Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research

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In view of disease heterogeneity of multiple sclerosis and limited access to ex vivo specimens, different approaches must be undertaken to better understand disease pathogenesis and new therapeutic challenges. Here, we critically discuss models of experimental autoimmune encephalomyelitis (EAE) that reproduce specific features of the histopathology and neurobiology of multiple sclerosis and their shortcomings as tools to investigate emerging therapeutic approaches. By using EAE models we have understood mechanisms of T-cell mediated immune damage of the CNS, and the associated effector cascade of innate immunity. Also, the importance of humoral components of the immune system for demyelination has been delineated in EAE, before it was applied therapeutically to subtypes of multiple sclerosis. Yet, similar to multiple sclerosis, EAE is also heterogeneous and influenced by the selected autoantigen, species and the genetic background. In particular, the relevance of cytotoxic CD8 T cells for human multiple sclerosis has been underestimated in most EAE models, and no EAE model exists that mimics primary progressive disease courses of multiple sclerosis. Seventy years after the first description of EAE and the publication of >7000 articles, we are aware of the obvious limitations of EAE as a model of multiple sclerosis, but feel strongly that when used appropriately it will continue to provide a crucial tool for improving our understanding and treatment of this devastating disease.

Keywords: transgenic mice; autoimmunity; animal models; EAE

Abbreviations: APC = antigen-presenting cell; APP = amyloid precursor protein; AT-EAE = adoptive-transfer EAE; BBB = blood–brain barrier; EAE = experimental autoimmune encephalomyelitis; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; PLP = proteolipid protein; TCR = T-cell receptor; Th = T helper cell; TNF = tumour necrosis factor; TRAIL = tumour necrosis factor-related apoptosis-inducing ligand

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Introduction

Immunologists view multiple sclerosis as an autoimmune disease, in which T-lymphocytes specific for myelin antigens start an inflammatory reaction in the central nervous system, which ultimately leads to demyelination and subsequent axonal injury. This view of multiple sclerosis as a T-cell-mediated autoimmune disease is derived primarily from studies on a single animal model, experimental autoimmune encephalomyelitis (EAE). The origins of EAE date back to the 1920s, when Koritschoner and Schweinburg induced spinal cord inflammation in rabbits by inoculation with human spinal...
immunization of rhesus monkeys with CNS tissue (Rivers cations associated with rabies vaccination by repetitive researches attempted to reproduce the encephalitic compli-
cord (Koritschoner and Schweinburg, 1925). In the 1930s, 
the grey matter, in particular in the cortex (Bo 
progressive disease. Advances in molecular medicine have 
disease, the brain is affected in a more global sense, with 
progressive disease, or with steady progression of 
disease once the pathogenesis of EAE is elucidated, and that new 
progressing white matter lesions are heterogeneous, resulting in 
the grey matter, in particular in the cortex (Bo et al., 2003; 
the characteristic hallmarks in patients with acute and relapsing disease (Raine et al., 1997; Compston et al., 2005). In patients with progressive disease, the brain is affected in a more global sense, with 
diffuse but widespread (mainly axonal) damage in the normal 
clinical heterogeneity of multiple sclerosis has been recognized for many years, but it is now apparent 
that this heterogeneity extends to both the genetics of the disease and the pathomechanisms involved in lesion 
formation. Clinically the illness may present as a 
relapsing–remitting disease, or with steady progression of 
neurological disability. The subsequent course of disease is 
unpredictable, although most patients with a relapsing–remitting disease will eventually develop secondary pro-
clinical, pathological and immunological aspects of 
progressive disease, the brain is affected in a more global sense, with 
diffuse but widespread (mainly axonal) damage in the normal 
appearance white matter and massive demyelination also in 
the grey matter, in particular in the cortex (Bo et al., 2003; 
mechanisms of tissue injury in focal white matter lesions are heterogeneous, resulting in 
patterns of demyelination that vary between patients or 
patient subgroups (Lassmann et al., 2001). Furthermore, 
there is a high inter-individual variability in the extent of 
axonal damage as well as remyelination and repair. The rea-
son for this complex situation is largely unknown, although it is 
likely that genetic factors influencing immune-mediated 
inflammation as well as neuronal and glial survival may 
play a major role in modulating the phenotype of the disease 
(Compston, 2004).

There are major differences between EAE and multiple 
sclerosis. The first and most obvious is that multiple sclerosis is a spontaneous disease, while EAE is induced by active 
sensitization with brain tissue antigens (see below). Only
recently have spontaneous models of EAE been developed, 
but even these are dependent on the use of transgenic approaches to override the intrinsic regulatory mechanisms 
that normally suppress tissue-specific autoaggression 
(Waldner et al., 2000; Bettelli et al., 2003; Zehntner et al., 
2003; T. Hünig and R. Gold, in preparation). Furthermore, in 
most protocols, strong immune adjuvants were required to 
induce disease (see below) and it seems unlikely that similarly intense ‘immunological boosts’ occur under physiological 
conditions, even in infectious diseases. Also, for practical 
reasons and for the sake of reproducibility, EAE is studied 
mainly in inbred animals or in genetically homogeneous 
populations. Thus, the genetic heterogeneity, which is so 
critical in the multiple sclerosis population, is only reflected 
when multiple different models of EAE are studied in parallel.

For all these reasons, it seems naïve to believe that the whole spectrum of multiple sclerosis can be covered in a 
single or even in several different EAE models. Despite 
these limitations, most of our current knowledge regarding 
principal mechanisms of brain inflammation has been gathered from studies on EAE, and without this knowledge the 
understanding of the pathogenesis of multiple sclerosis and 
development of new therapies would not be feasible. In view 
of disease heterogeneity, the advantages and limitations of 
different acute and chronic EAE models will be handled. It is 
the aim of this review to summarize these findings and discuss their implications for multiple sclerosis.

Clinical and histopathological potential and 
limitations of EAE models for multiple 
sclerosis: from primates to rodent species

Diversity of disease courses and target 
antigens in different EAE models

Following the first description of EAE in primates by Rivers 
et al. (1933), there was steady progress in eliciting EAE in 
different species. This was facilitated by the development of a 
new mineral oil-based adjuvant by Jules Freund that, when 
combined with brain extracts, enabled Kabat’s group to fast-
track disease induction. The use of Freund’s adjuvant results 
in disease after only a single injection (Kabat et al., 1951), 
whereas Rivers’ approach required multiple injections (up to 
80 per animal) over a period of a year. In the 1950s, rats and 
guinea pigs became the standard species in which to study 
EAE, when the addition of heat-inactivated mycobacteria 
tuberculosis to the adjuvant (complete Freund’s adjuvant, 
CFA) was found to enhance the response to sensitization 
with CNS tissue. Since then EAE has been induced in a 
wide range of species, and a variety of well-characterized 
rodent and primate models are now available (Table 1) 
that reproduce specific aspects of the immunopathology of 
the human disease.

For many decades, rat and guinea pig models of EAE 
dominated research in autoimmune-mediated inflammation 
of the CNS. In these species, active immunization with CNS
tissue, myelin or myelin basic protein (MBP) in CFA results in a high incidence of disease with a reproducible clinical course. The first clinical signs of diseases are generally observed within 9–12 days of sensitization; however, subsequent disease activity is dependent on the species under investigation and the mode of sensitization. For example, MBP induces an acute self-limiting disease in guinea pigs, whilst immunization with CNS tissue homogenates results in chronic relapsing–remitting or progressive disease (Alvord, 1972; Raine, 1985). It should also be noted that many myelin antigens are components of both CNS and PNS myelin, and can, therefore, induce disease with significant peripheral involvement, as described in the spinal roots of Lewis rats, investigating T-cell function and regulation in neuro-inflammation and autoimmune disease. In the Lewis rat, clinical signs of disease are typically observed 3–4 days after the transfer of MBP-specific T-cell lines. Disease activity reaches a maximum within the following 48 h after which it rapidly declines, resulting in a complete clinical remission within a few days. This clinical recovery is associated with enhanced apoptosis of inflammatory T cells in the lesion [Pender et al., 1991; reviewed in Gold et al. (1997)]. However, whilst this rat model provided formal evidence that MBP-specific T cells can induce an autoimmune-mediated disease of the CNS, it is important to recognize that AT-EAE in the rat is not a complete model of multiple sclerosis. The critical limitations are that the disease course is monophasic unless the immune system is manipulated with cyclosporine (Pender et al., 1990), and that it is an inflammatory disease in which CNS demyelination is minimal, a pathology that reflects that of acute disseminated encephalomyelitis.

Table 1 Commonly used rodent EAE models

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Further comments</th>
</tr>
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<tbody>
<tr>
<td>Lewis rat Active EAE (CNS myelin, MBP, MOG, PLP)</td>
<td>T-cell inflammation and weak antibody response</td>
<td>Monophasic, little demyelination</td>
<td>Reliable model, commonly used for therapy studies. With guinea-pig MBP little demyelination</td>
</tr>
<tr>
<td>Adoptive-transfer EAE (MBP, S-100, MOG, GFAP)</td>
<td>Marked T-cell inflammation. Topography of lesions</td>
<td>Monophasic, little demyelination</td>
<td>Homogeneous course, rapid onset. Differential recruitment of T cells/macrophages depending on autoantigen</td>
</tr>
<tr>
<td>Active EAE or AT-EAE + co-transfer of anti-MOG antibodies</td>
<td>T-cell inflammation and demyelination</td>
<td>Only transient demyelination</td>
<td>Basic evidence for role of antibodies in demyelination</td>
</tr>
<tr>
<td>Congenic Lewis, DA, BN strains Active EAE (recombinant MOG aa 1–125)</td>
<td>Relapsing–remitting disorders, may completely mimic histopathology of multiple sclerosis and subtypes</td>
<td>No spontaneous disease</td>
<td>Chronic disease course, affection of the optic nerve, also axonal damage similar to multiple sclerosis</td>
</tr>
<tr>
<td>Murine EAE (SJL, C57BL/6, PL/J, Biozzi ABH) Active EAE (MBP, MOG, PLP and peptides)</td>
<td>Relapsing–remitting (SJL, Biozzi) and chronic-progressive (C57BL/6) disease courses with demyelination and axonal damage</td>
<td>No spontaneous disease</td>
<td>Pertussis (toxin) required for many strains, whilst it is often not needed for SJL and some Biozzi EAE models. Higher variability of disease incidence and course, often cytotoxic demyelination in C57BL/6. With rat MBP inflammatory vasculitis with little demyelination</td>
</tr>
<tr>
<td>Murine EAE in transgenic mice or knockout mice (mostly C57BL/6 background)</td>
<td>Specifically addresses role of defined immune molecules/neurotrophic cytokines/ neuroanatomical tracts</td>
<td>Most results obtained with artificial permanent transgenic or knockouts</td>
<td>Extensive backcrossing (&gt;10 times) on C57BL/6 background required. Future work with conditional (cre/loxP) or inducible (e.g. Tet-on) mutants</td>
</tr>
</tbody>
</table>
(ADEM) rather than multiple sclerosis. Moreover, the potential to induce this inflammatory pathology is not restricted to myelin-antigen-specific T-cell lines, as demonstrated by the adoptive transfer of disease by T cells specific for astrocyte and neuronal antigens [see review in Sospedra and Martin (2005)]. These observations demonstrated that although the T-cell arm of the autoimmune response plays a key role in the breakdown of the blood-brain barrier (BBB) and pathogenesis of EAE, this alone is insufficient in the rat to trigger extensive demyelination and chronic disease activity, the characteristic hallmarks of the human disease. The situation in the mouse is somewhat different, as in this species the adoptive transfer of myelin-specific T cells can induce demyelination, although the extent of primary myelin loss is minimal in comparison to that seen in patients with multiple sclerosis [reviewed in Iglesias et al. (2001)]. Despite these limitations, AT-EAE provides a very reproducible disease model to study the principal mechanisms involved in the pathogenesis of T-cell-mediated inflammation in the CNS and has provided many insights that proved highly relevant for the design of anti-inflammatory therapies.

**Introduction of myelin oligodendrocyte glycoprotein as target antigen for demyelination**

Substantial progress in reproducing the pathology and clinical course of multiple sclerosis in EAE followed the identification of myelin oligodendrocyte glycoprotein (MOG) as a key autoantigen involved in the development of demyelinating lesions in EAE induced by sensitization with CNS tissue homogenates (Lebar et al., 1986). MOG is a unique myelin autoantigen as it induces not only an encephalitogenic T-cell response in susceptible species but also a demyelinating autoantibody response. Demyelinating anti-MOG antibodies augment disease severity and initiate extensive demyelination in T-cell-mediated brain inflammation in mouse, rat and primate models of EAE (Schluessener et al., 1987; Linington et al., 1988; Genain et al., 1995), and in animals actively immunized with MOG; this combination of pathogenic T-cell and antibody-dependent effector mechanisms act to reproduce the complex range of pathological and clinical phenotypes associated with multiple sclerosis (Storch et al., 1998). Genetic and environmental factors that contribute to disease susceptibility, or which modulate the pathological response in the CNS in MOG-induced EAE, became evident when Olsson and colleagues began to investigate disease susceptibility in other rat strains (Becanovic et al., 2003), including congenic Lewis rats that harbour non-Lewis MHC genes on a Lewis genetic background (Wallstrom et al., 1997; Weisset et al., 1998; Becanovic et al., 2003). These studies also revealed that MOG induced EAE in strains such as the Brown Norway rat that were previously regarded as ‘resistant’. In this case, MOG-induced disease was hyperacute and the demyelinating lesions were associated with an eosinophilic infiltrate (Steffel et al., 1999), suggesting the possible involvement of T helper cell (Th)2 mechanisms similar to subtypes of multiple sclerosis such as Devic’s disease (see below).

EAE induced in the marmoset by immunization with CNS tissue homogenates or recombinant MOG (Genain and Hauser, 2001; T’Hart et al., 2004) provides a disease model that reproduces many of the pathological features of multiple sclerosis in a species that is phylogenetically closer to man than rodents. The mechanisms involved in lesion formation in this primate appear similar, if not identical, to those involved in the pathogenesis of MOG-induced EAE in the rat—a combined attack by encephalitogenic T cells and demyelinating autoantibodies on the CNS (von Budingen et al., 2004). However, the usefulness of marmoset EAE as a benchmark animal model to unravel the complex interactions between the immune and nervous system in multiple sclerosis is limited by a number of technical issues. These include the inability to genetically manipulate components of the immune and nervous systems involved in the pathogenesis of chronic inflammation and tissue damage, and the limited availability of reagents and probes for cellular, immunological and histological studies. Also, the incidence and clinical course of disease is more variable in this outbred species than in rodents, as is seen by the incidence of fulminant as opposed to relapsing–remitting disease induced by MOG. Nonetheless, marmoset EAE provides an important experimental tool that when used appropriately and in combination with rodent models will help in providing a better understanding of the human disease, in particular with respect to pre-clinical treatment and imaging studies.

**Murine EAE: a tool to investigate genetic elements in disease pathogenesis**

Although murine models of EAE were first described in the 1950s, their usefulness was limited by a lower disease incidence and a more heterogeneous disease course than that which was achieved in guinea pig and rat. These problems were resolved following the introduction of pertussis toxin to augment disease induction and the identification of more susceptible mouse strains [see, for example, Yasuda et al. (1975) and Bernard and Carnegie (1975)]. Standard mouse models that are now in general use (see Table 1) include PLP139-151 peptide-induced relapsing EAE in SJL mice, MBP-induced disease in PL/J mouse, chronic-progressive models of MOG protein or MOG35-55 peptide-induced disease in C57/BL6 mice and active immunization with CNS tissue homogenates or MOG that induces a relapsing–remitting disease in Biozzi ABH mice [reviewed in Amor et al. (2005)].

In general, the mouse CNS appears more sensitive to damage by T-cell-mediated inflammatory responses than is the case in either rat or marmoset. As a consequence, primary immune-mediated demyelination occurs in the context of far more extensive tissue injury, in particular axonal and
neuronal damage. However, it must be stressed that as in other species the pathology and clinical course of EAE in the mouse is determined by both genetic factors and immunogen/adjuvant used to induce disease. This becomes particularly important when discussing the role of humoral immune effector mechanisms in disease pathogenesis.

Adoptive transfer studies demonstrate that as in rat, antibodies to antigens such as MOG that are exposed at the surface of the myelin sheath can enhance demyelination and exacerbate disease severity in mouse models of EAE (Morris-Downes et al., 2002; Kanter et al., 2006). However, there is considerable variation between mouse strains with respect to both the efficacy of complement cascade and their ability to mount a demyelinating antibody response to MOG. This is particularly important when discussing MOG-induced EAE in C57BL/6 mice, the strain favoured for studies using transgenic approaches to dissect regulatory and immunopathomechanistic pathways in EAE. In this mouse strain, genes associated with the H-2b MHC haplotype selectively censor its ability to mount a demyelinating autoantibody response when challenged with either mouse or rat MOG (Bourquin et al., 2003). As a consequence, primary tissue damage in MOG-induced EAE does not involve a demyelinating autoantibody response in C57BL/6 mice, a factor that must be taken into account in studies dissecting the potential role of factors such as Fc receptors in disease pathogenesis. Such studies should either use alternative mouse strains (Abdul-Majid et al., 2002) or use human MOG to induce disease in C57BL/6 mice, as this antigen will induce a demyelinating antibody response (Oliver et al., 2003). The factors responsible for modulating the autoimmune response to autologous MOG in H2-b mice are still to be clarified, but this example demonstrates that genetic diversity can significantly influence the identity of the effector mechanisms responsible for lesion formation in the mouse.

The use of genetically modified mice has provided many novel and often unexpected insights into the mechanism involved in the pathogenesis of EAE, but, nonetheless, mouse EAE models also have their drawbacks. Even in models of MOG-induced disease the lesions are in general characterized by massive global tissue injury (including axonal and neuronal damage) with very little primary demyelination. With only few exceptions (Bourquin et al., 2003), tissue damage is accomplished by T cells and activated macrophages. The role of demyelinating autoantibodies in lesion formation appears considerably less important than in rat or guinea pig models of EAE, even when present at very high titres such as in anti-MOG B-cell transgenic mice (Litzenburger et al., 1998), possibly due to the low efficacy of the complement system in the mouse. Further experiments highlight the multifactorial and complex roles for B cells in EAE [see review by Cross et al. (2001) and Oliver et al. (2003)].

Despite these limitations, the mouse offers many opportunities for genetic manipulation owing to the availability of methodologies to generate knockout and transgenic mice (Madsen et al., 1999; Owens et al., 2001; Kuchroo et al., 2002; Bareyre et al., 2005) and provide an exciting tool to investigate immune tolerance, regulation of cytokine/chemokine networks and the pathophysiological outcome of inflammation on axonal survival and regeneration. The enormous body of literature in which transgenic approaches were used to investigate EAE is beyond the scope of this review, but they have provided many unexpected insights into the roles of specific molecules and signalling pathways in disease pathogenesis, such as the completely unexpected finding that ablation of the IFNγ gene exacerbates rather than suppresses disease activity (Ferber et al., 1996).

However, whilst transgenic mice are an invaluable experimental tool, care must be taken to ensure that they exhibit an appropriate level of genetic homogeneity. Many transgenic mouse strains are derived initially from 129 mice and are then backcrossed with C57BL/6 to provide transgenic strains that are susceptible to MOG35-55 peptide-induced disease. In this case, a minimum of at least six backcrosses should be performed before the offspring are used in experimental studies, and controls must include wild-type littermates.

**Which components of the adaptive immunity cause autoimmune CNS damage in EAE and multiple sclerosis: from T cells to autoantibodies**

EAE is mediated by the complex interplay of several different immune effector mechanisms, and it is increasingly apparent that multiple sclerosis exhibits a similar level of complexity. During the last decades most research focused on the role of the adaptive immune response as represented by T and B lymphocytes. Recently, components of the innate immune system, in particular macrophages and Toll-like receptors, have also been recognized to play an important role in disease pathogenesis (Takeda et al., 2003; Munz et al., 2005; Prinz et al., 2006). The following components of the immune system have been characterized in experimental systems, before they were at least partly studied in multiple sclerosis where additional insight was obtained.

The pathological role of T lymphocytes was confirmed in EAE by adoptive transfer of T cells specific for CNS autoantigens >20 years ago (Ben-Nun et al., 1981), but the network of interactions that control the expansion and pathogenicity of an encephalitogenic T-cell response in vivo is still the subject of intense research. The requirement for antigen-presenting cells (APC) to educate this T-cell response was shown in the 1990s. Professional APC belong to the dendritic cell lineage and are endowed with the complete repertoire of co-stimulatory molecules including members of the immunoglobulin-superfamily, such as CD28/B7 and ICOS, and the tumour necrosis factor (TNF) family such as OX40/OX40L and 4-1BB/4-1BBL that enable them to present antigen to, and fully activate naïve T cells (Dustin and
In contrast, non-professional APCs, (macrophages, and resident CNS cells such as microglia or astrocytes that can upregulate the expression of immune molecules during the inflammatory process) can activate memory but not naive T-cells. The outcome of antigen presentation is not restricted to a ‘simple’ activation of the T cell; it also triggers the secretion of an array of cytokines and chemokines. These soluble molecules play a crucial role in determining the functional outcome of an immune response as well as in modifying the local microenvironment within the target organ. Crucially, the balance of APC-derived cytokines determines the subset of regulatory or effector T cells into which a naive cell will differentiate, the classical example being the opposing roles of IFNγ and IL4 in the differentiation of naive CD4+ T lymphocytes into either Th1 or Th2 effector T-cell subsets (Mosmann and Sad, 1996; Janeway et al., 2001). T-cell differentiation is biased towards the generation of Th1 T cells in the presence of IFNγ, whilst IL4 favours the generation of Th2 subset T cells. These differentiation pathways are associated with distinct intracellular signalling pathways and result in T-cell subsets with very different effector functions [see review in Dalakas (2001)]. Early studies suggested a clear division of labour between these two T-cell populations in the pathogenesis of EAE, with Th1 T cells being identified as the cell population responsible for initiating the inflammatory response in the CNS, whilst Th2 T cells were regarded as counter-inflammatory. This distinction is now becoming somewhat blurred, as in certain circumstances neuroantigen-specific Th2 T cell responses can also damage the CNS (Lafaille et al., 1997; Stefferl et al., 1999). In addition, cytokines such as IL-17 do not fit well into the Th1/Th2 paradigm (Harrington et al., 2005).

In these experimental systems based on the initial methodologies developed by Ben-Nun et al., the addition of exogenous antigen in vitro favours the expansion of an antigen-specific CD4+ T-cell population. However, recent studies using murine rather than rat models have demonstrated that CD8+ T lymphocytes can also exhibit an encephalitogenic potential in vivo (Huseby et al., 2001; Sun et al., 2001; Cabarrocas et al., 2003; Ford and Evavold, 2005) and in vitro, attack and transect MHC class I expressing axons in an antigen-specific manner. This is particularly important, since CD8+ T cells are a major component of the inflammatory infiltrate in multiple sclerosis lesions (Babbe et al., 2000; Neumann et al., 2002). However, whilst myelin antigen-specific CD8+ T cells can induce a CNS pathology in the mouse, further studies are required to determine how closely this reproduces the pathology of the human disease [reviewed in Friese and Fugger (2005)]. What is clear from these experimental studies is that once the endogenous control mechanisms providing regulatory T cells (Reddy et al., 2004) or NK-cells is circumvented, a variety of neuro-antigen-specific effector T-cell subsets can initiate an inflammatory response in the CNS in rodents, and we anticipate that the same will be true for man.

As mentioned previously, these T-cell responses are, however, insufficient to initiate a ‘multiple sclerosis-like’ pathology in either rat or marmoset models of EAE. In these species, the formation of large demyelinating lesions is dependent on the additional generation of myelin-specific antibodies by B lymphocytes, and the same mechanism appears to be involved in the pathogenesis of lesion formation in both a subset of multiple sclerosis patients (Lucchinetti et al., 2000) and patients with Devic’s type of neuromyelitis optica (Lennon et al., 2004). With the exception of Devic’s disease (Lennon et al., 2005), the identity of the antigens targeted by pathogenic antibodies in these patients remains obscure, but to mediate tissue damage the target antigen must be accessible to antibody present in the extracellular milieu. The complete antigenic profile of the myelin surface is unknown, and as yet only three antigens are known that initiate a demyelinating autoantibody response in EAE, galactosyl ceramide (GC), sulphatide and MOG. Once these antibodies bind to the myelin surface, demyelination is then mediated ultimately by a combination of complement and antibody-dependent cellular cytotoxicity-dependent mechanisms. It should be noted that even sublytic activation of complement by low levels of antibody will enhance the local inflammatory response by generating pro-inflammatory signals such as C5a and arachidonic acid derivatives, further stimulating the recruitment and activation of effector cells into the developing lesion. In EAE these demyelinating antibodies are non-pathogenic in normal healthy animals, as the BBB does not allow them to reach their target in a sufficiently high concentration to mediate detectable tissue injury as demonstrated in transgenic mice that express high levels of demyelinating MOG-specific antibodies (Litzenburger et al., 1998).

The plethora of target autoantigens in EAE and multiple sclerosis: complexity of the dysregulated immune response and clinical manifestations

MBP was identified as an encephalitogenic component of CNS myelin in the early 1960s, and studies on this antigen dominated multiple sclerosis research for 20 years until it was finally accepted that proteolipid protein (PLP) was also encephalitogenic. Since then the list of autoantigens known to induce an encephalitogenic CD4+ T-cell response in susceptible species has grown to include not only many other myelin (MOG—Linnington et al., 1988; Piddlesden et al., 1993), MAG, CNPase, OSP, MOBP (Kaye et al., 2000) antigens but also antigens of astrocytic and neuronal origin (S-100—Kojima et al., 1994), GFAP, transaldolase, Ma (Pellkofer et al., 2004), and amyloid precursor protein (APP) (Furlan et al., 2003). It must now be assumed that in the context of an appropriate genetic background, any CNS autoantigen will elicit an encephalitogenic T-cell response [see review in Sospedra and Martin (2005)]. This must be
considered a potential risk in the development of therapeutic strategies based on the induction of beneficial/protective autoimmune responses in neurological diseases. Neglecting this possibility can have serious consequences, as demonstrated regrettably in a clinical trial of the effects of immunization with peptides from APP in Alzheimer’s disease (Hock et al., 2002). This study was based on the observation that the induction of an autoimmune response to APP significantly reduced the CNS burden of amyloid plaques in a murine model of Alzheimer’s disease. However, whilst the transgenic mouse strain used in this study failed to develop a significant encephalitogenic T-cell response, several of the patients developed severe meningoencephalitis. Only after the event was it demonstrated in genetically susceptible mice and by using appropriate adjuvants that this pathology was attributable to the induction of an encephalitogenic APP peptide-specific T-cell response (Furlan et al., 2003).

The demonstration that ‘encephalitogenicity’ is not restricted to the myelin-specific T-cell repertoire immediately raised a number of questions with respect to the role of T-cell specificity in the development of multiple sclerosis. In very general terms, the distribution of inflammatory infiltrates in AT-EAE reflects the anatomical distribution of the target antigen, in that myelin-specific T cells have a predilection to target the inflammatory response to myelinated tracts, whilst the adoptive transfer of SI100beta-specific T cells mediate particularly severe inflammation in grey matter. However, there are marked differences in the anatomical distribution and cellular composition of the inflammatory infiltrates induced by CD4+ T cells specific for different myelin antigens (Berger et al., 1997). In the Lewis rat, the adoptive transfer of MBP-specific T cells results in widespread inflammation of the spinal cord, but little forebrain involvement. In contrast, MOG-specific T cells induce a far higher density of lesions in the forebrain and optic nerves. These differences with regard to lesion location are of practical importance when EAE models are combined with specific imaging techniques such as ultrasound detection (Reinhardt et al., 2005) or MRI (Merkler et al., 2005). They will also influence the clinical score, which is based primarily on the development of motor deficits resulting from spinal cord lesions. Nonetheless, although many Lewis rats with MOG-induced AT-EAE were apparently completely healthy, the number of lesions in the spinal cord was comparable with that in animals with severe disease induced by MBP-specific T cells. Immuno-pathological studies revealed that this dichotomy between the lesion density and the intensity of the clinical deficit was due to the failure of MOG-specific T cells to recruit macrophages into the CNS (Linnington et al., 1993). Recent studies demonstrate that this was due to a failure of resident APCs to fully activate MOG-specific T cells as they invade the CNS (Kawakami et al., 2004). As a consequence, the infiltrating T cells fail to express cytokines such as IFNγ that are required to trigger the local expression of chemokines such as MCP-1 that are necessary to recruit macrophages/monocytes into the developing lesion. This block in the development of EAE can be overcome by introducing additional target antigen into the CNS compartment, suggesting that antigen/epitope availability plays an important role in determining the clinical outcome in AT-EAE. However, the mechanisms responsible for differences in the anatomical distribution of lesions in MOG- and MBP-induced AT-EAE remain obscure. It is notable that there is a predilection for lesions to develop in the optic tract in both MOG-induced EAE and multiple sclerosis, suggesting that loss of immunological self-tolerance to MOG is involved in the development of optic neuritis in early multiple sclerosis. Unfortunately, as yet there is no clinical evidence to support this hypothesis.

These studies suggest that there is a virtually unlimited pool of CNS autoantigens that can initiate an encephalitogenic T-cell response in EAE, proving that they can be processed within the CNS to provide epitopes that can be presented by the host’s class II MHC molecules. In contrast, MOG is the only protein known to induce a demyelinating autoantibody response in EAE. This reflects the necessity of the target antigen to express epitopes exposed to the extracellular milieu at the surface of the myelin sheath/oligodendrocyte, but it would be naïve to assume that MOG is the only antigen that satisfies these criteria, and it is anticipated that further targets for antibody-mediated demyelination will be identified in the near future. However, MOG provides an ideal tool to investigate the mechanisms and pathophysiological consequences of antibody-mediated demyelination in EAE. The passive transfer of demyelinating monoclonal anti-MOG antibodies into animals with AT-EAE resulted in widespread demyelination and enhanced clinical disease (Linnington et al., 1988; Piddlesden et al., 1993). The ability of antibody to induce these effects is itself dependent on pre-existing BBB damage mediated by the encephalitogenic T-cell response. This combination of effector mechanisms is the minimal requirement to form large demyelinating ‘multiple sclerosis-like’ lesions in rat and primate models of EAE, the pathology of which reproduces that seen in early multiple sclerosis patients with Pattern II lesions.

In experimental animals, this demyelinating response is restricted to a limited number of discontinuous/conformation-dependent epitopes formed by the extracellular IgG-like domain of MOG (Brehm et al., 1999; von Budingen et al., 2002, 2004). Why this pathogenic autoantibody response is restricted in terms of its epitope specificity is unclear. A recent study demonstrated that MOG exhibits a high degree of structural and sequence homology with the N-terminal IgG-like domains of butyrophilin gene family members. It was suggested that self-tolerance mediated by these butyrophilin gene products acts to reduce the complexity of the MOG-specific repertoire, as a consequence of molecular mimicry (Fujinami and Oldstone, 1989) between these structurally related proteins (Breithaupt et al., 2003). This has yet to be proven, although there is increasing evidence that there is functional immunological cross-reactivity between...
these proteins (Guggenmos et al., 2004; Mana et al., 2004). In addition, a number of butyrophilin genes are encoded in and adjacent to the MHC locus, which in H-2b mice contains as-yet-unidentified genes that selectively censor the ability to mount a conformation-dependent pathogenic autoantibody response to MOG, while leaving T- and B-cell responses to linear MOG epitopes intact (Bourquin et al., 2003).

EAE studies suggested that MOG could play a similar role as a target for antibody-mediated demyelination in multiple sclerosis, but as yet there is no consensus as to whether or not MOG-specific antibodies actually play a significant role in the human disease. Autoantibody/B-cell responses to MOG are enhanced in multiple sclerosis, but this response is not disease specific. Moreover, there is no evidence that MOG-reactive autoantibodies identified in multiple sclerosis sera, cerebrospinal fluid and multiple sclerosis lesions are able to bind to the native protein in the context of the membrane surface—a prerequisite if they are to mediate primary demyelination. To address this question, MOG-transfected cell lines have been used in an attempt to identify potentially pathogenic MOG-specific antibodies in multiple sclerosis sera (Haase et al., 2001; Lalive et al., 2006). These studies indicate that in the majority of patients the anti-MOG response is directed against linear peptide epitopes that are not accessible when the native protein is expressed at the cell surface. Only in a small percentage of cases were antibodies detected that recognize the native protein, an observation suggesting that pathogenic autoantibody responses to MOG may only play a significant role in demyelination in a small subset of the multiple sclerosis population (Haase et al., 2001).

Evidence from immunopathological studies suggests that antibody/complement-dependent mechanisms are involved in approximately 60% of multiple sclerosis cases, and at least a proportion of these respond to therapeutic plasma exchange (Keegan et al., 2005). Recent progress in understanding the immunopathogenesis of neuromyelitis optica (Devic’s disease) stresses how important it is to identify the antigenic targets involved in this aspect of multiple sclerosis. Autoantibodies to the aquaporin-4 water channel were recently identified as a serological marker for neuromyelitis optica (Lennon et al., 2005), a disease in which plasma exchange can have a dramatic effect on clinical disease activity (Weinshenker et al., 1999; Ruprecht et al., 2004). The clinical response to plasma exchange indicates the involvement of humoral immune mechanisms (Keegan et al., 2005), a concept supported by the extensive deposition of immunoglobulins and complement within the lesions (Luchinetti et al., 2002). Whether or not the antibody response to aquaporin-4 is pathogenic awaits clarification in appropriate animal models following Koch–Witebsky criteria, but if this is the case it will have important implications for multiple sclerosis. Aquaporin-4 is not a myelin component but is located in astrocytic foot processes at the BBB. In this case, tissue injury cannot be due to a direct antibody-mediated attack on the myelin sheath, but must involve other indirect (bystander) mechanisms.

**EAE as a tool to understand immune surveillance of the CNS, inductor and effector phase of the autoimmune attack: the basis for developing novel therapies against multiple sclerosis**

Whereas the preceding parts of the manuscript identified cellular elements and molecular targets of the dysregulated immune response, it still remains open how these components interact in a coordinated and sequential manner. It was in the 1980s when Wekerle coined the idea of constant immunosurveillance of the brain (Wekerle et al., 1986), which was supported by Hickey’s studies on cellular migration during EAE (Hickey and Kimura, 1988) and Perry’s work on inflammatory mechanisms in the brain [reviewed in Perry et al. (1995)]. Until then the CNS had traditionally been viewed as an immunoprivileged organ, not regularly patrolled by immune cells. This new concept of persistent immunosurveillance required several essential steps (Fig. 1). With the available molecular and imaging techniques, these sequential events can be confirmed in experimental models. First, autoreactive cells must escape selection in the thymus and occur naturally, despite the widespread expression of myelin autoantigens in the thymus gland (Kyewski and Derbinski, 2004). Naturally occurring MBP-reactive T cells were first detected in the repertoire of naive Lewis rats (Schlusener and Wekerle, 1985). Following findings from these EAE models, human research initially focused on autoreactive MBP-specific T-cell responses from multiple sclerosis patients. Surprisingly, many similarities between multiple sclerosis patients and healthy controls were observed (Ota et al., 1990; Pette et al., 1990), highlighting that control of autoimmune cells by regulatory elements is of critical importance. The principal proof of the relevance of MBP-specific human T cells in EAE models was found later by introducing human MHC class II restriction elements and the respective T-cell receptors (TCRs) into transgenic mice (Fridkis-Hareli et al., 2001).

A crucial control of autoreactive T cells is exerted by regulatory T cells, which shape and tune the recognition of self-antigens [reviewed in Kuchroo et al. (2002) and Sakaguchi (2000)]. Using transgenic mice bearing autoimmune TCRs, Lafaille et al. pointed out that even if all transgenic T cells bear myelin-specific TCRs, spontaneous EAE is still prevented by naturally occurring regulatory T cells, but will start as soon as the physiological mechanisms controlling T-cell homeostasis are disturbed (Lafaille et al., 1994). There is corresponding evidence that the T-cell regulation is disturbed in multiple sclerosis patients (Viglietta et al., 2004), once again demonstrating that findings obtained from experimental studies can advance our understanding of this disease.
As a second step, autoreactive T cells, present in the normal immune repertoire, must be activated and acquire the capacity to migrate across the BBB (Engelhardt and Ransohoff, 2005). These activated T cells express a set of molecules and enzymes, ready to traverse the BBB. For many years it remained unclear why in AT-EAE brain inflammation does not start immediately after intravenous transfer of T-cell blasts, but takes at least 3–5 days to develop. Only when retroviral techniques were developed to introduce green fluorescent protein (GFP) into autoreactive T cells was it possible to follow their fate during this incubation period. During this pre-clinical phase there are clear changes in the surface phenotype of antigen-specific effector T cells. In addition to the downregulation of several T-cell activation markers (e.g. CD25, OX-40), expression of chemokine receptors increases (Flugel et al., 2001). Although it has been formally shown that AT-EAE can be induced in splenectomized Aly mice lacking secondary lymphoid organs (Greter et al., 2005), these mice still have residual, unstructured lymphoid tissue that may exert the same role of re-programming injected T cells as in naïve mice. Then autoreactive T cells adhere at the endothelium via upregulated adhesion molecules. Here some players out of the diverse ligand pairs such as VLA-4/VCAM-1 seem to play a dominant role [reviewed in Butcher et al. (1999)]. This adhesion step at the endothelium can be followed by intra-vital microscopy of spinal cord vessels and can also therapeutically be manipulated (Vajkoczy et al., 2001). For the transmigration, enzymes such as matrix metalloproteinases are of crucial importance, based on their upregulation during EAE and therapeutic blockade (Kieseier et al., 1998). It is still an open question whether in multiple sclerosis the blockade of VLA-4/VCAM-1 interaction also affects immune cells in the brain parenchyma (Engelhardt and Ransohoff, 2005).

Third, as soon as the T cells have passed the barriers they start local interaction with APCs in the CNS (Flugel et al., 2001). Importantly, two-photon laser microscopy helped in performing live imaging of effector cells in the CNS, which appeared tethered and formed immune synapses (Kawakami et al., 2005). In contrast, non-autoreactive, ovalbumin-specific T cells moved through the brain and stopped only when ovalbumin was injected intracerebrally. Clinical disease in EAE develops only when T cells that have entered the CNS are sufficiently re-activated in the CNS environment (Kawakami et al., 2005). Again, non-autoreactive, ovalbumin-specific T cells moved through the brain and stopped only when ovalbumin was injected intracerebrally.

Fourth, the initial damage introduced by autoantigen-specific T cells is a strong stimulus for further recruitment of macrophages and the plethora of effector mechanisms, which include complement-mediated damage to oligodendrocytes (Scolding et al., 1989) and other structures in the CNS. Activated macrophages and microglia both produce a large number of deleterious soluble factors, which can induce functional blockade and/or structural damage in axons in vitro. Amongst these are nitric oxide, possibly in combination with reactive oxygen species (Redford et al., 1997), matrix metalloproteinases (Leppert et al., 2001; Lindberg
et al., 2004) and other molecules including excitotoxins (Smith et al., 2000a) and proteases (Anthony et al., 1998).

Finally, most T cells entering the brain are destroyed by apoptotic cell death, first recognized by Pender et al. (1991) and soon after described systematically by two of the authors (Schmied et al., 1993), taking advantage of novel molecular techniques for apoptosis detection (Gold et al., 1994). Until now the decisive death signals that induce T-cell apoptosis are only incompletely understood. There is a clear contribution of TNF-signalling (Bachmann et al., 1999), but also naturally occurring steroids may be involved (Gold et al., 1996).

Phagocytosis of these apoptotic T-cell fragments by microglial cells, so far characterized in detail in cell culture of rodent and human gial cells (Chan et al., 2003), seems to provide an elegant feedback loop to downregulate inflammation. In contrast to T cells, physiological elimination of monocytic cells from the inflamed brain seems to occur rather by migration and not by cellular apoptosis. The rare apoptotic macrophages observed in EAE (Nguyen et al., 1994) do not suffice to explain the regression of macrophage infiltration.

**Mechanisms of immune-mediated tissue injury in EAE: from rodents to primates and back to the multiple sclerosis plaque**

Multiple sclerosis is a complex disease with pathological features that are heterogeneous between patients and between different stages of disease evolution. This complexity is reflected in EAE only, when a large spectrum of models induced in different species by different sensitization techniques are analysed (Table 2). In patients with acute and relapsing disease, new focal white matter lesions dominate. In contrast, global diffuse white matter injury and extensive cortical demyelination are additional pathological hallmarks in patients with primary and secondary progressive multiple sclerosis (Kutzelnigg et al., 2005). Regardless of their phenotype, all multiple sclerosis lesions occur on a background of inflammation, composed of lymphocytes and activated macrophages or microglia.

The immunopathological classification of actively demyelinating focal plaques in acute and relapsing multiple sclerosis led to the definition of distinct pathological patterns in lesions [reviewed in Lassmann et al. (2001)]. Pattern I plaques are characterized by T-cell/macrophage-associated myelin damage (see Fig. 2). The main characteristic of Pattern II lesions is the precipitation of immunoglobulins and complement components at sites of active myelin breakdown. Pattern III lesions display signs suggestive of an oligodendrocyte dystrophy with a disproportionate loss of MAG and oligodendrocyte apoptosis, closely reflecting lesions in brain hypoxia (Aboul-Enein et al., 2003). Pattern IV lesions show degeneration of oligodendrocytes in a small rim of periplaque white matter adjacent to sites of active demyelination.

As stated above, all EAE models display certain aspects of the multiple sclerosis histopathology. The comparative studies of Berger et al. (1997) revealed that irrespective of the specificity or number of T cells transferred the major neuropathological correlate with disease severity was the absolute number of activated macrophages recruited into the CNS parenchyma. This reflects the situation seen in

<table>
<thead>
<tr>
<th>Feature of multiple sclerosis lesion</th>
<th>Most suitable EAE Model</th>
<th>References</th>
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<tr>
<td>CD4⁺ T-cell-mediated inflammation</td>
<td>AT-EAE in Lewis rat</td>
<td>Ben-Nun et al. (1981)</td>
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<tr>
<td>CD8⁺ T-cell-mediated inflammation</td>
<td>Passive transfer of CD8⁺ T cells in mice</td>
<td>Huseby et al. (2001), Cabarrocas et al. (2003), Sun et al. (2001)</td>
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<td>T-cell- and macrophage-mediated demyelination</td>
<td>Chronic EAE in C57BL/6 mice induced by MOG peptide 35-55</td>
<td>Mendel et al. (1995)</td>
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<td>T-cell- and antibody-mediated demyelination</td>
<td>Chronic EAE in DA and BN rats or in marmosets sensitized with recombinant MOG 1-125</td>
<td>Storch et al. (1998), T’Hart et al. (2004)</td>
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<td>Inflammation-induced hypoxia-like tissue injury</td>
<td>LPS injection into white matter</td>
<td>Felts et al. (2005)</td>
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<td>T-cell- and macrophage-mediated demyelination with increased oligodendrocyte susceptibility</td>
<td>Chronic EAE in CNTF-deficient mice sensitized with MOG 35-55</td>
<td>Linker et al. (2002)</td>
</tr>
<tr>
<td>Axonal injury in demyelinated plaques</td>
<td>All chronic EAE models in mice and rats Recombinant MOG 1–125 induced EAE in marmosets or in LEW 1.W and LEW 1.AR.I rats</td>
<td>Kornek et al. (2000), Pomeroy et al. (2005), T. M. Storch et al., unpublished data</td>
</tr>
<tr>
<td>Cortical demyelination</td>
<td>So far no model available</td>
<td></td>
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<tr>
<td>Global diffuse axonal injury in the normal appearing white matter</td>
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Pattern I lesions where macrophages appear to be the primary cell type responsible for tissue damage. A Pattern I ‘multiple sclerosis-like’ histopathology is seen in C57BL/6 mice with EAE induced by immunization with MOG35-55 (Calida et al., 2001). Introduction of genetic mutations that affect oligodendrocyte/myelin homeostasis can be shown to increase disease susceptibility in this model. Lack of ciliary neurotrophic factor (CNTF) increases the susceptibility of oligodendrocytes to TNF-α-mediated cell death, resulting in a corresponding increase in disease severity, myelin damage and oligodendrocyte apoptosis (Linker et al., 2002). Such a scenario in which autoimmune attack occurs in the context of compromised oligodendrocyte function may reflect the pathogenesis of Pattern IV. A type of demyelination resembling Pattern III lesions in multiple sclerosis patients (Aboul-Enein et al., 2003), has so far not been seen in any EAE model. However, very similar lesions can be induced by injection of lipopolysaccharide into the white matter (Felts et al., 2005).

Only recently have some of the characteristic features of progressive multiple sclerosis been identified in EAE models. Extensive cortical demyelination can be present in chronic EAE in a subset of marmosets, sensitized either with myelin or MOG (Pomeroy et al., 2005). Similar cortical lesions are also present in selected rat strains (Lewis 1.W and Lewis 1.AR1), which develop a slowly progressive disease following sensitization with MOG (T. M. Storch et al, unpublished). However, diffuse global axonal damage in the normal appearing white matter, similar to that prominent in primary or secondary progressive multiple sclerosis, has so far not been noted in EAE models.

Lessons from studying axonal and neuronal damage in EAE

The importance of axonal injury as a component of the multiple sclerosis lesion was rediscovered in the late 1990s having been first described almost a century earlier [Ferguson et al., 1997; Trapp et al., 1998; reviewed in Kornek and Lassmann (1999)]. Experimental studies soon showed parallel findings in EAE (Kornek et al., 2001; Wujek et al., 2002), and identified specific molecular abnormalities such as redistribution of ion channels on chronically demyelinated axons that may play an important role in the axonal pathology of multiple sclerosis (Kornek et al., 2001; Craner et al., 2004a, b). Changes in intra-axonal ionic homeostasis due to altered or
aberrantly expressed ion channels may indeed result in axonal damage in EAE, as axonal loss is significantly reduced by pharmacological agents that reduce sodium and calcium transport/entry across the axolemma (Kapoor et al., 2003; Bechtold et al., 2004). These observations are extremely important as they demonstrate that neuroprotection is a valid therapeutic approach in multiple sclerosis, which, if successful, may decrease the development of chronic disabilities. However, axonal loss, demyelination and inflammation in multiple sclerosis are intimately inter-related and therapeutic strategies must take this into consideration. Inflammatory mediators and cytokines must take this into consideration. Inflammatory mediators such as nitric oxide are per se deleterious to axonal function, and this is compromised further by demyelination that not only results in acute electrophysiological dysfunction but also increases susceptibility to inflammatory mediators and reduces long-term axonal survival by disrupting axonal/glial interactions. EAE provides an important tool to investigate how the interplay of these neurobiological and immune-mediated mechanisms results in axonal injury and ultimately in degeneration.

Acute axonal injury is now recognized as a normal response to inflammation in EAE that can occur in the absence of extensive myelin loss. In this case, activated macrophages (and microglia cells) are the obvious suspects responsible for mediating this pathology, as macrophages are the dominant effector cell population responsible for acute clinical disease in EAE: the number of macrophages infiltrating the CNS correlates broadly with disease severity (Berger et al., 1997; McQualter et al., 2001; Heppner et al., 2005). Macrophages also associate with dystrophic axons in both EAE and multiple sclerosis (Ferguson et al., 1997; Kornek et al., 2001). Activated macrophages and microglia both produce a large number of deleterious soluble factors, which can induce functional blockade and/or structural damage in axons in vitro. Nitric oxide, possibly in combination with reactive oxygen species, is one important candidate (Redford et al., 1997), but other molecules including excitotoxins (Smith et al., 2000a) and proteases (Anthony et al., 1998) may play equally important roles. These molecules are all involved in disease pathogenesis but it should not be forgotten that inflammatory cells including macrophages also produce neurotrophic factors such as BDNF that may provide a neuroprotective function [reviewed in Hohlfeld et al. (2000)].

Recent reports have demonstrated that CD8+ T cells may also interact directly with demyelinated axons to mediate axonal injury in an antigen-dependent manner. This was initially demonstrated in vitro (Medana et al., 2001). Multiple sclerosis lesions contain large numbers of CD8+ T cells. Laser microdissection and CDR3 spectratyping indicate that these infiltrating CD8+ T cells are clonally expanded with the CNS (Skulina et al., 2004), but the functional relevance of this response—immune regulation versus tissue damage—remains unknown. It should be remembered that animal experiments demonstrate that neuro-antigen-specific CD8+ T cells are not essential to cause axonal injury in EAE, as demonstrated in studies using EAE-susceptible β2-microglobulin knockout mice (Zhang et al., 1997). Indeed, Linker et al. (2005) reported that disruption of MHC class I-CD8+ interactions in these mice was associated with increased levels of axonal damage.

Axons, however, may also be destroyed by CD8+ cells in an indirect way. Adoptive transfer of CD8+ T cells lead to inflammatory brain lesions with extensive axonal injury and destruction (Huseby et al., 2001) even when these T cells were directed against a myelin antigen (MBP) instead of an axonal antigen. A mechanism by which T cells might mediate axonal injury non-specifically is via ligation of TNF-related apoptosis-inducing ligand (TRAIL) receptors (Aktas et al., 2005). In EAE, disease severity and neuronal apoptosis in brainstem motor areas were both substantially reduced upon brain-specific blockade of TRAIL, and in AT-EAE TRAIL-deficient myelin-specific lymphocytes showed reduced encephalitogenicity in comparison with wild-type T cells when transferred to wild-type recipients. In addition, TRAIL-receptor 2 is upregulated in the CNS during the course of EAE and, correspondingly, intracerebral delivery of TRAIL increased clinical deficits in animals with EAE, while having no effect in naïve mice. Once again it must be stressed that multiple T-cell-dependent pathways can damage neurons and this is not necessarily antigen-specific. Using two-photon microscopy, Nitsch et al. investigated the dynamic interaction between neurons and T cells in vitro using brain slices (Nitsch et al., 2004). They demonstrated that within the complex cellular network of living brain tissue myelin- and ovalbumin-specific T cells can both make contact with and induce calcium oscillations in neurons. This effect is MHC-independent and finally resulted in a lethal elevation in neuronal calcium levels that could be prevented by blocking both perforin and glutamate receptors.

In vivo studies on the efficacy of neuroprotective strategies on functionally defined tracts in EAE is difficult, as the lesions are widespread and occur throughout the CNS. This problem can, in part, be overcome by systematic analysis of the response of retinal neurons, which in response to immune-mediated demyelination and axonal damage in the optic nerve undergo early apoptotic degeneration (Meyer et al., 2001). Using this model, it was possible to demonstrate a neuroprotective effect of erythropoietin, a drug widely used in medicine (Sattler et al., 2004). Further neuroprotective strategies identified in EAE include the use of epigallocatechin-3-gallate derived from green tea (Aktas et al., 2004); the neurotrophic cytokine LIF, which supports survival of oligodendrocytes (Butzkueven et al., 2002) and which in contrast to other neurotrophic factors will reach the target organ after s.c. or i.v. application; and the blockade of glutamate receptors (Smith et al., 2000b) and sodium channels (Bechtold et al., 2004). However, in all these studies there are additional treatment effects that influence the local inflammatory response and it remains difficult to differentiate between an effect of the therapeutic agent that increases
the resistance of the axon to injury and effects that are secondary to a simple reduction of the intensity of the local inflammatory insult.

Until recently it was regarded unlikely that the CNS would have any substantial capacity to regenerate and functionally repair neuronal/axonal damage associated with inflammatory demyelination. In fact this was virtually impossible to investigate in standard models of EAE as inflammation and tissue damage are disseminated throughout the neuraxis, and the clinical deficit cannot be directly attributed to a defined tract system. This problem was overcome by the development of ‘targeted’ models of EAE in which single large inflammatory lesions develop in the dorsal columns of the spinal cord (Kerschensteiner et al., 2004b). These lesions show all the pathological hallmarks of multiple sclerosis plaques and lead to reproduceable and pronounced deficits in hind limb locomotion. This model allowed a detailed characterization of axonal changes that may contribute to recovery from lesions (Kerschensteiner et al., 2004a). In this experimental system, axons remodel at multiple levels in response to a single neuroinflammatory lesion demonstrating unexpected plasticity and ability to regenerate. Initially, sprouting of local interneurons was observed in the vicinity of the lesion; this was followed by the extension of new collaterals from descending corticospinal tract axons into proximal spinal cord segments, and finally remodelling of distribution of projection neurons in the motor cortex. These histological studies were complemented by behavioural tests that directly demonstrated the plastic functional response of the motor system to a single neuroinflammatory lesion (Kerschensteiner et al., 2005). Success and failure in using EAE models to validate new therapeutic strategies

Innovative concepts raised by novel findings in EAE can easily be translated into therapeutic approaches in these models. Also, systematic histopathological studies are possible, which cannot be routinely performed in human patients. This being said, we will focus our following comments on approaches touching these aspects (see also Fig. 1), selected out of thousands of EAE therapeutic studies.

Glucocorticosteroids and statins

The routine therapy of acute multiple sclerosis relapses includes glucocorticosteroid pulses, dating back to first reports from Milligan and Compston (Milligan et al., 1987). The optic neuritis trial (Beck et al., 1992) clearly showed that dose comparisons are difficult if not impossible because of the heterogeneity of multiple sclerosis. In EAE, it could be delineated that T-cell apoptosis linearly correlates to steroid dosage and severity of disruption of the BBB (Schmidt et al., 2000). Here, therapeutic steroids were given up to 50 mg/kg body weight and correlated with tissue levels as measured by HPLC. The efficacy of steroid delivery into sites of inflammation can be augmented by liposomal packaging (Schmidt et al., 2003b). Importantly, not only T-cell infiltration but also macrophage and microglia activation could be reduced by this approach (Schmidt et al., 2003b). It seems promising to follow this line of targeted and improved delivery during short-term clinical studies.

Years after introduction into medical therapy statins turned out to have a substantial influence on programming T-cell cytokine pattern. The first experimental studies from Dr Steinman’s group (Youssef et al., 2002) were soon followed by work pointing at microglial activation by lipid degradation products, downregulated by statin activity (Aktas et al., 2003). Again, transfer of experimental findings into human cellular systems suggests the therapeutic efficacy of statins also for multiple sclerosis (adjunctive) therapy (Neuhaus et al., 2002). These experimental findings have already stimulated first clinical observations (Vollmer et al., 2004). Currently, controlled studies have been initiated.

Antigen-specific therapies

In the late 1980s, the finding of limited TCR variable region usage by autoaggressive rodent T-cell lines (Chluba et al., 1989) stimulated many research lines, which also tried to transfer these findings to human disease [see review in Zamvil and Steinman (1990)]. This nourished hope of developing TCR-specific immunotherapies, such as specific vaccination (Sun et al., 1988). In the following years it could be shown that even for a single autoantigen in inbred rat strains a variety of TCRs are used (Gold et al., 1995), and of course even more in the outbred human population (Utz et al., 1994). Therefore, this concept has now been abandoned by most research groups [see review in Hafler et al. (1996)].

The subsequent sophisticated attempts to modulate the antigen by creating altered peptide ligand (APL) and bring them to human therapy (Bieleckova et al., 2000; Kappos et al., 2000) came to the surprising finding that the APLs were able to stimulate autoreactivity in some patients, while they dampen it in others. This is a clear example of how difficult it is to transfer experimental data, obtained in inbred species, into the human therapeutic setting. Currently, the only effective antigen-specific therapy is glatiramer acetate (Teitelbaum et al., 1971), developed as a bystander product in EAE studies and soon transferred into multiple sclerosis therapy (Abramsky et al., 1977). Besides its immunological actions (Duda et al., 2000), glatiramer acetate may also act through neuroprotection by stimulating BDNF secretion [reviewed in Hohlfeld et al. (2000)].

Cytokines

Therapeutic studies interfering with cytokines highlight the problems that may occur when premature results, obtained
in EAE models, are transferred into the human context. TNF-α has long been considered as a prime target for multiple sclerosis therapy because of its pro-inflammatory actions and its involvement in the induction of immune-mediated tissue injury. However, in the course of studies on antigen-induced T-cell apoptosis in EAE (Weishaupt et al., 2000), induction of apoptosis was found to be associated with release of TNF-α, indicating an additional role of TNF-α in the downregulation of inflammation. This may explain that despite promising results on TNF-blockade in EAE (Selmaj et al., 1991) the ultimate transfer into human therapy failed both by using monoclonal antibodies (Van Oosten et al., 1996) and recombinant TNF-receptor (The Lenercept Multiple Sclerosis Study Group, 1999). The patients in the verum group of the Lenercept Study even developed stronger inflammation in MRI and CSF, thus emphasizing the role of TNF for limitation of inflammation as described in studies with transgenic mice [see review in Probert et al. (2000)]. Together, these studies provide novel findings and at the same time shed light on the complex pathophysiology of TNF-α in CNS inflammation. Interpretation of these effects must also take into account the existence of two TNF-receptors, which may have opposing effects.

**Regulatory NK and T cells**

The increasing knowledge about regulatory elements in the immune system has been translated into biomedicine at several levels. The group of Dr Yamamura stimulated the regulatory properties of NK T cells by using synthetic glycolipids (Miyamoto et al., 2001). This was associated with a Th2 bias of autoimmune T cells. In addition to “classical” anti-CD28 antibodies, Tacke and Huenig described a ‘superagonistic’ anti-CD28 antibody, able to stimulate T cells without concomitant TCR engagement (Tacke et al., 1997). Later, it turned out that the superagonistic anti-CD28 binds to a different region of the molecule (Luhder et al., 2003) and can directly induce regulatory T cells, which suppress acute and chronic EAE, even upon passive transfer (Beyersdorf et al., 2005). In addition, preceding studies had revealed therapeutic activity also in experimental neuritis (Schmidt et al., 2003a). Reduced activity of regulatory T cells in multiple sclerosis has been shown by different investigators (Viglietta et al., 2004; Huan et al., 2005). A similar focus on regulatory elements of the immune system was put by studies on oral tolerance. Its efficacy seemed to be connected with secretion of TGF-β (Khoury et al., 1992; Miller et al., 1992), which was effective in blocking EAE in vivo (Racke et al., 1991). Owing to equivocal results in human studies, this approach waits for a reappraisal.

**Adhesion molecule blockade**

The 1990s saw increased efforts at therapeutic usage of adhesion molecule blockade. Soon the ligand pair VCAM-1/VLA-4 turned out to be crucial for egress of inflammatory T cells into the brain parenchyma (Yednock et al., 1992). This was a remarkable finding, and in the following years the transfer into multiple sclerosis studies using the humanized anti-VLA4 antibody natalizumab was fast and effective (Tubridy et al., 1999; Miller et al., 2003). It came as a great surprise that anti-VLA4 therapy is in some patients associated with increased blood levels and infectivity of JC-virus, leading to progressive multifocal leuencephalopathy in two multiple sclerosis patients who received combination therapy with IFN-β [see commentary in Berger and Koralnik (2005)]. Such a complication is unlikely to be foreseen from EAE models owing to the relatively short period of therapeutic intervention and the species differences in the susceptibility for certain virus infections. Currently, there are no unequivocal scientific explanations available: both breakdown of immune surveillance of the CNS and mobilization of virus-harbouring bone marrow cells are discussed (Ransohoff, 2005).

Thus, EAE models mimic some, but not all, aspects of brain inflammation in multiple sclerosis and proved to be useful for pre-clinical testing of anti-inflammatory therapeutic strategies. In addition, the mechanisms of tissue damage and, in particular, of axonal injury are similar in EAE and multiple sclerosis, and it can be expected that neuroprotective therapies, developed in EAE models, will in the future show a beneficial effect also in multiple sclerosis patients. Approaches for antigen-specific therapy or for correcting disturbances of immune regulation are less likely to be transferable into the human situation, owing to principal differences between the spontaneous nature of the human disease and the disease induction by active sensitization in EAE. Finally, it has to be acknowledged that EAE models so far do not sufficiently reflect the nature of tissue injury in primary and secondary progressive multiple sclerosis.

**Conclusions**

Autoimmune encephalomyelitis is, thus, an excellent tool for studying basic mechanisms of brain inflammation and immune-mediated CNS tissue injury, and for obtaining proof of principle, whether a certain therapeutic strategy has the potential to block these pathways. Whether they are relevant for multiple sclerosis patients in general and, if yes, for what subpopulation of patients has to be determined in respective clinical studies. Pitfalls arose when premature laboratory findings were translated too early into human therapy, or when the multiple feedback loops of cytokine networks were disregarded. Also, the dominance of CD4+ T cells in most EAE models used for therapeutic studies contrasts with the CD8+-shift observed in multiple sclerosis lesions. There is a clear need for more CD8+-based models, in analogy to human multiple sclerosis pathology. Finally, owing to the complexities of human diseases, it is obvious that there is no single EAE model, but rather a combination of different approaches that will finally help us to develop new and more effective therapeutic approaches.
Merits and limitations of EAE for multiple sclerosis

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