Animal models for autoimmune demyelinating disorders of the nervous system

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that takes a relapsing-remitting or a progressive course (reviewed in Refs 1,2). Its counterpart in the peripheral nervous system (PNS) is chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (reviewed in Ref. 3). In addition, there are acute, monophasic disorders, such as the inflammatory demyelinating polyradiculoneuropathy termed Guillain–Barré syndrome (GBS) in the PNS, and acute disseminated encephalomyelitis (ADEM) in the CNS. Both MS and GBS are heterogeneous syndromes. In MS different exogenous assaults together with genetic factors can result in a disease course that finally fulfills the diagnostic criteria. In both diseases, axonal damage can add to a primarily demyelinating lesion and cause permanent neurological deficits. No single animal model exists that mimics all the features of human demyelinating diseases; rather, the available models reflect specific facets. Here, we focus on experimental autoimmune encephalomyelitis (EAE) and its neuritis (EAN) as models in rat and mouse strains, and discuss their distinct histopathology and the roles played by different autoantigens.

Experimental autoimmune encephalomyelitis

Experimental aspects of EAE

EAE can be reliably elicited in a number of different species by immunization with either CNS tissue or with purified components of CNS myelin, for instance myelin basic protein (MBP) and proteolipid protein (PLP). This type of model is called ‘actively induced EAE’. Sensitized T-cell lines, propagated in vitro and then injected intravenously, generate ‘adoptive-transfer EAE’ (AT-EAE), giving conclusive proof of the pivotal pathogenic role of T cells. In contrast to human trials, which require inclusion of hundreds of patients to demonstrate statistically significant effects, experimental therapies can be evaluated in the inbred rodent models with only 6–12 animals per treatment group, depending on disease variability. This collectively depends on the antigen(s) used, the strain of rodent, the mode of immunization and the respective treatment. In the AT models, fewer animals are required because the disease runs a more synchronous course. Maximum disease expression of EAE in the Lewis rat is normally reached two to four days after onset, and severely affected rats can even die as a result of widespread inflammation in the brainstem. Recovery from the monophasic disease is then associated with enhanced apoptosis of inflammatory T cells in the lesion, which could also have therapeutic relevance (reviewed in Ref. 4). Only with immunopharmacological modulation, for example by low-dose cyclosporin A, can relapses of active EAE in Lewis rats be achieved.

Lessons from encephalitogenic T-cell lines

The generation of antigen-specific, autoreactive T-cell lines (i.e. T-cell lines that attack the host) was a major step towards better understanding immunological paradigms of EAE in rodents (reviewed in Refs 2,3). Recognition patterns of major epitopes of myelin proteins that give rise to encephalitogenic (encephalitogenic protein(s) were characterized in autoreactive-specific CD4+ T cells in rats and mice and then adapted to study human T-cell lines. In particular, the finding that these T cells used a limited range of disease-associated T-cell receptor (TCR) variable regions in different rodent strains raised hopes of developing TCR-specific immunotherapies. This concept has now been abandoned by most research groups because TCR usage turned out to be more heterogeneous than originally suspected. Furthermore, these T-cell lines and clones served as tools to characterize the secretion pattern of the proinflammatory mediators, cytokines and chemokines that orchestrate the autoimmune reaction in nervous tissue.

Newer EAE models and histopathology

The value of the Lewis rat EAE model for the elucidation of the pathogenesis of MS is limited by the lack of spontaneous relapses, and also by the absence of primarily demyelinating lesions. In contrast to MS, the histopathological features of EAE that is induced by immunization with MBP or by transfer of specific T cells are dominated by inflammation and by axonal damage in the spinal cord. A significant amount of demyelination is only seen when co-transfer of antibodies specific for myelin-oligodendrocyte glycoprotein (MOG), together with encephalitogenic T cells, is performed, reflecting the contribution of autobody and complement deposits to demyelination in MS (Ref. 6). Despite these limitations, the Lewis rat has still helped us to define important disease patterns in which a variety of putative autoantigens apparently determine the topography of inflammatory lesions in the CNS. It came as a great surprise that even proteins expressed in glial cells, such as S-100, which are not CNS-specific but are also found in thymus and peripheral nerve, can serve as autoantigens. S-100 EAE results in widespread T-cell mediated inflammation with pathology in the optic nerve, a prominent feature of early MS15.
There is increasing evidence to suggest that it is not the major myelin proteins like MBP and PLP, but rather the minor constituent MOG, that plays a key role in CNS autoimmunity. The whole spectrum of MS pathology was closely reflected in MOG-induced EAE in susceptible rat strains. Even clinical and histopathological subforms of MS, such as neuromyelitis optica (Devic’s disease), could reproducibly be induced in this model. These findings have already been extended to the analysis of concomitant axonal damage, which has recently attracted much interest in MS. Finally, the role of genetic and environmental factors that contribute to susceptibility to MS became evident when MOG-EAE was transferred to congenic Lewis rat strains that harbour non-Lewis major histocompatibility complex (MHC) genes on a Lewis genetic background. Under these experimental conditions, a relapsing-remitting course could be induced in Lewis-AV1 strains, with histological evidence of demyelination in the spinal cord and other sites of the neuraxis (Fig. 1). These findings have already been extended to the analysis of concomitant axonal damage, which has recently attracted much interest in MS. Finally, the role of genetic and environmental factors that contribute to susceptibility to MS became evident when MOG-EAE was transferred to congenic Lewis rat strains that harbour non-Lewis major histocompatibility complex (MHC) genes on a Lewis genetic background. Under these experimental conditions, a relapsing-remitting course could be induced in Lewis-AV1 strains, with histological evidence of demyelination in the spinal cord and other sites of the neuraxis (Fig. 1).

**EAE in mice**

EAE in mice shares many features with MS in humans because it can have a chronic, relapsing course and also shows histopathological evidence of demyelination (Table 1). Both actively-induced EAE and AT-EAE can be investigated. Susceptible strains such as SJL/J mice or PL/J mice exhibit a high degree of heterogeneity in their disease course. With the newer variants of rat EAE (see above) in mind, the main advantage of mouse EAE is that genetically engineered mutants can be bred. Thus, the influence of genetics on susceptibility, disease course and remyelination can be studied. For instance, transgenic mice expressing MBP-specific TCR genes have revealed that the balance between effector and regulatory mechanisms is critical for development of disease. Thus, the development of autoimmune disease models

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**Figure 1.** (a) Individual disease courses of relapsing EAE in Lewis (LEW) A-V1 rats induced by immunization with 50 μg recombinant human myelin-oligodendrocyte glycoprotein (MOG). Note that rats 2 and 3 died during the second relapse as indicated by †, whereas, in rat 1, a third relapse occurred. Immunohistochemical analysis of T-cell (b) and macrophage (c) infiltration in the brainstem of a LEW A-V1 rat (a congenic Lewis rat strain) in combination with myelin stain by Luxol fast blue. Note severe macrophage infiltration (brown reaction product) with concomitant demyelination in this early brainstem lesion. Arrows indicate the margin of ongoing demyelination. Scale bar for (b) and (c) = 50 μm.
disease depends not only on the existence of self-reactive T cells, but also on the presence of the appropriate number of functional regulatory T cells. Furthermore, in some cases, such as Bcl-2 knockout mice, the genetic defect can interfere with the ability to mount a significant T-cell response after active induction and require the use of adoptive-transfer models to generate pathology.

EAE in primates

The identification of mechanisms that cause autoimmune disorders of the CNS is essential for the successful application of novel immunotherapies. Because of the species barrier, non-human primate models were developed that more closely reflect human MS. The general fundamental principles obtained in rodent EAE have been confirmed and thus underscore the importance of EAE for the evaluation of novel immunotherapies (reviewed in Ref. 9). Recently, detailed histopathological analyses have also shown striking parallels between autoantibody deposition in primate EAE and in human MS lesions.

Table 1. Antigen-induced animal models for autoimmune demyelinating disorders

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<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Further comments</th>
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<tr>
<td>Lewis rat</td>
<td>T-cell inflammation and weak antibody response</td>
<td>Axonal damage, monophasic; secondary demyelination</td>
<td>Reliable model, commonly used for therapeutic studies</td>
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<tr>
<td>Adoptive-transfer EAE (MBP, S-100)</td>
<td>T-cell inflammation; topography of lesions</td>
<td>Axonal damage (MBP)</td>
<td>Homogenous course, rapid onset; differential recruitment of T cells and macrophages depending on autoantigen</td>
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<tr>
<td>Active EAE + co-transfer of anti-MOG antibodies</td>
<td>T-cell inflammation and demyelination</td>
<td>Only transient demyelination</td>
<td>First model to demonstrate pathogenic role of antibodies</td>
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<tr>
<td>Active EAN (PNS myelin, P2, PMP22)</td>
<td>Strong demyelination</td>
<td>With myelin re-isolation; also CNS inflammation</td>
<td>Only mild disease with PMP-22</td>
</tr>
<tr>
<td>Adoptive-transfer EAN (P2, P0)</td>
<td>Severe T-cell inflammation</td>
<td>Only moderate demyelination</td>
<td>Superimposed ischemic Wallerian degeneration</td>
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Abbreviations: EAE, experimental autoimmune encephalomyelitis; EAN, experimental autoimmune neuritis; MBP, myelin basic protein; MOG, myelin-oligodendrocyte glycoprotein; PLP, proteolipid protein; MS, multiple sclerosis.

Models of virus-induced demyelination

Theiler’s murine encephalomyelitis viruses (TMEV) belong to the Picornaviridae and are natural pathogens of mice. In susceptible strains, such as SJL or DBA/2, persistent infection of the CNS with TMEV leads to chronic progressive, immune-mediated demyelination (reviewed in Ref. 1). Histological features of the pathological lesion in TMEV infection and MS are very similar, although chronic demyelination in TMEV is induced in the absence of antemyelin responses by B cells. The Theiler’s virus model has served as a useful tool to study immunotherapies targeted at remyelination.

Experimental autoimmune neuritis

The first successful attempt to induce experimental autoimmune neuritis (EAN) was conducted by B. Waksman’s immunization of rabbits with PNS tissue in adjuvant (reviewed in Ref. 3). Like EAE, well-defined neuritogenic components of PNS myelin, such as P2 protein or P0 protein, were then defined concomitantly with the description of an AT-EAN. The methodology of isolating encephalitogenic T-cell lines could be easily transferred to establish P2 specific, neuritogenic T cells. Nevertheless, EAN has never reached the popularity of EAE; this might also be due to the lower incidence of GBS and CIDP compared with MS.

In our opinion, EAN
serves as an invaluable adjunct to EAE to characterize mechanisms of T-cell mediated autoimmunity. Like EAE in the Lewis rat, EAN is monophasic. A relapsing disease course is only observed when immunopharmaceutical modifiers such as cyclosporin A are given, or different strains are challenged. In EAN, specific neurophysiological methods can be applied to yield functional information on myelin integrity and axonal damage, which is similar to GBS and CIDP. This allows for a better monitoring of immunotherapies, which can be directed at the induction or at the effector phase of the disease. Chronic EAN induced by immunization of rabbits with galactocerebroside underscores the pathogenetic contribution of circulating autoantibodies for the induction of demyelinating lesions.

Concluding remarks
In the CNS, interest is focused on MS as a chronic demyelinating disease, whereas, in the PNS, the monophasic acute form GBS attracts greatest attention. Animal models for autoimmune demyelinating disorders provide a rational basis for studying mechanisms of pathogenesis and new immunotherapeutic strategies for MS, GBS and CIDP (reviewed in Refs 3,9). Owing to the complexities of human disease, it is apparent that there is no single model (Table 1). Instead, the adaptation of different approaches will finally help us to develop new and more effective therapeutic strategies.

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