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Experimental immunotherapies for multiple sclerosis

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Summary. Multiple sclerosis (MS) is a chronic demyelinating disease affecting the central nervous system (CNS) principally in young adults. Although its etiology is as yet unknown current evidence suggests that tissue damage is mediated by autoimmune T cells. The examination of an experimental animal model for MS, experimental allergic encephalomyelitis (EAE), has demonstrated that myelin basic protein (MBP)- or proteolipid protein (PLP)-specific T cells mediate the destruction of CNS myelin. In recent years, elegant studies in EAE have shown that encephalitogenic T cells recognize short peptides of MBP or PLP in the context of MHC/HLA-class II molecules, express a restricted number of T cell receptor (TCR) molecules and secrete interferon-γ and tumor necrosis factor-α/β. Understanding the pathogenetic steps in lesion development at the molecular level led to highly specific immunotherapies for EAE targeting each individual molecule. It has been the hope of many investigators that immunological events resembling those in EAE can be found in patients with MS and that the specific immunotherapies effective in EAE could also be applied to MS. However, to date, the evidence for a unique immunological abnormality in MS is not strong. Although MBP- and PLP-specific T cells with properties similar to those that are encephalitogenic in animals can be isolated from patients, they are not specific for MS and occur with similar frequency in controls. In addition, the variability in specificity and TCR usage has raised questions regarding the relevance of these cells in patients. The importance of the T cell responses to myelin antigens in MS may not be established until the effects of abrogating their activity through specific therapies targeting the trimolecular complex (TMC) have been demonstrated. Consequently, attention has begun to focus on modifying the biology of the MS lesion rather than targeting the initiating event at the level of the TMC, and the success of this approach is reflected by the effect of interferon-β on lesion development in MS. The recent approval for the use of interferon-β for the treatment of relapsing-remitting MS has raised great interest in examining novel strategies for immunotherapies in MS. The

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basic concepts as well as the current candidates for such new immunotherapies will be outlined in this short review.

Introduction

The etiology of multiple sclerosis (MS) is not known, but it is currently believed that autoimmune T cells specific for myelin antigens are involved in its pathogenesis [77]. Although the evidence for an immunological mechanism is largely circumstantial, it is becoming increasingly convincing. The pathology of MS resembles that associated with immunologically mediated diseases [100], and, in some patients, a beneficial effect of immunosuppressive therapy is seen. Further, the similarities between MS and the experimental autoimmune demyelinating disease, experimental allergic encephalomyelitis (EAE) support an autoimmune process in MS. While it is clear that the different forms of EAE that can be induced in different susceptible animal strains do not reflect all the clinical, histopathological and immunological features of MS, EAE is nevertheless an excellent model that has led to a better understanding of the pathogenetic steps leading to demyelination. In the chronic-relapsing form of EAE in the SJL mouse, the disease exacerbates and remits as in MS [99], and the pathology, characterized by inflammation and demyelination with lesions of different ages, bears a strong resemblance to that seen in MS [108, 109]. It is crucial to remember, however, that, while studies using the EAE model may provide important clues regarding potential treatment of MS, experimental treatments in EAE are mostly initiated during the period of disease induction. Even treatments that ameliorate the subsequent course when used after the disease is established operate in conditions considerably different from those in MS, where therapy is usually begun long after the disease has started. Evidence derived from magnetic resonance imaging (MRI) studies indicates that even at the time of the initial clinical episodes, many patients show multiple lesions, indicating considerable previous central nervous system (CNS) inflammation [46]. Based on evidence from the EAE model, some generalizations regarding the cause and potential therapy of MS can be made. First, it is possible that MS, like EAE, is caused by T cells recognizing antigens found in the CNS. Although studies of EAE have focused on two encephalitogenic proteins of myelin, myelin basic protein (MBP) [36] and proteolipid protein (PLP) [140], several other antigens, including proteins that are present predominantly in astrocytes and not in myelin, have been found to cause encephalitis [5, 60] although sometimes without demyelination. In addition, the T cell response to viruses which can persist in the CNS such as Theiler's virus [74], Corona virus [147] or a rat-adapted measles virus [71], can also produce an EAE-like picture. In MS, it is uncertain whether the immune response to one antigen triggers the disease or whether the relevant antigen varies from patient to patient. Further, in EAE, an immune response to multiple myelin antigens may occur as disease progresses and myelin breakdown occurs [86]. Thus, in MS an autoimmune response directed at multiple CNS myelin antigens could contribute to disease.

Since the general concepts about the activation of myelin-specific T cells and lesion development have been derived from EAE and the study of MS pathology, these findings shall be briefly summarized. It has been known for a long time that outbred animals such as mice are highly resistant to the induction of EAE, whereas disease can easily be induced in certain inbred rodent strains [36]. The factors that are most closely associated with disease susceptibility are members of the major his-

tocompatibility complex (MHC) class II gene family, i.e., certain IA- and IE-MHC molecules (e.g., IA^5 in the SJL mouse) [37, 87]. Further examination of the influence of these MHC class II molecules has demonstrated that particular peptides derived from myelin antigens are preferentially recognized and are encephalitogenic in the various animal strains [36]. The portion of a specific myelin protein (i.e., MBP) that is encephalitogenic differs between species and strains and is influenced by the MHC makeup of the animal. Another important finding in EAE has been that encephalitogenic T cells in Lewis rats and B10.PL and PL/J mice preferentially use a very limited set of T cell receptor (TCR) chains ($V\beta 8.2$ [19, 25] and $V\alpha 4.3$, PL/J mice [1] or $V\alpha 4.2$ and -2.3 in B10.PL mice [141]) in the recognition of the complex formed by disease-associated MHC class II molecules and encephalitogenic peptide. This relationship between disease and the trimolecular complex (TMC) comprised of TCR, MHC molecule and autoantigenic peptide has led to innovative therapeutic approaches targeting each of the components specifically. Blocking the MHC molecule with a nonstimulatory modified encephalitogenic peptide [156] or an unrelated peptide with high binding affinity [68] as well as T cell vaccination [11] or TCR peptide vaccination [48, 144] have been successful in the EAE model. Whether similar approaches will be successful in MS is not certain since the relationship between a specific TCR and disease is not yet as clear in MS as it is in some of the EAE models.

Considering the events that result in the induction of an autoimmune reaction, it is most likely that myelin-specific T cells are activated in the periphery. This conclusion is supported by the fact that T cells with specificity for a number of myelin antigens have been demonstrated in animals before induction of EAE and also in healthy human individuals [20, 76, 98]. Furthermore, it has been shown that encephalitogenic T cell lines (TCL) can be generated by repeated stimulation with MBP from lymph node cells of healthy Lewis rats [118] and marmosets [40]. These findings not only demonstrate that autoreactive T cells are a component of the T cell repertoire in healthy individuals, but also that they have the potential of mediating a demyelinating disease and are usually either actively suppressed or do not encounter their target autoantigen in the proper co-stimulatory environment such as the sufficient expression of CD28 and B7 antigens. Although the natural stimuli that lead to activation of myelin-reactive T cells in MS are as yet poorly understood, either viruses or bacterial superantigens could result in the activation of autoantigen-specific T cells. Viruses could also contribute to tissue tropism by inducing or up-regulating the expression of necessary adhesion and co-stimulatory molecules in the target organ.

Following activation the first event in lesion development appears to be the adhesion of activated T cells to endothelial cells in postcapillary venules preferentially in the periventricular white matter [110]. The relative importance of interactions between cell adhesion molecules (CAM) and selectins expressed on endothelial cells and their ligands on encephalitogenic T cells is not yet completely understood. The majority of studies demonstrate an up-regulation of constitutive expression of CAM and selectins on endothelial cells during the early phases of lesion development [9, 21, 34, 93, 152]. The need for up-regulation of these CAM as a requirement for T cell adherence and transmigration is currently unresolved [110]. The ability of activated T cells to transmigrate through the endothelial cells into the brain parenchyma has been clearly demonstrated in the rat [149] and mouse [30, 31], indicating that whichever CAM are expressed constitutively they are sufficient for this process. A relationship between encephalitogenicity and expression of very late antigen (VLA)-4 has been identified on PLP- [62] and MBP-specific T cells [9] and antibody to

VLA-4 reduces the severity of EAE [158]. Expression of vascular CAM (VCAM)-1 is, however, not usually high in acute active lesions but is increased in chronic active lesions suggesting that it occurs during a later stage of lesion evolution. Intercellular adhesive molecule (ICAM)-1, the ligand for LFA-1 on lymphocytes, is expressed at low levels constitutively [128]. Efforts to block disease with antibody to ICAM-1 or LFA-1 have met with variable success [22, 151]. Some investigators have reported no effect, while others have observed worsening of disease [22, 150]. The effect of anti-ICAM-1 and anti-LFA-1 seems to be related to the dose (M. Racke, unpublished results) and timing when antibody was given. Most likely, interactions with selectins and ICAM-1 are sufficient for the T cell to allow initial adhesion to the endothelial cell. Whether the T cell can then transmigrate through the endothelial cell barrier or whether some very local up-regulation of additional cell CAM such as VCAM-1 occurs is not known. Both interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which are secreted by activated encephalitogenic T cells, can up-regulate CAM and MHC class molecules [22, 151]. Endothelial cells can present MBP to MBP-specific T cells in vitro, which results in lysis of the endothelial cell after MHC expression on these cells is up-regulated by IFN-γ [80]. The importance of antigen presentation at the level of the endothelial cell in EAE or MS is unknown.

Cells that have encountered their target antigen remain in the CNS and initiate an inflammatory response. Adoptive transfer of MBP-sensitized T cells labeled with ^{14}C [30] indicate that these cells can be found in the lesion during the early stage of lesion development. The labeled cells tend to remain in the perivascular cuff suggesting that antigen presentation takes place in the perivascular area [30]. These results are consistent with histochemistry and polymerase chain reaction (PCR) amplification studies of TCR expressed by encephalitogenic T cells in affected tissue, which have demonstrated the presence of specific cells early during the course of inflammation [10, 18, 54]. As the lesion progresses, secondarily recruited cells far outnumber the encephalitogenic T cells [10, 54]. Thus, the T cells that enter the CNS initially and encounter antigen induce a cascade of proinflammatory cytokines, which leads to activation of the endothelium and recruitment of additional inflammatory cells including macrophages. Among the factors secreted in the tissue by autoantigen-specific T cells, TNF-α/β (lymphotoxin; TNF-α/LT) and IFN-γ are probably the most important ones. Both TNF-α and IFN-γ are involved in the differentiation of Th1 CD4 T cells, in the up-regulation of MHC antigens and adhesion molecules [111, 151] on endothelial cells and putative antigen-presenting cells (APC) in the CNS, and the recruitment and activation of other T cells and mononuclear phagocytes. Furthermore, TNF-α can mediate direct toxicity to myelin membranes and oligodendrocytes [120]. The actual effector mechanisms causing demyelination are uncertain, but could include the abovementioned effects of TNF-α and IFN-γ and reactive oxygen and nitrogen intermediates (O_2^- and NO) that are secreted by macrophages upon activation. Finally, myelin-specific autoantibodies that may enter the inflammatory site through an open blood-brain barrier (BBB) and be secreted locally by plasma cells may mediate the attachment of activated macrophages to myelin molecules such as myelin-associated glycoprotein (MAG) or myelin oligodendrocyte glycoprotein (MOG) that are integral parts of the myelin membrane. Some experimental evidence for each of these mechanisms exists [73].

The inflammatory response in the CNS parenchyma is probably terminated by apoptosis of infiltrating antigen-specific and nonspecific T cells in situ [119, 133].

Table 1. Pathogenetic steps and factors involved in inflammatory demyelinating diseases

Sensitization/activation of myelin-specific T cells
EAE
Encephalitogenic T cells specific for myelin and glial antigens or viruses persistently infecting the CNS
Disease susceptibility linked to MHC class II makeup
Disease mediated by $CD4^+$, MCH class II-restricted, Th1 T cells
Restricted TCR usage seen in some animal strains but encephalitogenic T cells not limited to those TCR even in those strains
MS
T cell specificity of disease-mediating T cells unknown
Limited evidence for restricted TCR usage
Disease associated with HLA-class II makeup, but no absolute linkage
Entry of T cells into the CNS
EAE
Up-regulation of cell adhesion molecules may be required
Entry of T cells nonspecific and related to level of T cell activation
MS
Role of adhesion molecules uncertain
Parenchymal effector phase (induction of inflammatory response)
EAE
Disease correlated with Th1-type T cells
Secretion of proinflammatory cytokines associated with acute lesions
Lesion development related to secondary recruitment of T cells and monocytes
Induction of Th2-type cytokines reverses or prevents lesion development
MS
Acute lesions associated with both Th1- and Th2-type cytokine production
Demyelination
EAE
Oligodendrocytes preserved in acute lesions
Ultrastructural evidence for macrophage-myelin interaction
Demyelination enhanced by transfer of antibody to MOG
Cytokines such as TNF/LT may directly damage oligodendrocytes
Reactive oxygen and nitrogen intermediates may contribute to tissue damage
MS
Ultrastructural evidence for macrophage-myelin interaction
Oligodendrocytes probably not targeted in acute lesions
Oligodendrocytes lost in chronic lesions
Remyelination
EAE
Incomplete remyelination found in some animal strains
Can be augmented in some animal models using antibodies
MS
Incomplete remyelination in acute lesions

EAE, Experimental allergic encephalomyelitis; MS, multiple sclerosis; CNS, central nervous system; TCR, T cell receptor; MOG, myelin, oligodendrocyte glycoprotein; TNF/LT, tumor-necrosis factor-α/lymphotoxin-α

Remyelination is usually incomplete and the myelin protein composition of remyelinated plaques changes considerably during the course of the disease [86].

Thus, several stages of lesion development can be targeted therapeutically and are listed in Table 1. The discussion of current directions in the treatment of MS will be examined with respect to the phase of lesion development targeted by the treatment.

Therapies employing general immunosuppression

Since the specificity of the immune response producing disease is not known, the use of general and, in most cases, drug-induced immunosuppression was and continues to be an important approach in the treatment of MS. A large number of general immunosuppressive treatments have or are being studied. These are listed in Table 2. The evidence for significant effectiveness of any of these agents is marginal and often controversial. Many of the trials of immunosuppressive drugs that have been reported as showing effectiveness have been questioned because of the methods applied. The problems in identifying the usefulness of immunosuppressive drugs may be more a question of difficulties in conducting clinical trials in MS than a real lack of effectiveness of these drugs. Most immunosuppressive drugs have been tested in trials involving patients with chronic progressive MS (CP-MS) since the relationship between the potential risks and possible benefits is thought to be clearer. However, outcome measures in patients who already suffer from considerable disability are difficult and progression of disease in this patient population may also be related to mechanisms such as axonal degeneration further complicating the assessment of the results. Difficulties such as these may be overcome by trials involving larger numbers of patients and long periods of treatment, both of which are usually impossible because of the expense. Of interest, the recent meta analysis of the various trials using azathioprine indicate that the drug has some effectiveness in reducing progression of disability [159].

Since the role of antigen-specific suppression is uncertain because of our inability to identify a causative agent or the specific myelin antigen that is pathogenetically relevant in an individual patient, generalized immunosuppression may remain an important part of therapy in MS. Hopefully, studies using MRI as an outcome measure will permit assessment of these drugs over shorter study periods, thus limiting the potential risks of the treatment so that their effectiveness in patients with milder relapsing remitting MS (RR-MS) can be examined [83].

Table 2. Unspecific anti-inflammatory and immunosuppressive treatments

Corticosteroids
Azathioprine
Cyclophosphamide
Mitoxantrone
Cyclosporine
Methotrexate
Deoxyspergualine
2-Chlorodeoxyadenosine
Total body irradiation

Therapies targeting disease-producing T cells and the TMC

Although our understanding of the antigens and cells involved in myelin-specific T cell responses has greatly increased in recent years, the antigen or antigens which are the target of the immunopathological process in a specific patient or group of MS patients are still poorly understood. Some experimental therapies have built on the probability that the proteins with strong encephalitogenic potential in experimental animals such as MBP or PLP are likely candidate antigens in MS and on the increased number of MBP- and PLP-specific T cell in the peripheral blood and cerebrospinal fluid of MS patients [91, 92, 94, 163]. Based on this assumption, therapies designed to block or eliminate this response have been undertaken or are being designed (Table 3).

Therapies targeting the TMC can lead to a number of potential outcomes including blocking activation, induction of anergy or tolerance rather than activation, induction of programmed cell death (apoptosis) or change in functional characteristics of the T cell, so that it either no longer mediates disease or possibly even targets suppressive cells to the lesion. Specific therapies can be directed at each of the components of the TMC, i.e., the antigenic peptide, the TCR or the MHC class II molecule that presents the antigenic peptide to the TCR. Attempts to inactivate MBP-specific T cells in EAE by stimulating a potentially existing regulatory or suppressive network date back to the early 80s when Cohen and coworkers administered inactivated encephalitogenic T line cells [11] as a T cell vaccination. It was later shown that T cell vaccination may either be directed against molecules specific for the encephalitogenic cells, most likely the TCR idiotype [48, 132, 144] or against activation molecules on encephalitogenic T cells (anti-ergotypic therapy; [75]). The presence and in vivo activity of $CD8^+$ T cells that directly lyse encephalitogenic T line cells and suppress disease was demonstrated later [64, 132]. Recent studies in MS patients demonstrated that the administration of inactivated MBP-specific T cell clones not only induce populations of $CD8^+$ idiotype-specific T cells, but also result in a reduction of precursor frequency numbers of MBP-specific T cells [162].

Shortly after the demonstration of regulatory T cells in EAE in vitro, it became clear that encephalitogenic T cells in Lewis rats as well as in B10.PL and PL/J mice, although recognizing different MBP peptides in the context of different restriction elements, express a restricted TCR repertoire with the predominant expression of a particular β chain, Vβ8.2, and also a limited number of α chains [1, 19, 25, 141]. This observation has not only led to the successful application of monoclonal antibodies directed against Vβ8.2 [1, 19, 141, 160], but also allowed the development of therapies that employed the administration of peptides derived from these TCR chains rather than the injection of inactivated whole T cell populations [48, 144]. Such TCR peptides have been either derived from a region of the variable chain of the Vβ8.2 molecule (complementarity determining region 2, CDR2; [144]) or from the junctional region (CDR3) of the TCR [48]. The rationale for using a CDR2 peptide was to immunize with a peptide chain that is shared by all $V\beta8.2^+$ cells; this bears the inherent problem that every immune response that employs Vβ8.2 would be affected. The second approach, using a CDR3 peptide, assumed that targeting the area of the TCR that is known to interact with the antigenic peptide (CDR3; [53]), rather than with the MHC molecule (CDR2 and CDR1), would be more suitable and tailored specifically for the cells that react with the known encepalitogenic peptide. Both strategies were effective in EAE and the therapy that is based on immunization with the CDR2 peptide of Vβ8.2 presumably acts through the induction of $CD8^+$ regulatory T cells and TCR-

Table 3. Therapies targeting the trimolecular complex

1. Therapies unspecifically modifying the activation and function of antigen-specific CD4 T cells:

- Copolymer-1; interferes with antigen-presentation
- CD4 analogue peptides; interfere with T cell – APC conjugation
- Antibodies against HLA-class II antigens or CD4; interfere with antigen-presentation to class II-restricted T cells
- MHC-blocking peptides; interfere with antigen presentation

2. Antigen-specific inactivation of T cell responses against the encephalitogenic portions of myelin proteins:

- Antibodies against complexes of disease-associated MHC class II antigen and encephalitogenic peptide; exact mechanism of action not known
- Immunization with peptides derived from the third hypervariable region of disease-associated MHC class II antigen; induction of antibodies against this portion of the disease-related MHC class II antigen

3. Induction of anergy or tolerance:

- T cell vaccination and TCR peptide vaccination; induction of regulatory T cells of $CD4^+$ or $CD8^+$ phenotype as well as antibodies against portions of the TCR
- Anergy induction with peptide/MHC complexes, with membrane fractions of APC that had been prepulsed with encephalitogenic antigen or with fixed APC coupled with myelin antigen
- Oral tolerance; dose-dependent induction of anergy and/or active suppressive mechanisms including IL-4- and TGF-β secreting T cells
- Administration of altered peptide ligands based on the encephalitogenic peptide; induction of anergy or functional antagonism

4. Induction of apoptosis:

- High-dose i.v. injection of myelin antigen leads to apoptotic death of preactivated encephalitogenic T cells

6. Treatments inducing functional changes in encephalitogenic T cells:

- Altered peptide ligands based on the the encephalitogenic or immunodominant peptide may include phenotypic switches in encephalitogenic T cells from Th1 to Th2 cells

APC, antigen-presenting cell; IL, interleukin; TGF-β, transforming growth factor-β

specific antibodies [39, 89]. In addition to the induction of $CD8^+$ T cells, the presence of $CD4^+$ TCR peptide-specific T cells involved in specific regulatory network has been reported [65]. Unfortunately, only a few studies have shown that T cells that had been induced by either T cell or TCR peptide vaccination specifically recognize either the immunizing cell or the cell expressing the region from which the CDR3 TCR peptide was derived [64, 132]. Although the effectiveness of these approaches has been debated [56], it is now generally agreed that TCR peptide vaccination is effective in EAE. A clinical trial in MS that is based on the observation that Vβ5.2 and Vβ6.1 are overrepresented in MBP-specific TCL in MS patients [61] is currently under way [14, 27] and has shown that the administration of CDR2 peptides is well tolerated and, in some patients, results in a reduction of precursors for MBP-specific T cells and in T cell immunity against the immunizing peptides [14, 27]. The approach of TCR peptide and T cell vaccination has fascinated immunologists because of its extreme specificity in inducing an immune response only against the pathogenetically relevant cells. It relies, however, on restricted TCR usage by autoantigen-specific T cells, and that the particular TCR usage is known. Based on the current data, it seems unlikely that human autoantigen-specific T cells use a restricted TCR repertoire [42,

78]. Since we do not know which antigen and which T cell population is most relevant in an individual MS patient, we can currently only speculate whether TCR peptide vaccination will ever be used in MS. If so, it might be necessary to tailor an individual therapy for each patient. There is, however, some evidence that both the specificities and TCR usage of, for example, MBP-specific T cells in subgroups of patients such as those that are DR15 $Dw2^+$, show similarities [90, 94, 157]. Furthermore, the number of epitopes recognized and the heterogeneity of TCR expressed is probably smaller during the early stages of disease [124, 143]. Finally, the findings of restricted TCR usage in the brains of MS patients [90] as well as the skewing in the TCR repertoire that was observed in identical twins discordant for MS [142] argue for the involvement of T cells in the pathogenesis of MS and suggest that specific immunotherapies targeting subsets of T cells might be feasible at certain stages of the disease and in well-defined subgroups of patients.

Even before the TCR usage of encephalitogenic T cells was studied, it became clear that susceptibility for developing EAE in various animal strains was largely related to their MHC class types [37, 87]. Furthermore, depending on the disease-associated MHC class II phenotype, different regions of the encephalitogenic protein, such as MBP, were encephalitogenic in the different strains (for example, amino acids, aa, Ac1-11 in B10.PL and PL/J mice, aa 89–101 in SJL/J mice and aa 68–86 in Lewis rats; [36]). Based on these observations, a number of elegant studies have shown that modifications of those encephalitogenic peptides that are capable of binding to the restriction element with high affinity, but which do not induce an encephalitogenic response themselves, can block EAE [127, 156]. This could also be achieved with high affinity, binding peptides completely unrelated to the encephalitogen [68]. However, this approach has a number of problems. Theoretically, blockade of all available MHC class II molecules, for example HLA-DR2, in a homozygote individual could result in general immunosuppression. Although this seemed not to be the case in experimental therapies with MHC-blocking peptides, it has been recently shown that the peptide concentrations that are necessary to achieve MHC blockade in vivo can not be maintained over long periods of time [49]. Recently, a large multicenter trial [137] examined the effect of copolymer-1 (COP-1), a polypeptide composed of the four aa, tyrosine, lysine, alanine and glutamic acid in random sequence. This trial in early RR-MS patients showed a small, but significant therapeutic effect. Earlier reports claimed that COP-1 blocks EAE via the induction of MBP-specific suppressor cells [136]. Recent data suggests that it blocks antigen-presentation by interfering with binding of antigenic peptide to disease-associated MHC-class II molecules in vitro [103], although additional evidence that this mechanism accounts for the effects on disease in vivo is needed. In addition, monoclonal antibodies against disease-related MHC class II antigens have successfully been applied [131], but the problems mentioned above and the induction of xenogeneic antibodies have prevented this approach in humans. A recent study in which animals were vaccinated with a peptide derived from the third hypervariable region of the disease-associated MHC class II antigen (IA^s β chain 58–78; [139]) was effective in treating EAE via the induction of autoantibodies specific for the IA^s β chain. Finally, a monoclonal antibody that was generated against the antigen-IA complex also blocked disease [3].

Other experimental therapies have also successfully targeted the TMC in EAE by either including inactivated carrier cells such as spleen cells that had been coupled with antigen [57, 58, 134, 135] or by soluble MHC-peptide complexes [126] or by the administration of membrane fragments of cells that have been prepulsed with protein

[13]. These methods have led to an anergic state since encephalitogenic T cells are not properly activated in the absence of co-stimulatory signals.

A state of transient or long-term unresponsiveness resulting in anergy could also be achieved by intrathymic injection of antigen either in neonatal [28] or adult [44] animals. Administration of bacterial superantigen can result in either deletion or activation of T cells using TCR that are recognized by this superantigen [15, 106, 113, 117]. Treatment with superantigen reduced disease when it was injected before EAE was initiated [106]. These strategies are obviously not applicable to humans. However, anergy can also be induced by intraperitoneal [38] administration of encephalitogenic peptide and results in an improved course of EAE after rechallenge with antigen.

Recently, it has been shown that EAE can be influenced therapeutically by the induction of programmed cell death in encephalitogenic T cells [29]. This therapeutic strategy is based on the finding that preactivated T cells that are vigorously proliferating in the presence of IL-2 undergo apoptosis when they are rechallenged with antigen. This observation provided the basis for treating animals after the induction of EAE with high doses of MBP [29]. A therapeutic effect, due to apoptotic death of preactivated encephalitogenic T cells, could be demonstrated in this situation, but timing and dose of the administration of the apoptotic signal proved to be critical for the outcome.

It is obvious from the various therapeutic strategies that have been outlined above that some are too specific in that they only target one specific T cell clone bearing one TCR, and that others are too nonspecific and result in general immunosuppression. The solution to some of these problems may be found in a therapeutic concept called TCR antagonism. About 2 years ago, Sette and coworkers [33] observed that modified antigenic peptides are able to partially block or completely antagonize the stimulatory signal that is mediated through the recognition of the native peptide presented in the context of an appropriate MHC. The previous view that T cells receive either a positive signal (on) once their TCR engages the peptide-MHC complex, or no signal (off) if the complex is not recognized, did not explain a number of findings that followed this first observation. In the meantime, several studies have confirmed these results and have shown that TCR antagonists or altered peptide ligands (APL) can either mediate no signal, if the major TCR contact sites of an antigenic peptide are modified or cause a partial response. In the latter situation, APL peptides were able to induce lymphokine release or cytolysis in Th2 or Th1 cells, respectively, without concomitant proliferation (for review see [35]). Certain substitutions even cause phenotypic switches, i.e., Th1 cells no longer secrete IFN-γ and TNF/LT, but do produce IL-4 or other Th2 cytokines instead ([153], Vergelli et al., in preparation). The great advantages that TCR antagonism offers over the blockade of disease-associated MHC antigens by high-affinity binders and over TCR vaccination, are its effects in molar ranges that are orders of magnitude below those used for MHC blockade and that it is not specifically targeting one T cell population, but a broad range of T cells [63]. The latter notion has already been confirmed by the study of Kuchroo et al. [63] in SJL mice that showed that TCR antagonist peptides that are based on the encephalitogenic PLP peptide 138–153 can block EAE induced by a number of encephalitogenic PLP 138–153-specific T cell hybridomas although these cells express different TCR molecules. TCR antagonism has also been achieved in Lewis rat EAE by injection of soluble APL peptides [55]. Although the biological effects that are caused by these peptides are not yet entirely understood, they could act through

the induction of anergy, by antagonizing the stimulus by the native peptide and by inducing a phenotypic switch in the response to the encephalitogenic peptide.

Another approach that has recently been studied intensively originates from the observation that the route of administration such as oral feeding or inhalation of antigen results in an immune response that is drastically different from that after intravenous, intramuscular or subcutaneous immunization [12, 45, 84, 85]. Oral administration of MBP, for example, establishes a state of tolerance that, depending on the dose of protein that is fed, is due to T cell anergy at higher doses [45] or to the induction of regulatory T cells that secrete transforming growth factor-β (TGF-β), IL-4 and IL-10 at lower doses [24, 85]. In addition, oral administration of MBP was able to cross-protect against PLP-induced EAE in SJL mice [4, 24]. Oral tolerization is currently being tested for a number of experimental and human autoimmune diseases including uveitis, insulin-dependent diabetes, rheumatoid arthritis and MS. Preliminary results from a pilot trial of oral tolerance with bovine myelin in MS patients were reported as suggesting some efficacy in a group of $DR2^-$ male patients [148]. Since the duration of the trial was short and treatment efficacy was not controlled by MRI, it is too early to draw conclusions from this data. A large placebo-controlled, double-blind multicenter trial is currently under way to confirm these interesting experimental data.

Although not directly involved in the TMC. CD4 serves an important accessory function in the recognition of antigen by $CD4^+$ T cells. Therefore, some time ago, attempts were made to interfere with the $CD4^+$ encephalitogenic T cells using monoclonal antibodies against the CD4 molecule, which stabilizes the conjugation between $CD4^+$ T cells and the MHC molecule on APC. Monoclonal antibodies against CD4 were able to block EAE [17, 46], and a humanized monoclonal is currently being tested in MS patients in Europe. Initial results of these trials show that the antibody is well tolerated, that the formation of interfering antibodies is not a problem and that the antibody successfully depletes $CD4^+$ T cells from the peripheral blood of the patients [72]. A recently published study employed a CD4 analogue that was designed with D-amino acids [51]. This approach was also able to block EAE. It is, however, anticipated that any therapy that will interfere with a molecule involved in general helper T cell function will eventually result in a very broad immunosuppression.

Therapies targeting T cell homing, adhesion to and migration through the BBB

Evidence derived from studies of the EAE model and examination of the pathology of acute lesions as well as MRI studies of MS patients indicate that the initial event of the development of a new MS lesion is breakdown of the BBB. This event is most likely associated with the transmigration of lymphocytes that have been activated in the periphery into the brain or spinal cord parenchyma. The relative importance of the properties of T cells compared to the expression of adhesion molecules on endothelial cells for the regions where the inflammatory infiltrate preferentially develops (i.e., around small postcapillary venules in the periventricular white matter) is unresolved. Evidence from studies in the rat and mouse indicate that activated T cells can transmigrate into the brain regardless of their specificity [30, 47, 149]. T cells specific for myelin or glial antigens that encounter their target autoantigen in the CNS are retained while T cells with other specificities tend to leave. Sequential immunohistological and PCR studies that followed the expression of TCR chains in the CNS tissue after adoptive transfer of well-defined myelin-specific TCL have demonstrated that

encephalitogenic T cells and their TCR are found first, before a massive recruitment of cells with other receptors and presumably other specificities occurs [10, 18, 54]. Cells expressing the TCR of the encephalitogenic TCL stay longer and either leave or die in situ by apoptosis [199, 133].

Activated T cells express increased levels of several adhesion molecules and selectins [9, 21, 34, 62, 152]. For example, a relationship was described between encephalitogenicity of T cells raised against encephalitogenic determinants of MBP and PLP and the expression of VLA-4, the ligand of VCAM-1 [9, 62]. Only T cells expressing high levels of VLA-4 were able to transfer EAE without other manipulations of the mouse. Presumably, these cells cross the BBB more easily. Further, antibody to $\alpha 4\beta 1$ integrin can diminish the clinical and pathological severity of EAE [9, 158]. If interaction between VLA-4 on T cells and VCAM-1 is important in transmigration and encephalitogenicity, the expression of VCAM-1 must be up-regulated since VCAM-1 is expressed at only low levels on endothelial cells. Although constitutively expressed at higher levels on CNS endothelial cells, ICAM-1 is also up-regulated during the initial stages of lesion development and could contribute to T cell adhesion and subsequent transmigration. However, treatment of EAE with antibodies to ICAM-1 and its ligand, LFA-1, have produced variable results [7, 22, 149]. Depending on the timing and dose of monoclonal antibodies against ICAM-1 and LFA-1, the outcome was either beneficial, unaltered or deleterious [7, 22, 150]. It is also important to note that these CAM may contribute to cell-cell interactions other than those with endothelial cells or that engagement of these structures may itself provide an activation signal to T cells, such as stimulation by purified LFA-3 and ICAM-1 [123]. Before such treatments are considered for patients, carefully designed studies in experimental systems should address these questions and one should also determine whether there are phenotypic or functional differences in the various CD4 T cell populations that have been cultivated, for example, after stimulation with MBP, from MS patients and controls. Our own studies that compared MBP-specific cytotoxic and proliferative TCL could only find a slightly higher expression of LFA-1, but no differences for VLA-4 and LFA-3 (Vergelli et al., unpublished observations). Although human MBP-specific TCL in our hands are largely Th1 cells [145], like those mediating EAE in the various EAE systems [6, 62], it is not clear whether a specific subpopulation is pathogenetically more important than another.

With respect to treatment trials in MS, the use of humanized antibodies against different adhesion molecules has now entered or will enter clinical testing (see Table 4). Since treatments of this type would be expected to affect the occurrence of new lesions, MRI should provide a valuable tool for monitoring the effectiveness of these therapies.

Table 4. Therapies targeting the adhesion of autoantigenic T cells to the brain endothelium and their entry into the parenchyma

Antibodies against ICAM-1
Antibodies against LFA-1
Antibodies against VLA-4
Antibodies against VCAM-1

Therapies targeting proinflammatory cytokines

There is now overwhelming evidence that EAE can be mediated solely by the transfer of a $CD4^+$ Th1 T cell [67, 161]. These cells that mediate delayed-type hypersensitivity responses and tissue inflammation in various infectious diseases are characterized by their lymphokine secretion profile, i.e., the secretion of IFN-γ, TNF-α and lymphotoxin-α (LT) and IL-12, as well as by their ability to lyze antigen-presenting target cells [6, 16, 62]. As mentioned before, the encephalitogenic T cells are Th1 cells [6, 16, 62], and most of the human MBP-specific T cells probably also have this phenotype [76, 112, 145]. Th1-derived lymphokines contribute to the amplification of the local inflammatory response and to tissue damage in a number of ways. It is well known that IFN-γ up-regulates MHC antigen and adhesion molecule expression on a number of cells including endothelial cells, glial cells and cells of the lymphoid lineages themselves [81, 154, 155]. In addition, IFN-γ drives the differentiation of effector $CD4^+$ and $CD8^+$ T cells as well as macrophages and stimulates the release of cytocidal reactive oxygen (O_2^- and H_2O_2) and nitrogen (NO) intermediates from phagocytic cells and astrocytes [69]. Besides IL-12, IFN-γ is the most important molecule in skewing a $CD4^+$ T cell response in the direction of a Th1 response. Another Th1 lymphokine family, consisting primarily of macrophage-derived TNF-α and T cell-derived LT, also contributes to the inflammatory process [116, 120, 121]. Besides mediating effects similar to those of IFN-γ, TNF/LT has been shown to damage endothelial cells, resulting in changes to the BBB and oligodendroglial cells leading to demyelination [120]. TNF/LT is produced locally in inflammatory plaques in the CNS in EAE and MS. With respect to plaque development, it is currently hypothesized that encephalitogenic T cells adhere to CNS endothelial cells, up-regulate class II antigen and adhesion molecule expression at the BBB, and thus facilitate transmigration. In addition, they may directly damage endothelial cells [80], allowing the influx of serum components that contribute to tissue damage and then further enhance the local inflammatory process via promoting recruitment and activation of other incoming and resident cells. As mentioned before, TNF/LT has been shown to damage oligodendroglial cells in vitro and thus may contribute to demyelination. The importance of TNF/LT has been underlined by the effect of monoclonal antibodies against TNF/LT that effectively blocked EAE [116, 121]. Other strategies aimed at inactivating Th1 lymphokines include the administration of soluble TNF receptor-immunoglobin fusion proteins [8] or monoclonal antibodies against IL-12 [70], or targeting a macrophage-derived proinflammatory cytokine, IL-1β that is up-regulated during a Th1 response by administering soluble IL-1 receptors [50]. Each of these approaches has been successful in treating EAE.

The importance of proinflammatory cytokines in MS stems from a number of observations in patients. Therapy with IFN-γ resulted in an increase of exacerbations [96], and levels of TNF/LT have been reported to be increased in the blood and CSF of MS patients during relapses [26, 125]. The latter observation is, however, still controversial, since some studies have not confirmed the findings. Furthermore, it is known that soluble TNF is very difficult to determine in body fluids and tissue samples, since secretion of TNF rapidly induces the shedding of TNF receptors and consequently the formation of TNF-TNF receptor complexes. In this situation it is hard to determine the lymphokine level reliably.

Since the administration of monoclonal antibodies is costly and causes a number of problems during long-term treatment, there has been considerable interest in iden-

tifying other approaches to interfere with the production of TNF/LT and IFN-γ. It has been shown that phosphodiesterase (PDE) inhibitors are able to block lymphocyte activation and the secretion of TNF-α and IFN-γ via the increase of intracellular cAMP levels [122, 129]. This group of agents can be separated into phosphodiesterase inhibitors with relatively nonspecific action such as pentoxifylline and those that specifically block certain PDE types. A member of the latter group, the PDE type IV inhibitor rolipram, is particularly attractive for a number of reasons. In contrast to the unspecific PDE inhibitors, the PDE type IV is almost exclusively expressed in the CNS and in lymphoid cells and thus has significantly less side effects. The drug was originally developed and tested as an antidepressant but also has a potent suppressive effect on TNF and, to a lesser extent, on IFN-γ secretion [41, 129, 130]. Pentoxifylline was effective in EAE [88, 114]. The PDE type IV inhibitor rolipram could block TNF- and IFN-γ secretion in human MBP-specific T cells in a stereospecific manner and also inhibited EAE in the Lewis rats [129], in SJL mice (Sommer et al., in preparation) and also in the marmoset EAE model [41]. Since the substance has few side effects and can be administered orally it provides an attractive alternative to the treatments with monoclonal antibodies or soluble TNF receptors.

Another interesting approach that interferes with TNF employs inhibitors of metalloproteases [43]. These enzymes, which are usually matrix bound, as for example gelatinase B, cleave membrane-bound TNF from the cell membrane and thus release it at the inflammatory site. Inhibitors of metalloproteases are capable of inhibiting EAE and represent an attractive alternative to the above-mentioned strategies that interfere with Th1 lympkokines [43].

IFN-β, which was recently approved for the treatment of RR-MS, also belongs to the group of treatments that interfere with proinflammatory cytokines or their action at different levels, including the expression of adhesion molecules and MHC antigens, the recruitment of cells into the lesion, and the process of antigen presentation at the induction and effector stages [97, 138]. These therapies are summarized in Table 5.

Table 5. Therapies that modify the effector function of autoantigen-specific CD4 T cells (i.e., up-regulation of adhesion and MHC class molecule expression; activation of macrophages and resident glial cells) or unspecifically interfere with their activation

Interferon-beta (IFN-β)
IL-4
Transforming growth factor-beta (TGF-β)
IL-13
Soluble TNF receptor immunoglobulin fusion proteins
Antibodies against TNF/LT
Phosphodiesterase inhibitors (rolipram; pentoxifylline)
Inhibitor of the inducible nitric oxide synthase (aminoguanidine)
Metalloprotease inhibitors

Therapies employing regulatory cytokines

A number of cytokines have been identified which appear to down-regulate inflammation. One of the most potent is TGF-β. TGF-β has been shown to have immunosuppressive effects on T cells, B cells and natural killer cells and decreases leukocyte transmigration from blood vessels at inflammatory sites. Treatment of animals immunized with myelin antigens with antibodies to TGF-β have demonstrated accentuated disease [102]. TGF-β has also been examined for its ability to directly modify the clinical and pathological course of EAE. When administered prior to the onset of clinical disease in mice adoptively immunized with T cells sensitized to MBP, TGF-β significantly reduces the severity of clinical disease and produces a marked reduction of inflammation and demyelination pathologically [52, 66, 101, 104]. Further, immunohistochemistry indicates a decrease in expression of MHC class II molecules and proinflammatory cytokines such as TNF-α [101]. Administration of TGF-β after the onset of disease also reduces the severity and number of subsequent relapses [101]. It has also been demonstrated that amelioration of disease through feeding antigen (oral tolerance) induces T cells that secrete TGF-β [24, 85]. Together, these findings indicate that TGF-β can have a profound immunoregulatory effect on T cell-mediated autoimmune diseases such as EAE. Despite the encouraging initial results using TGF-β in the EAE, extrapolation into human disease must be done with caution since TGF-β has been thought to augment some inflammatory conditions and tissue fibrosis and may up-regulate expression of some CAM. A clinical phase I trial in MS patients to assess the safety and potential efficacy of systemically administered TGF-β has just begun at the National Institutes of Health.

In addition to TGF-β, other cytokines produced by Th2 T cells such as IL-4 [105] and IL-10 [115] and IL-13 [23] appear to down-regulate and antagonize Th1-mediated inflammation. Administration of IL-4 in the adoptive transfer EAE model in SJL mice has been shown to reduce clinical disease severity and to reduce production of proinflammatory cytokines produced by Th1 T cells [105]. Diets enriched for retinoic acids also ameliorated EAE in the mouse [107] and rat [79], and recent evidence suggests that the mechanism may involve the up-regulation of Th2-type cytokines. At present, it is not clear if the mechanism of retinoic acids is entirely due to increased production of IL-4. Along these lines, IL-10 expression increases several times in the CNS of mice that recover from an exacerbation of EAE [59], indicating that endogenous secretion of this Th2 lymphokine is critical for the recovery from CNS inflammation.

The modulation of experimental autoimmune diseases by immunoregulatory cytokines reflects their possible usefulness in modifying human diseases such as MS in a manner that is rather immunomodulatory than generally immunosuppressive.

Therapies targeting the effector phase of demyelination

As outlined above, activated macrophages and toxic radicals (NO and O_2^-) secreted by these cells as well as cytokines produced by macrophages and lymphocytes, in particular TNF/LT, are responsible for demyelination. A number of the approaches that have been mentioned to interfere with Th1 lymphokines will also act at this level and decrease the activation of macrophages, and consequently, also the extent of demyelination. The administration of immunoglobulins intravenously may not only

antagonize TNF [2], but also interfere with myelin-specific antibodies such as anti-MOG antibodies and the engulfment of myelin membranes into macrophages via coated pits. Aminoguanidine, an inhibitor of the inducible NO synthase suppresses the secretion of the toxic nitrogen intermediate NO and is effective in treating EAE [37].

Conclusions

Until recently most attempts to treat MS have focused on general immunosuppressive drugs since the disease was presumed to be immunologically mediated. Although some of these drugs may have effects on the disease process, their clinical trials were often inconclusive and discouraging. In the last years, attention has been directed at therapies which can modify the TMC in a very specific manner similar to that seen in the experimental model EAE. However, since the target antigen or antigens in MS are not known and since the restricted TCR usage often found in experimental animals is generally not found in MS, these approaches have been difficult to initiate for MS. Thus, more recent treatment approaches have begun to focus on modifying the biology of the MS lesion.

Despite our inability to identify the cause of MS, our understanding of events involved in the evolution of the MS lesion is increasing. This, in turn, has led to a number of innovative therapies of MS which target the processes involved in lesion development, rather than the precise cause of the disease or early steps important during the activation of autoreactive T cells. These approaches range from attempts to alter events at the BBB, down-regulation of proinflammatory cytokines, or enhancement of cytokines thought to down-regulate and delete the effector mechanisms in the lesion. Currently, studies examining the effect of blocking TNF-α/LT or administering the regulatory cytokine TGF-β are under way. Finally, despite the fact that a unique trimolecular interaction has not yet been demonstrated in the pathogenesis of MS, there is evidence that immunodominant peptides of autoantigens are recognized in the context of disease-associated HLA class II molecules. Particularly during the early phases of disease, the T cell response may be primarily directed against these MHC peptide complexes, and studies of the requirements of T cell activation indicate that modifications of amino acids important for TCR contact on encephalitogenic peptides can therapeutically modify the T cell response. This approach has the potential for targeting T cells, which may have regulatory or suppressive functions at the lesion, and down-regulating inflammatory activity into the lesion, regardless of the initiating antigen. A similar mechanism may be achieved by oral tolerance. Thus, despite continued uncertainty regarding the cause of MS, our improved understanding of the pathogenetic process during EAE and MS has resulted in innovative therapies that hold hope for effective treatment of the disease.

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