



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2009

Modeling multiple sclerosis in laboratory animals

Schreiner, B ; Heppner, F L ; Becher, B

DOI: <https://doi.org/10.1007/s00281-009-0181-4>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-26538>

Journal Article

Published Version

Originally published at:

Schreiner, B; Heppner, F L; Becher, B (2009). Modeling multiple sclerosis in laboratory animals. *Seminars in Immunopathology*, 31(4):479-495.

DOI: <https://doi.org/10.1007/s00281-009-0181-4>

Modeling multiple sclerosis in laboratory animals

Bettina Schreiner · Frank L. Heppner ·
Burkhard Becher

Received: 12 June 2009 / Accepted: 13 August 2009 / Published online: 3 October 2009
© Springer-Verlag 2009

Abstract Inflammatory demyelinating disease of the central nervous system is one of the most frequent causes of neurological disability in young adults. While in situ analysis and in vitro models do shed some light onto the processes of tissue damage and cellular interactions, the development of neuroinflammation and demyelination is a far too complex process to be adequately modeled by simple test tube systems. Thus, animal models using primarily genetically modified mice have been proven to be of paramount importance. In this chapter, we discuss recent advances in modeling brain diseases focusing on murine models and report on new tools to study the pathogenesis of complex diseases such as multiple sclerosis.

Keywords Autoimmunity · Immunopathology · EAE · TH17 · Cytokines · TH1 · IL-12 · IL-23 · IL-18 · CD8 · CTL · Gene targeting · Transgenic · *Mus musculus* · Animal models · In vivo · Multiple sclerosis

Experimental autoimmune encephalomyelitis—the gold standard

Mode of induction

Experimental autoimmune encephalomyelitis (EAE) was first described and established as an important model of

central nervous system (CNS) autoimmune inflammation over 50 years ago [1]. EAE can be actively induced in susceptible inbred mice like SJL mice by immunization with mouse spinal cord homogenate [2], myelin basic protein (MBP; [3]), proteolipid protein (PLP; [4]) or peptides corresponding to the major encephalitogenic regions of MBP (MBP_{84–104}), PLP (PLP_{139–151} or PLP_{178–191}), or myelin oligodendrocyte glycoprotein (MOG_{92–106}) in complete Freund's adjuvant (CFA). MOG makes up less than 0.5% of all myelin proteins and is situated on the surface of myelin sheaths. MOG_{35–55} peptide is strongly encephalitogenic in C57BL/6 mice, the strain providing the genetic background of most transgenic mice. Disease induction with intact MBP or MBP_{84–104} in SJL mice, and likewise with MOG_{35–55} in C57BL/6 mice, requires the use of pertussis toxin (PTX) as part of the induction regimen. PTX has been hypothesized to facilitate immune cell entry into the CNS [5, 6]; however, PTX has other biological effects that could contribute to its activity in EAE, such as breaking T cell tolerance and promoting clonal expansion and cytokine production by T cells [7–9]. In most murine EAE models, after a prodromal interval of 10–20 days, paralysis of tail and hind legs, progressing to the forelimbs, and weight loss develop, reflecting preferential targeting of inflammation to the spinal cord and to some extent the cerebellum (referred to as “classic EAE”). Accordingly, the scoring system is based primarily on motor deficits resulting from spinal cord lesions. However, enhanced brain inflammation and atypical clinical variations have been described in murine models using certain strain/antigen combinations or a background of genetic interferon (IFN)- γ deficiency [10, 11]. Factors that can influence the manifestation of disease are age, gender, and season at immunization [12, 13], the preparation and physical

B. Schreiner · B. Becher (✉)
University of Zurich,
Zurich, Switzerland
e-mail: burkhard.becher@neuroimm.uzh.ch

F. L. Heppner
Charité-Universitätsmedizin Berlin,
Berlin, Germany

structure of antigen/adjuvant emulsions [14, 15], the species origin of the antigen [16], and epigenetic factors [17]. The disease course of EAE depends on the strain, immunogen, and use of PTX [18]. In the SJL (H-2^s) mouse strain, EAE displays a relapsing–remitting clinical course (RR-EAE), while in C57BL/6 (H-2^b) or PL/J and B10.PL (H-2^u) mouse strains, the disease is chronic–progressive (C-EAE) or acute monophasic, respectively. Alternatively to direct induction, EAE can also adoptively be transferred to naïve mice by injection of in vitro neuroantigen-activated T cells isolated from primed donors [19]. The most common adoptive transfer models involve major histocompatibility complex (MHC)-class-II-restricted CD4⁺ T helper (T_H) cells [20], while only a few reports describe EAE transfer by CD8⁺ T cells [21, 22]. Many of the pathogenic CD4⁺ T_H cells respond to activation by secreting IFN- γ and tumor necrosis factor (TNF)- α , but not interleukin (IL)-4 and IL-5, qualifying them as T_H1 cells. Recent studies, however, suggest that this population includes a subset of CD4⁺ T_H cells that preferentially produces IL-17 (T_H17 cells); by modifying T cell effector functions in vitro before transfer, Langrish and colleagues confirmed that IL-23-driven IL-17⁺ T cells are highly encephalitogenic [23]. It therefore currently appears that both T_H1 and T_H17 cells have pathogenic potential [24]. Another way of manipulating the myelin-specific T cells in vitro before transfer is the labeling by genetic markers or fluorescent proteins/dyes, so that the autoreactive T cells can be traced in the recipient [25, 26].

No spontaneous and naturally occurring animal models of spontaneous EAE exist. However, T cell receptor (TCR) transgenic mice have been generated that do not require peptide priming and strong immune adjuvants but spontaneously develop EAE with variable incidence. Although the immunological repertoire in TCR transgenic mice is significantly altered and biased towards enhanced antigen-specific responses and alternative mechanisms of immune regulation [27, 28], studies using such transgenic models have contributed significantly to the understanding of the pathogenesis of CNS autoimmunity. Various TCR transgenic models of EAE on different genetic backgrounds exist, including humanized mice carrying human leukocyte antigen (HLA) class II molecules associated with multiple sclerosis (MS) susceptibility and TCR [29–31]. Interestingly, HLA-DRB1*0401-restricted MBP_{111–129}-specific humanized TCR transgenic mice display clinical signs of inflammatory demyelination in brain stem and cranial nerve roots, like dysphagia in addition to ascending paralysis typical of EAE [31]. 19G B10.PL (H-2^u)/MBP_{Ac1-11}, [27], 5B6 SJL (H-2^s)/PLP_{139–151} [32], and 2D2 C57BL/6 (H2-^b)/MOG_{35–55} TCR transgenics [33] are spontaneous models utilizing mouse MHC class II restriction elements. The clinical spectrum displayed

varies from classical hind limb paralysis to isolated optic neuritis (2D2 TCR transgenics in the C57BL/6 strain). A large percentage of MOG_{35–55} TCR transgenic mice crossed to MOG-specific immunoglobulin heavy chain knock-in animals spontaneously develop a Devic-like, optico-spinal disease [34, 35].

Pathogenesis (priming and CNS invasion)

Classical models of EAE are mainly driven by MHC-class-II-restricted CD4⁺ T lymphocytes [36–38]. Active and passive induction protocols both activate peripheral myelin-specific CD4⁺ T cells that have escaped immune tolerance and circulate in the periphery of naïve animals (reviewed in [39]). During the induction phase of actively induced EAE, myelin-reactive CD4⁺ T cells are primed and expand in the peripheral lymphoid organs. In contrast, in the adoptive transfer model of EAE, disease is induced by the peripheral introduction of a preactivated population of myelin-epitope-specific CD4⁺ T cells to a naïve mouse. The effector phase involves migration of activated myelin-specific T cells to the CNS, where they cross the blood–brain barrier (BBB). CNS-infiltrating CD4⁺ T cells require myelin peptides presented by local antigen-presenting cells (APCs) for full reactivation [40, 41]. Recently, also dendritic cells (DCs) have been demonstrated to be the initial APCs for encephalitogenic T cells to recognize their target antigen (Ag)/organ [42, 43]. The reactivated CD4⁺ T cells then initiate a cascade of events, including the secretion of chemokines, that recruit predominantly macrophages to the site of inflammation. Proinflammatory cytokines secreted by macrophages, such as TNF- α and IL-1, are important for both perpetuating inflammation and contributing to CNS tissue damage [44]. Microglia are also activated in EAE and blocking microglial activation suppresses the development of EAE, supporting their pathogenic role in CNS autoimmune disease [45–48]. The function of astrocytes in disease pathogenesis remains unclear, as they show proinflammatory activity by producing neurotoxic mediators, cytokines, and chemokines but also exert neuroprotective functions by providing neurotrophic factors (reviewed in [49–51]). After CNS tissue is damaged, epitope spreading is believed to occur by the release and subsequent presentation of endogenous myelin epitopes by local APCs [26, 52] and has been shown to mediate clinical relapses in certain EAE models [53, 54].

Mouse, rat, monkey background

EAE is mainly studied in the highly reproducible murine model, as mice are inbred and a genetically homogenous population. Furthermore, transgenic and targeted gene deletion murine models are abundant,

and numerous reagents are available that can be used to dissect the pathogenic mechanisms in EAE. Nevertheless, EAE has been replicated in a wide range of species, and the different species and strains offer different advantages. EAE induced in rabbits or guinea pigs with CNS myelin is similar to MS in that inflammation occurs in both the brain and spinal cord [55, 56]. To investigate the contribution of demyelinating antibodies, EAE models in guinea pig [57], common marmoset [58], and certain rat and mouse models [16, 59] have been proven to be useful.

EAE is induced in the marmoset by immunization with CNS tissue homogenate or recombinant MOG in CFA (reviewed in [60]) and provides a disease model that reproduces several clinical and pathological features of MS. Adoptive and passive transfer experiments indicate that, similar to MOG-induced EAE in the rat, synergism between encephalitogenic T cells and demyelinating antibodies is required for full lesion formation [58, 61]. In spite of variable disease induction in the outbred marmoset species and limited availability of reagents, the marmoset model may have some value for drug testing considering that it is phylogenetically closer to humans than rodents.

Histopathological findings—man versus mouse

Histopathological changes seen in EAE appear to represent a stereotypic response of the CNS to autoimmune damage. While EAE, as many other disease models, certainly is not a full mirror image of MS, it comes pretty close to what can be seen in human MS lesions. However, the clinical features, including the pattern of disease progression and the histopathology, may vary depending on (1) the species in which disease is provoked as well as (2) the approach of EAE induction (for review, see [62]).

Early and/or acute MS lesions in humans are characterized by demyelination (Fig. 1a, b), extensive macrophage invasion (Fig. 1c, d), perivascular and parenchymal T cell infiltrates (Fig. 1g, h), and few B cells/plasma cells as well as relative axonal preservation. While ongoing remyelination next to demyelination is a typical feature of active MS lesions [63], based on the myelin loss, extent of oligodendrocyte preservation, and composition of the inflammatory infiltrates, four distinct—admittedly descriptive—histopathological patterns of acute MS lesions have been suggested [64] and await final clinical validation. In brief, common feature of these acute lesions is the predominance of macrophages, which are ~10 times more frequent than, e.g., T cells (the latter are mainly of the CD8+ phenotype). On top of which, some lesions (referred to as pattern II) display activated complement on dying myelin sheaths and in macrophages, while others (so-called pattern III) are described to have a

preferential loss of the myelin-associated glycoprotein (for review, see [65]). Recent data also point to a crucial role of DCs, which due to their close proximity to invading T cells adjacent to CNS vessels (Fig. 1f, g, h) seem to be crucial APCs in conferring CNS inflammation of encephalitogenic T cells [42]. In contrast to acute MS lesions, chronic MS plaques display extensive and sharply demarcated white matter lesions with variable axonal loss and significant fibrillary astrogliosis; due to only few residual lymphocytes and macrophages/microglia, these chronic lesions appear to be “burnt out” (for review, see [65]).

Acute EAE lesions in mice—the species which we primarily refer to—histopathologically resemble the perivascular macrophage-/microglia-rich inflammatory pattern of acute/chronic active MS lesions in humans (Fig. 2), while severe demyelination is seen rather in chronic relapsing EAE models (for review, see [62]).

Due to the high reproducibility of EAE lesions with respect to cellular composition and histopathological appearance, animal models of EAE are most valuable in studying pathogenetically relevant consequences of defined manipulations. Moreover, such lesions can be studied at any time point, which explains why EAE models also in terms of histopathology are a most precious addition to the repertoire of modern MS research tools.

Use of transgenic mice to unravel pressing questions about the pathogenesis of CNS inflammation; knockout mice

Specific mouse genes can routinely be overexpressed as transgenes or eliminated by gene targeting (“knockouts”), which allows a “clean” and direct assessment of the causative roles of certain molecules in disease pathogenesis. Various transgene constructs involving targeted expression of MHC/costimulatory molecules or alterations of the TCR repertoire towards a highly autoreactive population have been designed to challenge the immune system’s mechanisms of self-tolerance [66]. Studies in transgenic and knockout mice have implicated a number of effector molecules such as cytokines, chemokines, and other proteins in MS [67]. The conventional approach has several disadvantages; transgene expression may have direct toxic effects on the target tissue and be insensitive to regulatory networks, and in particular, normal developmental kinetics cannot be mimicked, as expression is either “on” or “off” from time of developmental onset (reviewed in [68–71]). Further (conditional) techniques have been developed to limit manipulated gene expression to defined cell types (Cre-loxP system of site-specific recombination [72]) and specific time points. Commonly used examples for such a spatial and temporal control of genes of interest are the

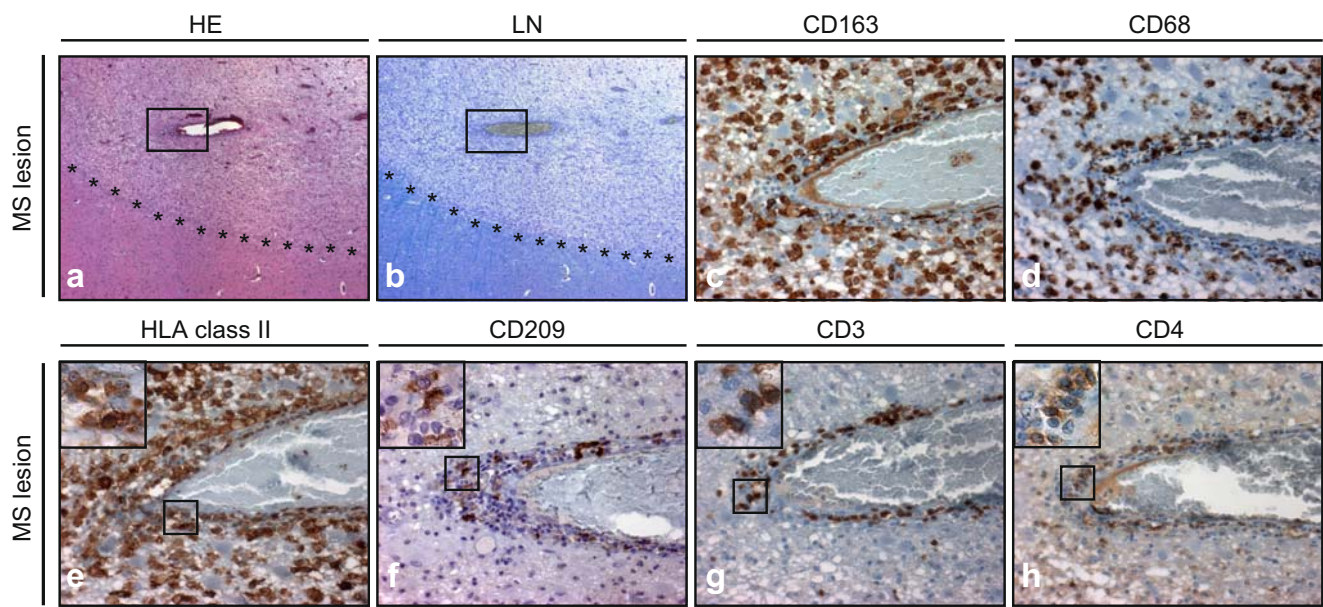


Fig. 1 Histopathology of acute and/or chronic active MS lesion: severe demyelination (H&E (**a**); LN, Luxol-Nissl (**b**)) and numerous CD163+ (**c**) or CD68+ (**d**) macrophage and microglia-rich inflammatory infiltrates including prominent perivascular inflammation are predominant histopathological findings. Gray and white matter in **a** and **b** is separated by *asterisks*. *Rectangles* represent the area chosen for

higher-magnification analysis throughout. While strong HLA class II immunoreactivity (**e**) was present throughout the MS lesion, CD209+ cells (**f**) were mainly restricted to vessels and seen in close proximity to invading CD3+ (**g**) and CD4+ (**h**) T cells. *Scale bar* 400 μ m (**a**, **b**), 100 μ m for all other panels. Adapted from [42]

tetracycline-controlled transactivator system [73] and the tamoxifen-controlled expression of Cre (CreERT2, [74]). CNS-specific expression can be induced, e.g., by promoter regions encoding glial fibrillary acidic protein (GFAP), neurofilament, MBP, or MOG which are expressed in astrocytes, neurons, and oligodendrocytes, respectively. The nestin-Cre transgene mediates excision of loxP-flanked sequences in early neuronal precursors during embryonic life, resulting in target gene inactivation in all neuroectodermal cells of the CNS [75]. CNS-targeted

manipulations of cytokine/chemokine expression offer a relatively noninvasive technique for assessment of their role (reviewed in [68, 76]). In vivo cell lineage ablation, e.g., of MOG⁺ oligodendrocytes, has recently been improved by stable mouse lines, carrying conditional expression constructs for diphtheria toxin (DT) or DT receptor (DTR) that could be activated upon Cre-mediated recombination and the application of DT, respectively [77]. CD11b-herpes simplex virus thymidine kinase (HSVTK) transgenic mice, which express HSVTK in macrophages/microglia

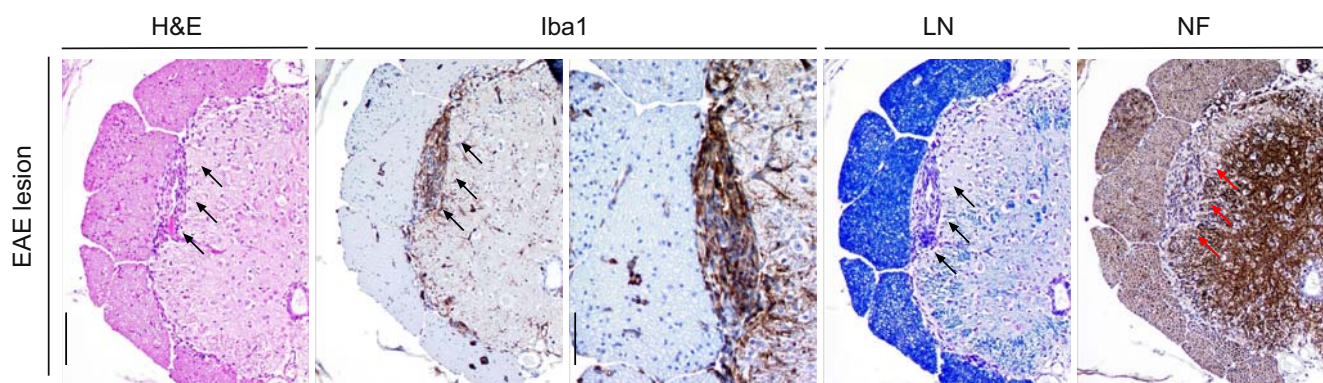


Fig. 2 Spinal cord cross sections of wild-type mice displaying an acute perivascular EAE lesion (H&E, *arrows*), mainly consisting of activated Iba1+ macrophages/microglia (*second* and, at higher magnification, *third* row). Impairment of myelinated (Luxol-Nissl, LN; *fourth* row) and axonal structures (neurofilament, NF, *fifth* row),

while indicated due to the early state is not yet fully developed (and better visible in chronic lesions). *Scale bars* 100 μ m for *first*, *third*, *fourth*, and *fifth* column; 100 μ m for *second*, *fourth*, and *sixth* column. Adapted from [96]

have been generated as a tool to study the impact of microglial activation on CNS disease in vivo. Expression of HSVTK renders preferentially proliferating cells sensitive to ganciclovir, as the active metabolite competes with thymine for DNA synthesis [45]. To dissect APC functions in the CNS parenchyma versus the periphery, gene targeting can also be combined with bone marrow chimerism [42]. Astrocyte activation during EAE in vivo was monitored by Luo and colleagues by the use of bioluminescence imaging in mice expressing luciferase under the control of a GFAP promoter [78]. “Knock-ins” of functional cytokine genes have been ligated to green fluorescent protein (GFP) for cell tracking of cytokine-producing cells [79]. Based on the linked expression of GFP to Foxp3⁺ T cells, two groups have generated Foxp3gfp.KI mice in order to reliably monitor regulatory T cells (T-reg) in vivo [80, 81]. Taken together, the true strength of the mouse model is its capacity to intervene with the pathogenesis of inflammatory CNS disease at the molecular level and to prove or mostly dismiss hypothesis about the involvement of specific molecules in the inflammatory cascade.

Cytokines and mediators of inflammation

Upon cognate antigen recognition on an activated APC, T cells become polarized towards an effector type depending on the quality of priming immune synapse and the cytokines present. Signaling of certain signature cytokines results in lineage commitment towards one of the three effector types, termed T_H1, T_H2, or T_H17. The classic paradigm, proposed by Mosmann and Coffman in 1986, strictly divided effector T_H cells into T_H1 cells, directing cell-mediated immunity, and T_H2 cells, regulating B cell activity and humoral immunity [82]. T_H1 cells produce primarily IFN- γ and IL-2, while IL-4 and IL-5 are produced by T_H2 cell. Lineage commitment employs autocrine loops for “self-propagation” and reciprocal inhibition via their cytokines. In addition to the T_H1-promoting effects of IFN- γ itself, T_H1 cells are predominantly polarized by the APC-derived factors IL-12 and IL-18 [83]. The T_H1/T_H2 paradigm provided a conceptual framework which shaped our understanding of communication pathways during adaptive immune response. Over the years, T_H1 cells were found to be the main culprits behind CNS inflammation, primarily based on the fact that inflammatory CNS lesions and invading T cells produced IFN- γ . In MS patients, elevated levels of IL-12p40 mRNA were detected in the CNS [84], while in the animal model EAE such T_H1 cells were shown to actually infiltrate the CNS in great numbers and produce their effector cytokines when CNS inflammation occurred. Purified T_H1 cells could be transferred into naive animals and mediate autoimmune disease. Conversely, T_H2 cells were shown to exert beneficial effects in EAE (reviewed in [85]).

Given the overwhelming case for T_H1 cells and IFN- γ , it was surprising at first that in mice none of the T_H1 and T_H1-inducing cytokines (IFN- γ , IL-12, IL-18, TNF- α) could account for the proposed importance of the T_H1 effector cells in the disease [86–89]. Even though functional studies could hardly verify the pivotal role of T_H1 cells in autoimmunity, the model was not abandoned but clearly dominated common belief for decades. A tangible alternative was proposed with the discovery that the IL-12 family member IL-23 instead of IL-12 itself is a prerequisite for the development of EAE [87, 90, 91]. The central role of IL-23 in EAE was proven by Sedgewick and colleagues by the deletion of the p19 subunit of IL-23 [91]. The particular impact connected to this finding was the description of a novel T_H cell effector type, which showed IL-23-dependent expansion and expression of the proinflammatory cytokine IL-17A [23]. They were therefore coined T_H17 cells. IL-23- and IL-6-deficient mice showed impaired T_H17 polarization. In consequence, it seems that such loss rendered the mice resistant to EAE [24]. A plethora of data was generated that correlated T_H17 cells and IL-17A expression with the development of autoimmune inflammation in mice and humans (reviewed in [92]). Almost forgotten was the fact that in most respects T_H1 cells and T_H1 cytokines correlate just as well with autoimmune disease and that adoptive transfer of T_H1 cells for the passive induction of EAE was the standard procedure for years.

Surprisingly, IL-17A neutralization in EAE in C57BL/6 mice by Hofstetter et al., however, only revealed a very mild effect of treatment with either monoclonal antibodies against IL-17A or with the soluble receptor of IL-17A and F [93]. IL-17A^{-/-} mice were generated by the group of Iwakura and found to be fully susceptible to EAE after active immunization but demonstrate an alleviated course of clinical EAE in the chronic phase of disease [94]. Even more surprisingly, these findings were then interpreted by others to represent the clear and solid proof that IL-17A is the key player in CNS autoimmune inflammation in mice and probably in men as well. The slight change in the course of EAE found upon deletion of IL-17A certainly confirms its proinflammatory properties at the site of an active autoimmune lesion but, nonetheless, fails to mark it as an essential encephalitogenic factor. Haak et al. and Kreymborg et al. have by now dismissed the T_H17 cytokines IL-17A, IL-17F, and IL-22 as critical players in the EAE pathogenesis [95, 96]. After an initial confusion in the field, it remains a solid fact that IL-23 is vital for EAE development while T_H17 cells and their cytokines display a great deal of redundancy. Recently, McGeachy et al. and Gyölvézi et al. reported that IL-23 promotes the CNS tropism of encephalitogenic T cells [97, 98]. The mechanistic underpinnings of this phenomenon remain to be established.

Hence, while we have clear evidence that a certain quality of T cell effector function permits encephalitogenic T cells access into the CNS, the precise molecular signature remains to be established.

BBB biology and leukocyte trafficking to the CNS

Under physiological conditions, a limited number of leukocytes enter the CNS (referred to as immune surveillance; [99]). In the course of EAE, BBB dysfunction is associated with increased leukocyte extravasation across CNS postcapillary venules and subsequent leukocyte accumulation in the perivascular space forming inflammatory cuffs. In postcapillary venules, the endothelial cell monolayer and underlying basement membrane, ensheathing astrocyte end feet, leptomeningeal cells, and their associated parenchymal basal membrane all contribute to the BBB [100]. Therefore, EAE presents an excellent model to study leukocyte migration across the BBB into the CNS during autoimmune inflammation. Several techniques have been developed to assess the disruption of the BBB in situ, like immunohistochemical analysis for tight junction proteins, serum-derived proteins (fluorescein isothiocyanate albumin leakage assay, [101]), or Evans Blue diffusion [102, 103]. In vivo tracing studies using genetically or fluorescently labeled leukocytes have been combined with intravital microscopic analysis to study interactions with superficial brain microvasculature [104]. Finally, to further elucidate molecular mechanisms of CNS trafficking neutralizing antibodies, small molecule inhibitors or (endothelial specific) transgenic/knockout models of trafficking determinants like adhesion molecules can be used [105].

In EAE, intravital fluorescence videomicroscopy has revealed an integrin α_4 - and P-selectin-dependent rolling of leukocytes along CNS vessels, without the involvement of E-selectin as in other tissues [106] and an integrin $\alpha_4\beta_1$ (very late antigen-4, VLA-4)-mediated, G-protein-independent capture of T cells at the endothelial cell surface [104, 107]. The integrin $\alpha_4\beta_1$ ligand, vascular cell adhesion molecule (VCAM-1), as well as intercellular adhesion molecule 1 (ICAM-1), and activated leukocyte cell adhesion molecule (ALCAM) are well-described adhesion molecules expressed on inflamed BBB endothelial cells and seem to be important for leukocyte transmigration [108, 109]. The realization that integrin $\alpha_4\beta_1$ /VCAM-1 interactions play a crucial role in leukocyte adhesion to the BBB has led to the development of natalizumab, a humanized anti- α_4 -integrin antibody, as an effective novel drug for the treatment of MS [110, 111]. More recent data indicate that $\alpha_6\beta_1$ integrin, the major receptor of laminin 4, mediates T lymphocyte migration across the endothelial basement membrane. Elimination of laminin 4 or $\alpha_6\beta_1$ integrin

resulted in reduced T cell infiltration and EAE severity [112]. Functional expression of CCL19 and CCL21 occurs in CNS venules surrounded by inflammatory cuffs, and these lymphoid chemokines have been implicated in T lymphocyte migration across the endothelial cell monolayer [113]. Previous studies have suggested that subsequent migration out of the perivascular cuff and into the CNS parenchyma requires the classical inflammatory chemokines, including CCL2 [114–116]. Further, leukocytes use focal matrix metalloproteinase (MMP-2 and MMP-9) activity to penetrate the parenchymal basement membrane [117].

Models of CD8-mediated EAE

Attention has long been focused on CD4+ T cells because susceptibility of MS is associated with MHC class II genes [118], and CD4 T cells are critical to the induction in most EAE models [119]. However, CD8+ T cells have also been implicated in MS by their higher number and invasiveness into the parenchyma compared to mainly perivascularly clustered CD4+ T cells in MS lesions [65, 120–122], their clonal expansion based on TCR analysis [123, 124], and their pathogenicity in certain EAE models [22, 125, 126], whereas in other models, CD8+ T cell may play a regulatory role and protect against the disease development [123, 127–129]. Data showing that CD8+ T cells can, in some conditions, directly attach to and transect axons support the idea that CD8+ T cells mediate damage [130].

CD4 bias after immunization

Due to the method of induction of classical EAE with CNS-derived antigen emulsified in adjuvants which favors a CD4+ T cell bias in the population of responding myelin-specific T cells, EAE is not an ideal model to investigate the contribution of CD8+ T cells to disease. The most important component in the adjuvant CFA is heat-inactivated *Mycobacterium tuberculosis*, which induces a prominent MHC-class-II-restricted CD4+ T_H1 response [131, 132].

Myelin-specific CD8+ T cells can adoptively transfer EAE, thereby establishing a pathogenic role for CD8+ T cells in EAE and providing a model for analysis of CD8+ T cells in MS. In a study by Huseby et al., adoptive transfer of MBP-specific CD8+ T cells isolated from wild-type C3H mice mediated severe CNS autoimmunity [21]. The symptoms resembled many features of MS not seen in CD4+ T-cell-mediated EAE models, such as ataxia, spasticity, hyperreflexiveness, and loss of coordinated movements. CNS demyelinating lesions predominated in the brain versus the spinal cord, which was quite distinct

from most CD4⁺ T-cell-mediated models of EAE in which the lesions are mainly found in the spinal cord. Perivascular cuffs were composed of lymphocytes, macrophages, and a few neutrophils. CD8⁺ T-cell-mediated CNS autoimmunity was independent of CD4⁺ T cells and largely inhibited by neutralizing IFN- γ activity. Two other groups successfully induced CD8⁺ T-cell-mediated EAE in C57BL/6 mice by transferring CD8⁺ T cells specific for MOG_{37–46} into syngeneic mice [22, 126].

Transgenic models (auto versus neo Ag)

Several transgenic mouse models have been developed to study the pathogenic role of CD8⁺ T cells in autoimmune CNS inflammation. Brisebois and colleagues have recently shown that endogenous CD8⁺ T cells were necessary for the development of full-blown neuroinflammation in mice overexpressing the costimulatory ligand CD86 on microglia cells [133]. The majority of T cells in the CNS of mouse mutants that overexpress PLP in oligodendrocytes expressed CD8 [134].

Other systems have employed TCR transgenic T cells recognizing natural or model brain autoantigens in an MHC-class-I-restricted manner. In GFAP–hemagglutinin (HA) \times CL4-TCR double transgenic mice, in which the viral (neoself) antigen influenza HA is specifically expressed in astrocytes and enteric glia and in which most CD8⁺ T cells are HA specific, early death due to an attack on enteric glial cells precluded the analysis of EAE development [135]. Adoptive transfer of HA-specific CD8⁺ T cells resulted in immune cell infiltration in the brains of transgenic mice, expressing HA in astrocytes, but failed to induce clinical CNS disease [136]. In H-2K-restricted MBP_{79–87} TCR transgenic mice, MBP-specific CD8⁺ T cells sufficed to induce CNS autoimmunity, but immune tolerance prevented these autoreactive cells from causing disease [137]. Transgenic mice expressing a Borna disease virus (BDV) antigen in either neurons or astrocytes were also tolerant to the neoself antigen. In this model, however, adoptive transfer of activated BDV-specific CD8⁺ T cells was sufficient to induce disease, which was attributed to the high viral antigen concentrations in the CNS [138].

Mice in which the model antigen influenza HA is selectively expressed in oligodendrocytes have been generated (DKI) to test whether autoreactive CD8⁺ T cells can contribute to the loss of oligodendrocytes as observed in MS plaques. Crossing these mice to CL4-TCR transgenic mice did not result in spontaneous neuroinflammation, and CL4-TCR transgenic CD8⁺ T cells rather seemed to remain “indifferent” to the neoself antigen sequestered in the CNS. However, transfer of preactivated HA-specific CD8⁺ T cells in DKI mice led to inflammatory lesions in the optic

nerve, spinal cord, and brain [139]. In another study, intracerebral injection of the influenza virus HA_{512–520} peptide in soluble form in CL4-TCR transgenic mice initiated CNS infiltration by the circulating HA-specific CD8⁺ T cells. It was suggested that peptide-loaded MHC class I molecules expressed on the luminal side of endothelial cells at the BBB might have triggered transmigration [140]. A similar model for CNS-specific autoimmunity employed ovalbumin (OVA) sequestered as a neoself antigen in the cytosol of oligodendrocytes. Introduction of the MHC-class-I-restricted, OVA-specific OT-I TCR as a second transgene led to spontaneous fulminant demyelinating EAE with MS-like lesions, affecting cerebellum, brainstem, optic nerve, and spinal cord. Endogenously generated OT-I TCR transgenic CD8⁺ T cells were highly encephalitogenic, which seemed to depend on a BBB, permeable for naïve CD8 T cells during the first 10 days of life and on the production of IFN- γ . In contrast, OVA-specific CD4⁺ T cells (OT-II), remained fully ignorant of the self-antigen unless OT-I TCR Tg CD8⁺ T cells first released OVA from oligodendrocytes for peripheral presentation [141]. Recently, a role for HLA-A-restricted CD8⁺ T cells in the pathogenesis of an MS-like disease was also demonstrated in humanized mouse models [142, 143].

Infection-induced CD8 expansion

CD8⁺ T cells are key mediators in the immune response to many viral infections, and a role for CD8⁺ T cells in demyelination has been illustrated in viral models of CNS inflammation. CD8⁺ T cells are essential for clearance of virus but might also become pathogenic and damage CNS tissue. Several mechanisms have been described to explain how viruses might trigger autoimmune disease including adjuvant effects and the provision of viral antigens that are similar to self-antigens and stimulate cross-reactive immune responses (molecular mimicry; reviewed in [144]). Models of molecular identity, although artificial, showed that an immune response to a viral infection could recognize identical antigens presented as self in the CNS, resulting in overt CNS autoimmune disease [145, 146]. During lymphocytic choriomeningitis virus (LCMV) infection, LCMV-specific CD8⁺ T cells expand greatly [147, 148]. Evans and colleagues generated transgenic mice that expressed the nucleoprotein or glycoprotein of LCMV as self in oligodendrocytes. After peripheral (but not CNS) infection with LCMV, the virus was efficiently cleared, but a chronic CNS autoimmune disease developed characterized by T cell inflammatory lesions with mainly CD8⁺ T cells, areas of focal myelin loss, marked upregulation of MHC class I and II molecules, and clinical motor dysfunction. Autoimmune responses in the CNS developed

despite the fact that LCMV was not detectable in the CNS, demonstrating that autoimmunity can occur at a site distal to the initiating infection [145]. In other models of molecular identity/mimicry, autoimmune disease is induced by infection with a nonpathological Theiler's murine encephalomyelitis virus (TMEV) variant that was engineered to express the immunodominant self-epitope from myelin PLP peptide (PLP_{139–151}) [146] or viral peptides that mimic PLP_{139–151} [149]. Other studies focusing on viral infections of the CNS have revealed that T cells under certain circumstances are able to bypass recognition of peptide–MHC complexes and mediate tissue destruction without TCR engagement [150, 151]. Haring and colleagues used transgenic TCR/RAG2^{-/-} mice with only T cells that are not specific for mouse hepatitis virus to show that activated CD8⁺ T cells are able to cause bystander tissue damage even in the absence of cognate antigen in the CNS [150].

Theiler virus and models of demyelination

TMEV-induced demyelinating disease

TMEV, murine hepatitis virus, and Semliki Forest virus are useful models for understanding the potential viral etiology of MS (reviewed in [152]). Intracerebral infection of susceptible strains of mice, such as SJL, with natural TMEV leads to either rapidly fatal encephalomyelitis (high-neurovirulence strains) or persistent CNS infection and immune-mediated demyelination (low-neurovirulence strains, BeAN, or DA; [153, 154]). Gait disturbances, spastic hind limb paralysis, and urinary incontinuity start 30–40 days after infection. Intracerebral injection of virus leads to persistent CNS infection. The level of infectious virus is low during the chronic phase, but abundant amounts of viral RNA and antigen can be detected throughout the lifetime of the mouse [155–157]. During the acute phase of the disease, virus replication is mainly in neurons, whereas during the chronic phase, TMEV persists predominantly in macrophages and glia [155, 158–160]. Virus particles have been identified in oligodendrocytes which have a “dying back” pathology [161]. TMEV virus is known to cause demyelination in infected nude mice that cannot generate mature T lymphocytes [162], also pointing to a direct viral effect on myelin damage.

Upon TMEV infection, a variety of chemokines and cytokines are induced in primary astrocytes via the nuclear factor κ B pathway including TNF- α , IL-1, IL-6, CCL2, and CCL5 [163–165]. Inflammatory responses to TMEV infection seem to depend on toll-like receptor (TLR)3 and TLR2 [166, 167], as well as protein kinase R, signaling

[168]. The immune response is initially directed against persistent viral antigens, but the chronic phase of the disease is dependent on de novo activation of autoimmune CD4⁺ T cell responses against CNS myelin that develop locally as a result of the release of myelin antigens, i.e., epitope spreading [26, 169–171]. In SJL mice, reactivity appears to multiple myelin peptides starting with the immunodominant epitope and spreading later to other subdominant myelin determinants in a hierarchical manner [170, 172]. Resistance to persistent CNS infection by TMEV is controlled by multiple genetic loci, with the strongest linkage to the class I H-2D MHC region [173, 174], which indicates a role for CD8⁺ T cells. Most evidence points towards a protective function of CD8⁺ T cells via the clearance of TMEV from the CNS [175–178]; however, there is also evidence that MHC-class-I-restricted CD8⁺ T might be directly cytotoxic to axons and mediate injury [179, 180].

Cuprizone and lyssolecithin, demyelination without autoimmunity

Several toxin-based models of demyelination, including cuprizone and lyssolecithin, while not attempting to accurately mimic the pathogenesis of autoimmune CNS inflammation, have proven very useful to study the mechanisms of demyelination and remyelination. In contrast to the systemically administered cuprizone, the membrane-solubilizing, glia-toxic lyssolecithin has been used to create focal areas of demyelination by direct injection into defined CNS white matter tracts, which allows for a better control of lesion size and location (reviewed in [181]).

Feeding of cuprizone (bis(cyclohexanone)-oxaldihydrazone) to young adult mice induces a consistent, synchronous, and anatomically reproducible demyelination. Furthermore, removal of cuprizone of the diet of mice leads to remyelination (reviewed in [182]). Cuprizone is a chelator that binds copper, an essential component of metalloenzymes, like the mitochondrial cytochrome oxidase and monoamine oxidase. It has been assumed although not proven that copper deficiency results in specific damage to oligodendrocytes in the CNS and subsequent demyelination. The specific susceptibility of oligodendrocytes has been attributed to the high metabolic demand of these glial cells required to maintain a vast expanse of myelin and the resulting vulnerability to a disturbed energy metabolism.

Genetic factors and gender influence susceptibility to cuprizone-induced demyelination [183]. Cuprizone can induce demyelination in different strains of mice, among them in 8–10-week-old C57BL/6 mice, the background most commonly used for knockout and transgenic mice. A 0.2% dose of cuprizone is usually well tolerated. If

cuprizone is overdosed, (mitochondrial) hepatopathy and weight loss are serious toxic effects [184]. The regional pattern of demyelination includes white matter tracts preferentially the corpus callosum, as well as the cerebellar peduncles [185]. More recently, cortical demyelination has been detected [186], and demyelination in the hippocampal formation has been associated with seizures in the cuprizone model [187, 188]. Substantial demyelination is present starting after 3 weeks of cuprizone, and by 4–5 weeks more than 90% of axons will be demyelinated. Under continued exposure of cuprizone, about 50% of axons recover and are remyelinated by 6 weeks. If cuprizone is discontinued by this time, recovery continues, so that by 10 weeks 90% of the axons are remyelinated. If, in contrast, the cuprizone challenge is retained, demyelination again dominates so that few myelinated fibers remain [182]. During cuprizone toxicity, there is also demyelination-associated axonal loss which is more prominent in aged mice [189]. Reduction in average axon caliber and some dystrophic neurites are observed [190, 191]. Demyelination/remyelination is preferentially quantitated by ultrastructural analysis performed by electron microscopy, which is best to evaluate the number of myelinated axons, thickness of the myelin sheath, and relationship to the axon size. Histochemical lipid staining with Luxol Fast Blue–periodic Schiff or immunostaining for myelin proteins allows us to estimate and screen for myelination semiquantitatively. Biochemical (lipid) assays analyze the content of the myelin sheath [192]. Magnetic resonance imaging (MRI) has been used to map the pattern of demyelination noninvasively [193]. During administration of cuprizone, the expression of myelin genes in the brain decreases, and most of the mature oligodendrocytes in the corpus callosum, the site of particular profound demyelination, undergo apoptosis [194]. Studies of changes in oligodendrocyte precursor and mature oligodendrocyte dynamics suggest that oligodendrocyte precursors drive the repopulation of oligodendrocytes in the remyelination phase [194]. Oligodendrocyte precursor cells are detectable in the corpus callosum, presumably proliferate, and convert to mature glutathione S-transferase (GST)-II⁺ oligodendrocytes during remyelination [194], sequentially expressing myelin proteins [195].

In contrast to EAE, the BBB seems to remain intact [196, 197]; T cells are almost completely absent, and microglia/macrophages accumulate and predominate in the demyelinated areas [198, 199]. Bone marrow studies indicate that resident microglia outnumber peripheral macrophages that are recruited in cuprizone-induced CNS demyelination [197]. Studies using lymphocyte-deficient RAG-1 mice have demonstrated that lymphocytes may not play a role in the cuprizone model [200, 201]. Microglia/macrophages are the main cell type responsible for clearance

of myelin debris and are able to secrete a wide variety of cytokines. Analysis of MHC-II-null mice showed reduced inflammation, delayed remyelination, and regeneration of oligodendrocytes, whereas removal of MHC I had little effect [201, 202]. Macrophage depletion by treatment with clodronate liposomes throughout the remyelination phase significantly decreases the extent of oligodendrocyte remyelination following lyssolecithin-induced demyelination [203]. Various inflammatory products, including IFN- γ , IFN- β , leukemia inhibitory factor, lymphotoxin- α , lymphotoxin- β , IL-1 β , nitric oxide, insulin-like growth factor, and macrophage inflammatory protein 1 α , have been studied in the cuprizone model and have been shown to have a deleterious or protective role [204–214]. TNF- α seems to be protective, promoting proliferation, differentiation of oligodendrocyte progenitors (bromodeoxyuridine-labeled NG2⁺ cells), and subsequent remyelination [200]. Thus, induction of MHC II by TNF- α has been suggested as an important regulatory event in remyelination, emphasizing the active inflammatory response in brain regeneration after brain injury [202].

Neuropathological changes observed during cuprizone treatment correlate with alterations in the neurological function such as hyperactive behavior and mild motor dysfunction detectable by sensitive motor tests [215, 216].

Model of lipopolysaccharide-induced demyelination

A number of studies have examined the effects of lipopolysaccharide (LPS) injections into the CNS [217–220]. Typically, a local inflammatory reaction is evoked but substantially muted when compared with similar injections in other tissues [217]. Felts et al. described an inflammatory reaction and after a delay of 5–7 days a demyelinating lesion that arise following the injection of LPS into rat spinal white matter [219, 220] and that persist between 9 and 14 days. Besides activation of resident microglia, CNS-infiltrating phagocytes (neutrophils and monocytes) seem to contribute to the inflammatory response [221], although injecting LPS or leukocyte chemotaxis into the CNS seems to induce a lower myelomonocytic recruitment compared to other tissues [217, 222]. Microglial TLR4 is required for leukocyte recruitment into the brain in response to LPS [223]. The exact mechanism of LPS-induced demyelination is unknown. In primary mixed glial cultures, LPS induces a selective loss of oligodendrocyte precursors, and activation of microglia, but not astrocytes, is required for LPS toxicity [224, 225]. Conditioned media from LPS-exposed microglia (or astrocytes) injures oligodendrocyte progenitors [226]. TLR4 is necessary for LPS-induced oligodendrocyte injury in the CNS, although mRNA for the LPS receptors TLR4/CD14 is either absent or only found at very low levels

in oligodendrocyte precursors [224]. Thus, LPS might not be directly toxic to oligodendrocytes but cause demyelination via factors derived from activated microglia/macrophages (or astrocytes). Accordingly, recent evidence suggests that micro-

glial peroxynitrite (the reaction product of nitric oxide and superoxide anion), or in the presence of astrocytes activation of TNF/TNFR signaling, play a key role in LPS-induced damage of developing oligodendrocyte in vitro [225, 227].

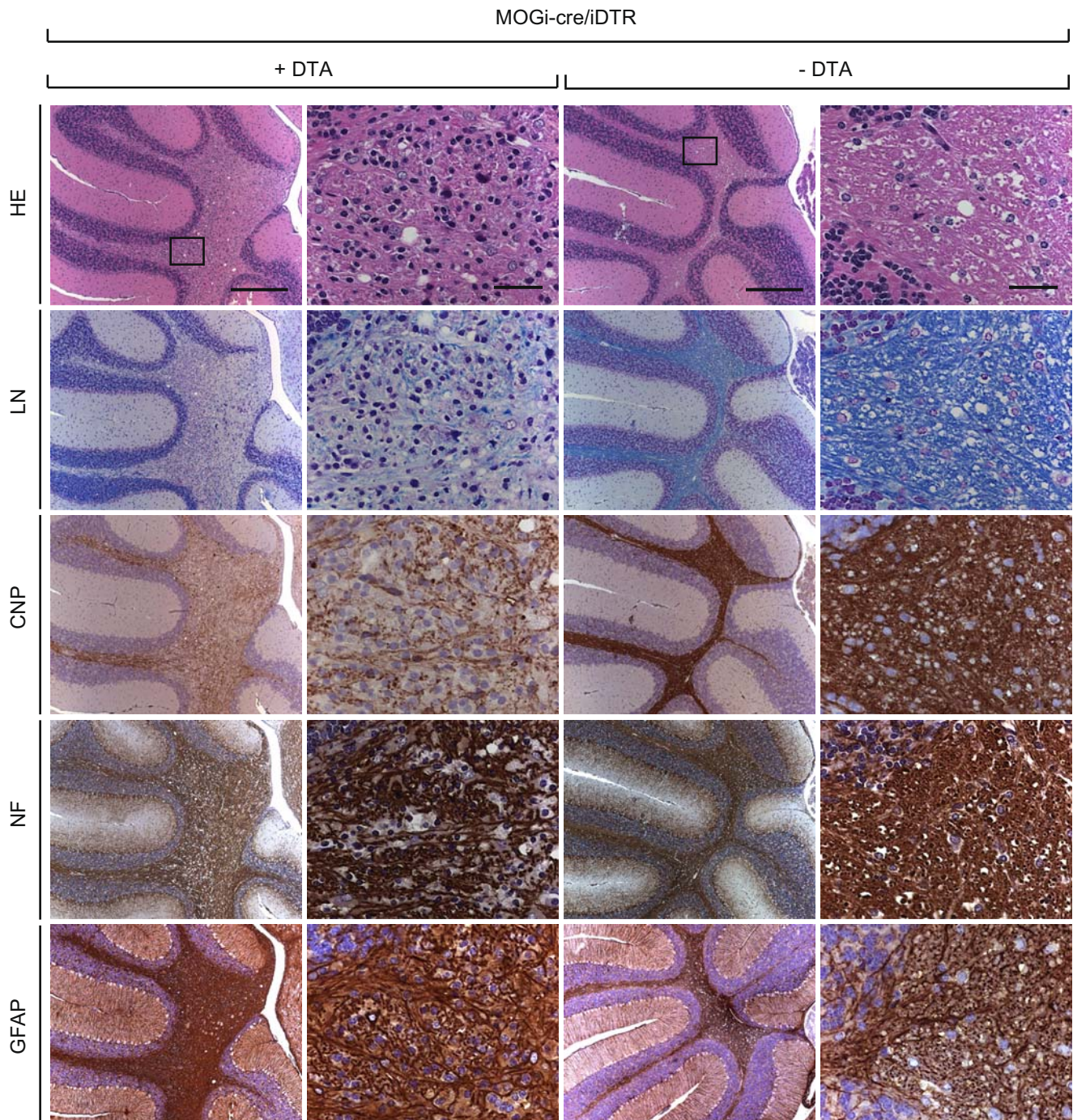
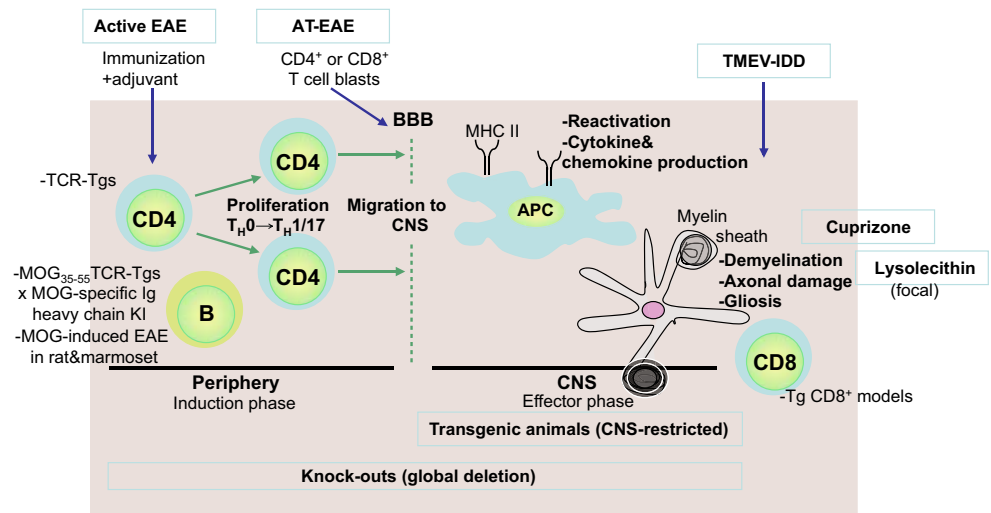


Fig. 3 DT-mediated ablation of oligodendrocytes in MOGi-Cre/iDTR mice: cerebella of DT-treated (+DT, *first* and *second* column) MOGi-Cre/iDTR mice present with a severe destruction of myelinated structures, as visualized by Luxol-Nissl (LN) and CNPase stains. Untreated (–DT) iMOG-Cre/iDTR mice (*second* and *third* column) were unaffected. Axonal integrity was affected in DT-treated iMOG-

Cre/iDTR mice (neurofilament immunohistochemistry) and some inflammatory cells (HE) and a mild reactive astrogliosis (GFAP) were visible in DT-treated iMOG-Cre/iDTR mice. *Rectangles* indicate the adjacent high-magnification image. *Scale bar* 500 μ m for *first* and *third* column; 100 μ m for *second* and *fourth* column. Adapted from [77]

Fig. 4 Induction and effector stage of MS/murine models. Abbreviations: *EAE*, experimental autoimmune encephalomyelitis; *AT*, adoptive transfer; *TMEV-IDD*, Theiler’s murine encephalomyelitis virus-induced demyelinating disease; *TCR*, T cell receptor; *Tg*, transgenic; *KI*, knock-in, *KO*, knockout; *T_H*, T helper cell; *BBB*, blood–brain barrier; *MOG*, myelin oligodendrocyte glycoprotein; *MHC*, major histocompatibility complex



oDTR mice

The means for specific in vivo cell lineage ablation have recently been improved by stable mouse lines, carrying conditional expression constructs for DT or DTR that could be activated upon Cre-mediated recombination and the application of DT, respectively [77, 228]. In Cre-inducible DTR transgenic mice (iDTR) Cre-mediated excision of a STOP cassette renders cells sensitive to DT. DT crosses the BBB and promotes cell ablation in the CNS. Injecting DT intraperitoneally into MOGi-Cre/iDTR double transgenic mice expressing Cre recombinase under the control of the oligodendrocyte-specific promoter MOG results in a severe myelin loss throughout the CNS [77]. Axonal integrity subsequently also appears to be affected, and some reactive astrogliosis as well as inflammatory cells are seen (Fig. 3). After approximately 30 days, the DT-treated MOGi-Cre/iDTR mice present clinically with tremor, hind limb paralysis, and weight loss (Fig. 4; Table 1).

Concluding remarks

Taken together, there is now a plethora of different animal models available, each of them serving the purpose to study specific aspects of the pathogenesis of MS in rodents. Whereas these models are most useful and have provided a wealth of knowledge otherwise unavailable to us, there are a number of limitations in regards to interpreting the data. A number of questions cannot be directly answered in animal models including the initial trigger of the MS pathogenesis (vaccination with autoantigen is hardly to blame), the driving self-Ag, the genetic predisposition, and others. Therefore, none of the described animal models can truly serve for drug testing or replace clinical trials. To this day, the animal models described here are the state-of-the-art and the very best modeling system available to us. We need to be cautious not to overinterpret the findings and to continue to create better models to accurately study certain aspects of the pathogenesis of neuroinflammatory disease.

Table 1 Commonly used MS models

Model	Mode of induction	Pathomechanism	Phenotype
EAE	-Immunization	CD4 ⁺ T-cell-mediated	Myelitis (>cerebellitis)
	-Adoptive transfer (CD4 ⁺)	”	”
	-Spontaneous → (humanized) TCR Tgs (MHCII-restricted)	”	”
CD8 ⁺ AT	•2D2 MOG ₃₅₋₅₅ TCR Tgs × MOG-specific IgH KI	CD4 ⁺ T/B-cell-mediated	Optic neuritis [33]
	-In rats/marmosets (MOG) [in (conditional) Tg/KO mice]	T cell/antibody-mediated	Devic-like disease [34]
	-Adoptive transfer (CD8 ⁺)	CD8 ⁺ T-cell-mediated	Encephalomyelitis
TMEV-IDD	•TCR Tgs (MHCI-restricted) × auto/neo brain ag [spontaneous]	”	Encephalomyelitis
	-Intracerebral infection	Virus-induced demyelination	Encephalomyelitis
Cuprizone	-Feeding	Toxin-based demyelination	Corpus callosum, cerebellar peduncles [185]
Lysolecithin	-Local CNS injection	”	Focal white matter lesion

References

1. Olitsky PK, Yager RH (1949) Experimental disseminated encephalomyelitis in white mice. *J Exp Med* 90:213–224
2. Brown AM, McFarlin DE (1981) Relapsing experimental allergic encephalomyelitis in the SJL/J mouse. *Lab Invest* 45:278–284
3. Fritz RB, Chou CH, McFarlin DE (1983) Induction of experimental allergic encephalomyelitis in PL/J and (SJL/J x PL/J)F1 mice by myelin basic protein and its peptides: localization of a second encephalitogenic determinant. *J Immunol* 130:191–194
4. Trotter JL, Clark HB, Collins KG, Wegeschiede CL, Scarpellini JD (1987) Myelin proteolipid protein induces demyelinating disease in mice. *J Neurol Sci* 79:173–188
5. Linthicum DS, Frelinger JA (1982) Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. *J Exp Med* 156:31–40
6. Kerfoot SM et al (2004) TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. *J Immunol* 173:7070–7077
7. Kamradt T, Soloway PD, Perkins DL, Gefer ML (1991) Pertussis toxin prevents the induction of peripheral T cell anergy and enhances the T cell response to an encephalitogenic peptide of myelin basic protein. *J Immunol* 147:3296–3302
8. Waldner H, Collins M, Kuchroo VK (2004) Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. *J Clin Invest* 113:990–997
9. Shive CL, Hofstetter H, Arredondo L, Shaw C, Forsthuber TG (2000) The enhanced antigen-specific production of cytokines induced by pertussis toxin is due to clonal expansion of T cells and not to altered effector functions of long-term memory cells. *Eur J Immunol* 30:2422–2431
10. Muller DM, Pender MP, Greer JM (2000) A neuropathological analysis of experimental autoimmune encephalomyelitis with predominant brain stem and cerebellar involvement and differences between active and passive induction. *Acta Neuropathol* 100:174–182
11. Abromson-Leeman S et al (2004) T-cell properties determine disease site, clinical presentation, and cellular pathology of experimental autoimmune encephalomyelitis. *Am J Pathol* 165:1519–1533
12. Teuscher C et al (2004) Gender, age, and season at immunization uniquely influence the genetic control of susceptibility to histopathological lesions and clinical signs of experimental allergic encephalomyelitis: implications for the genetics of multiple sclerosis. *Am J Pathol* 165:1593–1602
13. Smith ME, Eller NL, McFarland HF, Racke MK, Raine CS (1999) Age dependence of clinical and pathological manifestations of autoimmune demyelination. Implications for multiple sclerosis. *Am J Pathol* 155:1147–1161
14. Fillmore PD et al (2003) Genetic analysis of the influence of neuroantigen-complete Freund's adjuvant emulsion structures on the sexual dimorphism and susceptibility to experimental allergic encephalomyelitis. *Am J Pathol* 163:1623–1632
15. Maatta JA, Nygardas PT, Hinkkanen AE (2000) Enhancement of experimental autoimmune encephalomyelitis severity by ultrasound emulsification of antigen/adjuvant in distinct strains of mice. *Scand J Immunol* 51:87–90
16. Oliver AR, Lyon GM, Ruddle NH (2003) Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice. *J Immunol* 171:462–468
17. Sobel RA (2000) Genetic and epigenetic influence on EAE phenotypes induced with different encephalitogenic peptides. *J Neuroimmunol* 108:45–52
18. Tsunoda I, Kuang LQ, Theil DJ, Fujinami RS (2000) Antibody association with a novel model for primary progressive multiple sclerosis: induction of relapsing–remitting and progressive forms of EAE in H2s mouse strains. *Brain Pathol* 10:402–418
19. Pettinelli CB, McFarlin DE (1981) Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement for Lyt 1+ 2– T lymphocytes. *J Immunol* 127:1420–1423
20. Zamvil S et al (1985) T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature* 317:355–358
21. Huseby ES et al (2001) A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J Exp Med* 194:669–676
22. Sun D et al (2001) Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 166:7579–7587
23. Langrish CL et al (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201:233–240
24. Stromnes IM, Cerretti LM, Liggitt D, Harris RA, Goverman JM (2008) Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat Med* 14:337–342
25. Flugel A et al (2001) Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. *Immunity* 14:547–560
26. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD (2005) Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat Med* 11:335–339
27. Lafaille JJ, Nagashima K, Katsuki M, Tonegawa S (1994) High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell* 78:399–408
28. Olivares-Villagomez D, Wang Y, Lafaille JJ (1998) Regulatory CD4(+) T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. *J Exp Med* 188:1883–1894
29. Madsen LS et al (1999) A humanized model for multiple sclerosis using HLA-DR2 and a human T-cell receptor. *Nat Genet* 23:343–347
30. Ellmerich S et al (2005) High incidence of spontaneous disease in an HLA-DR15 and TCR transgenic multiple sclerosis model. *J Immunol* 174:1938–1946
31. Quandt JA et al (2004) Unique clinical and pathological features in HLA-DRB1*0401-restricted MBP 111–129-specific humanized TCR transgenic mice. *J Exp Med* 200:223–234
32. Waldner H, Whitters MJ, Sobel RA, Collins M, Kuchroo VK (2000) Fulminant spontaneous autoimmunity of the central nervous system in mice transgenic for the myelin proteolipid protein-specific T cell receptor. *Proc Natl Acad Sci U S A* 97:3412–3417
33. Bettelli E et al (2003) Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J Exp Med* 197:1073–1081
34. Bettelli E, Baeten D, Jager A, Sobel RA, Kuchroo VK (2006) Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. *J Clin Invest* 116:2393–2402
35. Krishnamoorthy G, Lassmann H, Wekerle H, Holz A (2006) Spontaneous optico-spinal encephalomyelitis in a double-transgenic mouse model of autoimmune T cell/B cell cooperation. *J Clin Invest* 116:2385–2392
36. Waldor MK et al (1985) Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. *Science* 227:415–417

37. Sriram S, Steinman L (1983) Anti I-A antibody suppresses active encephalomyelitis: treatment model for diseases linked to IR genes. *J Exp Med* 158:1362–1367
38. Jameson BA, McDonnell JM, Marini JC, Korngold R (1994) A rationally designed CD4 analogue inhibits experimental allergic encephalomyelitis. *Nature* 368:744–746
39. Seamons A, Perchellet A, Goverman J (2003) Immune tolerance to myelin proteins. *Immunol Res* 28:201–221
40. Tompkins SM et al (2002) De novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. *J Immunol* 168:4173–4183
41. Hickey WF, Kimura H (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* 239:290–292
42. Greter M et al (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med* 11:328–334
43. Becher B, Bechmann I, Greter M (2006) Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain. *J Mol Med* 84:532–543
44. Becher B, Prat A, Antel JP (2000) Brain-immune connection: immuno-regulatory properties of CNS-resident cells. *Glia* 29:293–304
45. Heppner FL et al (2005) Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat Med* 11:146–152
46. Becher B, Durell BG, Miga AV, Hickey WF, Noelle RJ (2001) The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. *J Exp Med* 193:967–974
47. Becher B, Durell BG, Noelle RJ (2003) IL-23 produced by CNS-resident cells controls T cell encephalitogenicity during the effector phase of experimental autoimmune encephalomyelitis. *J Clin Invest* 112:1186–1191
48. Prinz M et al (2006) Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. *J Clin Invest* 116:456–464
49. De Keyser J, Zeinstra E, Frohman E (2003) Are astrocytes central players in the pathophysiology of multiple sclerosis? *Arch Neurol* 60:132–136
50. Ridet JL, Malhotra SK, Privat A, Gage FH (1997) Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci* 20:570–577
51. Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 6:626–640
52. Bailey SL, Schreiner B, McMahon EJ, Miller SD (2007) CNS myeloid DCs presenting endogenous myelin peptides ‘preferentially’ polarize CD4⁺ T(H)-17 cells in relapsing EAE. *Nat Immunol* 8:172–180
53. McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD (1995) Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J Exp Med* 182:75–85
54. Vanderlugt CL et al (2000) Pathologic role and temporal appearance of newly emerging autoepitopes in relapsing experimental autoimmune encephalomyelitis. *J Immunol* 164:670–678
55. Waksman BH, Adams RD (1956) A comparative study of experimental allergic neuritis in the rabbit, guinea pig, and mouse. *J Neuropathol Exp Neurol* 15:293–334
56. Waksman BH (1960) The distribution of experimental auto-allergic lesions. Its relation to the distribution of small veins. *Am J Pathol* 37:673–693
57. Lebar R, Boutry JM, Vincent C, Robineaux R, Voisin GA (1976) Studies on autoimmune encephalomyelitis in the guinea pig. II. An in vitro investigation on the nature, properties, and specificity of the serum-demyelinating factor. *J Immunol* 116:1439–1446
58. Genain CP et al (1995) Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. *J Clin Invest* 96:2966–2974
59. Schluesener HJ, Sobel RA, Lington C, Weiner HL (1987) A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J Immunol* 139:4016–4021
60. Hart BA et al (2004) Modelling of multiple sclerosis: lessons learned in a non-human primate. *Lancet Neurol* 3:588–597
61. Genain CP et al (1994) In healthy primates, circulating autoreactive T cells mediate autoimmune disease. *J Clin Invest* 94:1339–1345
62. Baxter AG (2007) The origin and application of experimental autoimmune encephalomyelitis. *Nat Rev Immunol* 7:904–912
63. Bruck W, Kuhlmann T, Stadelmann C (2003) Remyelination in multiple sclerosis. *J Neurol Sci* 206:181–185
64. Lucchinetti C et al (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 47:707–717
65. Kuhlmann T, Lassmann H, Bruck W (2008) Diagnosis of inflammatory demyelination in biopsy specimens: a practical approach. *Acta Neuropathol* 115:275–287
66. Miller SD, Karpus WJ (2007) Experimental autoimmune encephalomyelitis in the mouse. *Curr Protoc Immunol* 15:15.1
67. Brodmerkel CM, Vaddi K (2003) Transgenic animals in inflammatory disease models. *Curr Opin Biotechnol* 14:652–658
68. Owens T, Wekerle H, Antel J (2001) Genetic models for CNS inflammation. *Nat Med* 7:161–166
69. Morel L (2004) Mouse models of human autoimmune diseases: essential tools that require the proper controls. *PLoS Biol* 2:E241
70. Leiter EH (2002) Mice with targeted gene disruptions or gene insertions for diabetes research: problems, pitfalls, and potential solutions. *Diabetologia* 45:296–308
71. Steinman L (1997) Some misconceptions about understanding autoimmunity through experiments with knockouts. *J Exp Med* 185:2039–2041
72. Yu Y, Bradley A (2001) Engineering chromosomal rearrangements in mice. *Nat Rev Genet* 2:780–790
73. Thomas MK et al (2001) Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. *J Clin Invest* 108:319–329
74. Hirrlinger PG, Scheller A, Braun C, Hirrlinger J, Kirchhoff F (2006) Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. *Glia* 54:11–20
75. Tronche F et al (1999) Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* 23:99–103
76. Krishnamoorthy G, Holz A, Wekerle H (2007) Experimental models of spontaneous autoimmune disease in the central nervous system. *J Mol Med* 85:1161–1173
77. Buch T et al (2005) A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat Methods* 2:419–426
78. Luo J et al (2007) Glia-dependent TGF-beta signaling, acting independently of the TH17 pathway, is critical for initiation of murine autoimmune encephalomyelitis. *J Clin Invest* 117:3306–3315
79. Mohrs M, Shinkai K, Mohrs K, Locksley RM (2001) Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* 15:303–311
80. Bettelli E et al (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441:235–238
81. Fontenot JD et al (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 22:329–341

82. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136:2348–2357
83. Gutcher I, Becher B (2007) APC-derived cytokines and T cell polarization in autoimmune inflammation. *J Clin Invest* 117:1119–1127
84. Comabella M et al (1998) Elevated interleukin-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy. *J Clin Invest* 102:671–678
85. O'Garra A, Steinman L, Gijbels K (1997) CD4+ T-cell subsets in autoimmunity. *Curr Opin Immunol* 9:872–883
86. Willenborg DO, Fordham S, Bernard CC, Cowden WB, Ramshaw IA (1996) IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J Immunol* 157:3223–3227
87. Becher B, Durell BG, Noelle RJ (2002) Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J Clin Invest* 110:493–497
88. Gutcher I, Urich E, Wolter K, Prinz M, Becher B (2006) Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation. *Nat Immunol* 7:946–953
89. Frei K et al (1997) Tumor necrosis factor alpha and lymphotoxin alpha are not required for induction of acute experimental autoimmune encephalomyelitis. *J Exp Med* 185:2177–2182
90. Gran B et al (2002) IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. *J Immunol* 169:7104–7110
91. Cua DJ et al (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421:744–748
92. Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. *Annu Rev Immunol* 27:485–517
93. Hofstetter HH et al (2005) Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. *Cell Immunol* 237:123–130
94. Komiyama Y et al (2006) IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 177:566–573
95. Haak S et al (2009) IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. *J Clin Invest* 119:61–69
96. Kreymborg K et al (2007) IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* 179:8098–8104
97. McGeachy MJ et al (2009) The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 10:314–324
98. Gyölvézi G, Haak S, Becher B (2009) *Eur J Immunol* 39(7): 1864–1869
99. Hickey WF (2001) Basic principles of immunological surveillance of the normal central nervous system. *Glia* 36:118–124
100. Bechmann I, Galea I, Perry VH (2007) What is the blood–brain barrier (not)? *Trends Immunol* 28:5–11
101. Suidan GL, McDole JR, Chen Y, Pirko I, Johnson AJ (2008) Induction of blood brain barrier tight junction protein alterations by CD8 T cells. *PLoS ONE* 3:e3037
102. Parathath SR, Parathath S, Tsirka SE (2006) Nitric oxide mediates neurodegeneration and breakdown of the blood–brain barrier in tPA-dependent excitotoxic injury in mice. *J Cell Sci* 119:339–349
103. Yepes M et al (2003) Tissue-type plasminogen activator induces opening of the blood–brain barrier via the LDL receptor-related protein. *J Clin Invest* 112:1533–1540
104. Coisne C, Mao W, Engelhardt B (2009) Cutting edge: natalizumab blocks adhesion but not initial contact of human T cells to the blood–brain barrier in vivo in an animal model of multiple sclerosis. *J Immunol* 182:5909–5913
105. Doring A, Wild M, Vestweber D, Deutsch U, Engelhardt B (2007) E- and P-selectin are not required for the development of experimental autoimmune encephalomyelitis in C57BL/6 and SJL mice. *J Immunol* 179:8470–8479
106. Kerfoot SM, Kubes P (2002) Overlapping roles of P-selectin and alpha 4 integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis. *J Immunol* 169:1000–1006
107. Vajkoczy P, Laschinger M, Engelhardt B (2001) Alpha4-integrin-VCAM-1 binding mediates G protein-independent capture of encephalitogenic T cell blasts to CNS white matter microvessels. *J Clin Invest* 108:557–565
108. Bullard DC et al (2007) Intercellular adhesion molecule-1 expression is required on multiple cell types for the development of experimental autoimmune encephalomyelitis. *J Immunol* 178:851–857
109. Cayrol R et al (2008) Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat Immunol* 9:137–145
110. Polman CH et al (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 354:899–910
111. Rudick RA et al (2006) Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med* 354:911–923
112. Wu C et al (2009) Endothelial basement membrane laminin alpha5 selectively inhibits T lymphocyte extravasation into the brain. *Nat Med* 15:519–527
113. Alt C, Laschinger M, Engelhardt B (2002) Functional expression of the lymphoid chemokines CCL19 (ELC) and CCL 21 (SLC) at the blood–brain barrier suggests their involvement in G-protein-dependent lymphocyte recruitment into the central nervous system during experimental autoimmune encephalomyelitis. *Eur J Immunol* 32:2133–2144
114. Toft-Hansen H et al (2006) Metalloproteinases control brain inflammation induced by pertussis toxin in mice overexpressing the chemokine CCL2 in the central nervous system. *J Immunol* 177:7242–7249
115. Babcock AA, Kuziel WA, Rivest S, Owens T (2003) Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J Neurosci* 23:7922–7930
116. Glabinski AR, Tani M, Tuohy VK, Tuthill RJ, Ransohoff RM (1995) Central nervous system chemokine mRNA accumulation follows initial leukocyte entry at the onset of acute murine experimental autoimmune encephalomyelitis. *Brain Behav Immun* 9:315–330
117. Agrawal S et al (2006) Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. *J Exp Med* 203:1007–1019
118. Hafler DA et al (2007) Risk alleles for multiple sclerosis identified by a genome wide study. *N Engl J Med* 357:851–862
119. Steinman L (1996) Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 85:299–302
120. Hauser SL et al (1986) Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* 19:578–587
121. Booss J, Esiri MM, Tourtellotte WW, Mason DY (1983) Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J Neurol Sci* 62:219–232
122. Crawford MP et al (2004) High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood* 103:4222–4231

123. Jiang H, Braunstein NS, Yu B, Winchester R, Chess L (2001) CD8+ T cells control the TH phenotype of MBP-reactive CD4+ T cells in EAE mice. *Proc Natl Acad Sci U S A* 98:6301–6306
124. Babbe H et al (2000) Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 192:393–404
125. Ji Q, Goverman J (2007) Experimental autoimmune encephalomyelitis mediated by CD8+ T cells. *Ann N Y Acad Sci* 1103:157–166
126. Ford ML, Evavold BD (2005) Specificity, magnitude, and kinetics of MOG-specific CD8+ T cell responses during experimental autoimmune encephalomyelitis. *Eur J Immunol* 35:76–85
127. Najafian N et al (2003) Regulatory functions of CD8+CD28– T cells in an autoimmune disease model. *J Clin Invest* 112:1037–1048
128. Jiang H et al (2003) Regulatory CD8+ T cells fine-tune the myelin basic protein-reactive T cell receptor V beta repertoire during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 100:8378–8383
129. Jiang H, Zhang SI, Pernis B (1992) Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science* 256:1213–1215
130. Medana I, Martinic MA, Wekerle H, Neumann H (2001) Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. *Am J Pathol* 159:809–815
131. Su SB et al (2005) Essential role of the MyD88 pathway, but nonessential roles of TLRs 2, 4, and 9, in the adjuvant effect promoting Th1-mediated autoimmunity. *J Immunol* 175:6303–6310
132. Yip HC et al (1999) Adjuvant-guided type-1 and type-2 immunity: infectious/noninfectious dichotomy defines the class of response. *J Immunol* 162:3942–3949
133. Brisebois M, Zehntner SP, Estrada J, Owens T, Fournier S (2006) A pathogenic role for CD8+ T cells in a spontaneous model of demyelinating disease. *J Immunol* 177:2403–2411
134. Ip CW et al (2006) Immune cells contribute to myelin degeneration and axonopathic changes in mice overexpressing proteolipid protein in oligodendrocytes. *J Neurosci* 26:8206–8216
135. Cornet A et al (2001) Enterocolitis induced by autoimmune targeting of enteric glial cells: a possible mechanism in Crohn's disease? *Proc Natl Acad Sci U S A* 98:13306–13311
136. Cabarrocas J, Bauer J, Piaggio E, Liblau R, Lassmann H (2003) Effective and selective immune surveillance of the brain by MHC class I-restricted cytotoxic T lymphocytes. *Eur J Immunol* 33:1174–1182
137. Perchellet A, Stromnes I, Pang JM, Goverman J (2004) CD8+ T cells maintain tolerance to myelin basic protein by 'epitope theft'. *Nat Immunol* 5:606–614
138. Baur K et al (2008) Antiviral CD8 T cells recognize Borna disease virus antigen transgenically expressed in either neurons or astrocytes. *J Virol* 82:3099–3108
139. Saxena A et al (2008) Cutting edge: multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. *J Immunol* 181:1617–1621
140. Galea I et al (2007) An antigen-specific pathway for CD8 T cells across the blood–brain barrier. *J Exp Med* 204:2023–2030
141. Na SY et al (2008) Naive CD8 T-cells initiate spontaneous autoimmunity to a sequestered model antigen of the central nervous system. *Brain* 131:2353–2365
142. Mars LT et al (2007) CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A*0201-transgenic mice. *J Immunol* 179:5090–5098
143. Friese MA et al (2008) Opposing effects of HLA class I molecules in tuning autoreactive CD8+ T cells in multiple sclerosis. *Nat Med* 14:1227–1235
144. Munz C, Lunemann JD, Getts MT, Miller SD (2009) Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat Rev Immunol* 9:246–258
145. Evans CF, Horwitz MS, Hobbs MV, Oldstone MB (1996) Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. *J Exp Med* 184:2371–2384
146. Olson JK, Croxford JL, Calenoff MA, Dal Canto MC, Miller SD (2001) A virus-induced molecular mimicry model of multiple sclerosis. *J Clin Invest* 108:311–8
147. Butz EA, Bevan MJ (1998) Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. *Immunity* 8:167–175
148. Murali-Krishna K et al (1998) Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 8:177–187
149. Croxford JL, Ercolini AM, Degutes M, Miller SD (2006) Structural requirements for initiation of cross-reactivity and CNS autoimmunity with a PLP139–151 mimic peptide derived from murine hepatitis virus. *Eur J Immunol* 36:2671–2680
150. Haring JS, Pewe LL, Perlman S (2002) Bystander CD8 T cell-mediated demyelination after viral infection of the central nervous system. *J Immunol* 169:1550–1555
151. Dandekar AA, Anghelina D, Perlman S (2004) Bystander CD8 T-cell-mediated demyelination is interferon-gamma-dependent in a coronavirus model of multiple sclerosis. *Am J Pathol* 164:363–369
152. Ercolini AM, Miller SD (2009) The role of infections in autoimmune disease. *Clin Exp Immunol* 155:1–15
153. Dal Canto MC, Lipton HL (1975) Primary demyelination in Theiler's virus infection. An ultrastructural study. *Lab Invest* 33:626–637
154. Lipton HL (1975) Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. *Infect Immun* 11:1147–1155
155. Dal Canto MC, Lipton HL (1982) Ultrastructural immunohistochemical localization of virus in acute and chronic demyelinating Theiler's virus infection. *Am J Pathol* 106:20–29
156. Lipton HL, Twaddle G, Jelachich ML (1995) The predominant virus antigen burden is present in macrophages in Theiler's murine encephalomyelitis virus-induced demyelinating disease. *J Virol* 69:2525–2533
157. Trottier M, Kallio P, Wang W, Lipton HL (2001) High numbers of viral RNA copies in the central nervous system of mice during persistent infection with Theiler's virus. *J Virol* 75:7420–7428
158. Aubert C, Chamorro M, Brahic M (1987) Identification of Theiler's virus infected cells in the central nervous system of the mouse during demyelinating disease. *Microb Pathog* 3:319–326
159. Rodriguez M, Leibowitz JL, Lampert PW (1983) Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. *Ann Neurol* 13:426–433
160. Zheng L, Calenoff MA, Dal Canto MC (2001) Astrocytes, not microglia, are the main cells responsible for viral persistence in Theiler's murine encephalomyelitis virus infection leading to demyelination. *J Neuroimmunol* 118:256–267
161. Rodriguez M (1985) Virus-induced demyelination in mice: "dying back" of oligodendrocytes. *Mayo Clin Proc* 60:433–438
162. Roos RP, Wollmann R (1984) DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude mice. *Ann Neurol* 15:494–499
163. Kang MH, So EY, Park H, Kim BS (2008) Replication of Theiler's virus requires NF-kappa B-activation: higher viral replication and spreading in astrocytes from susceptible mice. *Glia* 56:942–953
164. Palma JP, Kim BS (2004) The scope and activation mechanisms of chemokine gene expression in primary astrocytes following infection with Theiler's virus. *J Neuroimmunol* 149:121–129
165. Palma JP, Kwon D, Clipstone NA, Kim BS (2003) Infection with Theiler's murine encephalomyelitis virus directly induces proin-

- flammatory cytokines in primary astrocytes via NF-kappa B activation: potential role for the initiation of demyelinating disease. *J Virol* 77:6322–6331
166. So EY, Kang MH, Kim BS (2006) Induction of chemokine and cytokine genes in astrocytes following infection with Theiler's murine encephalomyelitis virus is mediated by the Toll-like receptor 3. *Glia* 53:858–867
 167. So EY, Kim BS (2009) Theiler's virus infection induces TLR3-dependent upregulation of TLR2 critical for proinflammatory cytokine production. *Glia* 57:1216–1226
 168. Carpentier PA, Williams BR, Miller SD (2007) Distinct roles of protein kinase R and toll-like receptor 3 in the activation of astrocytes by viral stimuli. *Glia* 55:239–252
 169. Croxford JL, Olson JK, Miller SD (2002) Epitope spreading and molecular mimicry as triggers of autoimmunity in the Theiler's virus-induced demyelinating disease model of multiple sclerosis. *Autoimmun Rev* 1:251–260
 170. Miller SD et al (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat Med* 3:1133–1136
 171. Vanderlugt CL, Miller SD (2002) Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat Rev Immunol* 2:85–95
 172. Katz-Levy Y et al (2000) Temporal development of autoreactive Th1 responses and endogenous presentation of self myelin epitopes by central nervous system-resident APCs in Theiler's virus-infected mice. *J Immunol* 165:5304–5314
 173. Brahic M, Bureau JF, Michiels T (2005) The genetics of the persistent infection and demyelinating disease caused by Theiler's virus. *Annu Rev Microbiol* 59:279–298
 174. Lipton HL, Melvold R (1984) Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. *J Immunol* 132:1821–1825
 175. Borrow P, Tonks P, Welsh CJ, Nash AA (1992) The role of CD8+T cells in the acute and chronic phases of Theiler's murine encephalomyelitis virus-induced disease in mice. *J Gen Virol* 73 (Pt 7):1861–1865
 176. Fiette L, Aubert C, Brahic M, Rossi CP (1993) Theiler's virus infection of beta 2-microglobulin-deficient mice. *J Virol* 67:589–592
 177. Ure DR, Rodriguez M (2002) Preservation of neurologic function during inflammatory demyelination correlates with axon sparing in a mouse model of multiple sclerosis. *Neuroscience* 111:399–411
 178. Begolka WS et al (2001) CD8-deficient SJL mice display enhanced susceptibility to Theiler's virus infection and increased demyelinating pathology. *J Neurovirol* 7:409–420
 179. Howe CL et al (2007) CD8+ T cells directed against a viral peptide contribute to loss of motor function by disrupting axonal transport in a viral model of fulminant demyelination. *J Neuroimmunol* 188:13–21
 180. Howe CL, Adelson JD, Rodriguez M (2007) Absence of perforin expression confers axonal protection despite demyelination. *Neurobiol Dis* 25:354–359
 181. Woodruff RH, Franklin RJ (1999) Demyelination and remyelination of the caudal cerebellar peduncle of adult rats following stereotaxic injections of lysolecithin, ethidium bromide, and complement/anti-galactocerebroside: a comparative study. *Glia* 25:216–228
 182. Matsushima GK, Morell P (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol* 11:107–116
 183. Taylor LC, Gilmore W, Matsushima GK (2008) SJL mice exposed to cuprizone intoxication reveal strain and gender pattern differences in demyelination. *Brain Pathol* 19:467–479
 184. Suzuki K (1969) Giant hepatic mitochondria: production in mice fed with cuprizone. *Science* 163:81–82
 185. Blakemore WF (1973) Remyelination of the superior cerebellar peduncle in the mouse following demyelination induced by feeding cuprizone. *J Neurol Sci* 20:73–83
 186. Skripuletz T et al (2008) Cortical demyelination is prominent in the murine cuprizone model and is strain-dependent. *Am J Pathol* 172:1053–1061
 187. Hoffmann K, Lindner M, Groticke I, Stangel M, Loscher W (2008) Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in C57BL/6 mice. *Exp Neurol* 210:308–321
 188. Koutsoudaki PN et al (2009) Demyelination of the hippocampus is prominent in the cuprizone model. *Neurosci Lett* 451:83–88
 189. Irvine KA, Blakemore WF (2006) Age increases axon loss associated with primary demyelination in cuprizone-induced demyelination in C57BL/6 mice. *J Neuroimmunol* 175:69–76
 190. Mason JL, Langaman C, Morell P, Suzuki K, Matsushima GK (2001) Episodic demyelination and subsequent remyelination within the murine central nervous system: changes in axonal calibre. *Neuropathol Appl Neurobiol* 27:50–58
 191. Irvine KA, Blakemore WF (2008) Remyelination protects axons from demyelination-associated axon degeneration. *Brain* 131:1464–1477
 192. Jurevics H et al (2001) Cerebroside synthesis as a measure of the rate of remyelination following cuprizone-induced demyelination in brain. *J Neurochem* 77:1067–1076
 193. Wu QZ et al (2008) MRI identification of the rostral-caudal pattern of pathology within the corpus callosum in the cuprizone mouse model. *J Magn Reson Imaging* 27:446–453
 194. Mason JL et al (2000) Mature oligodendrocyte apoptosis precedes IGF-1 production and oligodendrocyte progenitor accumulation and differentiation during demyelination/remyelination. *J Neurosci Res* 61:251–262
 195. Lindner M et al (2008) Sequential myelin protein expression during remyelination reveals fast and efficient repair after central nervous system demyelination. *Neuropathol Appl Neurobiol* 34:105–114
 196. Kondo A, Nakano T, Suzuki K (1987) Blood-brain barrier permeability to horseradish peroxidase in twitcher and cuprizone-intoxicated mice. *Brain Res* 425:186–190
 197. McMahon EJ, Suzuki K, Matsushima GK (2002) Peripheral macrophage recruitment in cuprizone-induced CNS demyelination despite an intact blood-brain barrier. *J Neuroimmunol* 130:32–45
 198. Hiremath MM et al (1998) Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *J Neuroimmunol* 92:38–49
 199. Remington LT, Babcock AA, Zehntner SP, Owens T (2007) Microglial recruitment, activation, and proliferation in response to primary demyelination. *Am J Pathol* 170:1713–1724
 200. Arnett HA et al (2001) TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci* 4:1116–1122
 201. Hiremath MM, Chen VS, Suzuki K, Ting JP, Matsushima GK (2008) MHC class II exacerbates demyelination in vivo independently of T cells. *J Neuroimmunol* 203:23–32
 202. Arnett HA, Wang Y, Matsushima GK, Suzuki K, Ting JP (2003) Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. *J Neurosci* 23:9824–9832
 203. Kotter MR, Setzu A, Sim FJ, Van Rooijen N, Franklin RJ (2001) Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. *Glia* 35:204–212
 204. Mana P, Linares D, Fordham S, Staykova M, Willenborg D (2006) Deleterious role of IFN gamma in a toxic model of central nervous system demyelination. *Am J Pathol* 168:1464–1473

205. Lin W et al (2006) Interferon-gamma inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. *Brain* 129:1306–1318
206. Trebst C et al (2007) Lack of interferon-beta leads to accelerated remyelination in a toxic model of central nervous system demyelination. *Acta Neuropathol* 114:587–596
207. Marriott MP et al (2008) Leukemia inhibitory factor signaling modulates both central nervous system demyelination and myelin repair. *Glia* 56:686–698
208. Plant SR, Arnett HA, Ting JP (2005) Astroglial-derived lymphotoxin-alpha exacerbates inflammation and demyelination, but not remyelination. *Glia* 49:1–14
209. Plant SR et al (2007) Lymphotoxin beta receptor (Lt beta R): dual roles in demyelination and remyelination and successful therapeutic intervention using Lt beta R-Ig protein. *J Neurosci* 27:7429–7437
210. Mason JL, Suzuki K, Chaplin DD, Matsushima GK (2001) Interleukin-1 beta promotes repair of the CNS. *J Neurosci* 21(18):7046–7052
211. Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE (2003) Insulin-like growth factor (IGF) signaling through type I IGF receptor plays an important role in remyelination. *J Neurosci* 23(20):7710–7718
212. Linares D, Taconis M, Mana P, Correcha M, Fordham S, Staykova M, Willenborg DO (2006) Neuronal nitric oxide synthase plays a key role in CNS demyelination. *J Neurosci* 26(49):12672–12681
213. Arnett HA, Hellendall RP, Matsushima GK, Suzuki K, Laubach VE, Sherman P, Ting JP (2002) The protective role of nitric oxide in a neurotoxicant-induced demyelinating model. *J Immunol* 168(1):427–433
214. McMahon EJ, Cook DN, Suzuki K, Matsushima GK (2001) Absence of macrophage-inflammatory protein-1alpha delays central nervous system demyelination in the presence of an intact blood-brain barrier. *J Immunol* 167(5):2964–2971
215. Franco-Pons N, Torrente M, Colomina MT, Vilella E (2007) Behavioral deficits in the cuprizone-induced murine model of demyelination/remyelination. *Toxicol Lett* 169:205–213
216. Liebetanz D, Merkler D (2006) Effects of commissural de- and remyelination on motor skill behaviour in the cuprizone mouse model of multiple sclerosis. *Exp Neurol* 202:217–224
217. Andersson PB, Perry VH, Gordon S (1992) The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. *Neuroscience* 48:169–186
218. Bell MD, Perry VH (1995) Adhesion molecule expression on murine cerebral endothelium following the injection of a proinflammagen or during acute neuronal degeneration. *J Neurocytol* 24:695–710
219. Felts PA et al (2005) Inflammation and primary demyelination induced by the intraspinal injection of lipopolysaccharide. *Brain* 128:1649–1666
220. Marik C, Felts PA, Bauer J, Lassmann H, Smith KJ (2007) Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? *Brain* 130:2800–2815
221. Ji KA et al (2007) Resident microglia die and infiltrated neutrophils and monocytes become major inflammatory cells in lipopolysaccharide-injected brain. *Glia* 55:1577–1588
222. Andersson PB, Perry VH, Gordon S (1992) Intracerebral injection of proinflammatory cytokines or leukocyte chemoatins induces minimal myelomonocytic cell recruitment to the parenchyma of the central nervous system. *J Exp Med* 176:255–259
223. Zhou H, Lapointe BM, Clark SR, Zbytniuk L, Kubes P (2006) A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. *J Immunol* 177:8103–8110
224. Lehnardt S et al (2002) The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J Neurosci* 22:2478–2486
225. Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA (2005) Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc Natl Acad Sci U S A* 102:9936–9941
226. Pang Y, Cai Z, Rhodes PG (2000) Effects of lipopolysaccharide on oligodendrocyte progenitor cells are mediated by astrocytes and microglia. *J Neurosci Res* 62:510–520
227. Li J et al (2008) Tumor necrosis factor alpha mediates lipopolysaccharide-induced microglial toxicity to developing oligodendrocytes when astrocytes are present. *J Neurosci* 28:5321–5330
228. Brockschneider D, Pechmann Y, Sonnenberg-Riethmacher E, Riethmacher D (2006) An improved mouse line for Cre-induced cell ablation due to diphtheria toxin A, expressed from the Rosa26 locus. *Genesis* 44:322–327