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REVIEW

# Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS)

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Experimental autoimmune encephalomyelitis (EAE) is the most commonly used experimental model for the human inflammatory demyelinating disease, multiple sclerosis (MS). EAE is a complex condition in which the interaction between a variety of immunopathological and neuropathological mechanisms leads to an approximation of the key pathological features of MS: inflammation, demyelination, axonal loss and gliosis. The counter-regulatory mechanisms of resolution of inflammation and remyelination also occur in EAE, which, therefore can also serve as a model for these processes. Moreover, EAE is often used as a model of cell-mediated organ-specific autoimmune conditions in general. EAE has a complex neuropharmacology, and many of the drugs that are in current or imminent use in MS have been developed, tested or validated on the basis of EAE studies. There is great heterogeneity in the susceptibility to the induction, the method of induction and the response to various immunological or neuropharmacological interventions, many of which are reviewed here. This makes EAE a very versatile system to use in translational neuro- and immunopharmacology, but the model needs to be tailored to the scientific question being asked. While creating difficulties and underscoring the inherent weaknesses of this model of MS in straightforward translation from EAE to the human disease, this variability also creates an opportunity to explore multiple facets of the immune and neural mechanisms of immune-mediated neuroinflammation and demyelination as well as intrinsic protective mechanisms. This allows the eventual development and preclinical testing of a wide range of potential therapeutic interventions.

## LINKED ARTICLES

This article is part of a themed issue on Translational Neuropharmacology. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.164.issue-4

## Abbreviations

ADEM, acute disseminated encephalomyelitis; ADNP, activity dependent neuroprotective protein; AHR, aryl hydrocarbon receptor; APC, antigen-presenting cells; APL, altered peptide ligand; AT, adoptive transfer; C1 and CB2 receptors, cannabinoid receptors 1 and 2; CIS, clinically isolated syndrome; CNS, central nervous system; DA, dark agouti; DMT, disease-modifying treatment; EAE, experimental autoimmune (allergic) encephalomyelitis; EAN, experimental autoimmune (allergic) neuritis; EBV, Epstein–Barr virus; GA, glatiramer acetate; IFN, interferon; IL, interleukin; IL-1RA, interleukin 1 receptor antagonist; JCV, John Cunningham virus; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NABT, normal appearing brain tissue; NAWM, normal appearing white matter; NMO, neuromyelitis optica; NK1 receptor, neurokinin 1 receptor; PGE, prostaglandin E; PLP, proteolipid protein; PP, primary progressive; PR, progressive relapsing; ROR, retinoid orphan receptor; RR, relapsing-remitting; SP, secondary progressive; TCR, T-cell receptor; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumour necrosis factor; Treg, regulatory T cell; VLA, very late antigen

## Introduction

### *Multiple sclerosis*

Multiple sclerosis (MS) is the prototypical inflammatory demyelinating disease of the central nervous system (CNS). It is estimated to affect up to two million people worldwide and some 100 000 people in the United Kingdom (Compston and Coles, 2008). Its clinical manifestations begin typically in the third and fourth decade of life, and it affects women preferentially, with a female : male ratio approaching 3:1. Thus, MS represents a prime cause of neurological disability in young adults and has wide health, psychological, economical and social consequences.

Clinically, MS manifests itself as neurological deficits that frequently exhibit a relapsing and remitting pattern and can resolve completely or leave residual deficits. The deficits can involve any part of the CNS alone or in combination. Somatosensory, pyramidal-motor and visual manifestations, the latter due either to inflammatory demyelination in the afferent visual pathways (optic neuritis) or in the efferent visual pathways (ocular motility disorders such as internuclear ophthalmoplegia) are among the most common manifestations. Eventually, many people with relapse-onset MS have fewer clinically recognizable relapses and develop a gradual neurological progression.

In terms of the clinical course, there are several MS subtypes: relapsing-remitting MS (RRMS), with relapses (flare-ups) of disease separated by periods without clinical progression; secondary progressive, SPMS, which represents the phase of the disease where a gradual neurological deterioration (progression) follows a period of RR disease; primary progressive, PPMS, affecting approximately 15% of people with MS where the neurological deterioration is present from the onset, most frequently without superimposed relapses. The rare variant where a few acute exacerbations are superimposed on the gradual PPMS-like course is called progressive-relapsing MS (PRMS) (Lublin and Reingold, 1996).

Individuals who have experienced a single typical episode of inflammatory demyelination suggestive of being the first attack of MS but have not had a second event are said to have clinically isolated syndrome (CIS).

There are four key pathological features of MS: (a) inflammation, of complex pathogenesis, which is generally believed to be the main trigger of the events leading to CNS tissue damage in the majority of cases, although recent evidence suggests that initial damage to neuroglial elements can trigger secondary inflammation in some cases (Barnett and Prineas, 2004); (b) demyelination, the hallmark of MS, where the myelin sheath or the oligodendrocyte cell body is destroyed by the inflammatory process; (c) axonal loss or damage; and (d) gliosis (astrocytic reaction to CNS damage). There is a certain degree of remyelination, which offers hope for therapies aimed at enhancing endogenous repair mechanisms in various experimental models (see below) but is partial and its efficiency is limited.

In addition to the clinical heterogeneity there is pathological heterogeneity, in terms of the relative proportion of the above key pathological features and the components of cellular and humoral immune response elements that mediate the inflammation.

The pathological correlate of relapses is inflammation and disruption of the blood–brain barrier (BBB), clinical relapses being thought to correspond to fresh waves of inflammatory cell infiltration in the CNS. The pathological correlate of long-term disability and progression is irreversible axonal loss. The acute MS lesion is characterized by inflammatory infiltrates with various immune cells and active demyelination (macrophages with myelin debris in their cytoplasm); when this lesion becomes chronic, there is significant loss of myelin with few if any inflammatory infiltrates and gliosis, which gives lesions their 'plaque' appearance (Charcot, 1868).

Axonal loss is most severe in the chronic plaques, but it is also present in what is known as the normal-appearing white matter (NAWM), or normal-appearing brain tissue (NABT), to take into account pathological changes in the normal appearing gray matter as well (Trapp *et al.*, 1998; Peterson *et al.*, 2001).

### *Diagnosis*

As its name implies, the diagnosis of relapse-onset MS (also known as disseminated sclerosis) requires evidence of dissemination in time and space of the inflammatory lesions. Clinically, this has traditionally meant two or more demyelinating attacks and clinical evidence of two or more parts of the CNS being involved (Poser *et al.*, 1983). The advent of magnetic resonance imaging (MRI), however, has greatly facilitated the diagnosis (and as a consequence the early treatment) of MS. The introduction of the International Panel (MacDonald) diagnostic criteria, which are strongly based on MRI, allows early diagnosis by substituting the appearance of new lesions for the requirement for a second demyelinating event (McDonald *et al.*, 2001; Polman *et al.*, 2005).

The diagnostic hallmark of MS is the presence of hyperintense lesions on T2-weighted images; the typical location is periventricular but posterior fossa, juxtacortical and spinal lesions often coexist. The T2 lesions lack pathological specificity but are very useful for diagnosis. Acute lesions show enhancement after administration of gadolinium, a paramagnetic agent, on T1-weighted images. The pathological substrate of Gadolinium enhancing T1 lesions is inflammatory infiltration with recent breakdown of the BBB (Filippi *et al.*, 2002). Extensive evidence also shows that the brain and spinal cord undergo atrophy in MS, the pathological substrate for which is loss of axons (and myelin) (Lin *et al.*, 2004; Edwards *et al.*, 2007).

The above conventional MRI measures are widely used in clinical trials in MS as reliable outcome measures. In addition, a number of quantitative MRI methods have contributed to further understanding of the pathogenesis of MS. As in pathology, there is ample evidence that the NABT is also abnormal by sensitive MRI metrics. This partially explains the lack of tight correlation between clinical and MRI activity in MS.

MS is undoubtedly an immune-mediated disease with many features consistent with an autoimmune pathogenesis. In addition to its many similarities to experimental autoimmune encephalomyelitis (EAE) (discussed below), its response to immunosuppressive and immunomodulatory treatments (some of which are discussed below) and its association with other autoimmune diseases (Edwards and Constantinescu, 2004; Constantinescu and Gran, 2010), strong evidence in support of its immune mediation comes from genetics. While

its association with major histocompatibility complex (MHC) genes has been well known for a long time, recent advances in genome-wide association study methodology has allowed identification of approximately another 16 genes, virtually all of which are immune response genes (International Multiple Sclerosis Genetics Consortium, 2008). Not surprisingly, an enormous amount of work has been invested so far in finding pathogenic immune pathways and immune modulation strategies, both in EAE and in MS, relative to the amount of work aimed directly at neuroprotection, repair or remyelination.

As a result, some immune response–modifying therapies have entered clinical practice (many undergoing successful translation from EAE studies) and have thus revolutionized MS treatment, care and quality of life in the last two decades. Although they are practically entirely aimed at the relapsing stages of the disease where inflammation is a predominant pathogenic mechanism, they have made a major impact (Lim and Constantinescu, 2010b). In the progressive stages of disease, axonal/ neuronal loss partially dissociated from inflammation is more prominent, although low-grade inflammation persists. Immunomodulatory/ immunosuppressive drugs may have a marginal effect against such low-grade inflammation, but overall they have not shown success in reducing progression. Neuroprotective and reparative strategies need to be found for this stages (as well as for PPMS), and, as discussed below, a few studies are promising in EAE, but so far none of these has been translated into MS treatment.

The disease-modifying treatments (DMTs) for MS have been in large part based on the concepts of MS immunopathogenesis. These concepts and consequently the therapeutic targets have evolved with time, and we will soon witness the emergence of a third generation of MS DMT.

A detailed discussion of these established and emerging drugs is beyond the scope of this review. The readers are directed to recent reviews (Lim and Constantinescu, 2010b; Rejdak *et al.*, 2010; Yiu and Banwell, 2010).

The first line of treatment was represented by type 1 interferons (IFN) and glatiramer acetate (GA). Although initial studies showed success both with IFN-alpha and IFN-eta, the established DMT currently is IFN-beta (in several preparations, including IFNβ1a, Rebif and Avonex; and IFNβ1b, Betaferon, Betaseron, Extavia). Type I IFNs are natural antiviral molecules produced with immunoregulatory properties. GA (Copaxone), which was discovered due to studies in EAE (Teitelbaum *et al.*, 1971), is a copolymer of four amino acids present in myelin basic protein, namely glutamic acid, lysine, alanine and tyrosine. All first-generation DMT, while varying in route and frequency of administration and side effect profile, roughly reduce the relapse rate by 30% (or more if given in CIS). They have marginal or no effects in SPMS, PPMS or PRMS.

The currently approved DMT of a second generation is natalizumab (Polman *et al.*, 2006). This is a monoclonal antibody against VLA-4 integrin, which was shown in preclinical studies in EAE to be required for T-cell entry into the CNS (Yednock *et al.*, 1992). Due to success in phase II and III clinical trials, natalizumab was approved and is currently the most potent licenced drug for MS, reducing relapse rate by 70% and new MRI disease activity by 90% (Kappos *et al.*, 2007). Natalizumab has been associated with a severe complication, which prompted its transient removal from the market: progressive multifocal leukoencephalitis (PML), an opportunistic CNS infection with high mortality and morbidity, caused by the JC virus, a human specific polyoma virus (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould *et al.*, 2005).

Mitoxantrone is another drug licenced for MS. It is an anthracene dione used as a cancer chemotherapeutic agent and is also very effective in more aggressive MS, reducing relapses and showing a potential effect against progressive disease. Mitoxantrone has cumulative cardiotoxicity reducing its long-term use and is associated with a risk of promyelocytic leukaemia.

Azathioprine, a less potent immunosuppressive agent, has the advantage of oral administration and is effective in reducing relapse frequency and possibly disease progression (Casetta *et al.*, 2007). It also reduces the number of new brain inflammatory lesions (Massacesi *et al.*, 2005). It is well tolerated and is considered appropriate maintenance treatment for patients with frequent relapses requiring steroids (Casetta *et al.*, 2007).

Drugs that have successfully completed or in phase III studies and are promising DMT in the not so distant future, as well as some drugs successful in phase II studies and undergoing phase III studies are listed in Table 1.

Slightly further at the horizon are the future cellular therapies. The only such treatment that has entered clinical practice, albeit not in large controlled studies, is haematopoietic stem cell transplantation, thought to represent a drastic form of immunosuppression, which may reset an autoimmune-prone immune system, and has at least theoretical potential for neurorepair (Muraro and Uccelli, 2010). More than 400 patients with MS, in large part SPMS, have received this treatment within or outside of trials. This approach and its relationship with knowledge derived from EAE are discussed later in this review.

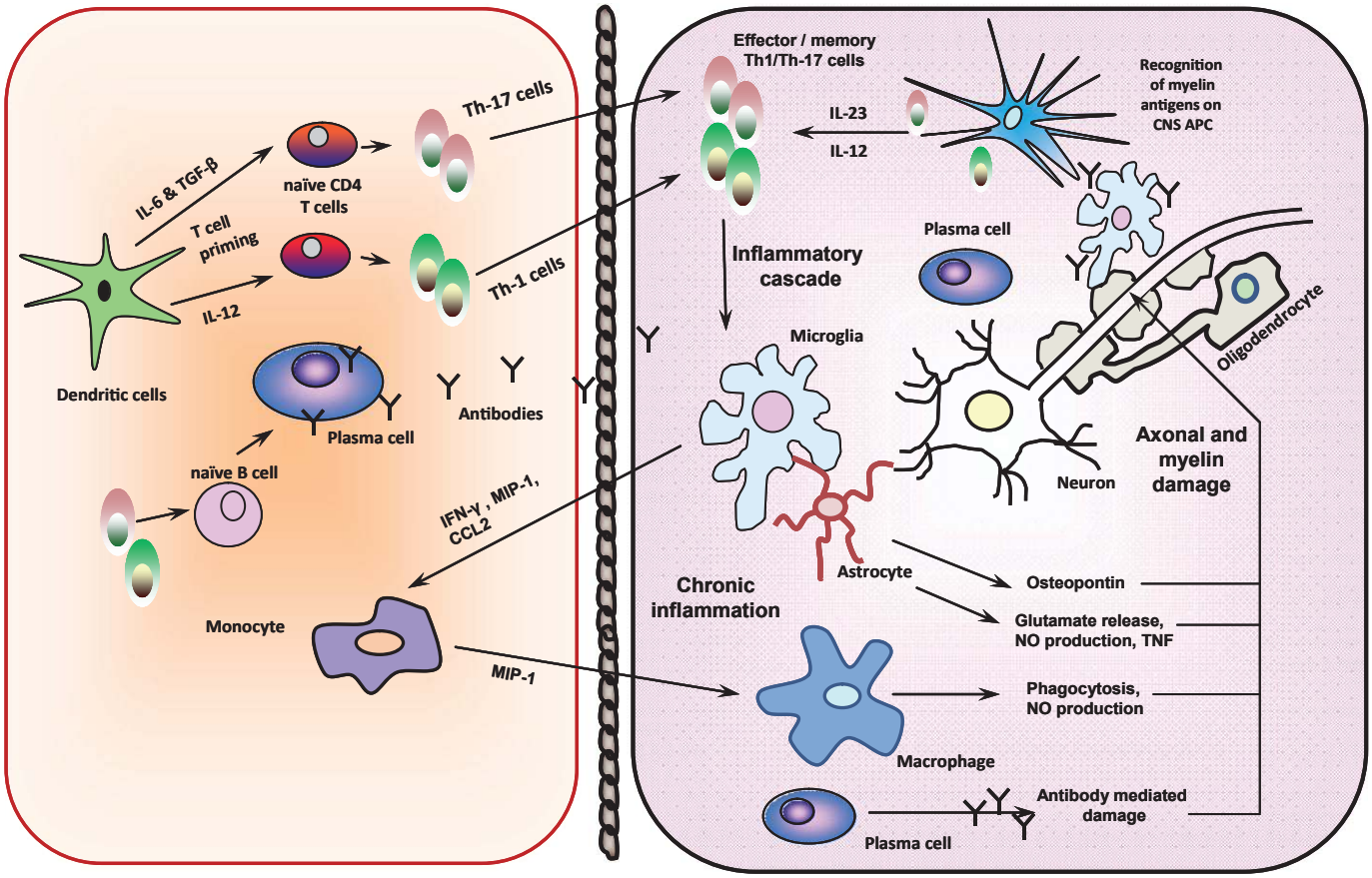
The interaction between multiple components of the immune system and all elements of the CNS determine the pathogenesis of MS. The most widely accepted current concepts are schematically represented in Figure 1.

Briefly, T cells in the periphery become activated by a viral or another infectious antigen or a superantigen. These show molecular similarity (mimicry) with some CNS antigen (Sospedra and Martin, 2005). These T cells are capable of producing inflammatory cytokines and may be differentiated or have the potential to differentiate on activation into Th1 (producing IFN-gamma) or Th17 cells (IL-17, IL-22, IL-21) or cells producing both (McFarland and Martin, 2007). Activated T cells up-regulate integrins such as VLA-4 and are capable of crossing the BBB. Through the permeabilized BBB, attracted by chemokine release, other immune cells including B cells and monocytes/macrophages migrate into the CNS. There, they encounter the cognate antigen, probably derived from myelin antigen, presented by CNS resident or immigrant antigen-presenting cells (APC). These can be macrophages/microglia and in certain instances dendritic cells or astrocytes. On encountering the antigen, such autoreactive T cells are reactivated and differentiate, producing their signature cytokines, which activate the neighbouring immune or neural cells and attract further inflammatory cells into the CNS. Of these, it is especially activated macrophages that are thought to indirectly and directly damage

## Table 1

Drugs in current or expected near-future use in MS

| | Route of administration in humans | Primary mechanism of action (elucidated via animal studies?) | Secondary/alternative mechanism of action (elucidated via animal studies?) | Animal studies in EAE | Reference |
|---|---|---|---|---|---|
| Cladribine | Oral | Immune cell depletion, apoptosis, cell cycle arrest; selectivity for lymphocytes (yes, in part) | Cytokine suppression; adenosine receptor effects (No) | None; studies in murine leukaemia and cancer xenografts; | (Giovannoni *et al.*, 2010) |
| Fingolimod | Oral | Sphingosine 1 phosphate partial agonist; blocks leukocyte migration from lymph nodes (yes) | Potential remyelination effect (yes) | yes | (Brinkmann *et al.*, 2002); (Kappos *et al.*, 2010); (Cohen *et al.*, 2010) (Foster *et al.*, 2007; 2009) |
| Fumarate | Oral | Potential antioxidant; inhibits inflammatory products of immune cells (yes) | Neuroprotective; potential antioxidant effect on other cells including astrocytes and oligodendrocytes (yes) | yes | (Rammohan and Shoemaker, 2010) |
| Alemtuzumab | i.v. | Anti-CD52 monoclonal antibody: depletion of immune cells bearing CD52 surface marker (No) | Immune system reset? (no) | None; transgenic mouse expressing CD52 generated recently | (Coles *et al.*, 2008; Jones *et al.*, 2009); (Hu *et al.*, 2009) |
| Rituximab | i.v. | Anti-CD20 monoclonal antibody: blockade/depletion of CD20+ B cells; (yes, partially) | Antibody depletion (including potentially anti-myelin antibodies); interference with B cell antigen presentation; depletion/blockade of EBV infected cells; preferential sparing of regulatory B cells (no) | Yes (murine equivalent) | (Hauser *et al.*, 2008); (Kap *et al.*, 2010b) |
| Teriflunomide | Oral | Pyrimidine analog (yes*) | Cytostatic effect, preferentially on proliferating lymphocytes | Yes (*parent compound leflunomide) | (Korn *et al.*, 2004); (O'Connor *et al.*, 2006); (Tallantyre *et al.*, 2008) |
| Laquinimod | Oral | (yes) | Cytokine modulation, inhibition of T cell migration, suppression of Th17 cells, induction of Th3 cells (yes) | Yes | (Wegner *et al.*, 2010); (Brunmark *et al.*, 2002); (Yang *et al.*, 2004); (Comi *et al.*, 2008); (Tselis, 2010) |




## Figure 1

Schematic diagram of some of the key pathological features of EAE pathogenesis. Activated Th1 cells and Th17 cells are thought to be the main culprit in EAE and MS. Th1 are IFN-γ producing, and Th17 are IL-17 producing T lymphocytes. They are primed outside the CNS by dendritic cells, then cross the blood–brain barrier and encounter CNS antigen-presenting cells. They produce inflammatory products and cytokines that damage the myelin and axons. They also activate the resident microglia and produce factors that attract further inflammatory cells to the CNS and perpetuate the inflammatory cascade. Antibodies and B cells can also enter the CNS, and plasma cells produce antibodies within the CNS. Antibody mediated damage contributes to the inflammatory demyelination and neurodegeneration.

the CNS. Myelin is phagocytosed by macrophages (Barnett *et al.*, 2006). Elements of the humoral immune response and soluble mediators also contribute to the pathology, via complement activation, direct cytokine cytotoxicity, nitric oxide, reactive oxygen and nitrogen species (Hemmer *et al.*, 2006). Plasma cells produce antibodies, which can bind and activate complement or induce antibody-dependent cytotoxicity. Th2 cells (producing IL-4) may enhance antibody production. CD8 (cytotoxic) T cells may enhance the damage through further cytokine production as well as granzyme and perforin production and can directly transect axons (Fletcher *et al.*, 2010). The resolution of inflammation, which can be partial and subject to recrudescence, occurs when anti-inflammatory cytokines (e.g. IL-10) and other immunoregulatory mechanisms such as regulatory T cells (Treg) or NK cells come into play. The consequence is that the myelin is destroyed and typically lacks full regeneration potential, especially after repeated injury, and the axons degenerate, in part because they are devoid of myelin and more exposed and deprived of trophic support, in part through wallerian degeneration and metabolic injury (Piaton *et al.*, 2009).

## EAE

Many elements of this cascade of events have been identified, tested or confirmed in EAE. From the pathogenesis point of view, therefore, EAE is a good model for studying MS mechanisms, even more so than for testing or developing drugs (Farooqi *et al.*, 2010).

A major difference between MS and EAE is that the latter requires an external immunization step to develop, whereas in humans, the sensitization to autoantigens is obviously not artificially induced (Gran *et al.*, 2007). Sensitization to myelin antigens in EAE typically occurs through the use of adjuvant, usually containing bacterial components highly capable of activating the innate immune system via pattern recognition receptors (Libbey and Fujinami, 2010). In EAE, the inducing antigens are known, whereas in MS, there is no unique identified antigen. Thus, important differences between these conditions may be due to how autoreactive T cells are primed and activated.

More recently, however, a refined model of pathogenesis has been put forward by t Hart and colleagues, in which three

rather than two compartments are considered critical to EAE and MS pathogenesis (t Hart *et al.*, 2009). The hypothesis was derived from work in a non-human primate model of MS (Kap *et al.*, 2010a). In this model, autoreactive T cells are actively induced by peripheral immunization (occurring in lymph nodes and spleen, 'afferent compartment') with antigen emulsified in an adjuvant (in EAE) or by infection with an as yet unidentified pathogen (in MS). Such T cells collect in the spleen (Flugel *et al.*, 2001) before migrating to the 'target compartment' (the CNS), where they recognize their cognate antigen on local APCs, are activated and start an inflammatory cascade leading to tissue injury. Tissue debris that are cleared from the CNS are then found in APCs within a third, 'CNS draining compartment', comprising the cervical and lumbar lymph nodes and the spleen (t Hart *et al.*, 2009). Draining is thought to occur by means of interstitial fluids and/or the cerebrospinal fluid. T-cell responses are then triggered in the lymph nodes of the third compartment leading to the generation of new autoreactive T-cell specificities. Such cells are then released into the afferent compartment, where they can either mitigate or exacerbate the ongoing autoimmune reaction (t Hart *et al.*, 2008; 2009). Similar observations were reported in a transgenic mouse model of EAE (Furtado *et al.*, 2008). Data show that APCs in the cervical lymph nodes that contain myelin breakdown products have an anti-inflammatory phenotype, whereas APCs containing neuronal antigen such as the light chain of neurofilament appear to have a pro-inflammatory phenotype. The anti-inflammatory nature of myelin-containing APCs is consistent with data showing a generally anti-inflammatory activity of myelin-fed macrophages. Both in EAE and in MS, increased numbers of professional APC-containing neuroantigens are seen in the CNS-draining lymphoid organs.

The three-compartment model of EAE pathogenesis can be extrapolated to MS immunopathogenesis. However, a specific peripheral trigger of autoreactive T cells in MS has been elusive. Thus, in accordance with the primary lesion hypothesis (Wilkin's hypothesis) (Wilkin, 1990), the attractive mechanism has been proposed whereby initial activation of encephalitogenic T cells in MS does not occur in the afferent compartment, such as in the EAE model, but in the draining compartment.

A further refinement of the primate model is the recent development of a marmoset model in which EAE can be induced by incomplete, rather than complete Freund's adjuvant (i.e. without *Mycobacterium tuberculosis*, a powerful inducer of pro-inflammatory cytokines) (Kap *et al.*, 2008). Although the use of incomplete Freund's adjuvant still involves external immunization, it more accurately reflects the human immune response. We discuss EAE in primates and its role in MS treatment development below.

## EAE induction

EAE is primarily used as an animal model of autoimmune inflammatory diseases of the CNS, and it resembles MS, the prototypical such disease, in many respects (Gold *et al.*, 2006; Steinman and Zamvil, 2005; 2006; Farooqi *et al.*, 2010). Some models are more similar to other, less common inflammatory CNS disorders, such as the monophasic acute disseminated encephalomyelitis (ADEM) or neuromyelitis optica (NMO, Devic's disease) (Furlan *et al.*, 2009). Increasingly, the use of EAE has expanded considerably beyond the laboratory study of MS and the development of MS therapeutics. EAE has also become a very well characterized model for organ-specific autoimmune disease in general. Indeed, several recent first reports of key novel functions of immunologically important molecules, or of a novel knockout mouse were published with EAE data as the *in vivo* validation model. Examples include the discovery of ROR-γ (RORC) as a master transcription factor for Th17 cell development (Ivanov *et al.*, 2006), the identification of the aryl hydrocarbon receptor (AHR) as an essential component in the development of both Treg and Th17 responses (Veldhoen *et al.*, 2008) and the differential role of the related molecules IL-12 and IL-23 in the susceptibility to autoimmune demyelination (Becher *et al.*, 2002; Gran *et al.*, 2002; Cua *et al.*, 2003).

EAE was first described over 75 years ago (Rivers *et al.*, 1933; Rivers and Schwentker, 1935) and is still a popular and widely used model. A PubMed search ['(experimental) autoimmune encephalomyelitis/encephalitis OR EAE OR experimental allergic encephalomyelitis/encephalitis'] identifies over 9000 citations, of which almost 6000 since 1990. Like all animal models, EAE has limitations when applied to human disease (Sriram and Steiner, 2005;Gold *et al.*, 2006; Steinman and Zamvil, 2006; Furlan *et al.*, 2009; Farooqi *et al.*, 2010); it is very heterogeneous in terms of induction methods, clinical and pathological features, and amenability to treatment, all of which add to its complexity. Therefore, its usefulness is critically dependent on using appropriate models to answer the specific scientific or clinical questions that are being addressed. If, for example, the pathogenesis of spontaneous recurrence of inflammation is studied, a relapsing rather than a monophasic EAE model should be used (Baker *et al.*, 1991; Miller *et al.*, 2007b).

Table 2 shows a list of potential uses of EAE to explore, develop and test general neuroscience and immunology concepts, developing general therapeutic strategies, and the potential uses of EAE to answer question specifically related to inflammatory demyelination and its consequences.

In terms of providing clues to the MS pathogenesis and allowing development of treatments, a most exciting and rewarding approach was that of the bidirectional translational studies pioneered by the group of L Steinman (Lock *et al.*, 2002; Robinson *et al.*, 2003; Steinman and Zamvil, 2003; Kanter *et al.*, 2006; Han *et al.*, 2008). This involved gene expression profiling in MS brain, identification of a number of plausible novel targets and then testing and validating these targets in EAE. Several such targets have been identified in this fashion, some supported by small previous studies in EAE, and these targets have a potential for being translated into MS treatment soon. Such targets include osteopontin, platelet-activating factor receptor, histamine receptors and alpha-B crystallin (Lock *et al.*, 2002; Han *et al.*, 2008; Steinman, 2009).

### *Induction of EAE in different strains of rodents and monkeys*

EAE can be induced in a multitude of species and strains. Interestingly, humans were the first species where sensitization with nervous tissue led to an inflammatory demyelinating CNS disease. This occurred as a rare complication of rabies

## Table 2

Uses of EAE

| | |
|---|---|
| General | Neuroprotective strategies<br>Immunosuppressive drugs<br>Neurotransmitters in inflammation<br>Channel function during inflammation, demyelination and remyelination<br>Immune responses in immunologically privileged sites<br>Effects of cytokines in the CNS<br>Blood–brain barrier function and dysfunction<br>Immunological tolerance<br>T-cell receptor restriction<br>Epitope spreading<br>Regulatory T cells |
| Specific to CNS inflammation, demyelination | Development, testing and validation of MS drugs: bioavailability, pharmacokinetics, preclinical efficacy, safety<br>Development and testing of drugs with possible dual action on the CNS and immune system, for development of drugs with both immunomodulatory and neuroprotective properties<br>Gene expression during demyelination a remyelination<br>Gene expression profiling for discovery and validation of new targets for MS treatment<br>Expression of genes associated with sparing of CNS elements or resolution of inflammation<br>Mechanisms of axonal damage and loss<br>Study of symptoms and symptomatic treatment for MS: e.g. spasticity and anti-spastic drugs, bladder dysfunction, pain |

vaccination with virus grown on rabbit spinal cord (Sabin and Wright, 1934). It was subsequently shown that the resultant encephalomyelitis was not due to rabies, but to an autoimmune response triggered by the spinal cord contaminant of the vaccine. Rivers in 1933 developed EAE in an attempt to understand better the pathogenesis of this post-vaccinal encephalomyelitis (Zinsser and Tang, 1926; Rivers and Stewart, 1928; no authors listed, 1931; Rivers *et al.*, 1933; Rivers and Schwentker, 1935).

Since then, EAE has been induced in a variety of rodents and monkeys, providing models of acute monophasic, relapsing–remitting and chronic progressive CNS inflammation. Optic neuritis, often a first sign of autoimmune demyelination, has also been modelled (Rao *et al.*, 1977; Raine *et al.*, 1980; Mendel *et al.*, 1995; Kornek *et al.*, 2000; Pomeroy *et al.*, 2005; Gold *et al.*, 2006)

'Active' EAE is induced by immunization with CNS tissue or myelin peptides, such as myelin basic protein (MBP) and proteolipid protein (PLP) in CFA, with high incidence of disease induced in susceptible animal strains (Stromnes and Goverman, 2006a). Disease onset typically occurs after 9–12 days and is followed by variable clinical and pathological outcomes as mentioned above. For example, acute self-limiting or chronic relapsing–remitting disease/ progressive disease can be induced in guinea pigs by immunization with MBP or with CNS tissue homogenate respectively (Raine *et al.*, 1977; Alvord *et al.*, 1985). By contrast, 'passive' or adoptive–transfer EAE (AT-EAE) can be induced in recipient animals by transferring pathogenic, myelin-specific CD4 T cells generated in donor animals by active immunisation (Stromnes and Goverman, 2006b). The latter type of EAE was instrumental in establishing the key role of myelin-reactive T cells in disease pathogenesis (Pettinelli and McFarlin, 1981). AT-EAE has enabled researchers to focus on variables associated with the 'effector phase' of disease and to 'bypass' its induction phase. Encephalitogenic T cells can also be manipulated *in vitro* to characterize the role of specific cytokines and other biological agents before adoptive transfer into recipients. These cells can be conveniently labelled to follow their localization, survival and interactions with other cell types in the recipient host. In addition, adoptive transfer of cells has made it possible to address the role of a variety of inflammatory molecules in different aspects of disease development and regulation through the use of gene-targeted donor or recipient animal strains (most frequently, C57BL/6 mice).

The pathology of lesions varies in different animal strains (Gran *et al.*, 2007; Lassmann, 2007). For example, in the C57BL/6 mouse, immunization with MOG35–55 in CFA can induce monophasic or a chronic, sustained form of EAE. The former is characterized by multifocal, confluent areas of mononuclear inflammatory infiltration and demyelination in the peripheral white matter of the spinal cord (Day, 2005). Macrophages and CD4+ T cells are the main cell types in the inflammatory infiltrate. In the brain, there is meningitis and perivascular inflammatory cuffing in the cerebellum and hindbrain white matter. The latter type of EAE, often induced with a 'booster' injection of the same myelin peptide 7 days after the initial immunisation (or with higher doses of peptide at the first immunization), shows similar pathology with reduced tendency to resolution of inflammation and demyelination after the peak of disease. These characteristics make this disease type a good model of chronic inflammatory demyelination, which approximates SPMS more closely (Bannerman *et al.*, 2005).

Another frequently used EAE model is induced in SJL/J mice by immunization with PLP139–151, leading to relapsing–remitting disease in which T-cell reactivity spreads to new myelin peptide determinants with each relapse (epitope spreading) (McRae *et al.*, 1995; Vanderlugt *et al.*, 2000). Typical lesions appear in the optic nerve, brainstem, spinal cord, cerebellum and cerebral cortex, initially with perivascular and meningeal lymphocyte and neutrophil infil-

tration, followed by resolution of the inflammatory infiltrate and at the same time progression of white matter damage and gliosis, demyelinated axons and myelin debris-containing macrophages. In the Lewis rat, active and passive EAE induced by MBP or transfer of MBP-specific T cells typically produces severe CNS inflammation, with little or no demyelination (Meeson *et al.*, 1994). Thus, the model is useful for the study of acute CNS inflammation. Interestingly, injection with antibodies to surface myelin antigens such as MOG leads to the appearance of demyelination and increased perivascular inflammation and clinical symptoms, usually followed by remyelination. In the Dark Agouti (DA) rat, syngeneic spinal cord tissue or recombinant rat MOG can be used to induce EAE characterized by demyelination and spinal cord lesions with perivascular and subpial inflammatory infiltration (Tanuma *et al.*, 2000). Demyelination tends to appear in the dorsal column of the spinal cord only during the second disease relapse. Whereas both TCRαβ-positive T cells and ED-1-positive macrophages are observed in the acute phase, in the chronic/relapsing phases, there is a predominance of macrophages over T cells (Tanuma *et al.*, 2000).

## Therapies in EAE

Immunologically (Table 3) or neurobiologically (Table 4) based interventions in EAE have allowed the exploration of pathogenesis pathways and the development of validation of certain targets for MS therapies.

Of note, some of the neuroimmune molecules listed above may have dual action, immunomodulatory and neuroprotective, and may be attractive candidates as therapeutic targets in MS. A few of the above have made their way into early clinical trials. These include cannabinoids, neuropeptides and ion channels, which are present and subject to modulation also in the immune system, and some neurotransmitters such as dopamine (Rog *et al.*, 2005; Nessler *et al.*, 2006; Vollmar *et al.*, 2009). This dual expression in the immune and nervous system not only certainly provides the opportunity for multiple therapeutic mechanisms but also invites caution: the favourable effects in one system should not be counteracted by detrimental effects in the other.

## Correlation between EAE and MS studies

As can be already observed from the above tables, a large number of EAE studies are corroborated by results in MS. Since the immune system and the immune mechanisms in EAE are very complex, some treatments that have been successful in EAE are yet to be assessed in MS.

In EAE, and to some extent in MS, unsuccessful studies may not be published, leading to publication bias. While this can be assessed when there are a substantial number of studies with the same or very similar compound, by plotting the standard error /deviation against the effect size (funnel plot), this is not feasible for a few small exploratory studies as are often done in EAE. However, for some of the studies discussed below, where such analysis is possible, there is no evidence of major (publication) bias (Farooqi *et al.*, 2010).

EAE studies may differ widely in terms of the experimental conditions. This includes the species, strain, and sex of the animals used; the age; specific induction method (including the neuroantigen, the type of adjuvant used, the active induction vs. AT EAE model); and the timing, frequency and dose of the therapeutic agent under study (Gold *et al.*, 2006). Most rodent EAE experiments are done in genetically identical groups of animals. This at least eliminates an important source of variation (although this variation does exist in humans). However, genetically identical animals may differ in their susceptibility to EAE depending on environmental factors, which may not easily be controlled. For example, the degree of colonization of the gut and the type of commensal flora can determine to a great degree the susceptibility to EAE (Yokote *et al.*, 2008).

Many of the above-discussed caveats may explain discrepancies between EAE studies.

Notwithstanding sources of variation, there are many examples of successful therapies in EAE that have also proven successful in MS.

Based on the congruence between the evidence in EAE and MS, studies of candidate DMT can be divided into several categories:

### *EAE and MS treatment success*

The most convincing correlations between EAE and MS therapeutic success are, reassuringly, those of the currently licensed and used DMT: IFN-beta (Abreu, 1982; Paty and Li, 1993), GA (Teitelbaum *et al.*, 1971; Johnson *et al.*, 1995) and the anti-VLA-4 antibody (Yednock *et al.*, 1992; Polman *et al.*, 2006). Their use in MS is discussed above. We have recently conducted a systematic review of all EAE studies looking at type 1 IFN, GA and anti-VLA-4 (natalizumab) (Farooqi *et al.*, 2010). The reader is referred to that review for more detail. The evidence and the role of EAE in the drug development and testing is discussed below.

*IFN-beta.* This is a good example of bidirectional dialogue between MS and EAE. The IFN evidence has developed almost in parallel, findings from the experimental model feeding into MS study and vice versa. In 1982, in EAE, Abreu found that IFN type 1 suppressed disease (Abreu, 1982), while Jacobs found that intrathecal IFN-beta was effective in 5 of 10 MS patients (Jacobs *et al.*, 1982).

A total of 25 therapeutic trials in EAE with IFN type 1 (both alpha and beta, as both have been used successfully in MS) have been identified during our systematic review Meta-analysis showed that the overall effect was beneficial, though in some studies, the results were equivocal and in a small number of studies there was an actual worsening (Farooqi *et al.*, 2010). Of note, many of the more recent studies were done with the purpose of exploring further the immunomodulatory effects of IFN-beta and elucidating mechanisms of action rather than confirming its clinical effects.

*GA.* The story of GA is tightly linked to EAE. As discussed above, GA a random copolymer of tyrosine, glutamate, alanine and lysine in ratios resembling myelin basic protein. It was developed in 1971 by Teitelbaum and colleagues in the laboratory of M. Sela, who was conducting extensive studies

## Table 3

Immunologically based therapies in EAE

| General approach class | Treatment type | Examples (EAE) | Used/tried in MS (comments) | Reference |
|---|---|---|---|---|
| Pharmacological (traditional pharmaceutical agents) | General immunosuppressive | Mitoxantrone | Yes (licenced) | (Ridge *et al.*, 1985; Levine and Saltzman, 1986; Lublin *et al.*, 1987; Tischner and Reichardt, 2007; Mangano *et al.*, 2010;) |
| | | Cyclophosphamide | Yes | |
| | | Glucocorticoids | Yes | |
| | Immunomodulatory drugs | IFN-beta | Yes (licensed, widely used) | (Abreu, 1982; Jacobs *et al.*, 1982; Hertz and Deghenghi, 1985; Abreu *et al.*, 1986; Paty and Li, 1993; Brod and Burns, 1994; Ruuls *et al.*, 1996; Yu *et al.*, 1996; Croxford *et al.*, 1998b; van der Meide *et al.*, 1998; Luca *et al.*, 1999; Yasuda *et al.*, 1999; Wender *et al.*, 2001; Schaefer *et al.*, 2006; Jaini *et al.*, 2006; Martin-Saavedra *et al.*, 2007) |
| | Other drugs | Captopril, losartan, pentoxyphyline, prazosin, antihistamines, and others | No (not as DMT) | (Babington and Wedeking, 1971; Brosnan *et al.*, 1985; Claudio and Brosnan, 1992; Nataf *et al.*, 1993; Constantinescu *et al.*, 1995; Dimitriadou *et al.*, 2000; Stegbauer *et al.*, 2009; Jadidi-Niaragh and Mirshafiey, 2010) |
| Immune deviation | Cytokines inducing Th1-Th2 shift | IL-4 | No | (Racke *et al.*, 1994; Young *et al.*, 2000) |
| | Immune decoy/antigen mimicry; interference with antigen presentation | Glatiramer acetate | Yes (licensed, widely used | (Teitelbaum *et al.*, 1971; 1973; 1974; 1996; 1999; 2004; Keith *et al.*, 1979; Lisak *et al.*, 1983; Aharoni *et al.*, 1993; 1997; 1998; Johnson *et al.*, 1995; 1998; Gran *et al.*, 2000; Gilgun-Sherki *et al.*, 2003; Illes *et al.*, 2004; Stern *et al.*, 2004; Giuliani *et al.*, 2005a,b; Jee *et al.*, 2007; Begum-Haque *et al.*, 2008; Stern *et al.*, 2008; Kala *et al.*, 2010) |
| (Brocke *et al.*, 1996; Bielekova *et al.*, 2000; Kappos *et al.*, 2000) | Altered peptide ligands | MBP | No (trial unsuccessful; some APL induced an immune response with exacerbation of the disease and/or anaphylactoid reaction) | |
| | | | Ongoing trial using ower doses | |
| Induction of immunological tolerance | i.v., p.o. administration of antigen | Oral tolerance to MBP, i.v. tolerance to MBP | No (trial of oral myelin showed interesting immune effects but largely unsuccessful) | (Weiner *et al.*, 1993; Chen *et al.*, 1994; Whitacre *et al.*, 1996) |
| | Blockade of second signal | CTLA4-Ig; non-depleting antiCD3 | No (trials unsuccessful or inconclusive) | (Croxford *et al.*, 1998a; Tran *et al.*, 2001) |
| | DNA therapy | cDNA 'vaccination' e.g. MBP, PLP | Yes (promising) | (Waisman *et al.*, 1996; Lobell *et al.*, 1998; Selmaj *et al.*, 2000) |
| Pathogenic (e.g. Th1, Th17) cytokine or cytokine developmental pathway blockade | Anti-cytokine monoclonal antibodies | Anti-IL-12/IL-23p40, IL-1RA, lenercept | No (worsening with lenercept, no effect with the others | (Constantinescu *et al.*, 1998; Chen *et al.*, 2006; Furlan *et al.*, 2007; Martin and Near, 1995) |
| | Cyokine receptor antagonists | | | |
| Targeting/depleting immune cells other than T cells | Macrophage/microglia depletion | Chlodronate | No | (Huitinga *et al.*, 1990; Jung *et al.*, 1993; Tran *et al.*, 1998) |
| | B cell depletion/blockade | AntiCD20/CD19 antibodies | Yes (promising) | (Hauser *et al.*, 2008; Matsushita *et al.*, 2008; Kap *et al.*, 2010b) |
| | Mast cell | Luteolin | No | (Theoharides, 2009) |
| Enhancing endogenous immune regulatory mechanisms | Up-regulation of Treg cells | Retinoic acid | Yes (small study plus IFN) | (Massacesi *et al.*, 1991; Vergelli *et al.*, 1997; Qu *et al.*, 1998) |
| | | Gut parasites | Phase I trials ongoing; Larger trials pending | (Sewell *et al.*, 2002; Sewell *et al.*, 2003; Correale and Farez, 2009) |
| | Enhancement of endogenous type 1 IFN | TLR ligands | Yes (polyI:C) | (Bever *et al.*, 1991) (Touil *et al.*, 2006; O'Brien *et al.*, 2010) |
| Cell-based therapies | Transfer of Treg | Transfer of CD4+CD25+ Treg cells | No | (Stephens *et al.*, 2009) |
| | Haematopoietic stem cell treatment | Immunosuppression and immune system renewal | Yes | (Karussis *et al.*, 1992; 1993; 1999; Burt *et al.*, 1998; van Bekkum, 2000; Burt *et al.*, 2009; Muraro and Uccelli, 2010; Pasquini *et al.*, 2010) |
| Cell trafficking/based approaches | Targeting adhesion molecules required for crossing the BBB | VLA4 antibody | Yes (licenced) | (Yednock *et al.*, 1992; Kent *et al.*, 1995; Leger *et al.*, 1997; Soilu-Hanninen *et al.*, 1997; Brocke *et al.*, 1999; Sheremata *et al.*, 1999; Theien *et al.*, 2001; 2003; Piraino *et al.*, 2002; van der Laan *et al.*, 2002; Cannella *et al.*, 2003; Leone *et al.*, 2003; Miller *et al.*, 2003; O'Connor *et al.*, 2004; 2005; Myers *et al.*, 2005; Polman *et al.*, 2006; Miller *et al.*, 2007a; Havrdova *et al.*, 2009) |
| | Targeting lymphocyte egress from the lymphoid organs | Fingolimod | Yes (licence awaited soon) | (Fujino *et al.*, 2003; Rausch *et al.*, 2004; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Balatoni *et al.*, 2007; Foster *et al.*, 2007; Foster *et al.*, 2009; Chiba *et al.*, 2011; Cohen *et al.*, 2010; Kappos *et al.*, 2010; Papadopoulos *et al.*, 2010) |
| Transgenic mice | Cytokine, chemokines, cell surface molecule knockouts; transgenic mice overexpressing immune molecules in CNS | Numerous | N/A but some have led to targeting immune molecules based on the results | (Glabinski *et al.*, 1999; Hilliard *et al.*, 1999; Izikson *et al.*, 2000; Furlan *et al.*, 2001; Huang *et al.*, 2001; Cua *et al.*, 2003; Park *et al.*, 2004; Elhofy *et al.*, 2005; Axtell *et al.*, 2006; Laouar *et al.*, 2008; Yang *et al.*, 2009) |

## Table 4

Neurobiologically/neuropharmacologically based therapies in EAE

| General approach class | Treatment type | Effects in EAE | References |
|---|---|---|---|
| Ion channel-based approaches | Na channel blockers (e.g. lamotrigine, flecainide), Na channel knockout mice | Improvement | (Bechtold *et al.*, 2004; 2006; Black *et al.*, 2006; O'Malley *et al.*, 2009) |
| | K Channel blockade | Improvement | (Judge *et al.*, 1997; Strauss *et al.*, 2000; Beeton *et al.*, 2001; Madsen *et al.*, 2005; Reich *et al.*, 2005) |
| | Ca channel blockade | Improvement | (Brand-Schieber and Werner, 2004; Tokuhara *et al.*, 2010) |
| | ASIC channel blockers (e.g. amiloride), ASIC knockouts | Improvement | (Friese *et al.*, 2007) |
| Neurotransmitter-based approaches | Dopamine signalling, increase in dopamine levels | Improvement | (Dijkstra *et al.*, 1994) |
| | Dopamine depletion (e.g. MPTP) | Worsening | (Balkowiec-Iskra *et al.*, 2007) |
| | Noradrenaline increase | Improvement | (Simonini *et al.*, 2010) |
| | Serotonin increase | Improvement | (Weistock *et al.*, 1977; Scott *et al.*, 1982; Vollmar *et al.*, 2009) |
| | Glutamate receptor antagonism | Improvement | (Wallstrom *et al.*, 1996; Paul and Bolton, 2002; Gilgun-Sherki *et al.*, 2003) |
| Neuropeptides | Substance P antagonism, Substance P receptor (NK1) knockout mice | Improvement | (Nessler *et al.*, 2006; Reinke *et al.*, 2006) |
| | Neuropeptide Y | Improvement | (Bedoui *et al.*, 2003; Luhder *et al.*, 2009) |
| Cannabinoids | CB1 and CB2 receptor agonism | Improvement | (Arevalo-Martin *et al.*, 2003; Pryce *et al.*, 2003; Zajicek *et al.*, 2003; Ni *et al.*, 2004; Maresz *et al.*, 2005; Rog *et al.*, 2005; Freeman *et al.*, 2006; Wissel *et al.*, 2006; Centonze *et al.*, 2007; Collin *et al.*, 2007; Palazuelos *et al.*, 2008; Zhang *et al.*, 2009) |
| Cell-based therapies | Neural stem cells | Improvement | (Picard-Riera *et al.*, 2002; Einstein *et al.*, 2003; Pluchino *et al.*, 2003; 2005; Einstein *et al.*, 2006; Makar *et al.*, 2008; Pluchino *et al.*, 2009; Yang *et al.*, 2009) |
| | Mesenchymal stem cells | Improvement | (Zappia *et al.*, 2005; Kassis *et al.*, 2008; Schafer *et al.*, 2008; Bai *et al.*, 2009; Lanza *et al.*, 2009; Lu *et al.*, 2009; Rafei *et al.*, 2009; Freedman *et al.*, 2010; Gordon *et al.*, 2010; Karussis *et al.*, 2010; Yamout *et al.*, 2010) |

of the immunogenicity of proteins. GA was developed initially as a putative encephalitogen; however, it showed the opposite action in that it effectively blocked EAE (Teitelbaum *et al.*, 1971; 1973; 1974).

Extensive subsequent studies were aimed mostly at elucidating mechanisms of actions of this versatile drug, but also at optimizing its pharmacology. Both pre- and post-licensing studies with GA in EAE have been successful. Our recent systematic review did not appear to detect a suggestion of publication bias (Farooqi *et al.*, 2010).

GA is a versatile compound. Although its mechanism of action is incompletely elucidated, it is likely to be multifactorial. The understanding of GA has paralleled the advances in our understanding of fundamental immunological principles and has thus evolved. From an immune decoy mechanism, through interference with antigen presentation, cytokine shift, induction of immunological tolerance, induction of immunoregulatory mechanisms (in the form the T suppressor cells to the Treg cells of today), antioxidant effects, to the more recent attention to its suppressive effects on Th17 development and effector function, and even to even more speculative attribution of neuroprotective and remyelinative properties, there is some evidence for a potential role of GA in all of these therapeutic mechanisms (Arnon and Aharoni, 2009). Most of these have come from studies in EAE and/or related demyelinating disease models.

*Altered peptide ligands.* Altered peptide ligands (APL) of MBP were initially developed to treat EAE based on the concept that substitution of one or more amino acids to change MHC or

T-cell receptor (TCR) binding characteristics would induce tolerance to the native peptide through mechanisms including T-cell antagonism and partial agonism as well as cytokine deviation (Evavold and Allen, 1991; Racioppi *et al*., 1993) and antagonism at the T-cell activation level (De Magistris *et al*., 1992). Brocke *et al*. induced EAE with a MBP87-99-specific T-cell clone and were able to induce tolerance *in vivo* by treating mice with an analogue of the native peptide (alanine substitution of the phenylalanine at residue 96). Paralysis was reversed, inflammatory infiltrates were regressed and brain T-cell infiltrates were depleted. Interestingly, it was also found that the simple administration of the native MBP peptide was equally effective, indicating that a 'tolerizing' injection before the 'immunizing' one was sufficient to prevent disease (Brocke *et al*., 1996). The approach was then tested in MS in a small phase II placebo-controlled trial, which had to be suspended because of hypersensitivity reactions in 9% of the patients. Secondary analysis of patients who completed the study showed a reduction of the volume and number of contrast-enhancing lesions in the treatment group. A regulatory Th2 response to the APL was also observed, which cross-reacted with the native peptide (Kappos *et al*., 2000). Major concerns, however, were raised by a parallel study by Bielekova *et al*. who clearly documented that APL could exacerbate MS. In a phase II clinical trial, they found that three patients developed exacerbations after administration of a MBP APL. In two patients, increased disease activity was shown to be linked to the APL immunologically and radiologically. Patients recovered well, and the therapeutic disappointment was at least partly compensated for by the scientific insight into the ability of modified MBP peptides to induce exacerbations in patients, thus confirming a long suspected link between autoimmunity to MBP and MS (Bielekova *et al*., 2000).

*Adhesion molecule blockade.* A solid body of evidence implicated VLA-4 (alpha4 beta 1 integrin) as an essential molecule in EAE, important for lymphocyte entry into the brain parenchyma. Steinman and Yednock showed that an antibody to VLA-4 suppressed EAE (Yednock *et al*., 1992). This eventually led to the development and production of the monoclonal antibody natalizumab (Tysabri®). Our recent meta-analysis of 19 EAE studies of VLA-4 antibodies or blockers that use clinical score as an outcome measure identified an overall beneficial effect (Farooqi *et al*., 2010). Interestingly, however, two studies (Theien *et al*., 2001; 2003) demonstrated a significant exacerbation of disease with anti-VLA-4 agents and advised caution in translation to MS. Of note, these two studies showed a discordant effect depending on the timing of administration of the agent (when administered either before EAE induction or at peak disease it induced an exacerbation), and one employed a small molecule VLA-4 antagonist. It is of interest that preliminary human studies with small molecule antagonists of integrins including VLA4 seem to be less effective than antibody blockade studies.

Clinical licence for Tysabri in MS was obtained in 2004 after remarkable success in phase II and III clinical trials (Miller *et al*., 2003; O'Connor *et al*., 2004). However, the drug was withdrawn soon thereafter following its rare association with progressive multifocal leukoencephalopathy (PML), an opportunistic infection due to the reactivation of latent John Cunningham virus (JCV) (Kleinschmidt-DeMasters and Tyler, 2005). The drug was cautiously re-introduced in 2006 after safety review, under a special prescription and observation programme, as the clinical benefits in people with aggressive RRMS are judged to outweigh the risks (Polman *et al*., 2006; Goodin *et al*., 2008). Indeed, natalizumab is currently considered the most potent licenced DMT.

Although natalizumab is another example of general concordance between EAE and MS studies, it does illustrate the potential risks of extrapolating all aspects of EAE results of a drug treatment to MS. As JCV only infects humans, side effects relating to it were not predicted on the basis of EAE experiments. This underscores the need for tailoring the appropriate experimental model experiments to the clinical/scientific question being addressed.

Generally, while hints at immune or neurobiological effects can often be provided by EAE experiments, alternative modelling may well be required for safety and pharmacokinetic/pharmacodynamic studies.

Other studies in EAE have investigated the inhibition of other adhesion molecules than VLA-4 and inhibitors other than monoclonal antibodies. None as yet have been translated to clinical use.

*Other compounds.* A substantial number of other studies have shown treatment success with concordant results in EAE and MS, using a variety of compounds. Some of these agents, like the immunosuppressants azathioprine (Hauser *et al*., 1983; Massacesi *et al*., 2005; Casetta *et al*., 2007; Elkhalifa and Weiner, 2010), mitoxantrone (Hartung *et al*., 2002; Weiner, 2004; Martinelli Boneschi *et al*., 2005) are licenced or well-established therapies for specific groups of patients with MS. Others, like laquinimod or fingolimod, have reached late phase clinical trials or are awaiting licencing decisions (please see Table 1 for examples). Table 5 lists some approaches with some degrees of evidence of success in MS with proof of concept/mechanisms supported by concordant success in EAE.

## *Discrepancies between EAE and MS treatment success*

The results outlined above suggest a reassuring degree of concordance between EAE and MS treatment. Moreover, this concordance is seen for the majority of the established or soon-to-be established DMTs for MS. However, there are many examples of EAE treatment success, which has not translated into similar MS success. This has been one of the most consistent criticisms of the EAE model (Sriram and Steiner, 2005). There are also, of course, a large number of therapeutic interventions that have shown positive results in EAE and that, for many reasons, have not yet been tested in MS. Depending of the strength of evidence from EAE results and on the practicability of application to human use in early stage trials, some of these may someday emerge into the field of MS. Nevertheless, there are many examples in the literature of treatments that were successful in EAE but not in MS (see below). Some of these examples are discussed below, although the list is incomplete. There is a possibility that negative results in EAE are not published, and thus, the EAE literature predominantly contains positive results. In MS, the current trend is to publish both positive and negative large

## Table 5

Studies of compounds used with some success in both EAE and MS

| Compound/class | EAE proof of concept, mechanistic hint, confirmation (references) | MS therapeutic success (references) |
|---|---|---|
| Hormones: | | |
| • Estriol<br>• Testosterone | (Kim *et al.*, 1999)<br>(Palaszynski *et al.*, 2004) | (Soldan *et al.*, 2003)<br>(Sicotte *et al.*, 2007) |
| Statins | (Youssef *et al.*, 2002) | (Vollmer *et al.*, 2004) |
| Quinolones | | |
| • Linomide<br>• Laquinimod | (Karussis *et al.*, 1993b)<br>(Brunmark *et al.*, 2002) | (Comi *et al.*, 2010) |
| Tolerizing DNA vaccines | (Waisman *et al.*, 1996; Lobell *et al.*, 1998; Selmaj *et al.*, 2000) | (Bar-Or *et al.*, 2007; Garren *et al.*, 2008) |
| Antibiotics with immunomodulatory and neuroprotective functions: minocycline | (Brundula *et al.*, 2002; Giuliani *et al.*, 2005a,b) | (Metz *et al.*, 2004; 2009; Zabad *et al.*, 2007; Zhang *et al.*, 2008) |
| Immunosuppressants | | |
| Azathioprine<br>Cyclophosphamide<br>Mitoxantrone | (Blaszczyk *et al.*, 1978)<br>(Mangano *et al.*, 2010)<br>(Ridge *et al.*, 1985; Lublin *et al.*, 1987) | Hauser *et al.*, 1983; Hafler *et al.*, 1991; Noseworthy *et al.*, 1993; van de Wyngaert *et al.*, 2001; Hartung *et al.*, 2002; Krapf *et al.*, 2005; Massacesi *et al.*, 2005; Smith *et al.*, 2005; Casetta *et al.*, 2007 |
| Haematopoietic stem cell transplantation | (Karussis *et al.*, 1992; Karussis *et al.*, 1993b; Burt *et al.*, 1995; Burt *et al.*, 1998; Karussis *et al.*, 1999; van Bekkum, 2000; Cassiani-Ingoni *et al.*, 2007) | (Muraro *et al.*, 2003; 2005; Burt *et al.*, 2009; Muraro and Uccelli, 2010; Pasquini *et al.*, 2010) |
| i.v. immunoglobulins | (Achiron *et al.*, 1994; Achiron *et al.*, 2000; Ephrem *et al.*, 2008) | (Achiron *et al.*, 1998; Haas, 2000; Strasser-Fuchs *et al.*, 2000; Katz *et al.*, 2006) |

studies, industry sponsors having an obligation to do so. However, small pilot studies, in particular older ones, that have not yielded conclusive results or that were negative may still remain unpublished.

Induction of immunological tolerance by oral administration of antigen has provided very important clues to the fundamental mechanisms of immune regulation and offered hopes for the treatment of autoimmune diseases such as MS and rheumatoid arthritis (Weiner, 2000). Oral tolerance has been shown to suppress EAE (as well as animal models of arthritis) (Benson *et al.*, 1999). A pioneering study in which oral myelin was given to patients with RRMS did not show efficacy on the clinical primary outcome measures, although it did show evidence of immune modulation that may, in the future, be harnessed more successfully to treat MS (Weiner *et al.*, 1993).

Deoxyspergualin is a xenobiotic with immunosuppressive properties. In the early 1990s, it was shown to be successful in several models of EAE (Yamamura *et al.*, 1987; Schorlemmer and Seiler, 1991). Despite this, when applied to a trial in MS with clinical MRI follow-up, it failed to show success on the primary outcome measure (Kappos *et al.*, 1996).

There is ample evidence of alteration of the balance between the pro- and anti-inflammatory cytokines in MS, with great potential opportunity for disease modulation. Indeed, the existing DMTs act, in part, via their cytokine-modulatory effects. Cytokine therapy, whereby pro-inflammatory cytokines are blocked by antibodies or their soluble receptors, has been successful in many models of autoimmune disease including many EAE studies. It has also made its way in human disease where it has revolutionized the treatment of rheumatoid arthritis (RA), for example, where anti-tumour necrosis factor (TNF) biologicals are now in well-established use and have made a great impact on the quality of life and the prevention of late complications in patients (Feldmann, 2002). The IL-1 receptor antagonist, anakinra, is also used as a treatment for RA, although not on such large a scale as TNF blockers (Mertens and Singh, 2009). TNF and MS are discussed below. Despite evidence for a role of IL-1 in EAE (Martin and Near, 1995), anakinra was unsuccessful in an MS trial.

A similar approach is to enhance anti-inflammatory cytokines. Both IL-10 and transforming growth factor (TGF)-beta were shown to suppress EAE (Santambrogio *et al.*, 1993; Rott *et al.*, 1994); however, attempts at treating a small number of MS patients with TGF-beta were unsuccessful due to side effects (Calabresi *et al.*, 1998).

Another immunological concept to which EAE studies have brought a major contribution is that of a restricted TCR use in immune responses (Hafler *et al.*, 1996). This has significant implications both for the presumed pathogenesis and for therapeutic opportunities. As regards the pathogenesis, such a restricted receptor use could imply an inciting or causative infectious agent such as a virus; it could also explain

why superantigens (which have predilection for specific TCRs) can trigger or exacerbate autoimmune disease. The finding of shared TCR usage across more than one model of autoimmune disease, for example EAE and its peripheral nerve counterpart, experimental autoimmune neuritis (EAN), led to the hypothesis that certain TCRs usage predisposes to autoimmune diseases in general. This also raised the possibility of immune intervention across the autoimmune spectrum. One approach was a trial of an antibody against the TCR most frequently used by MS patients, and that was unsuccessful (Killestein *et al.*, 2002). Another approach is inducing tolerance to the autoimmune-prone TCR by T-cell vaccination in MS, which is thought to trigger an anti-idiotypic immunoregulatory network (Vandenbark *et al.*, 2008). Such an approach is conceptually interesting but so far has not been translated into clinical success.

MS and EAE were long considered Th1-mediated diseases. Similar to other Th1/Th2 dichotomous experimental situations (e.g. the murine leishmaniasis model), modulation of both disease susceptibility and established disease activity was possible by manipulating the differentiation and maintenance of Th1/Th2 pathways. However, the discovery of IL-23, the IL-12-related cytokine that shares the p40 subunit with IL-23, led to the re-evaluation of all previous work done with neutralization of p40 or with p40 knockout mice and to the eventual description of the Th17 cells that are stimulated by IL-23 and produce IL-17 and other cytokines (IL-21, IL-22) (Cua *et al.*, 2003; Harrington *et al.*, 2005; Langrish *et al.*, 2005). This represented a paradigm shift in immunology. The developmental pathway of murine Th17 cells was also shown to involve TGF-beta and IL-6, and the *in vivo* system in which this was shown was EAE. Subsequent work has shown that, with some possible variation, these pathways also seem to be working in the human immune system in similar ways, with IL-23 having a role in stimulating and maintaining, if perhaps not inducing, Th17 responses (Korn *et al.*, 2009). It therefore became obvious that an intervention that targeted IL-12/23p40, thus down-regulating both Th1 and Th17 responses, is potentially beneficial in MS. The results of the clinical trial of ustekinumab, a human anti-p40 monoclonal antibody, in RRMS, were both surprising and disappointing in that respect (Segal *et al.*, 2008). The lack of clinical or MRI effect was shown despite the fact that there was evidence that the antibody did have an immunomodulatory effect. This study led to another rethinking, and consideration of therapeutic options in MS that might be beyond the Th1/Th2/Th17 split. Some potential options are considered in a recent review article (Steinman, 2010) and some are discussed below.

IFN-gamma has been one of the most poignant examples of discrepancy between MS and EAE and a major argument in the criticism of the EAE model. Moreover, the experience with this cytokine in MS and EAE and the studies showing its amenability to inhibition by type 1 interferons have contributed to the development of the latter compounds as DMTs. The role of IFNs in EAE and MS is discussed in more detail in other reviews (Sanvito *et al.*, 2010) but the evidence can be summarized as follows: treatment of EAE with IFN-gamma suppresses disease, while its blockade enhances disease in EAE. The opposite is true for MS, where intravenous IFN-gamma treatment in a clinical trial induced relapses in a substantial number of participants (Panitch *et al.*, 1987). A non-placebo controlled trial of an anti-IFN-gamma antibody showed that it suppressed MS, in contrast to an anti-TNF antibody, which did not (Skurkovich *et al.*, 2001).

There are more examples of discrepancies between therapeutic successes in EAE and MS, including some chemokine antagonists. The reasons for these translational failures are likely multiple and complicated but such results always offer the opportunity to rethink the pathogenesis of MS and the therapeutic approaches to it. Also, occasionally they provide new insights into fundamental immunological mechanisms.

## *Unpredicted EAE and MS treatment failure*

There is an informative category of studies where most of the results of the EAE studies concur with those in MS and are both negative, despite high biological plausibility of the intervention used as potentially beneficial in EAE and MS. This is usually when there are results in EAE that are discrepant or can be interpreted differently as beneficial or ineffective (for example a transient positive effect in EAE or a difference between antibody neutralisation and knockout mouse results). The most poignant example in this category is the work related to the role of TNF in EAE and MS (reviewed in Lim and Constantinescu, 2010a). In some papers addressing the deficits of EAE as a model for MS, TNF is often used as an example of inconsistency between MS and EAE results. In EAE, disease is said to be suppressed by antibody neutralisation of TNF, but in fact a closer look at the literature shows that this only occurs in adoptive transfer EAE models. In MS, trials of anti-TNF biological lenercept showed an unexpected worsening of the disease and further study of anti-TNF agents has been discontinued (The Lenercept Multiple Sclerosis Study Group, 1999). Moreover, demyelination has been reported in recipients of anti-TNF biologicals for other inflammatory diseases; these are contraindicated when these inflammatory conditions coexist with MS (Mohan *et al.*, 2001). The initial studies of anti-TNF biologicals in MS preceded the EAE studies with TNF (or TNF plus Lymphotoxin) knockout mice, and were only supported by passive transfer EAE studies, but not by active EAE studies. Even in a TNF knockout mouse model, a delay in EAE onset despite retained susceptibility led to the more emphasized conclusion that TNF is important for early disease, rather than that it is not absolutely required for EAE (Frei *et al.*, 1997). Anti-TNF antibody transiently delayed superantigen induced relapses in EAE; however, it was subsequently shown that other cytokines (IL-12/IL-23 and possibly IL-6) were more important in suppressing superantigen-induced relapses (Lim and Constantinescu, 2010a).

In conclusion, the results of TNF in EAE and MS are not incongruous, as they agree more than they disagree; on balance, they seem to argue against a significant and unique pathogenic role of TNF in demyelinating disease, and against TNF as a therapeutic target in MS.

## *Discrepancies in therapeutic effects of in different EAE models depending on the experimental conditions*

The above example with TNF neutralisation having an effect in passive but not active EAE illustrates that different results

## Table 6

Examples of conditions that determine variations in EAE outcome

| Condition | Examples | References |
|---|---|---|
| Strain | SJL/J, etc EAE susceptible; BALB/c etc EAE resistant | (Constantinescu *et al.*, 2001) |
| Sex | Females more susceptible | (Butterfield *et al.*, 1999) |
| Passive transfer versus active induction EAE | TNF neutralization effective in passive but not active | (Selmaj *et al.*, 1991)<br>(Teuscher *et al.*, 1990) |
| Early versus adult immunization | 3 week old mice resistant to EAE; adult mice susceptible | (Smith *et al.*, 1999) |
| Timing of intervention | • IL-12 during remission or after anti-CD40-CD40 ligand interaction blockade: induces relapse; IL-12 at immunization: suppresses EAE<br>• Pertussis toxin at immunization (adjuvant): induces EAE<br>• Pertussis toxin before EAE immunization or at peak disease: prevention/suppression | (Constantinescu *et al.*, 1999; Gran *et al.*, 2004)<br>(Stromnes and Goverman, 2006a,b)<br>(Ben-Nun *et al.*, 1993) |
| Environment | Specific pathogen-free: TCR transgenic mice develop EAE<br>Germ free environment: TCR transgenic mice protected | (Goverman *et al.*, 1993; Lafaille *et al.*, 1994) |
| Dose/affinity effects | High dose/affinity APL may worsen disease<br>Low dose/medium affinity APL (same sequence) can ameliorate disease | (Nicholson *et al.*, 1995)<br>(McCue *et al.*, 2004) |
| Exogenous administration versus endogenous blockade | IL-12/IL-23 administration versus anti-IL-12/IL-23p40 neutralisation at immunization | (Constantinescu *et al.*, 1998; Gran *et al.*, 2004; Touil *et al.*, 2010) |

are sometimes seen in different EAE models or even in the same model depending on the experimental conditions. There are many such examples. The differential susceptibility to EAE of male versus female mice is well known. Other examples are given in Table 6.

Besides the discrepancies that can result from the experimental conditions, other factors that must be taken into account when extrapolating from EAE to MS are the dual or multiple actions of the same target, and that some of these actions might be contradictory. Depletion of macrophages suppresses EAE but impedes nerve regeneration and remyelination (Huitinga *et al.*, 1990; Kotter *et al.*, 2001); a whole range of cytokines have opposing actions in EAE that need to be considered when designing MS treatments. A special issue of the Open Autoimmunity Journal is dedicated to the most representative of these cytokines (Gran and Becher, 2010).

Prostaglandin E-2 (PGE-2) enhances both Th1 and Th17 and thus augments EAE but also has BBB stabilizing effects that partially counteract the former effects (Esaki *et al.*, 2010). Osteopontin also enhances both Th1 and Th2 responses but also has a role in remyelination and is neuroprotective (Braitch and Constantinesco, 2010). These examples and others underscore the complexity of inflammatory demyelination and the numerous factors that come into play when considering therapeutic possibilities. Moreover, some of the subtle or delayed effects may be hidden, for example interference with neural repair or remyelination may be missed in a study dealing only with immunological aspects of EAE.

*Aspects of MS that cannot be tested in animal models.* Some treatments that have shown success in MS and are approaching widespread clinical application have not been tested or published in EAE. Cladribine, an immunosuppressant showing remarkable results from phase II and III studies in RRMS, is not reported to have been tested in EAE (Giovannoni *et al.*, 2010).

Alemtuzumab, a monoclonal antibody against human CD52, is an immunosuppressant that depletes immune cells achieving a highly suppressive effect on disease activity in RRMS (Coles *et al.*, 2008). Alemtuzumab does not cross-react with mouse CD52 and its effects in EAE have not been investigated. However, there is a recently developed transgenic mouse expressing human CD52, where effects of alemtuzumab have been tested with regard to its other applications in cancer (Hu *et al.*, 2009). Its effects in EAE would be very interesting.

Some targets cannot be tested in knockout mouse models because they are embryonic or neonatal lethal, for example TGF-beta or Retinaldehyde dehydrogenase type 3 knockout mice (Hines *et al.*, 1994; Dubinsky *et al.*, 2010). Also, activity dependent neuroprotective protein (ADNP) which is a neuroprotective molecule also amply expressed in the immune system and capable of immune modulation, has been reported to suppress EAE and its knockout mouse is neonatal lethal (Braitch *et al.*, 2010).

A very important situation where animal models such as EAE cannot answer MS-related question is related to compli-

cations restricted to humans, for example infectious diseases with humans as only hosts. We mentioned the JC virus example above (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould *et al.*, 2005; Clifford *et al.*, 2010).

Increasing epidemiological and biological evidence implicates the Epstein-Barr virus (EBV) in the pathogenesis of MS (Serafini *et al.*, 2007; Ascherio and Munger, 2010). Many hypotheses have been put forward about how EBV might modify the immune system and make people more prone to MS, including its ability to immortalize B cells and enhance their antigen presenting function. EBV is also a human specific virus, and all these mechanistic studies, as well as any potential studies of EBV-targeted therapies would not be appropriate in rodent EAE (unless one were dealing with a mouse with a human immune system).

MS can affect a variety of specific human factors such as fatigue or induce subtle cognitive disturbances. It also has wide psychological, social and economic implications. None of these can be modelled properly in experimental models.

*Transgenic and knockout mouse models.* The development of transgenic and knockout technology has made a major impact in the understanding of the pathogenesis of EAE and has contributed to the widespread use of the mouse models of EAE (Steinman, 1997; Gold *et al.*, 2006). Many of the emerging or future therapeutic interventions have been tested in these models, and the confirmation of a potential benefit of a (immuno)pharmacological blocking intervention through the appropriate knockout or transgenic model adds credence to the validity of the approach. However, caution is needed in interpreting the results of such studies, due to the well-known redundancy in the immune system and to some extent in the nervous system, or to some early developmental roles of some immune or neural molecules that might no longer be relevant for the adult rodent. In some instances conditional knockouts may be preferable (Korn *et al.*, 2008).

*Testing MS symptomatic treatment in EAE.* EAE has served as a model for developing and validating symptomatic treatments in MS as well. A study in EAE has shown the role of cannabinoids in controlling spasticity and tremor (Baker *et al.*, 2000). More recently, bladder symptoms of MS could be modelled in EAE and the utility of future drugs for neurogenic bladder dysfunction in MS could be tested in this model (Altuntas *et al.*, 2008; Al-Izki *et al.*, 2009).

*Pharmacokinetics.* Although rodent EAE has been an important model for understanding the pathogenesis and develop treatments for MS, relatively few published studies have included pharmacological evaluations of potential drugs, such as pharmacodynamic and pharmacokinetic studies. This is justified in part by the largely immune active rather than classical pharmaceutical repertoire of therapies used, but also by the difficulties extrapolating the results to humans. As discussed above, pharmacokinetic studies of a promising compound are sometimes done on larger animals and mechanistic studies are done in EAE. One notable study looking at retinoid modulation of EAE, however, has measured the exogenous retinoid pharmacokinetics in rats (Vergelli *et al.*, 1997).

*Requirements for translational success in MS of a putative treatment as predicted by EAE studies.* EAE results, especially those taken in relative isolation, cannot easily predict the success of a given therapeutic intervention in MS. For an immunological intervention in EAE to be matched in MS, the appropriate MS population (i.e. RRMS) needs to be tested. For example, photopheresis has positive effects on the inflammatory activity in rat EAE (Cavaletti *et al.*, 2001). In humans it was studied in progressive MS, where it was not successful (Rostami *et al.*, 1999); but a small study shows it to be successful in RRMS, which validates the EAE results (Cavaletti *et al.*, 2006). Many EAE treatments are tested at disease induction, therefore for a prophylactic effect, whereas, for obvious reasons, they are then tested in established MS. Of course, with the increased availability of first and second line DMTs, a major unmet need is developing effective treatments for progressive MS forms. EAE resembling secondary progressive MS can be established in some mouse models (Tsunoda *et al.*, 2005; Hampton *et al.*, 2008). However, the development of pure progressive EAE models to mimic primary progressive MS is problematic. Although a very interesting model of partial immunological tolerance induction shows that it cannot stop progressive neurodegeneration and thus resembles primary progressive MS (or one-attack progressive, or transitional MS) in terms of clinical phenomenology, it is not clear that is actually shares pathogenic mechanisms with primary progressive MS; however, it does create opportunities to study treatments targeted to the progressive neurodegeneration in MS (Pryce *et al.*, 2005).

Notwithstanding the importance of the right MS population being selected for translational studies, there are some aspects of the EAE studies themselves that may help to develop a successful MS drug.

Generally, corroboration of successful results from several EAE studies is a better predictor of translational success.

Listed below are several qualities of a putative drug that we consider, with the obvious variations depending on the drug being tested, requirements for translational success in MS:

1 Biological plausibility
2 Convincing, significant differences from appropriately chosen controls in initial studies
3 Evidence of biological effect (e.g. down-regulation of a known pathogenic pathway for the chosen EAE model)
4 Validation with congruent results in further EAE models/refinement of the same model (e.g. transfer EAE, EAE induced by another neuroantigen, EAE in another species/strain)
5 Clinical, immunological, histological (imaging) results are consistent
6 Efficacy in both males and females
7 Efficacy both as prophylactic and therapeutic (in established disease) treatment
8 Efficacy both in monophasic and relapsing EAE models
9 Synergistic effects of synergistic drugs/interventions
10 Antagonistic effects of antagonistic drugs/interventions
11 Where possible and appropriate, confirmation in transgenic/knockout mice
12 Ideally dual action, immunomodulatory and neuroprotective

13 Careful monitoring of CNS effects (effects on axon, myelin, brain/spinal cord volume, etc.) shows favourable effects/no adverse effects
14 Careful monitoring of long term effects, not only on immune/nervous system
15 Validation in another model of autoimmune disease if an immune-active drug; validation in another model of neurodegenerative disease if neurobiologically active drug
16 Where appropriate and possible, validation in primate MS model, where pharmacokinetics/ pharmacodynamics studies support use in MS; Clinical, immunological, histological (imaging) results are consistent; results favour treatment versus control despite clinical and genetic heterogeneity.

*Concluding remarks.* In conclusion, EAE has contributed to the development, validation, and testing of MS drugs and even more remarkably, to the understanding of the pathogenesis of MS. The multitude of models and results underscores the complexity both of MS and of this model. It also indicates that studies in the model need to be carefully tailored to the pathogenesis or therapy question, and that results showing a high degree of consistency between various models and experimental conditions are more likely to lead to translation into therapeutic success.

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