The value of animal models for drug development in multiple sclerosis

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The rodent model for multiple sclerosis, experimental allergic (autoimmune) encephalomyelitis (EAE), has been used to dissect molecular mechanisms of the autoimmune inflammatory response, and hence to devise and test new therapies for multiple sclerosis. Clearly, artificial immunization against myelin may not necessarily reproduce all the pathogenetic mechanisms operating in the human disease, but most therapies tested in multiple sclerosis patients are nevertheless based on concepts derived from studies in EAE. Unfortunately, several treatments, though successful in pre-clinical EAE trials, were either less effective in patients, worsened disease or caused unexpected, severe adverse events, as we review here. These discrepancies must, at least in part, be due to genetic and environmental differences, but the precise underlying reasons are not yet clear. Our understanding of EAE pathogenesis is still incomplete and so, therefore, are any implications for drug development in these models. Here, we suggest some potential explanations based on new thinking about key pathogenic concepts and differences that may limit extrapolation from EAE to multiple sclerosis. To try to circumvent these rodent–human dissimilarities more systematically, we propose that pre-clinical trials should be started in humanized mouse models.

Keywords: animal models; experimental allergic encephalomyelitis; immunomodulation; multiple sclerosis; treatments

Abbreviations: CFA = complete Freund’s adjuvant; EAE = experimental allergic (autoimmune) encephalomyelitis; HSCT = haematopoietic stem cell transplantation; MBP = myelin basic protein; PDEs = phosphodiesterases; PML = progressive multifocal leukoencephalopathy; PPARs = peroxisome proliferator-activated receptors; TCR = T-cell receptor; TH1 = T helper 1; TNF = tumour necrosis factor

Introduction

Multiple sclerosis is the commonest neurological disease of young adults, afflicting at least 350 000 individuals in North America and 500 000 in Europe (Hafler et al., 2005; Sospedra and Martin, 2005). Although multiple sclerosis does not usually shorten life expectancy, its socio-economic burden in young adults is second only to trauma (Sospedra and Martin, 2005). Its clinical signs and symptoms are very variable and depend on the parts of the CNS it affects, that is, the brain and spinal cord, and include motor, sensory, autonomic and cognitive disabilities (Noseworthy et al., 2000a). It can run at least three clinical courses: (i) relapsing–remitting (RR) multiple sclerosis, which is most frequent (~85%) and characterized by discrete attacks (exacerbations) and subsequent periods of clinical stability. In most relapsing multiple sclerosis patients, (ii) a secondary progressive (SP) phase ensues, with continuously increasing deficits. About 10–15% of multiple sclerosis patients develop...
steadily increasing neurological deficits from onset, (iii) the primary-progressive subtype (Noseworthy et al., 2000a).

Neuropathologically, CNS tissue from multiple sclerosis patients shows discrete lesions (predominantly in the white matter) with inflammatory infiltrates, demyelination, astroglisis and early axonal damage. Again, there is considerable heterogeneity in composition of cellular infiltrates and in involvement of antibodies and complement (Lassmann et al., 2001). Multiple sclerosis is widely considered an autoimmune demyelinating disease, and the inflammatory infiltrates as pathogenically primary events. Its aetiology remains a mystery, but infectious agents have long been suspected as triggers (Marrie, 2004). The evidence for an autoimmune reaction targeting myelin is strong but not definitive. There are, for example, descriptions of primary oligodendrocyte apoptosis with microglial activation in early multiple sclerosis lesions in the absence of lymphocytes or myelin phagocytosis (Barnett and Prineas, 2004). Further, the decreasing inflammatory activity that is seen by MRI during the SP phase has led to the assumption that the pathology is inflammatory at first and degenerative later. Despite these uncertainties, it is generally accepted that multiple sclerosis involves an autoimmune reaction by myelin-specific CD4+ T helper 1 (Th1) cells, which initiate the neuropathology (Hafler et al., 2005; Sospedra and Martin, 2005). This notion is based on the cellular composition of CNS- and CSF-infiltrating cells (Hauser et al., 1986), on genetic studies in multiple sclerosis (Dyment et al., 2004) and on one animal model of multiple sclerosis, experimental allergic (autoimmune) encephalomyelitis (EAE) (Zamvil and Steinman, 1990).

Dissecting the pathogenesis of a complex disease in man is fraught with many problems, particularly those associated with clinical and genetic heterogeneity. Not surprisingly, most of our current thinking about multiple sclerosis stems from EAE. This model originated from vaccination with rabies-infected rabbit spinal cord by Louis Pasteur (from 1885). About 1 in 1000 vaccinees had 'neuroparalytic incidents'; this acute demyelinating disorder later proved to be due to 'contamination' by spinal cord components in the inoculum. The EAE model has since evolved a long way; different variants, mice, rats or non-human primates are immunized with whole spinal cord, myelin proteins or even defined peptides, usually in complete Freund's adjuvant (CFA). This immunization leads to a disease that shares clinical and neuropathological changes with multiple sclerosis (Steinman, 1999). The course it takes ranges from acute monophasic (or even lethal) to chronic progressive or relapsing–remitting (Steinman, 1999). Typical CD4+ Th1 myelin-specific T cells have been implicated as the disease-initiating subset. In almost all models, they are sufficient to induce EAE; they can be isolated, cloned and used to transfer disease to naive healthy animals (Zamvil and Steinman, 1990). These various EAE models have been used to dissect molecular mechanisms of the autoimmune inflammatory response, and hence to devise and test new therapies for multiple sclerosis. It is clear, however, that the artificial induction of a myelin-specific immune response may by-pass key pathogenetic mechanisms operating in human disease, as we do not even know the key target auto-antigens in multiple sclerosis.

**Limitations of current EAE models**

Without doubt, EAE models are vital for studying general concepts as well as specific processes of autoimmunity, however rarely they predict success in clinical trials (see below). Nevertheless, their value is further challenged by our rudimentary understanding of the key pathogenetic mechanisms in EAE models, and their failure to forewarn us of adverse effects (reviewed below). As with other murine disease models, including the NOD model of type 1 diabetes (Roep et al., 2004), it appears much easier to prevent, reverse or ameliorate EAE in mice than multiple sclerosis in man.

Furthermore, since EAE almost always has to be induced, it cannot mimic a spontaneous disease. The most important component in the inducing adjuvant CFA is heat-inactivated Mycobacterium tuberculosis, which always induces a prominent CD4+ Th1 response by activating certain toll-like receptors (Su et al., 2005). This leaves little room for variability in disease pathways and certainly does not reflect heterogeneous inducing mechanisms in multiple sclerosis. Also, demyelination is not obvious in all models. Moreover, the time courses are very different. Since EAE develops over days in most models, they seem more similar to post-infectious acute demyelinating events (Steinman, 1999). Indeed, the mice are rarely monitored for late relapses and fatal adverse effects, such as those noted in marmosets (Genain et al., 1996). Nevertheless, the same treatment can have a different degree of efficacy or even opposite effects at different stages in EAE, such as those noted in marmosets (Genain et al., 1996). Nevertheless, the same treatment can have a different degree of efficacy or even opposite effects at different stages in EAE, as has also been reported for other autoimmune models such as in NOD mice (Shoda et al., 2005). In contrast, multiple sclerosis usually manifests insidiously over years, for example, in its relapsing–remitting and later chronic forms (Noseworthy et al., 2000a), by when antibodies and complement may also be more important than in most mouse models. Indeed, many patients present after much more protracted epitope spreading than is usually seen in EAE mice (Vanderlugt and Miller, 2002). These and other obvious mouse : human differences are summarized in Table 1.

Many aspects of pathology and immunology differ between multiple sclerosis and EAE. These differences are fundamental, as ongoing imbalances in immune regulation must be crucial for the progression of multiple sclerosis; such orders of complexity have not yet been recapitulated in EAE models.

**What can we learn from failures or successes in adapting therapies from EAE to multiple sclerosis?**

Only very few therapeutics that were successful in pre-clinical EAE trials have shown similar efficacy in multiple sclerosis.
patients; the majority of new treatments were either less effective in these patients, worsened disease or caused severe adverse events. In Table 2 we list a subset of these therapies reflecting this discrepancy.

**Antigen-specific therapies**

Only one licensed multiple sclerosis therapy (Glatiramer acetate, GA), a synthetic amino acid copolymer (Glu, Ala, Lys and Tyr), emerged from findings in EAE (Teitelbaum et al., 1971). It was designed to mimic encephalitogenic myelin basic protein (MBP) epitopes, but instead it suppresses EAE by other mechanisms in several species, and it reportedly reduces multiple sclerosis relapses by 30% (Johnson et al., 1995). GA has many biological activities including bystander suppression via induction of TH2 cells that partly cross-react with MBP, and/or upregulation of CNS growth factors (Arnon and Aharoni, 2004). However, its *in vivo* mechanisms are not clear and even its beneficial effects on the main outcome measures in multiple sclerosis (disease progression) have now been questioned in a systematic Cochrane review (Munari et al., 2004).

A more specific therapeutic approach in EAE and multiple sclerosis has been based on an altered peptide ligand of MBP 85–99 that was modified at its main T-cell receptor (TCR) contact sites (Broke et al., 1996). Despite promising effects in EAE, subcutaneous administration of altered peptide ligand at high doses led to multiple sclerosis exacerbations in some patients, which could be linked to this treatment (Bielekova et al., 2000). A trend towards improved MRI parameters was observed in another phase II trial (Kappos et al., 2000), and an additional phase II study is under way. Its success in EAE may depend on the stereotyped TH responses of inbred mice.

**Oral administration of myelin antigens** leads to specific immune hyporesponsiveness in mice. Different doses and feeding regimes have been demonstrated to induce different types of ‘oral tolerance’/degrees of immune suppression in different EAE models (Faria and Weiner, 2003). The key autoimmunizing antigen(s) are not known. However, a large double-blind phase III trial of a single oral dose of bovine myelin in RR multiple sclerosis did not show differences in the number of relapses between placebo and treated groups (Faria and Weiner, 2005). Treatment failure could have been due to the unexpectedly strong
### Table 2
Some immunomodulatory approaches of multiple sclerosis and their development from EAE or in vitro studies to clinical application

<table>
<thead>
<tr>
<th>Treatment approach</th>
<th>Based on clear hypothesis</th>
<th>Rationale confirmed</th>
<th>Efficacy in EAE</th>
<th>Efficacy in multiple sclerosis</th>
<th>Adverse event profile</th>
<th>Status of development</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glatiramer acetate</td>
<td>No</td>
<td>Yes</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>Approved</td>
<td>Johnson et al. (1995)</td>
</tr>
<tr>
<td>Oral myelin</td>
<td>No; i.d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bielekova et al. (2000), Kappos et al. (2000)</td>
</tr>
<tr>
<td>Anti-α4 integrin</td>
<td>Yes</td>
<td>Yes</td>
<td>++</td>
<td>–</td>
<td>+++</td>
<td>Not continued after phase III owing to lack of efficacy</td>
<td>Faria and Weiner (2005)</td>
</tr>
<tr>
<td>Anti-CD40L</td>
<td>Yes</td>
<td>Yes</td>
<td>+++</td>
<td>n.k.</td>
<td>±</td>
<td>Taken off the market</td>
<td>Miller et al. (2003)</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>Yes</td>
<td>No</td>
<td>++</td>
<td>±</td>
<td>–</td>
<td>Not continued after phase III owing to lack of efficacy</td>
<td>Dumont (2002)</td>
</tr>
<tr>
<td>Anti-CD52</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>Approved for phase II</td>
<td>van Oosten et al. (1997)</td>
</tr>
<tr>
<td>Anti-CD25</td>
<td>No</td>
<td>No</td>
<td>±</td>
<td>++; i.d.</td>
<td>++</td>
<td>Approved for phase II</td>
<td>Bielekova et al. (2004)</td>
</tr>
<tr>
<td>CTLA-4-Ig</td>
<td>Yes</td>
<td>Yes</td>
<td>++</td>
<td>n.k.</td>
<td>n.k.</td>
<td>In phase III</td>
<td>Kremer (2004)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>No</td>
<td>No</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Approved</td>
<td>Paty and Li (1993)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>No</td>
<td>No</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>Stopped in phase I</td>
<td>Panitch et al., (1987)</td>
</tr>
<tr>
<td>Anti-TNF antibodes</td>
<td>Yes</td>
<td>No</td>
<td>++(?)</td>
<td>–</td>
<td>–</td>
<td>Approved for other indication</td>
<td>van Oosten et al. (1996)</td>
</tr>
<tr>
<td>TNFR-lg fusion protein</td>
<td>Yes</td>
<td>No</td>
<td>++(?)</td>
<td>–</td>
<td>–</td>
<td>Approved for other indication</td>
<td>The Lenercept Multiple Sclerosis Study Group and The University of British Columbia multiple sclerosis/MRI Analysis Group (1999)</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>Yes</td>
<td>Yes</td>
<td>++</td>
<td>i.d.</td>
<td>–</td>
<td>Stopped in phase I</td>
<td>Calabresi et al. (1998)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Yes</td>
<td>No</td>
<td>±</td>
<td>i.d.</td>
<td>i.d.</td>
<td>Stopped in phase II</td>
<td>Wiendi et al. (2000)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Yes</td>
<td>Yes</td>
<td>+ (+)</td>
<td>–; i.d.</td>
<td>++</td>
<td>Not continued</td>
<td>Frank et al. (2002)</td>
</tr>
<tr>
<td>PDE4 inhibitors</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Not continued</td>
<td>R. Martin et al. (unpublished data)</td>
</tr>
<tr>
<td>PPARγ agonists</td>
<td>Yes</td>
<td>Yes</td>
<td>++</td>
<td>n.t.</td>
<td>n.a.</td>
<td>Not yet tested in multiple sclerosis</td>
<td>Diab et al. (2002), Feinstein et al. (2002)</td>
</tr>
<tr>
<td>Statins</td>
<td>Yes</td>
<td>Yes</td>
<td>+++</td>
<td>++; i.d.</td>
<td>+</td>
<td>Not yet tested in multiple sclerosis</td>
<td>Diab et al. (2002), Feinstein et al. (2002)</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>No</td>
<td>No</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>Approved</td>
<td>Harung et al. (2002)</td>
</tr>
<tr>
<td>Linomide</td>
<td>No</td>
<td>No</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>Phases III stopped due to cardiotoxicity</td>
<td>Noseworthy et al. (2000c)</td>
</tr>
<tr>
<td>Lasmimod</td>
<td>No</td>
<td>No</td>
<td>++</td>
<td>+</td>
<td>i.d.</td>
<td>In phase II</td>
<td>Polman et al. (2005)</td>
</tr>
<tr>
<td>Deoxypergualin</td>
<td>No</td>
<td>No</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>After phase II stopped owing to lack of efficacy</td>
<td>Wiendi and Hohlfeld (2002)</td>
</tr>
<tr>
<td>Sulphasalazine</td>
<td>Yes</td>
<td>No</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>In phase III</td>
<td>Noseworthy et al. (1998)</td>
</tr>
<tr>
<td>IVIG</td>
<td>No</td>
<td>No</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>In phase II</td>
<td>Home (2004), Sorensen et al. (2002)</td>
</tr>
<tr>
<td>Haematopoietic stem cell transplant</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
<td>++; i.d.</td>
<td>±; i.d.</td>
<td>In phase III</td>
<td>Mancardi et al. (2005), Tyndall and Saccardi (2005)</td>
</tr>
</tbody>
</table>

i.d., insufficient data; n.a., not applicable; n.k., not known; n.t., not tested; †The table depicts whether a therapeutic approach was developed for multiple sclerosis on the basis of a clear and pre-formed hypothesis, whether the rationale for its clinical/EAE testing had later been shown and whether the therapy was effective in EAE and/or multiple sclerosis. Both the clinical efficacy and the tolerability and safety are depicted by + or – signs. In the context of the adverse events, + indicates a favourable profile. The relative weighting reflects the subjective perception of the authors either from own experience or the published literature; †Reasonable safety profile, but one specific severe adverse event (PML). ‡Development of demyelinating episodes and diseases in RA- and Crohn’s patients; §Broad immunosuppressant; no specific target.

Effects in the placebo group, wrong dose or type of antigen, or route of administration.

### Adhesion molecules
Another promising strategy, using a blocking anti-α4 integrin humanized antibody (natalizumab), emerged from EAE evidence that α4β1 integrin is critical for T cell and monocyte homing to the CNS (Yednock et al., 1992). This mAb was highly effective in pre-clinical EAE studies and successfully completed phase II and III testing in large numbers of multiple sclerosis patients. Because of its remarkable efficacy in multiple sclerosis (Miller et al., 2003), natalizumab was approved by the Food and Drug Administration even before phase III trial data had been published, but was taken off the market four months later because of rare but very severe
adverse events. Three patients had developed progressive multifocal leukoencephalopathy (PML), an often lethal opportunistic infection of the CNS; two died, and one is recovering, though with considerable neurological deficits (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005; Van Assche et al., 2005). A large post facto analysis estimated the risk of PML for a 2-year treatment period to 1 in 1000 patients (Yousry et al., 2006). PML is caused by reactivation and mutation of the highly prevalent polyoma virus JC (JCV), which destroys oligodendrocytes. PML is almost exclusively observed in immunosuppressed individuals, and it is not clear what initiated its unexpected development under natalizumab treatment. JCV persists in kidneys and lymphoid organs, including bone marrow (Monaco et al., 1998). During immunosuppression, latent infection can be reactivated, and JCV disseminates to the CNS (Tornatore et al., 1992). That might have resulted either from compromised T-cell surveillance of the CNS or from mobilization of stem cells and JCV from the bone marrow (Papayannopoulou and Nakamoto, 1993; Ransohoff, 2005), where αβ integrin serves as a retaining signal (Simmons et al., 1992). Since JCV is not found in rodents, this adverse event could not have been anticipated from pre-clinical investigations. Therefore, this drug cannot be called a failure of prediction, especially as many thousand patients needed to be treated to unravel potential adverse effects. In addition, the recently published two-year phase III trials underline its compelling effects on relapse rate and clinical progression (Polman et al., 2006; Rudick et al., 2006). In March 2006, The Peripheral and CNS Drugs Advisory Committee, under The Food and Drug Administration, voted unanimously to recommend the return of natalizumab for the treatment of RR multiple sclerosis in a subset group of patients.

Co-stimulatory molecules

Despite its promise in EAE, anti-CD40 ligand (CD154) (Howard et al., 1999) was not developed because of its thromboembolic complications in man (Kawai et al., 2000), which result from its expression on human but not murine platelets. Anti-CD4 therapy was effective in EAE (Waldor et al., 1985), but not in human studies (van Oosten et al., 1997). Anti-CD52, which depletes both CD8+ and CD4+ T-cells (Coles et al., 1999b), was never evaluated in EAE, but is very effective against new lesions in multiple sclerosis, though ~30% of treated multiple sclerosis patients develop autoimmune hyperthyroidism (Coles et al., 1999a). On the other hand, IL-2 receptor blockade with the humanized anti-CD25 antibody (daclizumab) caused impressive reductions in MRI lesions and improvements in some clinical measures (Bielekova et al., 2004). In this case, the theoretical role of CD25 in promoting T regulatory cells, and equivocal EAE data (Engelhardt et al., 1989; Reddy et al., 2004), might have argued against its use in multiple sclerosis. Interestingly, there is little evidence that it perturbs T regulatory or T1f function; indeed it may act by expanding immunoregulatory NK cells (Bielekova et al., in review). CTLA-4-Ig interferes with co-stimulation from CD80/CD86 molecules on antigen-presenting cells (APCs) to the stimulatory or inhibitory ligands CD28 and CTLA-4 (Alegre et al., 2001). Data in EAE indicate that CTLA-4-Ig is much more effective as a preventive pre-treatment (Cross et al., 1995) than in therapy of ongoing disease (Cross et al., 1999). Treatment with CTLA-4-Ig is also effective in other autoimmune diseases such as rheumatoid arthritis (Kremer et al., 2003), and is currently being tested in a phase III trial in multiple sclerosis.

Cytokines

Cytokines have different effects at different stages of pathogenesis, for example, in the induction phase and the chronic/relapsing phase in EAE. These differences suggest a pleiotropic role in CNS inflammation and might explain some of the below-described discrepancies between EAE and multiple sclerosis.

Interferon-β (IFN-β), the first drug approved for multiple sclerosis, had not been previously tested in EAE. It exerts a wide variety of effects on the immune system: it inhibits both leukocyte proliferation and antigen presentation; it biases towards production of anti-inflammatory cytokines and it inhibits T-cell migration across the blood-brain barrier (Billiau et al., 2004). Although widely used in multiple sclerosis, its long-term effectiveness and side-effects are still uncertain (Filippini et al., 2003). With other cytokines, effects have seemed contradictory in EAE vis-à-vis multiple sclerosis. In the mid-1990s, it was found that IFN-γ knockout mice develop lethal EAE (Ferber et al., 1996), and IFN-γ administration in EAE showed a protective effect on disease severity (Kراكowski and Owens, 1996). By then, its use in multiple sclerosis patients had already led to a modest increase in disease exacerbations (Panitch et al., 1987). Although this study is limited, it is unlikely that IFN-γ will ever be tested again in multiple sclerosis.

In contrast, tumour necrosis factor-α (TNF-α) has long been considered a key mediator of multiple sclerosis pathogenesis (Sharief and Hentges, 1991), and its blockade by antibodies or soluble TNF receptors prevents or reverses disease in EAE models (Ruddle et al., 1990; Selmaj et al., 1991, 1995). Paradoxically, this approach worsens disease in multiple sclerosis patients and had to be discontinued (The Lenercept Multiple Sclerosis Study Group and The University of British Columbia Multiple Sclerosis/MRI Analysis Group, 1999; van Oosten et al., 1996). Indeed, a substantial number of cases developed their first demyelinating event while being treated with anti-TNF-α agents for other diseases such as rheumatoid arthritis or Crohn’s disease (Hyrich et al., 2004). Despite the data that TNF-α is an important component in the pathogenesis in EAE, a precise role for TNF-α in multiple sclerosis remains unclear. However, subsequent EAE experiments using TNF-α gene deleted mice (TNF-α−/−) surprisingly showed that TNF-α−/− mice displayed profound neurological impairment and high mortality with extensive
demyelination and monocytic cell infiltration in comparison with control mice (Liu et al., 1998). Conversely, treatment of the TNF-α−/− mice but also wild-type mice with recombinant TNF-α reduced disease severity in afflicted mice and prevented development of EAE in pre-treated mice. These studies suggested that TNF-α may be protective in the CNS during the development of demyelinating disease also in mice and may serve to limit the extent of immune-mediated inflammation, as was implicated by the study in multiple sclerosis patients. Further, we can only speculate on the reasons for the adverse outcome of TNF-blocking approaches in multiple sclerosis, but it has been demonstrated that TNF signalling is also important for remyelination (Arnett et al., 2001; Diemel et al., 2004).

Transforming growth factor (TGF)-β2 ameliorates EAE and is a very potent immunosuppressive cytokine. However, it has never been approved as a treatment in multiple sclerosis, as it is associated with nephrotoxicity in multiple sclerosis patients, which was not checked in EAE (Calabresi et al., 1998). Interleukin (IL)-10 is another important suppressive cytokine, produced mainly by regulatory CD4+ T cells (O’Garra et al., 2004). Its selective upregulation in the CNS during the recovery phase of EAE prompted the evaluation of IL-10 treatment in a Lewis rat EAE model. Systemic administration during the initiation phase suppressed EAE disease (Rott et al., 1994). Again IL-10 treatment in a phase II clinical trial in multiple sclerosis patients had to be stopped, owing to lack of efficacy (Wiendl et al., 2000).

**Neurotrophic factors**

Apart from cytokines, several neurotrophic factors have been proposed as a novel therapeutic in multiple sclerosis. These proteins regulate survival and differentiation of neurons by binding to specific neurotrophin receptors (Thoenen and Sendtner, 2002). They are involved in skewing the cytokine balance in the CNS from Th1 to Th2 responses (Villoslada et al., 2000), and neuroprotective effects have been proposed (Hohfeld et al., 2005). The clinical potential of one such factor, insulin-like growth factor-1 (IGF-1), was first studied in a rat EAE model, in which myelin regeneration and clinical recovery was enhanced (Yao et al., 1995). In contrast, there was no clear effect in one phase-I/II study in seven patients (Frank et al., 2002).

**Anti-inflammatory substances, immunosuppression and immunomodulation**

Cytokine secretion and proliferation of T cells are regulated by intracellular cyclic AMP (cAMP) levels, which are reduced by phosphodiesterases (PDEs). The type 4 PDE (PDE4) is a cAMP-specific phosphodiesterase expressed in cells of the immune system and the CNS (Engels et al., 1994). Inhibition of PDE4 activity by rolipram ameliorates EAE severity (Sommer et al., 1995). It was originally developed and evaluated in clinical studies as an anti-depressant (Zeller et al., 1984). Since rolipram and other PDE4 inhibitors (Dinter et al., 2000) demonstrated efficacy in various EAE models, it seemed a promising candidate for clinical use in multiple sclerosis patients. However, a phase II clinical trial was halted owing to lack of efficacy on the primary outcome measure, that is, the reduction of gadolinium-enhancing inflammatory CNS lesions by MRI (Martin, R., Bielekova, B., Sturzebecher, C.S., Richert, N., Frank, J.A., Ohayon, J., McCartin, J., McFarland, H.F., unpublished data).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcriptional factors that include receptors for steroids, thyroid hormone, vitamin D and retinoic acid (Mangelsdorf et al., 1995). PPAR-γ is expressed in adipose tissue, on macrophages, T cells, and endothelial and vascular smooth muscle cells. The natural 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) and the synthetic anti-diabetic thiazolidinedione are PPAR-γ ligands; their administration before and at the onset of clinical signs of EAE significantly reduced its severity (Diab et al., 2002). Another orally administered PPAR-γ agonist pioglitazone reduced the incidence and severity in C57BL/6 EAE and B10.Pl murine models. Pioglitazone also reduced clinical signs when given after disease onset (Feinstein et al., 2002). So far there is only casuistic information about PPAR-γ agonists in multiple sclerosis (Pershad Singh et al., 2004), but clinical trials are being planned.

Statins block the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase; they are widely used to lower cholesterol levels and prevent cardiovascular disease. They are highly effective in reversing EAE, shifting the pro-inflammatory Th1-type cytokine profile to a Th2-type pattern and also interfering with antigen presentation (Yossef et al., 2002). In this case, exploratory trials in multiple sclerosis patients have shown promising results with up to 44% reduction in the number of contrast-enhancing MRI lesions (Vollmer et al., 2004), so they are a solitary success.

Mitoxantrone is a potent anti-inflammatory cytostatic anthrancenedione, and suppresses both B and T lymphocytes and macrophages, resulting in down-modulation of the inflammatory cascade. Since it had already been approved for the therapy of malignancies, this immunosuppressant could logically have been assessed in multiple sclerosis without studying EAE. Not surprisingly, mitoxantrone has potent effects in EAE. It suppressed paralysis in acute EAE, prevented its development when administered during the induction period and still showed some effects when given after clinical signs and symptoms appeared (Ridge et al., 1985; Levine and Saltzman, 1986). These results paralleled recent proof of efficacy in multiple sclerosis (Hartung et al., 2002). However, cardiotoxicity was a reported adverse effect in humans; though not observed in the early EAE studies (Ridge et al., 1985; Levine and Saltzman, 1986), it was also detected in careful retrospective analysis in treated mice. Meanwhile, the more serious problem of inducing hematopoietic malignancies is predictable for a cytotoxic agent, and has already
been reported in multiple sclerosis patients, though not in rodents (Cohen and Mikol, 2004). An estimate of 0.07% has been reported on the basis of a review of over 1 300 patients (Ghali et al., 2002).

Linomide and laquinimod are immunoregulatory components with yet-unidentified mechanisms of action. Both are quinoline carboxamides with structural homology to tryptophan metabolites (Platten et al., 2005). Linomide (Karussis et al., 1993) and laquinimod (Brunmark et al., 2002) effectively inhibit and even reverse EAE, and show some efficacy in patients with multiple sclerosis (Noseworthy et al., 2000c; Polman et al., 2005). However, a phase II clinical trial of linomide in patients with multiple sclerosis was halted because of serious concerns that some quinoline carboxamides are cardiotoxic (Noseworthy et al., 2000c). A recently described additional orally active derivative of a tryptophan metabolite, N-(3,4-dimethoxycinnamoyl) anthranilic acid (3,4-DAA), reversed paralysis in mice with EAE (Platten et al., 2005). However, as it is proposed that these metabolites act similarly to quinoline carboxamides and, as tryptophan metabolism is an ubiquitous process, concerns remain about their use in multiple sclerosis.

FTY720 is a structural analogue of myricin, a metabolite of the ascomycete fungus *Isaria sinclairia*, with some structural resemblance to sphingosine, an endogenous lysolipid. Sphingosine phosphorylation by sphingosine kinase generates sphingosine-1-phosphate (S1P), the cognate ligand for the family of S1P receptors (S1PR) (Rosen and Goetzl, 2005). Their activation results in many different physiological actions such as chemotaxis, cellular differentiation, survival and growth, cell adherence and cell shape changes (Rosen and Goetzl, 2005). At least one action of S1P is to sequester circulating lymphocytes in peripheral lymph nodes, leading to a sustained lymphopenia (Schwab et al., 2005; Wei et al., 2005). FTY720 was able to suppress EAE development in different models (Brinkmann et al., 2002; Fujino et al., 2003; Webb et al., 2004). However, its success in EAE was not surprising as FTY720 is a broadly active immunosuppressant (Gonsette, 2004). It is now being tested in a 6-month phase I/II clinical trial in multiple sclerosis. As it suppresses the egress of lymphocytes from lymph nodes, one has to monitor these patients for signs of infection. In addition, S1PR is highly expressed in myocytes of the myocardium and regulates the heart rate (Sanna et al., 2004), so bradycardia is one adverse effect in treated patients (Budde et al., 2003), and has also been observed in mice (Sanna et al., 2004). For unknown reasons, FTY720 may also cause macular oedema.

Another immunosuppressive drug, deoxyspergualine (DSG), is an analogue of the bacterial product spergualin from the soil commensal *Bacillus lactosporus* (Maeda et al., 1993). The molecular mechanism underlying its pharmacological effects remains elusive; it is still not clear whether its inhibitory effect on pre-B- and pre-T-cell differentiation is due to the interference with NF-κB family members (Wang et al., 1996). DSG was tested in EAE models and has shown potent immunosuppressive actions, delaying onset and reducing severity of clinical symptoms (Schorlemmer and Seiler, 1991). However, its pronounced effect on pre-B- and pre-T-cell development (Wang et al., 1996) appears to cause serious problems with peripheral lymphodepletion. Again, a phase II clinical trial in multiple sclerosis was started but had to be stopped owing to lack of efficacy (Wiendl and Hohlfeld, 2002).

Sulphasalazine has been used for decades in the treatment of rheumatoid arthritis and inflammatory bowel disease, but its anti-inflammatory actions are still not clearly understood. Reports on its effects in EAE have been conflicting, ranging from beneficial (Prosiel et al., 1989, 1990) to neutral (Uitdenhaag et al., 1991) or deleterious (Correale et al., 1991). A randomized, double-blind, placebo-controlled phase III trial showed no significant improvement in multiple sclerosis progression (Noseworthy et al., 1998), while another phase III trial is currently under way.

The purified immunoglobulins administered intravenously as IVIG may act via Fcγ receptors and so deliver inhibitory signals to various immune cells (Nimmerjahn and Ravetch, 2006). Clearly effective in Guillain–Barre syndrome when given early, IVIG also had significant positive effects on both disease course and CNS inflammation in EAE, but only when administered at the time of immunization (Pashov et al., 1998; Achiorn et al., 2000). Positive effects have also been well described in RR multiple sclerosis (Sorensen et al., 2002). However, it did not reverse longstanding motor deficits in patients with established weakness (Noseworthy et al., 2000b) and had no significant effect in SP multiple sclerosis patients (Hommes et al., 2004).

**Transplantation and reconstitution**

Immune ablation plus autologous haematopoietic stem cell transplantation (auto-HSCT) have been proposed as an experimental therapy for patients with severe multiple sclerosis (Muraro et al., 2003; Fassas and Kimiskidis, 2004). As in other autoimmune diseases, the goal is to eliminate the pathogenic lymphocyte repertoire (Sykes and Nikolic, 2005) and ‘re-boot’ the immune system to restore immune tolerance, halt ongoing inflammatory activity and prevent further relapses. In practice, the chances of reconstituting thymopoiesis are higher before than after the age of 45 years old.

Susceptible mice can also be protected from autoimmune diseases by transfer of HSCT from resistant allogeneic strains; such allo-HSCT prevented the development of EAE (Sykes and Nikolic, 2005). The extent of remission of ongoing EAE was greater with allo- than with auto-HSCT (van Gelder and van Bekkum, 1996). At early stages, auto-HSCT produced nearly complete reversal of disease, but no effect was observed at chronic stages (Guillaume et al., 1998; Van Bekkum, 2003; Herrmann et al., 2005).

In multiple sclerosis, multiple clinical trials of high-dose immunosuppression/chemotherapy plus auto-HSCT have been started (Saccardi et al., 2005). Initial results appear
more promising than that would have been predicted from the findings in EAE (Mancardi et al., 2005; Tyndall and Saccardi, 2005). While the death rate was disturbingly high in one trial (5 out of 85 treated patients) (Tyndall and Saccardi, 2005), it can apparently be reduced by paying careful attention to the patient's disease stage and disability as well as the conditioning regime (Saccardi et al., 2004) and CD34+ cell preparation (R. Saccardi et al., manuscript submitted).

As shown in Table 2, there have been significant problems in multiple sclerosis with virtually all the agents that appeared beneficial in EAE. Even when efficacy has been replicated in the human disease, additional unexpected adverse effects appeared. These have precluded successful clinical application in nearly every case, as often happens in the development of any drug. The recent fatal adverse events in clinical trials of natalizumab (Kleinschmidt-DeMasters and Tyler, 2005; Van Assche et al., 2005) seem particularly tragic in a disease of young adults that is not life-threatening and runs a very long course. Adequate prediction of toxicity remains a fundamental issue for all forms of drug development, and current rodent EAE models cannot be expected to eliminate these problems.

Worse still, however, many of the EAE results have been actively misleading. Therefore, it is difficult to claim any predictive value from positive pre-clinical findings in 'conventional' EAE models. Although outbred humans and inbred mice may only differ by ~300 genes (Waterston et al., 2002), their immune systems must have evolved differently because of their different ecology and lifestyles, their different pathogenic challenges and their different longevity (Mestas and Hughes, 2004). The many similarities between the two immune systems have lulled us into overlooking these differences and into assuming identical functions for certain molecules in each species (Table 1). As one relevant example, steroid-sensitivity is ~100 times greater in mice than in humans, where it clearly also varies greatly between subjects (Glaman, 1972).

Rethinking pathophysiological concepts of EAE and multiple sclerosis

One crucial question is how well EAE models replicate the induction and pathogenic mechanisms in multiple sclerosis. Obviously, these would need to be accurately understood before optimal models could be developed. Furthermore, since EAE almost always has to be induced, it is of very limited value as a model for a spontaneous human demyelinating disease.

It now transpires that the currently favoured therapeutic concepts in multiple sclerosis—which nearly all depend on targeting CD4+ T_{H1} cells—are based on debatable foundations that demand to be re-examined. While it has been accepted for many years that EAE is largely a CD4+ T_{H1}-mediated disease (Zamvil and Steinman, 1990), it probably reflects the use of CFA in nearly all models. It is now also challenged by the observation that other T-cell populations including T_{H2} CD4+ T cells (Lafaille et al., 1997) and CD8+ myelin-specific T cells (Huseby et al., 2001) can also mediate EAE, depending on the model used. In addition, a new CD4+IL-17+ subset has been shown to mediate EAE after adoptive transfer (Langrish et al., 2005). This cell subpopulation is distinct from T_{H1} and T_{H2} populations, and its differentiation might be driven by APCs expressing IL-23 (Harrington et al., 2005; Langrish et al., 2005; Park et al., 2005), while others did not find a role for IL-23 in differentiation but report a combination of TGF-β and IL-6, amplified by IL-1β and TNF-α to be instrumental (Veldhoen et al., 2006); after selective depletion of CD4+IL-17+ T cells, the remaining cells no longer induce EAE after transfer (Langrish et al., 2005). Thus, its apparently exclusive role in EAE pathogenesis may help explain some contradictory results, if it also proves to predominate in a wider range of EAE models.

While these findings challenge the current dogma that EAE is mediated solely by T_{H1}-cells, those are still implicated by many data from multiple sclerosis patients—for example, by their increased numbers and higher antigen avidity (Sospedra and Martin, 2005). However, other subsets such as CD8+ T cells outnumber CD4+ T cells 3–10-fold in multiple sclerosis plaques (Hauser et al., 1986; Babbe et al., 2000), and oligoclonal CD8+ T-cell populations are present in multiple sclerosis brain, blood (Babbe et al., 2000) and CSF (Jacobson et al., 2002); moreover, IL-17 is found in multiple sclerosis plaques (Lock et al., 2002). While there is already much evidence to incriminate CD8+ T cells in multiple sclerosis (Friese and Fugger, 2005), it still needs more support. Indeed, it remains possible that different subsets play key roles at different stages, and that some of these may differ between multiple sclerosis and many EAE models. Thus, we suspect that CD4+ T_{H1} or CD4+IL-17+ cells are indeed crucial for initiation, whereas CD8+ T cells may be more important in the effector phase. Although certain findings show clear similarities in multiple sclerosis and EAE, translating the above knowledge on the involvement of novel T-cell populations to multiple sclerosis will pose new challenges due to species-specific differences between mouse and man—which clearly demands further studies.

New concepts for development of EAE models

To develop improved animal models that reflect the pathogenesis of multiple sclerosis better, one has to remember that multiple sclerosis is highly heterogeneous in its genetic basis, environmental effects, clinical course, pathological mechanisms (Lassmann et al., 2001) and treatment responsiveness. This heterogeneity needs to be comprehended in any ideal animal model (Box 1). Currently, they almost invariably use inbred strains, although the molecular interactions that
Box 1. Future considerations for improving animal models for multiple sclerosis

- Validate therapeutic effect in more than one model
- Consider dissimilarities in immune systems of rodents versus humans
- Consider treatment after disease onset
- Record disease course for as long as possible (feasible)
- Establishment of spontaneous disease models
- Establishment of a two-stage disease course in EAE (first relapsing-remitting, later chronic-progressive)
- Incorporate human disease risk factors
- Incorporate human immune system and modifying human CNS factors

determine the efficacy, metabolism and safety of drugs during prolonged follow-up of thousands of patients are orders of magnitude more complex. Thus, it is likely that genetic heterogeneity, at least in part, accounts for many inter-patient differences in clinical benefits and side-effects of various drugs.

Potential therapeutics need to be tested in models that, together, mimic both this heterogeneity and the main pathological mechanisms including inflammation, demyelination, axonal damage and glial scarring. That is seldom attempted in current models; at best, one inbred strain might correspond to one multiple sclerosis patient, perchance with a comparable genetic background.

Recently developed partially humanized mouse models are a significant first step towards addressing this genetic variability and disease complexity (Gregersen et al., 2004). Partially humanized mice, transgenic for human MHC-class II and a TCR from a multiple sclerosis patient’s myelin-specific TH clone, are beginning to reproduce some of the various clinical manifestations and corresponding CNS lesions of multiple sclerosis. For example, disease appears spontaneously in ~4% of mice transgenic for both HLA-DR2 (DRB1*1501) and the TCR from multiple sclerosis patient Ob, which is specific for HLA-DR2 (DRB1*1501)-bound immunodominant MBP 84-102 peptide (Madsen et al., 1999), so do mice transgenic for the other DR2 allele, DRB5*0101, and the multiple sclerosis patient-derived MBP 83-99-specific TCR 3A6 (Quandt, J., Yao, K., Huh, J., Baig, M., Kawamura, K., Bryant, M., McFarland, H., Martin, R., Ito, K., unpublished results). After backcrossing onto a Rag2−/− background (for recombination-activating gene 2), the incidence of spontaneous disease was much higher (Madsen et al., 1999), hinting at differences in regulation. Moreover, on both backgrounds, it affected the brain relatively more than the spinal cord, unlike in conventional EAE. A different clinicopathological picture is seen in another model instead using HLA-DRB1*0401 and the TCR from T-cell clone MS2-3C8 isolated from a multiple sclerosis patient reactive to MBP 111–129 (Quandt et al., 2004). These mice show dysphagia with restricted jaw and tongue movements and abnormal gait, and correspondingly more specific T-cell infiltrates and inflammatory lesions in the brainstem and cranial nerve roots in addition to the spinal cord and spinal nerve roots (Quandt et al., 2004). Thus, models that incorporate multiple susceptibility factors may reproduce the clinical heterogeneity of multiple sclerosis better, and perhaps improve identification of promising therapeutic approaches. Since they are still far from giving a complete picture, they need to be extended.

Stem cell technology may allow more complete humanization; for example, using human haematopoietic stem cells to reconstitute the immune system in mice (Gimeno et al., 2004; Traggiai et al., 2004; Shultz et al., 2005) may advance modelling of multiple sclerosis and improve pre-clinical development. Eventually, after due ethical reflection, it might become possible to develop murine models in which both the immune system and at least some components of the CNS (e.g. oligodendrocytes, endothelium) share human origins. It might be wise first to assemble ‘generically humanized’ strains with a human haematopoietic system, and then to incorporate multiple sclerosis-specific susceptibility alleles and TCRs.

By reducing species-imposed restrictions in identifying drug targets, such mice might enable us to test larger numbers of drugs than in the existing models. They should also be susceptible to many more human pathogens, and may therefore help us in investigating their involvement in triggering disease in susceptible individuals, in shaping its course and/or in causing severe adverse events, if kept in a non-specific pathogen-free environment.

Conclusions

In multiple sclerosis, at least two main components appear important for the disease process: immunological abnormalities, and susceptibility of the target tissue. Both components can interact with a variety of different pathogenic causes, depending on the environment and genetic background, resulting in the substantial heterogeneity that we observe at every level of the disease. At present, it remains unclear exactly why pre-clinical EAE studies predict treatment efficacy so poorly in multiple sclerosis. ‘Conventional EAE’ will continue to play an important role as a first-line model system in the development of novel treatment approaches, especially for addressing very specific mechanistic questions, as long as the rodent–human similarities or dissimilarities are borne in mind. To approach the complexity of multiple sclerosis patients, current progress in humanizing the entire immune system in rodents may offer substantial advantages for exploring novel immunomodulatory approaches in better suited models, especially after incorporation of multiple sclerosis-specific susceptibility alleles. While such models should substantially help in identifying promising therapies, we will probably always need carefully designed early clinical trials that include imaging outcome measures and mechanistic studies to assess their actions in patients.
The value of animal models for drug development in multiple sclerosis

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