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REVIEW ARTICLE

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

cord (Koritschoner and Schweinburg, 1925). In the 1930s, researchers attempted to reproduce the encephalitic complications associated with rabies vaccination by repetitive immunization of rhesus monkeys with CNS tissue (Rivers *et al.*, 1933). Since then EAE was elicited in many different species, including rodents and primates, and from these studies it became clear that EAE can reproduce many of the clinical, neuropathological and immunological aspects of multiple sclerosis (Hohlfeld and Wekerle, 2001). This led to the common belief that we will understand multiple sclerosis once the pathogenesis of EAE is elucidated, and that new therapeutic strategies developed in EAE should be automatically beneficial in patients. Unfortunately, this simplistic view has led to major disappointments and a growing feeling that EAE is no longer an appropriate model for multiple sclerosis.

Multiple sclerosis is a complex disease with heterogeneous clinical, pathological and immunological phenotype that might better be described as a syndrome rather than a single disease entity—a concept that has important implications with respect to the development of effective therapeutic strategies. The 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 progressive disease. Advances in molecular medicine have clearly demonstrated the heterogeneity of multiple sclerosis (Lassmann *et al.*, 2001). Its pathology is, in part, reflected by the formation of focal inflammatory demyelinating lesions in the white matter, which are 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 appearing white matter and massive demyelination also in the grey matter, in particular in the cortex (Bo *et al.*, 2003; Kutzelnigg *et al.*, 2005). The 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 reason 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

Table 1 Commonly used rodent EAE models

Model	Similarities to human disease	Differences from human disease	Further comments
Lewis rat Active EAE (CNS myelin, MBP, MOG, PLP)	T-cell inflammation and weak antibody response	Monophasic, little demyelination	Reliable model, commonly used for therapy studies. With guinea-pig MBP little demyelination
Adoptive-transfer EAE (MBP, S-100, MOG, GFAP)	Marked T-cell inflammation. Topography of lesions	Monophasic, little demyelination	Homogeneous course, rapid onset. Differential recruitment of T cells/macrophages depending on autoantigen
Active EAE or AT-EAE + co-transfer of anti-MOG antibodies	T-cell inflammation and demyelination	Only transient demyelination	Basic evidence for role of antibodies in demyelination
Congenic Lewis, DA, BN strains Active EAE (recombinant MOG aa 1–125)	Relapsing–remitting disorders, may completely mimic histopathology of multiple sclerosis and subtypes	No spontaneous disease	Chronic disease course, affection of the optic nerve, also axonal damage similar to multiple sclerosis
Murine EAE (SJL, C57BL/6, PL/J, Biozzi ABH) Active EAE (MBP, MOG, PLP and peptides)	Relapsing–remitting (SJL, Biozzi) and chronic-progressive (C57BL/6) disease courses with demyelination and axonal damage	No spontaneous disease	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
Murine EAE in transgenic mice or knockout mice (mostly C57BL/6 background)	Specifically addresses role of defined immune molecules/neurotrophic cytokines/ neuroanatomical tracts	Most results obtained with artificial permanent transgenic or knockouts	Extensive backcrossing (>10 times) on C57BL/6 background required. Future work with conditional (cre/loxP) or inducible (e.g. Tet-on) mutants

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 immunized with MBP (Pender *et al.*, 1995).

Until the 1980s, the intrinsic variability of EAE induced by immunization caused many researchers to define 'A' as 'allergic', since the autoimmune origin of this disease model was still under debate. Traditionally, the term 'allergic' is linked to any hyperreactivity of the immune system towards (exogeneous) antigens. A major milestone to confirm the central role of autoimmune reactions for EAE was achieved when Ben-Nun, Cohen and Wekerle developed techniques to propagate antigen-specific T cells *in-vitro* in the rat and demonstrated that the adoptive transfer of MBP-specific T-cell lines induced EAE in naïve syngeneic recipients (Ben-Nun *et al.*, 1981). The final proof for transforming 'allergic' into 'autoimmune' was obtained by selecting autoaggressive T lymphocytes from naïve rats (Schluesener and Wekerle, 1985). These data provided strong evidence in favour of the presence of potentially CNS-autoaggressive T clones in the normal immune system, which forms the basis for an autoimmune reaction.

Since the initial report of adoptive-transfer EAE (AT-EAE), these models have become a major experimental tool for investigating T-cell function and regulation in neuroinflammation 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

(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 (Schluesener *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 (Wallström *et al.*, 1997; Weissert *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 (Stefferl *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 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 $PLP_{139-151}$ peptide-induced relapsing EAE in *SJL mice*, MBP-induced disease in *PL/J mouse*, chronic-progressive models of MOG protein or MOG_{35-55} 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 MOG_{35-55} 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

Cooper, 2000; Dalakas, 2001). 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 naïve 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 naïve cell will differentiate, the classical example being the opposing roles of IFNγ and IL4 in the differentiation of naïve $CD4^+$ 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 neuro-antigen-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 exogeneous 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 endogeneous 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 millieu. 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 archadonic 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—Linington *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 S100beta-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. Immunopathological 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 (Linington *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 (Linington *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 (Lucchinetti *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 (Schluesener 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.

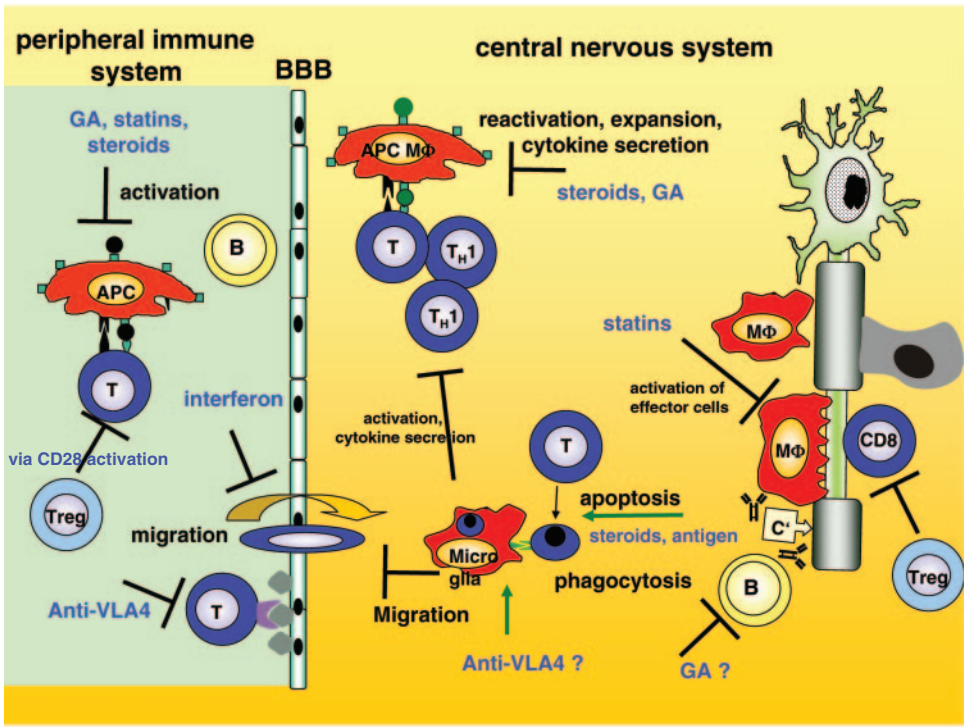

Fig. 1 Inductor and effector stages of the immune reaction in EAE/multiple sclerosis and therapeutic manipulation. Therapeutic intervention is indicated by black bars. Abbreviations: APC = antigen-presenting cell, BBB = blood–brain barrier, C = complement, GA = glatiramer acetate, MΦ = macrophage, Treg = regulatory T cells.

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.*, 2000*a*) 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 glial 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. The heterogeneity of the four patterns was observed between different patients, but not within multiple active demyelinating lesions from a single patient. These differences may have important consequences not only for understanding disease mechanisms but also for a subtype-based therapy in multiple sclerosis.

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 2 Pathological features of multiple sclerosis and the most suitable EAE models

Feature of multiple sclerosis lesion	Most suitable EAE Model	References
$CD4^+$ T-cell-mediated inflammation	AT-EAE in Lewis rat	Ben-Nun *et al.* (1981)
$CD8^+$ T-cell-mediated inflammation	Passive transfer of $CD8^+$ T cells in mice	Huseby *et al.* (2001), Cabarrocas *et al.* (2003), Sun *et al.* (2001)
T-cell- and macrophage-mediated demyelination	Chronic EAE in C57BL/6 mice induced by MOG peptide 35-55	Mendel *et al.* (1995)
T-cell- and antibody-mediated demyelination	Chronic EAE in DA and BN rats or in marmosets sensitized with recombinant MOG 1-125	Storch *et al.* (1998), T'Hart *et al.* (2004)
Inflammation-induced hypoxia-like tissue injury	LPS injection into white matter	Felts *et al.* (2005)
T-cell- and macrophage-mediated demyelination with increased oligodendrocyte susceptibility	Chronic EAE in CNTF-deficient mice sensitized with MOG 35-55	Linker *et al.* (2002)
Axonal injury in demyelinated plaques	All chronic EAE models in mice and rats	Kornek *et al.* (2000)
Cortical demyelination	Recombinant MOG 1–125 induced EAE in marmosets or in LEW 1.W and LEW 1.AR1 rats	Pomeroy *et al.* (2005), T. M. Storch *et al.*, unpublished data
Global diffuse axonal injury in the normal appearing white matter	So far no model available	

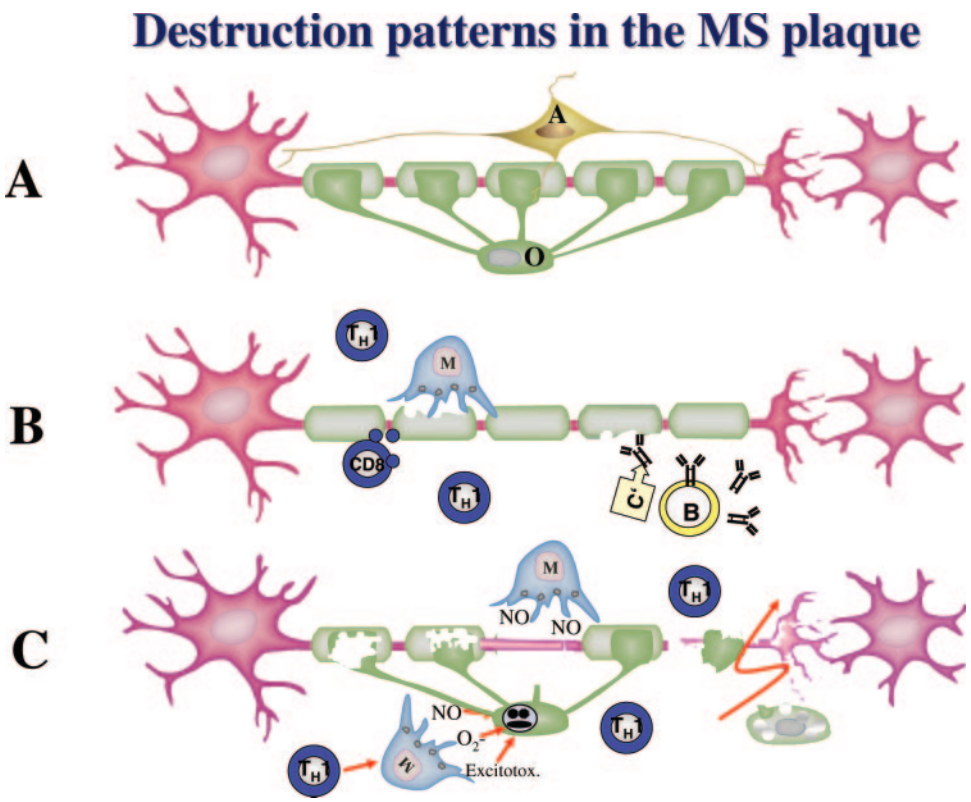

Fig. 2 Destruction patterns in the multiple sclerosis plaque. (**A**) In the healthy CNS oligodendrocytes ensheathe the axon and form myelin internodes of regular size. (**B**) Cytotoxic attack can destroy the myelin sheath via T-cell and macrophage inflammation. Cytotoxic T cells secrete perforin and granzyme as effector molecules directed towards the target (left). Humoral factors destroy the myelin sheath via local deposition of antibodies, which then activate complement as shown here (right) or phagocytic effector cells via ADCC (not shown). (**C**) Damage towards the oligodendrocyte and the axon is mediated via cytotoxic products of macrophages/microglia (left), with nitric oxide (NO) as one of the major constituents. Note that the oligodendrocyte shows typical morphology of apoptosis. On the right side, the diffuse pattern of axonal and myelin destruction is illustrated, where as yet no unequivocal pathogenetic mechanism has been identified.

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 MOG_{35-55} (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.*, 2004*a*, *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 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.*, 2000*a*) 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.*, 2000*b*) 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.*, 2004*b*). These lesions show all the pathological hallmarks of multiple sclerosis plaques and lead to reproducible 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.*, 2004*a*). 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. In an elegant follow-up paper, axonal damage in this model was monitored by *in vivo* imaging, an approach that is rapidly changing our concepts of the intrinsic dynamics of axonal degeneration and growth (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.*, 2003*b*). Importantly, not only T-cell infiltration but also macrophage and microglia activation could be reduced by this approach (Schmidt *et al.*, 2003*b*). 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 (Bielekova *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.*, 2003*a*). 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 leucencephalopathy 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.

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Also, we have avoided extensive discussion of $CD8^+$ T cells in EAE/MS because this topic has recently been handled in Friese and Fugger (2005).

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