autoimmune diseases such as MS. Thus, antigenic competition has been thought to influence the role of infection on autoimmunity and allergy (38). This concept suggests that the immune response to an antigen is decreased by a concomitant response against an unrelated antigen (38). It is considered that antigenic competition can affect both antibody production, cell-mediated immune responses, and autoimmune responses. Superantigens, which are components of bacterial or viral products, may in some cases have immunosuppressive properties. This mechanism is thought to involve modulation of the T-cell receptor V gene (57). Evidence for superantigens in protection against autoimmune disease is provided by the observations that murine EAE is prevented by treatment with staphylococcal enterotoxin B (58). In addition, enterotoxin B also inhibits the development of lupus nephritis in mice (59). Generalized immunosuppression is sometimes associated with measles and parasitic infections. In this respect, measles-induced immunosuppression is considered to be mediated by the direct effect of two virus proteins on cytokine-producing mononuclear cells (60). The passive transfer of maternal antiviral antibodies to newborns may have a role in the susceptibility of the child to subsequently develop autoimmune diseases. Zinkernagel suggested that decreased exposure of women to particular viruses before pregnancy may subsequently reduce the degree of protection against the virus and could provoke an immune response that could ultimately lead to an autoimmune disease in the child (61). In contrast, increased exposure before pregnancy counteracted this probability.

Finally, orally administered autoantigens (such as MBP) suppress autoimmunity in several animal models, including EAE, arthritis, diabetes, myasthenia gravis, and several more (62). When properly administered, oral antigens are known to induce antigen-specific regulatory T-cells in the gut (such as in Peyers Patches). It is considered that this approach may have potential for treatment in humans with autoimmune-type disorders.

Potential Role for the Innate Immune System

Among the innate immune protective mechanisms against susceptibility to autoimmune disorders are included the toll-like receptors (TRs), which are cellular receptors for various bacterial and viral ligands. When TRs are bound by specific ligands, mononuclear cells are stimulated to produce cytokines, some of which down regulate allergic and autoimmune responses (63). Toll-like receptors are considered by some to be an important link between innate and acquired immunity.

Fredrikson *et al.* (64) found that cord blood (CB) cells and a small number of normal human peripheral blood cells secrete anti-MBP and anti-AChR IgG antibodies. They concluded that autoantibodies against nervous tissue antigens are naturally occurring and may occur in a minority of healthy people. It was also found that CB has high numbers of T-cells recognizing nervous system myelin proteins and considered that this may be important for the mechanism of tolerance induction (64).

Recent evidence suggests that the innate (natural) immune system may help keep certain disease states in check and may actually suppress active expression of some diseases. For example, Rodman *et al.* (65–67) have identified a set of natural antibodies that show, *in vitro*, reactivity with the Tat protein of HIV, and which are present in normal human sera but are significantly reduced or absent in HIV patients with active disease. Furthermore, HIV-positive patients who do not develop active disease for prolonged periods of time (long-term nonprogressors) have near-normal titers of the antibodies and a fall in the antibody titer leads to relapse. Similar Tat-reactive natural antibodies are found in chimpanzee sera but not in sera of other animals. It is also significant that chimpanzees are highly resistant to HIV-induced disease. Characterization of epitopes has revealed that specific arginine rich motifs (ARMs) on the Tat protein are required for infectivity and activity of HIV. Reactivity of natural antibody specifically with the ARMs of the Tat protein is thought to markedly decrease the transcription of HIV. Therefore, specific ARMs appear to be required for infectivity and expression of HIV, and natural antibodies may provide resistance to the disease unless their levels are depleted.

Many microorganisms (bacteria/viruses) contain ARMs within their cell coat, core protein, or accessory proteins, and these ARMs are thought to play a role in the attachment, infectivity, replication, cellular interactions, and entry of these agents into mammalian cells. Such could be the case for the presence of organisms in the etiology of MS. These organisms could promote T-cell activation, which leads to demyelination and sclerotic plaque formation. It appears that some people are more susceptible to certain diseases such as MS, whereas others may have some natural resistance possibly due to naturally occurring antibodies. Therefore, the potential role for the innate immune system in MS remains to be clarified. It is possible that expression or exacerbation of the disease may be modulated or repressed by natural antibodies and the complement system and by biochemical/immunological manipulation that in turn influences the mechanism of cell activation, bystander suppression, and eventual demyelination.

Therapeutic Prospects

Several therapeutic agents for the treatment of MS have been tested and studied, but the management of the disease still remains complex and unreliable. Some of the older agents include Imuran, Cytoxan, Methotrexate, Cladribine, and more recently IFN-β and novel strategies, such as the

use of T-cell receptor peptide immunization. Anti-CD4 MoAb treatment has been of interest because CD4 helper T-cells are essential components for the induction of autoimmune antibodies. It is thought that treatment with such anti-CD-4 antibody interferes with the formation of the antibodies and myelin destruction (68, 69). Preliminary trials have been well tolerated and are still in progress.

In the past several years, many investigators have utilized the concept of oral tolerance for the treatment of autoimmune diseases in animals by feeding such animals the autoantigens. Based on successful animal models and the safety of the oral tolerance approach, human trials were initiated for MS, using MBP as the fed protein (70, 71). Early trials indicated that continuous oral administration of antigen was required to achieve some limited favorable responses with little or no toxicity. Other antigens are also being considered for potential therapeutic use in MS. At the present time, IFN-β and glatiramer acetate (copolymer 1, copaxone) are major approved medications for treatment of relapsing remitting MS. However, these drugs are only partially effective in MS treatment, and IFN-β can be associated with toxic side effects.

Glatirmer acetate (GA) is a synthetic copolymer composed of four basic amino acids and was initially designed to mimic MBP. Moreover, injection of GA into animals does not induce EAE, whereas injection of MBP does. As first demonstrated in animals, GA suppresses EAE induced by various encephalitogens (including MBP) and induces suppressor T-cells, which are deficient in MS, and is relatively nontoxic (72, 73). It preferentially affects T-cells specific for CNS autoantigens, alters their antigen/MHC recognition, and induces populations of GA-reactive Th2 regulatory cells that provide bystander suppression in the CNS (induction of cross-reactive Th3-, Th1-, or Th2-type regulatory cells) (72, 74). GA acts against the immunodominant epitope 82-100 of MBP by T-cell receptor antagonism and binds strongly with purified MS-associated HLA-DR2 (DRB1*1501) molecules of the MHC (73, 75, 76). This interferes with the recognition of self-antigens by autoreactive T-cells and results in the suppression of EAE.

The IFN-B exerts several beneficial effects in an antigen-nonspecific manner. It induces IL-10 secretion and suppresses IFN-γ-inducible MHC Class II up regulation on antigen presenting cells (APC). Combined therapy using GA and IFN-β are presently being contemplated (77, 78).

MS patients go into remission during pregnancy, and fewer relapses are observed during this period, which is marked by an increase in the production of sex hormones. Ovine type interferon, or IFN-τ (tau), has been found to promote a Th2 cell bias and enhance suppression of EAE by oral GA given to mice (77). IFN-τ, first described as a pregnancy hormone in ruminants, possesses antiviral and immunoregulatory properties (79). It induces T-cell secretion of IL-10, suppresses IFN-γ-inducible Class II up regulation on APC, is not toxic, and is equally effective either orally or parenterally (80, 81). It is considered to be a potential candidate for use as a single agent or in combination with GA for treatment of MS. Presently, clinical trials are in progress and show some positive results (81, 82). In other experiments, oral feeding of mice with a semisynthetic estrogen, ethinyl estradiol, was found to suppress EAE, down regulate inflammatory factors, and inhibit recruitment of inflammatory cells into the CNS of mice (83). It is now considered that ethinyl estradiol may be a potential candidate for therapy of MS.

A totally different approach to the management of MS with a monoclonal antibody has recently been reported. The antibody, natalizumab (Antegren; Biogen Inc., PLC, Dublin, Ireland), is a humanized monoclonal antibody directed against α 4-integrin. It disrupts adhesion molecule interactions and inhibits migration and trafficking of leukocytes through the BBB. Short-term clinical results show a clear reduction in the number of active brain lesions and fewer relapses (84, 85). Longer trials with more patients are required before a therapeutically beneficial role is more clearly defined. A therapeutic role for this antibody in the management of Crohn's Disease is also being investigated (86).

Protocols for controlled studies in progressive refractory MS have been designed in the last few years using high-dose immunosuppressive therapy and hematopoietic stem-cell transplantation (HSCT) (87). Although studies are preliminary and more patient data are needed, autologous HSCT has provided some early positive results in the management of progressive severe refractory MS and may be therapeutically feasible in certain cases (87–89).

Concluding Remarks

It is clear that several different treatment modalities for MS have been and are continuing to be attempted; however, the basic mechanism of the disease still remains elusive for more specific long-term treatment. For example, a better understanding of the mechanisms by which immunoregulatory molecules induce their effects, such as the intracellular signaling pathways they can activate, such as STATs (signal transducers and activators of transcription) and other gene modifiers, could provide information on the regulation of the innate immune response, tolerance, and the development of autoimmune disease (90). Furthermore, the mechanism by which Th2 regulatory cells can be induced to provide bystander suppression of autoantigens and response to MBP peptides needs to be more fully investigated. Little is known about the significance of the innate immune system and development/prevention of autoimmune diseases. More studies with a humanized animal model for MS may help clarify some of the differences between human MS and EAE in animals (91). Intravenous immunoglobulin (IVIG) administration (92), oral administration of interferon (93), and treatment with the immunosuppressive agent FTY720 (94) may also offer future approaches to the management of MS. It remains necessary to better understand the multiple

basic genetic and immunological mechanisms and the mechanisms by which they are controlled in order to manipulate events by new therapeutic tools that will help prevent the development of MS.

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