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REVIEW

# Transgenic mouse models of multiple sclerosis

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**Abstract** Multiple sclerosis (MS) is an inflammatory demyelinating disease affecting the central nervous system (CNS) and a frequent cause of neurological disability in young adults. Multifocal inflammatory lesions in the CNS white matter, demyelination, oligodendrocyte loss, axonal damage, as well as astrogliosis represent the histological hallmarks of the disease. These pathological features of MS can be mimicked, at least in part, using animal models. This review discusses the current concepts of the immune effector mechanisms driving CNS demyelination in murine models. It highlights the fundamental contribution of transgenesis in identifying the mediators and mechanisms involved in the pathophysiology of MS models.

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**Abbreviations**

| | |
|---|---|
| AICD | Activation-induced cell death |
| APC | Antigen presenting cell |
| BBB | Blood–brain barrier |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| DC | Dendritic cell |
| EAE | Experimental autoimmune encephalomyelitis |
| EBV | Epstein–Barr virus |
| GFAP | Glial fibrillary acidic protein |
| GFP | Green fluorescent protein |
| GTP | Guanosine tri-phosphate |
| HA | Hemagglutinin |
| HI | *Haemophilus influenzae* |
| LCMV | Lymphocytic choriomeningitis virus |
| MBP | Myelin basic protein |
| MOG | Myelin oligodendrocyte glycoprotein |
| mAb | Monoclonal antibody |
| MS | Multiple sclerosis |
| NF-L | Neurofilament light |
| NF-M | Neurofilament medium |
| NSE | Neuron-specific enolase |
| ODC | Oligodendrocyte |
| OVA | Ovalbumin |
| PLP | Myelin proteolipid protein |
| ROR | Retinoic acid receptor-related orphan nuclear receptor |
| TMEV | Theiler's murine encephalomyelitis virus |
| TNFRI | TNF receptor I |

## Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that affects over 2 million people worldwide [1, 2]. It is a major cause of clinical disability among young adults. The clinical evolution of this disease is heterogeneous. Its initial course is either relapsing-remitting (over 80% of cases), or primary progressive (~15% of cases). About half of the initially relapsing-remitting cases of MS evolve over time to a secondary progressive form with or without superimposing relapses [1, 2]. MS is thought to be driven by an autoimmune process in which autoreactive T and B cells foster inflammatory lesions in the brain and spinal cord [3]. These lesions cause local tissue damage, including demyelination, loss of oligodendrocytes, axonal degeneration and neuronal loss, leading to progressive neurological disability [4]. Variations in the anatomical localization and the histopathological characteristics of the lesions underlie the heterogeneous clinical expression of MS [5, 6]. The etiology of MS is unknown, but both genetic and environmental factors contribute to disease predisposition. Several genes of immune relevance are associated with disease susceptibility, including genes involved in antigen-presentation (HLA-DR, HLA-A, and HLA-C), cytokine receptors (IL-2RA and IL-7R), cell-adhesion (CD58), and signaling (IRF8, TYK2, Vav1, CBLB) [7–11]. In addition, two genes implicated in neuronal function (ACCN1, KIF1B) have been suggested to increase the risk of developing MS [12, 13], but confirmation of these data is still needed [14]. The environmental factors that predispose to MS include cigarette smoking [15, 16], lack of sunlight, or vitamin D deficiency which might impact negatively on immune regulation [17]. Furthermore, the role of microbial, in particular viral, agents, is receiving renewed credence as an environmental trigger of MS [18].

### Classical animal models of MS

Animal models have provided an indispensable contribution to our understanding of the immune mechanisms driving CNS tissue damage [19]. These models mimic either virus infection as an environmental factor in the etiology of MS, or its plausible autoimmune nature. In rodents, various viruses, including murine hepatitis virus (strain JHM) and Semliki Forest virus, can induce inflammatory demyelination [20]. Particular interest has been given to Theiler's murine encephalomyelitis virus (TMEV). This pathogen, discovered in 1937 by Max Theiler, belongs to the *Cardiovirus* genus and naturally infects mice [21, 22]. Upon intracerebral inoculation TMEV infects neurons and glial cells leading to acute encephalomyelitis, which is lethal when using neurovirulent strains (GDVII and FA) [23]. The inoculation of non-virulent strains of TMEV (DA or BeAn) causes an initial encephalomyelitis lasting for up to 2 weeks after which the virus is either cleared or persists chronically in the CNS [22]. Susceptibility is largely genetically determined, notably by the MHC haplotype. In experimental mice, the resistance is mapped to the H-2D locus [24] and the H-2D$^b$ allele confers dominant resistance to congenic B10 mice [25–27]. The resistance is mediated by the anti-viral CD8$^+$ T cell response [28]. Indeed, in H-2D$^b$ mice, CD8$^+$ T cells specific for the immunodominant H-2D$^b$-restricted TMEV epitope (VP2$_{121–130}$) clear the virus from the CNS [29, 30]. Interestingly, this epitope is conserved in all TMEV strains, suggesting that TMEV cannot evade immune recognition by mutating this particular epitope [22]. It is thought that altering this antigenic sequence within the VP2 capsid protein would destabilize the virion, thereby compromising TMEV viability [22]. In contrast, in susceptible mice, such as BALB/c or SJL/J, the immune response fails to clear infection and TMEV persists in glial cells of the spinal cord white matter [31]. This process is most extensively studied in SJL mice, in which white matter viral persistence causes focal inflammatory lesions comprising CD4$^+$ and CD8$^+$ T cells, B cells, and activated microglia/macrophages. These inflammatory lesions are associated with demyelination, glial scarring, and axonal damage reminiscent of the inflammatory tissue damage observed in MS lesions [22, 32].

Since its first description in 1933 in monkeys, the paradigm of experimental autoimmune encephalomyelitis (EAE) is thought to model the autoimmune process implicated in MS [33]. EAE develops in response to active immunization with CNS homogenates, myelin, or myelin-derived antigens, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or myelin proteolipid protein (PLP) emulsified in adjuvant or by the adoptive transfer of myelin-reactive T cells [34, 35]. Susceptible rodents commonly develop a paralytic disease that starts with tail atony followed by reduced tonicity in the hind legs that gradually aggravates to hind limb paralysis and quadriplegia [36]. Gold et al. [37] recently nicely reviewed the various EAE models. To illustrate the variation in clinical evolution, a chronic EAE course characteristically develops in C57BL/6 mice immunized with the immunodominant peptide of MOG, MOG$_{35–55}$ [38]. In Lewis rats immunized with MBP, EAE is characterized by a monophasic disease with an acute paralytic episode followed by complete recovery [39, 40]. Immunizing SJL/J mice with PLP causes a relapsing-remitting EAE, which involves multiple cycles of attack with full or partial recovery in-between [41]. Each of these models represents a different part of the heterogeneous human

disease MS and the choice of the model largely depends on the aspect that has to be analyzed [42].

Homologous recombination and transgenesis have provided the unique possibility to selectively induce or invalidate the expression of a particular gene and study its function [43]. This approach has revolutionized biomedical research in permitting the understanding of the function of a gene product in complex organisms, and is being extensively used to more finely decipher the mechanisms underlying CNS inflammation. This review will first focus on transgenic mouse models targeting the dominant lymphocytes found in inflammatory lesions of MS patients: $CD4^+$ T cells, $CD8^+$ T cells and B cells. Secondly, we will review the application of transgenesis to study the cellular and molecular mechanisms driving chronic autoimmune inflammation in the CNS. Lastly, we will discuss the transgenic models allowing cytokine and chemokine overexpression in the CNS, thereby promoting or modulating local tissue inflammation. With transgenic mice, new insights into the cellular and molecular mechanisms of EAE, and also likely of MS, were gained leading to a better understanding of the complexity of the disease.

## Transgenic models of CNS autoimmunity

### CNS autoimmunity in transgenic mice expressing a MHC class II restricted CNS-specific TCR

One of the very first self-reactive TCR transgenic mouse models underlined the ability of $CD4^+$ T cells to induce CNS autoimmune disease. Lafaille and colleagues constructed mice ($H\text{-}2^u$) expressing a transgenic TCR specific for $MBP_{Ac1–9}$ on $CD4^+$ T cells that developed spontaneous paralytic EAE with a frequency of about 14% [44]. To prevent expression of endogenous antigen receptors in B and T cells, these TCR transgenic mice were crossed to homozygosity with Rag-deficient mice. These Rag-deficient TCR transgenic mice developed EAE with greater incidence (100%) and severity revealing two aspects. First, a monospecific $CD4^+$ T cell response targeting myelin can cause EAE independently of other adaptive immune cells. Second, the disease exacerbation indicated that regulatory elements exist within the polyclonal T and B cell repertoire able to prevent EAE. Later it was shown, that $CD4^+CD25^+$ T cells are responsible for this regulation as they can prevent development of EAE after transfer in the Rag-deficient TCR transgenic mice [45]. Transgenic mice expressing different TCR myelin specificities were then developed (Table 1).

PLP is the most abundant integral membrane protein of myelin [46]. In SJL mice ($H\text{-}2^s$), the immunization with whole CNS homogenate induces relapsing EAE during which the autoreactive $CD4^+$ T cell response is focused on the $PLP_{139–151}$ peptide [47]. These $PLP_{139–151}$-specific $CD4^+$ T cells encompass two functionally distinct repertoires: pathogenic clones able to transfer EAE and non-pathogenic clones [48]. Interestingly, all of the pathogenic T cell clones recognized the tryptophan at position 144 (W144) of PLP as primary TCR contact residue. The non-pathogenic T cells used leucine and glycine at positions 141 and 142 as additional TCR contact residues permitting more leniency to W144 [49–52]. Three TCR transgenic mice were generated from both $PLP_{139–151}$ reactive T cell pools, to provide insight into the pathogenicity of these compartments. The 5B6 and 4E3 TCR require tryptophan at position 144 as TCR contact residue for activation, even though the original 4E3 T cell clone was non-pathogenic [53]. These two W144-dependent TCR transgenic mice (4E3 and 5B6) developed spontaneous fulminant EAE in SJL mice. The 4E3 line also developed disease in B10.S mice, which share the $H\text{-}2^s$ haplotype with the SJL strain, albeit with lower incidence and severity indicating that MHC-independent susceptibility genes influence EAE severity in TCR transgenic mice. This relative resistance can be overcome by activation of APCs via triggering TLR9 or TLR4 [54]. By contrast, a third TCR, Q1.1B6, was derived from the alternative $PLP_{139–151}$ reactive T cell pool that is more lenient towards the W144 TCR contact residue [55]. These Q1.1B6-TCR transgenic mice failed to develop spontaneous EAE in SJL mice, although $PLP_{139–151}$ immunization generated subclinical inflammatory lesions in the CNS [49, 56]. These observations indicate that the $PLP_{139–151}$ reactive T cell compartment in SJL mice contains distinct repertoires that vary in pathogenic potential.

MOG is a minor component of the myelin sheath accounting for 0.1% of total myelin protein [57]. Its position on the outermost lamellae of the myelin sheath renders MOG directly accessible to specific antibodies that are detected in MS patients [58–60]. In addition to being a candidate autoantigen for MS, MOG is highly pathogenic in rodent models [61]. The 2D2-TCR transgenic mice express on $CD4^+$ T cells a TCR specific for the $MOG_{35–55}$ peptide presented in the context of $I\text{-}A^b$ [62]. In the original study, 30% of 2D2-TCR mice developed spontaneous optic neuritis with inflammation and demyelination of the optic nerves without progression to clinical EAE. Only 4% of the 2D2-TCR mice developed clinical EAE with inflammatory and demyelinating lesions in brain and spinal cord. The susceptibility to EAE can be augmented by active immunization with the $MOG_{35–55}$ peptide. Immunized 2D2-TCR transgenic mice developed a more severe EAE characterized by a mortality of 40%, while immunization of non-transgenic C57BL/6 mice induced non-lethal EAE [62]. These data implement a role for MOG-specific $CD4^+$ T cell responses in the induction of optic neuritis and suggest

**Table 1** TCR transgenic mice

| TCR specifity | Name/clone | Genetic background | Model | Phenotype | References |
|---|---|---|---|---|---|
| $MBP_{Ac1-9}$ | 19 | PL/J or B10.PL | $CD4^+$ TCR tg | 14% paralytic EAE; 100% in RAG-deficient animals | [44] |
| $MBP_{Ac1-11}$ | 172.10 | B10.PL | $CD4^+$ TCR tg | 14–44% paralytic EAE | [260] |
| $MBP_{111-129}$ | MS2-3C8 | Humanized Tg (HLA-DR4) | Human $CD4^+$ TCR tg with DRB1*0401 | Paralytic EAE and dysphagia after adoptive transfer | [76] |
| $MBP_{84-102}$ | Ob.1A12 | Humanized Tg (HLA-DR2b) | Human $CD4^+$ TCR tg with DRA*0101/DRB1*1501 | 4% paralytic EAE; 100% in RAG-deficient animals | [70] |
| $MBP_{85-99}$ | Hy.2E11 | Humanized Tg (HLA-DR2b and/or HLA-DR2a) | Human $CD4^+$ TCR tg with DRA1*0101/DRB1*1501, DRA1*0101/DRB5*0101 | No spontaneous EAE (DR2a)<br>86% paralytic EAE (DR2b)<br>55% paralytic EAE (DR2a/DR2b) | [74] |
| $MBP_{85-99}$ | Ob.1A12 Line 7 | Humanized Tg (HLA-DR2b) | Human $CD4^+$ TCR tg with DRA1*0101/DRB1*1501 | 60% paralytic EAE; 100% in RAG-deficient animals | [72] |
| $PLP_{139-151}$ | 5B6 | SJL/J | $CD4^+$ TCR tg | 40% paralytic EAE (SJL/J) | [53, 54] |
| | | B10.S | | 4% paralytic EAE (B10.S) | |
| $PLP_{139-151}$ | 4E3 | SJL/J | $CD4^+$ TCR tg | 60–83% paralytic EAE | [53] |
| $PLP_{139-151}$ | Q1.1B6 | SJL/J | $CD4^+$ TCR tg | No spontaneous EAE; inflammation after immunization | [55] |
| $MOG_{35-55}$ | 2D2 | C57BL/6 | $CD4^+$ TCR tg | 4% paralytic EAE; 30% optic neuritis | [62, 108] |
| | | | | 18% paralytic EAE | |
| $MOG_{35-55}$ | 2D2 | C57BL/6 | $MOG^{-/-} \times CD4^+$ TCR tg | 18% paralytic EAE | [64] |
| $PLP_{45-53}$ | 2D1 | Humanized Tg (HLA-A3 and/or HLA-A2) | Human $CD8^+$ TCR tg with HLA-A*0301 and/or HLA-A*0201 | No spontaneous EAE (HLA-A2 or HLA-A2/HLA-A3) | [95] |
| | | | | 4% mild motor deficits; 71% mild initial motor deficits and 25% relapsing EAE after immunization (HLA-A3) | |
| | | | | Co-expression of HLA-A2 protects from disease | |
| Neo-self antigen OVA | OTI | C57BL/6 | ODC-OVA × OT-I $CD8^+$ TCR tg | 90% paralytic EAE; 100% in RAG-deficient animals | [88] |
| Neo-self antigen HA | CL4 | Balb/c | GFAP-HA × CL4 $CD8^+$ TCR tg | 100% early deadly colitis, 100% CNS inflammation with astrocyte loss without bystander damage after adoptive transfer | [84] |
| Neo-self antigen HA | CL4 | Balb/c | MOG-HA × CL4 $CD8^+$ TCR tg | Ignorance, 40% weight loss, and reduced mobility with 100% inflammation and demyelination after adoptive transfer | [86] |

that the optic neuritis can be a prelude for EAE. Similarly, in about one-third of the cases, optic neuritis is the first clinical manifestation of MS [63]. Due to the generous distribution of 2D2-TCR mice worldwide, several laboratories have reported higher incidence of spontaneous EAE in their local 2D2-TCR colonies, suggesting that differences in the local environment or in the genetic background of the C57BL/6 mice used to maintain the line can influence the prevalence of spontaneous EAE [62, 64].

Genetic studies have associated the HLA-DR2 haplotype (DRA*0101-DRB5*0101-DRB1*1501-DQB1*0602) with susceptibility to MS [65–67]. Myelin-reactive T cell clones isolated from HLA-DR2 patients were shown to respond to the immunodominant $MBP_{84–102}$ peptide, which is conserved in mouse MBP [68, 69]. The advent of transgenesis introduced the possibility to express a human TCR of interest together with the corresponding HLA restriction molecule and the matching human co-receptor in mice. As such, these humanized mice permit the direct investigation of the in vivo pathogenicity of human myelin-specific TCRs derived from MS patients.

Humanized mice were constructed expressing the human CD4 co-receptor, an MS-derived $MBP_{84–102}$:DR2-specific TCR (Ob.1A12), and the human HLA-DR2b molecule (DRA*0101/DRB1*1501) [70]. In these mice, 80% of the T cells expressed the transgenic TCR beta-chain but only less than 1% reacted functionally to the MBP peptide. Four percent of the mice spontaneously developed EAE; this disease incidence could be increased to 100% by either $MBP_{84–102}$ immunization or by introducing a Rag2-mutation [70]. No disease was observed in the absence of the $MBP_{84–102}$:DR2-specific TCR [71]. A second group generated an independent humanized mouse model expressing the same $MBP_{85–99}$:DR2-specific TCR (clone Ob.1A12) concomitantly with DR2b. In these mice, a high proportion of $CD4^+$ T cells (97%) expressed the transgenic human TCR beta-chain, which predisposed to spontaneous EAE with much higher penetrance (60% of mice by 6 months of age). These mice developed a classical EAE characterized by an ascending flaccid paralysis with inflammation and myelin loss in spinal cord and cerebellum [72]. Together, these humanized MBP-specific mice revealed the importance of the expression level and functionality of the transgenic TCR for the development of spontaneous autoimmunity.

The HLA-DR2 haplotype encodes the HLA-DR2a (DRA1*0101/DRB5*0101) and HLA-DR2b (DRA1*0101/DRB*1501) isoforms, but their strong linkage disequilibrium makes it difficult to discriminate their individual contribution in MS. The human TCR-Hy.2E11 recognizes $MBP_{85–99}$ when presented by the HLA-DR2b molecule as well as an EBV peptide presented by the HLA-DR2a molecule, this cross-reactivity does not apply to the Ob.1A12-TCR [73]. Humanized mice co-expressing the Hy.2E11-TCR and the HLA-DR2a molecule do not develop disease, whereas 86% of the mice co-expressing the TCR and the HLA-DR2b molecule spontaneously developed severe EAE (on average 182 days after birth). The addition of the HLA-DR2a transgene in the latter model reduced EAE prevalance (55%) and severity, and delayed onset by 40 days. This was achieved by activation-induced cell death (AICD) that decreased the number of autoreactive $CD4^+$ T cells in the periphery [74]. These results suggest that the HLA-DR2b allele favors EAE and possibly MS, whereas the HLA-DR2a allele functions as a genetic modifier of disease susceptibility.

$MBP_{111–129}$ is the immunodominant epitope in the context of HLA-DRB1*0401 (HLA-DR4) [75]. This epitope is not fully conserved in mice as the arginine at position 122 is replaced by lysine. The T cell clone MS2-3C8, which was isolated from the blood of a patient with relapsing-remitting MS, can respond to both human and murine MBP peptides, with higher sensitivity to the human epitope [75]. In the transgenic mice expressing HLA-DR4 and the $MBP_{111–129}$-specific MS2-3C8-TCR, no spontaneous development of EAE was detected. The transfer of in vitro generated $Th_1$ cells from the $MBP_{111–129}$-specific TCR transgenic mice into irradiated HLA-DR4 transgenic recipients induced EAE with classical ascending paralysis, as well as atypical clinical signs, such as head-tilt or lingual paralysis [76]. Histological analysis demonstrated inflammation in spinal cord but also brainstem and cerebellum leading to demyelination and axonal damage. These clinical and pathological features different from other transgenic MBP-specific models were suggested to be caused by the varying distribution patterns of the MBP isoforms and the distinct affinity of the myelin-specific T cells to the peptide–MHC complex.

These pioneering studies revealed that the pathogenicity of human self-reactive T cells can be efficiently tested in vivo by introducing the TCR of interest together with the matching co-receptor and HLA allele. Indirectly, these studies indicate the compatibility between the murine antigen-processing machinery and human MHC molecules. Humanized mouse models therefore represent a powerful tool to link auto-antigen specificity in human T cells with pathogenicity. These models will therefore undoubtedly contribute to the better understanding of the pathophysiology of MS.

## CNS autoimmunity in transgenic mice expressing a MHC class I restricted CNS-specific TCR

Accumulating evidence suggest an active pathogenic role for $CD8^+$ T cells in MS [77]. For instance, histological

analysis of MS samples revealed a predominance of $CD8^+$ T cells in active demyelinating lesions [79]. The striking oligoclonal expansion of $CD8^+$ T cells in the cerebrospinal fluid [80] and lesions [79] of MS patients suggest in situ antigen-driven activation of $CD8^+$ T cells during the course of MS. An active role for $CD8^+$ T cells in CNS autoimmunity is further strengthened by observations in EAE, as disease can be induced by the adoptive transfer of myelin-specific $CD8^+$ T cells [81, 82]. Transfer of MBP-specific $CD8^+$ T cells into syngeneic mice induced rapid and severe EAE. Clinical signs such as ataxia, and loss of coordinated movement, reflecting lesions in the brain and not in the spinal cord, suggested clear differences between $CD4^+$ and $CD8^+$-mediated CNS autoimmunity [81]. Similarly, after adoptive transfer of $MOG_{35–55}$-specific $CD8^+$ T cells, a severe and permanent EAE developed [82].

Although these studies established the pathogenicity of myelin-specific $CD8^+$ T cells, the effector mechanisms by which $CD8^+$ T cells contribute to lesion formation remain to be determined. To address these issues, several transgenic mouse models have been developed (Table 1). Our group expressed the hemagglutinin (HA) of influenza virus as a 'neo-self' antigen under the control of the 'astrocyte-specific' GFAP promoter. To study the impact of HA-specific $CD8^+$ T cells, the GFAP-HA mice were crossed with clone 4 (CL4) TCR transgenic mice in which most $CD8^+$ T cells recognize the immunodominant $HA_{512–520}$ peptide in the context of $H\text{-}2K^d$ [83]. This potent self-reactivity led to spontaneous autoimmune disease. Interestingly, this response was not directed against CNS astrocytes, but to a related cell-type called enteric glia that resides in the enteric nervous system. Consequently, the mice developed a fulminant enterocolitis, which caused premature death [84]. However, selective depletion of CNS astrocytes without bystander damage to the myelin sheath could be observed when HA-specific $CD8^+$ T cells were differentiated in vitro into cytotoxic $CD8^+$ T cells that produce TNF-$\alpha$, IFN-$\gamma$, as well as Granzyme B and transferred into unirradiated GFAP-HA mice [85].

Another set of transgenic mice in which HA expression was driven to oligodendrocytes using the MOG promoter provided the opportunity to study the mechanisms by which oligodendrocyte-specific $CD8^+$ T cells can induce CNS demyelination. Crossing the MOG-HA mice with the CL4 TCR transgenic mice resulted in immune ignorance [86]. Despite the overt autoreactivity in these mice the HA-specific $CD8^+$ T cell compartment exhibits a mainly naive phenotype and retains full proliferative capacity in response to the HA peptide. None of the mice developed histological or clinical signs of CNS inflammation. To test if effector $CD8^+$ T cells could overcome tolerance, we transferred HA-specific cytotoxic T cells into MOG-HA recipients [86]. Activated, but not naive HA-specific $CD8^+$ T cells, induced an overt monophasic disease peaking at day 8–10 with mild weight loss but no paralysis in 40% of the MOG-HA recipients. Strikingly, all MOG-HA recipients, but none of the non-transgenic recipients, exhibited inflammatory demyelination lesions that initiated by day 5 in the optic nerve and progressed by day 9 to the spinal cord and brain. Using GFP transduction to trace the transferred oligodendrocyte-specific $CD8^+$ T cells, we showed that $GFP^+$ HA-specific $CD8^+$ T cells dominate the inflammatory lesions. In the CNS white matter, these $GFP^+$ $CD8^+$ T cells readily co-localized with oligodendrocytes and occasionally the $GFP^+$ $CD8^+$ T cells polarized their cytotoxic Granzyme $B^+$ granules towards juxtaposed oligodendrocytes. In response to this interaction oligodendrocytes underwent apoptosis ultimately leading to oligodendrocyte loss, demyelination, microglial activation and axonal damage. Our study, therefore, indicated that oligodendrocyte-specific $CD8^+$ T cells can induce demyelinating lesions reminiscent of those observed in MS, by killing oligodendrocytes as a likely consequence of direct antigen recognition.

In parallel, the laboratory of Thomas Hünig [87, 88] expressed ovalbumin (OVA) as a 'neo-self' antigen in the cytosol of oligodendrocytes under the control of the proximal part of the MBP promoter (ODC-OVA mice). Similar to the MOG-HA mice, OVA expression in ODC-OVA mice is restricted to the CNS with no detectable expression in the thymus or periphery. Crosses with TCR transgenic mice indicated that OVA-specific $CD8^+$ T cells (OT-I) are highly encephalitogenic in the ODC-OVA mice in contrast to OVA-specific $CD4^+$ T cells (OT-II) that ignore the self-antigen [87]. Clinical signs of EAE appeared early in ODC-OVA × OT-I double transgenic mice (day 12–19), with severe locomotor deficits and ascending paralysis, but also uncoordinated limb movements and tremor revealing a cerebellar involvement. Ninety percent of the ODC-OVA × OT-I mice were affected, rising up to 100% upon crossing with Rag1-deficient mice, suggesting that the OVA-specific $CD8^+$ T cells are directly involved in disease development. Adoptive transfer in ODC-OVA mice showed that access of naive OT-I $CD8^+$ T cells to OVA-expressing oligodendrocytes is only possible within the first 2 weeks of life, before the blood–brain barrier (BBB) becomes impermeable to naive T cells. By using IFN-$\gamma$-deficient OT-I cells, the authors also demonstrated that the CD8-mediated EAE in ODC-OVA mice strongly depends on IFN-$\gamma$ production by the OT-I cells. Later they could show that oligodendrocyte-specific $CD8^+$ T cells cause collateral apoptosis of neurons in the grey matter of the CNS due to a spillover of perforin and Granzyme(s) [89, 90]. Interestingly, to test the potential for antigen-specific

immunotherapy, the authors used a mAb that specifically recognizes the $OVA_{257–264}$:H-$2K^b$ complexes [91]. Administration of this competing mAb, even after disease onset, prevented the activation and proliferation of the oligodendrocyte-specific $CD8^+$ T cells and the lysis of oligodendrocytes, indicating a therapeutic potential of such an approach [92].

Population-based genetic data indicate that independently of the DR-associated effect, expression of HLA-A*0301 (encoding HLA-A3) predisposes individuals to MS, whereas HLA-A*0201 (encoding HLA-A2) is protective [93, 94]. Friese et al. [95] generated humanized mice expressing the HLA-A3 and/or HLA-A2 molecules and co-expressing a human $PLP_{45–53}$:HLA-A3-specific TCR (2D1) isolated from an MS patient. As expected, introduction of the HLA-A3-restricted 2D1-TCR into HLA-A2 transgenic mice was innocuous. In contrast, the 2D1-TCR on the appropriate HLA-A*03012 background resulted in a mild motor deficit in 4% of the mice, providing the novel observation that human PLP-specific $CD8^+$ T cells cause CNS pathology. Immunization with $PLP_{45–53}$ induced mild disease in 71% of these mice, followed in 25% of the transgenic mice by a severe disease, characterized by hind limb paralysis. This secondary progression was dependent on epitope spreading involving endogenous polyclonal T or B cells as introducing a Rag2-deficiency prevented the secondary progression phase. More remarkable, this $CD8^+$-mediated autoimmune disease in 2D1-TCR × HLA-A*0301 transgenic mice can be fully prevented by introducing the HLA-A*0201 transgene. This striking abrogation of disease is due to the thymic deletion of the 2D1-TCR expressing $CD8^+$ T cells mediated by HLA-A2 [95]. This indicates that the 2D1-TCR is polyspecific recognizing both the $PLP_{45–53}$:HLA-A3 complex as well as an HLA-A2 complex with currently unknown self-peptide(s) that impose deletional tolerance. Precedents of such a mechanism exist, in that an immunoglobulin derived peptide conveys tolerance by purging polyspecific T cells from the repertoire that co-recognize a cornea self-antigen [96]. More generally, this study provides a functional scenario for the protective effect of the HLA-A*0201 allele in MS.

Taken together, these studies have established the pathogenic potential of myelin-specific $CD8^+$ T cells in driving CNS tissue damage and present $CD8^+$ T cells as novel therapeutic targets for MS. Strategies aimed to this effect can be efficiently tested in the pre-clinical setting provided by these transgenic mouse models.

## Transgenic mice expressing a CNS-specific BCR

MS was originally thought to be a T cell-mediated disease, but B cells, plasma cells, as well as antibodies and proteins of the complement system are found in and around active MS lesions [97, 98]. The role of autoantibodies in demyelination was demonstrated [99–101] and autoantibodies against myelin components are present in the serum and CSF of MS patients. Several studies have identified clonally expanded B cells in the CNS and CSF of MS patients [102–104] that correspond to the oligoclonal bands detected in the CSF of MS patients [105]. Treatment of patients with relapsing-remitting MS with a B cell-depleting anti-CD20 antibody (rituximab) reduced inflammatory brain lesions and clinical relapses for 48 weeks, providing strong evidence for B-cell involvement in MS [78]. In addition, plasmapheresis benefits patients with the MS variant Devic's disease characterized by anti-aquaporin IV autoantibodies [106]. Lately, proteolytic activity towards neural antigen was detected in the fraction of antibodies purified from mice with EAE and from MS patients but not in fractions of healthy controls [107]. These antibody enzymes (abzymes) degradated MBP specifically, suggesting a destructive role of antibodies in EAE and MS.

To better analyze the role of autoreactive B cells and the interaction of myelin-specific B cells with myelin-specific T cells, the previously mentioned $MOG_{35–55}$:I-$A^b$-specific 2D2-TCR transgenic mice were crossed with MOG-specific Ig heavy-chain knock-in mice ($IgH^{MOG}$ mice) [99], in which 20–30% of B cells express a BCR specific for MOG. Notably, 60% of 2D2-TCR × $IgH^{MOG}$ mice, but none of the $IgH^{MOG}$ mice, spontaneously developed severe EAE with a selective distribution of inflammatory lesions in the spinal cord and optic nerves, closely resembling the human Devic's disease. The presence of MOG-specific T cells led to a high production of MOG-specific IgG1 antibodies. The contribution of MOG-specific antibodies in the spontaneous EAE is not clear. However, MOG-specific B cells enhanced the proliferation and activation of MOG-specific T cells illustrating the potency of B cells as antigen presenting cells. These data revealed an active cooperation between the CNS antigen-specific T and B cells in inducing autoimmune disease resulting in the development of a spontaneous and severe form of optico-spinal EAE [62, 108].

The first model for spontaneous relapsing-remitting EAE was developed in SJL/J mice, expressing a transgenic $MOG_{92–106}$-reactive TCR ($TCR^{1640}$) in 99% of the $CD4^+$ T-cells [109]. These mice were crossed with the previously described $IgH^{MOG}$ mice. $TCR^{1640}$ × $IgH^{MOG}$ and $TCR^{1640}$ mice spontaneously developed EAE with overall comparable clinical and histological signs, but the disease itself was extremely variable in course and clinical expression in both the single and double transgenic settings. The relapsing-remitting disease course often permitted full recovery prior to the first relapse. The first attack was characterized by ataxia, while the relapses instigated classical signs of EAE with progressive flaccid paralysis. A sex

bias towards females was observed for the relapsing-remitting EAE. By contrast, males predominantly developed progressive EAE without relapses. The ataxic mice displayed large lesions in cerebellum and brain stem, whereas mice with conventional EAE had lesions distributed throughout the spinal cord, brain stem, and optic nerve. The immune attacks against different CNS parts in the relapsing model are not due to epitope spreading, as reported for actively induced relapsing-remitting EAE. The lesions in all mice were characterized mainly by $CD4^+$ T cells ($Th_1$, $Th_{17}$ and $Foxp3^+$ regulatory cells) and B cells together with a low amount of activated macrophages and $CD8^+$ T cells as well as IgG antibodies. In single $TCR^{1640}$ transgenic mice, the autoreactive MOG-specific B cells were expanded from the endogenous repertoire, accounting for the lack of difference between the two transgenic mouse models. The expansion of MOG-specific B cells requires the presence of the target autoantigen, as *Mog*-deficient $TCR^{1640}$ transgenic mice neither produced anti-MOG antibodies nor developed spontaneous EAE. The importance of MOG-reactive B cells in this relapsing-remitting EAE model was shown by the nearly complete suppression of EAE with a depleting anti-CD20 mAb [109]. B cells can act in opposing ways in EAE: initiating EAE by presenting the antigen to MOG-reactive T cells or limiting EAE by production of IL-10 and by influencing the function of regulatory T cells [110–112]. The exact contribution of the MOG-specific B cells in this model remains to be elucidated, but this transgenic mouse model is the first eliciting a spontaneous relapsing-remitting form of EAE corresponding to the most frequent type of MS [109]. Collectively, the TCR and BCR transgenic models have delivered a wealth of scientific information as well as provided new tools to investigate specific aspects of the pathophysiology of CNS autoimmunity. This well-characterized reductionist approach has clearly contributed to the characterization of the pathogenic potential of autoreactive $CD4^+$, $CD8^+$, and B cells both in fully murine and, importantly, in humanized models. The respective migratory and encephalitogenic properties of $CD4^+$ or $CD8^+$ T cells sharing the same TCR but exhibiting different effector functions can now be finely investigated. In addition, the combined pathogenic potential of the different arms of the autoimmune response can be accurately studied. As a first approach, models using both autoreactive TCR and BCR transgenes illustrate the synergy between T and B cells during CNS autoimmunity.

Moreover, the transgenic spontaneous models of EAE open new horizons in the field. In particular, these models offer a unique tool to study the impact of environmental factors, but also genetic polymorphisms, on spontaneous disease development and course. In addition, they provide much-needed spontaneous disease models to test at the pre-clinical level new immune intervention strategies for MS.

## Transgenic modulation of CNS autoimmunity

### Pathogenic $CD4^+$ T cell subsets involved in CNS autoimmune diseases

In order to respond optimally to the plethora of different pathogens, T cells have acquired the capacity to differentiate into functionally distinct subsets [113]. This discovery spawned active investigation concerning the implication of each subset in the generation of CNS autoimmune diseases. EAE has long been regarded as a prototypical $Th_1$ disease in which IL-12-driven $Th_1$ cells that produce IFN-$\gamma$ and TNF-$\alpha$ cause tissue damage [34, 114]. Observations from transgenic mice have significantly altered this dogma. Indeed, mice deficient for $Th_1$ effector cytokines IFN-$\gamma$ and TNF-$\alpha$, or for the IFN-$\gamma$-receptor still developed EAE, with at least similar severity [115–117]. This paradox was further emphasized by the observation that treatment with recombinant IFN-$\gamma$ or TNF-$\alpha$ ameliorated EAE [118, 119]. The issue was resolved when studying the importance of IL-12 (p40/p35 heterodimer) and IL-23 (p40/p19 heterodimer) in the immunopathogenesis of EAE. Through the use of mice deficient in either IL-12 ($p35^{-/-}$), IL-23 ($p19^{-/-}$), or both ($p40^{-/-}$), it was revealed that in fact deficiency in IL-23 individually led to resistance to EAE [120]. IL-12-deficient mice, as well as IL-12 receptor-deficient mice remained susceptible to EAE [120, 121]. Consequently, IL-23 rather than IL-12 is the critical cytokine driving pathogenic T cells in EAE. These observations led to the identification of a novel pathogenic T cell subset that depends on IL-23. Gene expression profiling identified that IL-23 stimulation of myelin-specific $CD4^+$ T cells rendered them highly encephalitogenic and promoted the expression of IL-17A, IL-17F, IL-21, and IL-22 [122, 123]. These $Th_{IL\text{-}17}$ cells or $Th_{17}$ cells have since been recognized as a novel T cell subset critical for mucosal and epithelial host defense against extracellular bacteria and fungi [124]. In mice, TGF-$\beta$ synergizes with IL-6 and IL-21 to promote IL-23 receptor expression favoring $Th_{17}$ lineage differentiation [125–128]. The transcription factor retinoic acid receptor-related orphan nuclear receptor (ROR)-$\gamma$t was proven to direct $Th_{17}$ differentiation by using mice in which GFP was introduced into the *Ror-γt* locus [129]. Consequently, heterozygous *Ror-γt*$^{gfp/+}$ can be used to trace and isolate ROR-$\gamma$t expressing $Th_{17}$ cells, permitting their detailed biological analysis. *Ror-γt*$^{gfp/gfp}$ mice serve as a functional knock-out and were shown to be resistant to EAE [129].

The current consensus, therefore, is that both autoreactive $Th_1$ and $Th_{17}$ cells are individually pathogenic in EAE

[130, 131]. The inflammatory composition and therefore the mechanisms of tissue damage differ, which is consistent with the distinct cytokine and chemokine profile inherent to the $Th_1$ and $Th_{17}$ subsets. Macrophages predominate in $Th_1$-mediated CNS inflammation whereas neutrophils are recruited during $Th_{17}$-mediated disease [130, 131]. Both Th subsets also differentially impact on inflammation in the brain versus spinal cord. IFN-$\gamma$ has been suggested to protect the brain from local inflammation. This was demonstrated by using MBP-specific TCR transgenic mice in which the spontaneous EAE preferentially targeted the brain rather than the spinal cord when the IFN-$\gamma$ gene was disrupted [132]. Furthermore, transferring $MOG_{35–55}$-specific pathogenic $Th_1$ cells into wild-type mice causes predominantly spinal cord inflammation, whereas IFN-$\gamma$ receptor knock-out recipients develop EAE specifically in the brain [133]. In the same spirit, it was proposed that the $Th_1/Th_{17}$ balance among encephalitogenic T cells critically impacts on their capacity to inflict damage to the brain. Indeed, brain inflammation only occurred when $Th_{17}$ cells outnumbered $Th_1$ cells [131, 133].

Do other $CD4^+$ T cell subsets contribute to EAE? $Th_2$ cells producing IL-4 have little or no impact on EAE as evidenced by the similar disease severity observed in IL-4-deficient mice [134]. Th cells producing IL-9 can be induced by the combined stimulation with TGF-$\beta$ and IL-4 during T cell priming [135, 136]. MOG-specific $Th_9$ cells are encephalitogenic, causing CNS lesions characterized by massive parenchymal mononuclear cell infiltrates and extensive demyelination after adoptive transfer into syngeneic recipients [137]. However, as their differentiation depends on IL-4 their implication in active EAE appears non-essential given the unaltered disease course in IL-4-deficient mice [134]. It is therefore likely that the pathogenic potential of the autoreactive T cells is retained within the $Th_1$ and $Th_{17}$ compartments. Nevertheless, controversy remains regarding the effector mechanisms that are being employed. GM-CSF is an effector cytokine essential for the pathogenicity of $Th_1$ cells [138], explaining the resistance of GM-CSF-deficient mice to EAE [139]. Despite the non-redundant requirement of either IL-23, ROR-$\gamma$t, or IL-6 for the induction of EAE, blocking or overexpressing effector cytokines from the $Th_{17}$ lineage fails to measurably affect EAE induction [140, 141].

## Influence of epitope spreading on chronic disease processes in the CNS

Inflammatory responses target sites of microbial infection to eradicate the invading microbe at the expense of tissue damage. This inflammatory milieu carries the intrinsic risk of generating secondary autoimmune disease. This transition towards auto-aggression has been predominantly explained by two mechanisms, epitope spreading and molecular mimicry [142] (next section).

Epitope spreading is the process by which reactivity to epitopes distinct from, and non-crossreactive with, the disease-inducing epitope are elicited during chronic inflammatory processes [143, 144]. During inflammatory tissue damage, activated antigen presenting cells (APCs) present self-antigens derived from debris and/or from the uptake of dying cells [145]. This permits the priming of additional T and B cells and allows the immune response to 'spread' to new self-epitopes within the same protein (intra-molecular spreading) or from distinct proteins (inter-molecular spreading) [146]. As such, epitope spreading can account for the induction of secondary autoimmune aggression or for the chronicity of an ongoing autoimmune disease.

TMEV infection of SJL mice leads to secondary autoimmune demyelination by the de novo priming of autoreactive T cells to myelin-derived antigens [147]. This mechanism relies on epitope spreading rather than molecular mimicry as no cross-reactivity between TMEV and myelin-derived antigens is observed. After 40 days, CNS APCs from TMEV-infected mice start presenting, in addition to viral antigens, myelin antigens released by local tissue damage [148]. Approximately 50 days post-infection, myelin-specific $CD4^+$ T cell responses develop that contribute to the pathology. The epitope spreading to myelin antigens progresses in a hierarchical order dependent on immunogenicity. The first response targets the immunodominant $PLP_{139–151}$ peptide, followed by $PLP_{178–191}$ and $PLP_{56–70}$ (intra-molecular spread) and later $MOG_{92–106}$ (inter-molecular spread) [147, 149]. Nevertheless, it currently remains unclear if secondary autoimmune phenomena can persist independent of the initiating viral infection [22]. To model the importance of this hierarchy in mediating autoimmune demyelination, SJL mice were immunized with $PLP_{139–151}$ generating relapsing-remitting EAE with a similar pattern of epitope spreading. Importantly, the secondary $PLP_{178–191}$ response was shown to be pathogenic as tolerization with $PLP_{178–191}$ before disease onset permitted $PLP_{139–151}$ EAE, but prevented relapses [150]. Blocking co-stimulation with a neutralizing anti-CD80 antibody abrogated epitope spreading after $PLP_{139–151}$ immunization, consistent with the interpretation that epitope spreading involves a naive myelin-specific T cell repertoire [150]. Using the $PLP_{139–151}$-specific 5B6 TCR transgenic mice as donors of traceable naive $CD4^+$ T cells the kinetics and anatomical location of epitope spreading was assessed in $PLP_{178–191}$-induced EAE and in TMEV-induced secondary autoimmune demyelination. In each of these models, naive $PLP_{139–151}$-specific 5B6 T cells get activated selectively in the CNS, and not in the cervical lymph nodes or other

secondary lymphoid organs [151]. F4/80$^{-}$CD11c$^{+}$CD45$^{hi}$ dendritic cells (DCs) efficiently present endogenous antigen to naive $PLP_{139–151}$-specific 5B6 T cells in vitro, suggesting that these DCs may be crucial in driving epitope spreading in the inflamed CNS. No data are available on whether the activation of naive T cells by the less immunogenic self-peptides can similarly occur within the CNS via epitope spreading. Together, these data demonstrated the pathogenic role of epitope spreading that is probably taking place directly in the CNS.

Epitope spreading is also observed in the humanized mice co-expressing the MS-derived $MBP_{85–99}$:DR15-specific TCR and HLA-DR2b that develop spontaneous EAE with 60% disease incidence [72]. In diseased animals, the CNS-derived T cells responded not only to $MBP_{85–99}$ but also to other HLA-DR2-restricted epitopes of MBP, MOG, and αB-crystallin, showing inter- and intramolecular epitope spreading also in this spontaneous model. Two of these epitopes were described to elicit T cell responses in HLA-DR15 MS patients [152], suggesting that these observations in humanized transgenic mice might be of relevance to the clinical setting.

Indeed, when the MBP-specific T cell repertoire in MS was analyzed longitudinally over a 6-year observation period, two patients revealed that their initially focused T cell repertoire had broadened to recognize multiple MBP-derived epitopes [153]. These observations suggest that the T cell repertoire is a composite over time and that the mechanism of epitope spreading is of likely importance in perpetuating the chronic inflammatory response that is characteristic of MS.

## Molecular mimicry

The existence of structural homology between pathogen-derived and host protein sequences was first observed in 1964 [154]. The concept of molecular mimicry posits that, due to this homology, effective anti-microbial immune responses can inadvertently react to self-antigens and thereby evoke autoimmune tissue damage [142, 155–157]. This provides a likely scenario by which environmental factors can promote autoimmunity.

Structural similarities between an immunodominant MBP peptide and microbial peptide sequences suggested a role for molecular mimicry in MS [158]. Seven viral (herpes simplex, adeno-, human papilloma, Epstein–Barr, influenza, and reo-virus) and one bacterial (*Pseudomonas aeruginosa*) peptides were able to activate MBP-specific CD4$^{+}$ T cell clones established from MS patients. Thus, molecular mimicry between microbial and oligodendrocyte proteins may be important in the etiology of a human CNS autoimmune disease such as MS. This idea is reinforced by the induction of CNS inflammation [142] or even full-blown EAE [159] with microbial peptides.

To assess whether molecular mimicry can provoke autoimmune tissue insult, specific transgenic models were designed. Evans et al. [157] generated a transgenic model in which the nucleoprotein or the glycoprotein of lymphocytic choriomeningitis virus (LCMV) were expressed as ‘neo-self’ antigens in oligodendrocytes using the MBP promoter. To determine if molecular identity between a viral antigen and an oligodendrocyte ‘neo-self’ antigen can predispose to autoimmunity, these mice were inoculated with LCMV. The immune system efficiently cleared viral infection but a chronic CNS inflammation ensued. Self-reactive T cells were activated in the periphery by LCMV and trafficked into the CNS to cause focal T cell inflammation. Microglia/macrophages were activated and MHC class I and II molecules upregulated within the lesions. However, no loss of oligodendrocytes could be observed. Interestingly, disease aggravation was induced with LCMV re-infection, but also after infection with unrelated viruses. These data indicate that a chronic autoimmune demyelinating disease can be initiated by a virus that shares antigenic epitopes with CNS antigens.

The BeAn strain of TMEV was genetically modified to express either $PLP_{139–159}$, or a mimic sequence of PLP from the bacteria *Heamophilus influenzae* (HI) [160, 161]. The sequence coding for the bacterial serine protease IV ($HI_{574–586}$) has a limited sequence identity but shares structural homology with $PLP_{139–151}$. Importantly, the sequence homology preserves the primary TCR contact and MHC-binding residues. Inoculation of mice with the unmodified TMEV BeAn strain caused chronic progressive demyelinating disease starting around day 30–35. Infection with BeAn encoding the native PLP peptide (PLP-BeAn) induces a severe disease with earlier onset (10–14 days post-infection) that is driven by $PLP_{139–151}$-specific $Th_1$ cells, indicating that molecular identity can aggravate disease [160, 161]. To assess the impact of molecular mimicry, mice were inoculated with BeAn expressing the $HI_{574–586}$ sequence (HI-BeAn). These animals exhibited an earlier disease onset and enhanced disease severity compared to BeAn-infected animals. This disease aggravation was due to the activation of pathogenic $PLP_{139–151}$-specific $Th_1$ cells by the $HI_{574–586}$ mimic sequence [49, 51, 162]. These data demonstrate that neurotropic viruses can cause autoimmune demyelination via molecular mimicry with myelin antigens.

Myelin-specific T cells from MS patients also revealed evidence for polyspecificity. The human T cell clone TCR-Hy.2E11 was derived from a DR2-positive MS patients [163]. This clone functionally recognizes $MBP_{85–99}$ in the context of HLA-DR2b and an EBV DNA polymerase in the context of HLA-DR2a, due to the strong resemblance in

TCR contact residues [73]. Furthermore, the previously described $MBP_{84–102}$:DR2b-specific TCR Ob.1A12 [70] has since been shown to react to at least five different DR2b-binding microbial peptides [164]. Re-analysis of the identified protein sequences revealed that the *Escherichia coli* and *Haemophilus influenza* peptides belong to a highly conserved bacterial guanosine tri-phosphate (GTP)-binding protein, engA [165]. The immunization of the humanized mice expressing the Ob.1A12 TCR and HLA-DR2b with three microbe-derived peptides, including engA, caused a severe autoimmune demyelinating disease. Crystallographic data indicate that the Ob.1A12 TCR focuses on the P2-His and P3-Phe residues which are conserved among the mimic peptides. Surface plasmon resonance analysis revealed that the Ob.1A12 TCR binds the $MBP_{84–102}$:DR2b complex with low affinity ($K_d \sim 47$ μM), and that the affinity of the TCR for the mimic peptide:DR2b complexes is even lower ($K_d$ ranging from 220 to 670 μM) [165]. These data indicate that microbial peptides shared by common bacteria can activate myelin-specific $CD4^+$ T cells with low affinity due to their preservation of essential TCR contact residues.

### Cumulative autoimmunity

While studying the pathogenic traits of the MOG-specific $CD4^+$ T cell response using the 2D2-TCR transgenic mice in which over 95% of $CD4^+$ T cells are specific for $MOG_{35–55}$:I-$A^b$, we recently observed that molecular mimicry also exists between two neural self-antigens. Indeed, we made the paradoxical observation that the 2D2-TCR mice crossed with MOG-deficient animals exhibited the same frequency of spontaneous EAE as their 2D2 MOG-sufficient counterparts [64]. Using a proteomics approach, the alternative autoantigenic target for the 2D2 T cells was identified as neurofilament medium (NF-M), a cytoskeletal protein expressed in neurons [64, 166–168]. $NF\text{-}M_{15–35}$ peptide was shown to share the same TCR-binding residues as $MOG_{35–55}$ peptide when presented in the context of I-$A^b$, and could stimulate $CD4^+$ T cells from 2D2-TCR × $RAG^{-/-}$ mice in vitro. Importantly, this cross-reactivity is relevant to the pathogenesis of MOG-induced EAE. We used a convenient adoptive transfer model in which in vitro differentiated 2D2 $Th_1$ cells cause lethal EAE in all syngeneic C57BL/6 mice, which express both MOG and NF-M. MOG-deficient recipients developed lethal EAE with a delayed disease onset demonstrating the importance of MOG as a target autoantigen. The remaining disease in the MOG-deficient animals targets NF-M, as recipient mice lacking both MOG and NF-M ($MOG^{-/-}$ $NF\text{-}M^{-/-}$ double-deficient) were fully resistant to EAE. These data revealed that molecular mimicry between the neural antigens $MOG_{35–55}$ and $NF\text{-}M_{15–35}$ allows a clonal $CD4^+$ T cell response to target more than one cognate autoantigen simultaneously and thereby participate to disease severity. We coined this observation cumulative autoimmunity.

Given their potential to investigate with high resolution the immune response at the clonal level, there is little doubt that transgenic models will further be used to zoom in on the different steps of disease development. Ex vivo analysis using 'omic' approaches will document the metabolic changes of autoreactive transgenic T and B cells at each step of CNS autoimmunity. Furthermore, intravital imaging of CNS-invading lymphocytes will certainly make use of the homogenous T and B cell populations from TCR and BCR transgenic mice, which can be coupled with transgenes revealing distinct functional properties of these cells.

In addition, the mechanistic understanding of T and B cell tolerance towards CNS antigens can benefit from the unambiguous identification of autoreactive lymphocytes provided by transgenic models. This allows studies of immune tolerance at the cellular and molecular levels [66, 169, 170]. As such, transgenic models should help reveal and analyze in depth immunological phenomena relevant for MS pathophysiology.

## Transgenic models of CNS inflammation

### CNS inflammation induced by primary neurodegeneration

Histological analysis of MS patients' CNS during or shortly after a relapse were in some cases consistent with a primary oligodendrocytic damage with some activated microglial cells and little or no lymphocytes within the area of the active lesion thereby suggesting a secondary involvement of the immune system [171–173]. Interestingly, similar observations have been generated using a transgenic mouse overexpressing a normal PLP gene in oligodendrocytes. Homozygous animals developed late-onset and slowly progressing demyelination associated with axonopathic changes [174, 175]. This primary demyelination predisposed to local inflammation. Few B cells and $CD4^+$ T cells infiltrated the CNS, but the number of $CD8^+$ T cells and $CD11b^+$ macrophages/microglia dominated in adult PLP transgenic mice. This coincided with a marked increase of MHC class I expression in the white matter. Approximately 25–30% of the T cells were in close contact with MHC class I-positive oligodendrocytes, suggesting that $CD8^+$ T cells directly attack oligodendrocytes. By crossing the PLP transgenic mice with Rag1-deficient mice, the demyelinating phenotype could be ameliorated. Worsening occurred upon transfer of $CD8^+$ T cells but not $CD4^+$ T cells, confirming the view that $CD8^+$

T cells are the pathogenic immune cells [176]. These data indicate that primary demyelination can predispose to secondary $CD8^+$-mediated tissue damage, possibly via a mechanism analogous to epitope spreading.

### CNS inflammation by local co-stimulation

Modifying the homeostasis of the CNS can predispose to secondary autoimmune aggression. Transgenic mice have been generated in which the co-stimulatory molecule CD86 is expressed under the H-$2K^b$ promoter [177] and fortuitously, one subline constitutively expressed CD86 on microglial cells [178]. Ascending flaccid paralysis caused premature death in all these CD86 transgenic mice [178]. The animals spontaneously developed demyelinating lesions in the spinal cord that are dominated by $CD8^+$ T cells but also contain $CD4^+$ T cells and MHC class II expressing cells. The lesions were observed in the grey and white matter with axonal damage. This spontaneous pathology is T cell-dependent, as CD86 transgenic mice crossed on a TCR$\beta^{-/-}$ background, to remove $\alpha\beta$ T cells, did not develop disease [178]. Importantly, T cell reconstitution with bone marrow chimeras induced disease only when the bone marrow was derived from CD86 transgenic mice, and not from wild-type mice. The disease could be similarly restored by the transfer of CD86 transgenic T cells indicating that the expression of CD86 on T cells is important for the generation of spontaneous disease. $CD4^+$ T cells did not contribute to this spontaneous disease. Reconstituting CD86 transgenic TCR$\beta^{-/-}$ with bone marrow from I-$A^{b-/-}$ CD86 or $CD4^{-/-}$ CD86 transgenic mice accelerated disease onset and aggravated disease severity [179]. $CD8^+$ T cells are therefore proposed as the effector immune cell type. Activated oligoclonal $CD8^+$ T cells infiltrate the CNS prior to disease induction and exert their damage in an IFN-$\gamma$-dependent mechanism, as CD86 transgenic IFN-$\gamma^{-/-}$ mice remained healthy. Clarifying the nature of the targeted CNS autoantigens will provide further insights into the mechanisms by which T cell co-stimulation controls $CD8^+$-mediated CNS tissue damage.

### Overexpression of cytokines in the CNS

The local release of pro-inflammatory cytokines is thought to contribute to axonal damage and oligodendrocyte loss, characteristic of the MS lesions [180, 181]. To assess the impact of such cytokines, transgenic models have been created that impose the secretion of a given cytokine locally in the CNS (Table 2).

TNF-$\alpha$ is expressed by both the pathogenic $Th_1$ and $Th_{17}$ cells and can be produced by innate immune cells in the CNS [182]. Consistently, TNF-$\alpha$ levels are elevated in the CNS during both MS and EAE. Transgenic mice have been generated driving expression of TNF-$\alpha$ to neurons, astrocytes, and oligodendrocytes [183–186]. The first model resulted from the fortuitous integration of a TNF-$\alpha$ transgene in a locus that directs expression to CNS neurons [184, 185]. These transgenic mice develop spontaneous CNS inflammation causing ataxia, seizures, as well as motor deficits [184]. Histological examination of the inflammatory infiltrate revealed the presence of $CD4^+$ and $CD8^+$ T cells, as well as macrophages in the leptomeninges and the CNS parenchyma causing local glial activation and focal demyelination. These results demonstrated that overexpression of TNF-$\alpha$ in the CNS can recruit blood-borne immune cells and cause structural as well as functional defects in the nervous system. Further studies directed TNF-$\alpha$ expression to astrocytes [186] or oligodendrocytes [183] using the GFAP or MBP promoter, respectively. Mice expressing TNF-$\alpha$ in astrocytes developed a chronic inflammatory disease causing focal loss of myelin and axonal damage in the lumbar spinal cord [186]. In contrast, no spontaneous disease was detected in the model expressing TNF-$\alpha$ in oligodendrocytes [183]. Nevertheless, upon MBP immunization, these animals developed EAE with greater severity than wild-type mice, indicating that the expression of TNF-$\alpha$ in the CNS favors autoimmune demyelination. The local effect of TNF-$\alpha$ in mice expressing TNF-$\alpha$ in CNS glial cells depends on signaling through the p55 TNF receptor I (TNFRI) [185]. On the contrary, the absence of spontaneous disease in the MBP-TNF-$\alpha$ mice might reflect the more restricted distribution of oligodendrocytes and/or variations in promoter activity resulting in lower production of TNF-$\alpha$. Taken together, these studies underline the detrimental role of TNF-$\alpha$ in CNS demyelination.

IFN-$\gamma$ is the hallmark cytokine of $Th_1$ cells, and is readily produced by cytotoxic $CD8^+$ T cells, NK, and NKT cells [187]. To test the impact of IFN-$\gamma$ on the local CNS environment, this pro-inflammatory cytokine was expressed by oligodendrocytes using the MBP promoter [188]. These transgenic animals, in which IFN-$\gamma$ is expressed early in life, exhibited profound pathological defects to the myelin sheath, with severe hypomyelination and premature death. In another study using IFN-$\gamma$ cDNA driven by the MBP promoter, the transgenic mice presented shaking, hind-limb weakness that resulted in premature death of all male mice by 8 weeks of age, whereas 50% of the transgenic females presented delayed clinical signs [189]. Primary demyelination can be observed in these transgenic animals concomitantly to activated macrophages/microglia. Furthermore, the presence of myelin degradation products within phagocytic cells was indicative of an active process. Both studies provide evidence of the detrimental impact of IFN-$\gamma$ on the integrity of the CNS underlining the potential impact of this cytokine in CNS inflammatory

**Table 2** Transgenic mice expressing cytokine or chemokine in the CNS

| Molecule | Promoter | Target | Phenotype | References |
|---|---|---|---|---|
| *Cytokines* | | | | |
| TNF-α | Unknown | GP[g] | Ataxia, seizures, motor defects. CNS infiltration: $CD4^+$, $CD8^+$ T cells, macrophages. Histopathology: demyelination, moderate axonal damage | [184, 185] |
| TNF-α | GFAP[a] | Astrocytes | TNF: chronic inflammatory disease, neurodegeneration, astrocytosis and microgliosis/With unclearable transmembrane form of TNF-α: clinic similar than with TNF, Histopathology: demyelination and loss of neuronal axons, infiltration, widespread astrocytosis and microgliosis | [186] |
| TNF-α | NFL[b] | Neurons | TNF: chronic inflammation/with a membrane form of TNF-α: no pathology | [186] |
| TNF-α | MBP[c] | ODC[h] | No clinical or histological signs. Increased susceptibility to active EAE | [183] |
| INF-γ | MBP[d] | ODC | Shaking/tremor. CNS infiltration: macrophages. Histopathology: hypomyelination, reactive gliosis, microglia activation | [188] |
| INF-γ | MBP[d] | ODC | Shaking, hind-limb weakness, hunched posture. CNS infiltration: $CD8^+$ T cells, macrophages. Histopathology: demyelination, microglia activation | [189] |
| IFN-α | GFAP[e] | Astrocytes | Ataxia, premature death. CNS infiltration: $CD4^+$, $CD8^+$, and B cells. Histopathology: microglia activation, neurodegeneration, astrogliosis | [261] |
| TGF-β1 | GFAP[e] | Astrocytes | Motor disease, no spontaneous inflammation, increased EAE | [262] |
| IL-3 | GFAP[e] | Astrocytes | Strong IL-3 expression: early onset of acute neuroinflammatory disease<br>Low IL-3 expression: late onset of chronic progressive motor disorder<br>Histopathology: demyelination, microglia/macrophage activation | [218] |
| IL-6 | GFAP[e] | Astrocytes | Runting, tremor, ataxia, seizures. Histopathology: inflammation (diffuse), neurodegeneration, astrocytosis | [209] |
| IL-6 | NSE[f] | Neurons | No clinical signs. Histopathology: reactive astrocytosis, gliosis, no neuronal damage | [263] |
| IL-12 (p35 + p40) | GFAP[e] | Astrocytes | Ataxia, muscle atrophia. CNS infiltration: T and NK cells. Histopathology: neurodegeneration, reactive astrocytosis. Enhanced susceptibility to active EAE | [203] |
| IL-12 (p40) | GFAP[e] | Astrocytes | No clinical or histological phenotype. Reduced susceptibility to active EAE | [203] |
| *Chemokines* | | | | |
| CXCL1 | MBP[d] | ODC | Progressive neurological dysfunction. CNS infiltration: neutrophils. Glial activation: microglia, astrocytes | [253] |
| CXCL10 | GFAP[e] | Astrocytes | No clinical signs. CNS infiltrate: dominated by neutrophils and macrophage. T cells present to a lesser extent (CXCL10-dose and age-related) | [249] |
| CCL2 | MBP[d] | ODC | No clinical signs. CNS infiltrate: monocytes and macrophages. Reduced susceptibility to active EAE | [247, 264] |
| CCL2 | GFAP[e] | Astrocytes | No clinical signs. CNS infiltrate: monocytes (diffuse). Reduced susceptibility to active EAE | [248] |
| CCL19 | MBP[d] | ODC | No clinical or histological phenotype | [252] |
| CCL21 | MBP[d] | ODC | Loss of reflex, tremor, ataxia, premature death. CNS Infiltrate: neutrophils, macrophages, occasionally eosinophils. Glial activation: Microglia, astrocytes | [252] |

[a] Glial fibrillary acidic protein (GFAP) promotor [265, 266]

[b] Neurofilament-light (NF-L) promotor [267, 268]

[c] Promoter/distal enhancer of MBP [269]

[d] Proximal promoter/enhancer of MBP [270]

[e] Glial fibrillary acidic protein (GFAP) promotor [271]

[f] Rat neurospecific enolase (NSE) promoter

[g] Glial progenitors (GP)

[h] Oligodendrocytes (ODC)

diseases. This is unlikely due to a direct impact of IFN-$\gamma$ on oligodendrocytes as IFN-$\gamma$ is not directly cytotoxic for cultured oligodendrocytes [190]. It is conceivable that this phenotype is partly mediated by the induction of MHC class I molecules. One possible mechanism could involve the induction of MHC class I expression, as transgenic mice in which overexpressing MHC class I in oligodendrocytes exhibited a neurological phenotype characterized by hypomyelination of the CNS without inflammation [191, 192], resembling the phenotype of the IFN-$\gamma$ transgenic mice [188, 189].

IL-12 is a pivotal factor in $Th_1$ differentiation and activation of NK cells [193–195]. Expression of IL-12 in the CNS is found in a number of neuro-inflammatory states, including MS [196] and EAE [197, 198]. Besides being produced by CNS-infiltrating immune cells, IL-12 can also be produced by CNS-resident cells such as microglia [199–202] and astrocytes [199, 202]. To determine the consequences of local CNS production of IL-12, a transgenic line expressing IL-12 (p35 and p40) in astrocytes under the control the GFAP promoter has been generated [203]. The transgenic mice exhibited clinical signs including hunchback, ataxia and muscle atrophy. Histological examination revealed perivascular and parenchymal inflammatory lesions involving mostly the cerebellum with loss of cerebellar Purkinje cells and neurons from both granular and molecular layers. The inflammatory infiltrates were mainly composed of $CD4^+$ and $CD8^+$ T cells together with NK cells. These data showed that localized expression of IL-12 by astrocytes could promote the spontaneous development of an inflammatory response in the CNS.

IL-6 is a pluripotent cytokine that is produced by many cell types, including leukocytes and CNS resident cells [204]. The expression of IL-6 is induced in the CNS during EAE and MS [205, 206]. Functionally this cytokine is essential for EAE as IL-6 knock-out mice are highly resistant to disease induction [207, 208]. To assess the individual contribution of IL-6 to CNS inflammation, transgenic mice were generated, expressing IL-6 under the GFAP promoter [209]. In these mice, IL-6 is predominantly expressed by astrocytes in the cerebellum [210]. Clinically, the GFAP-IL-6 mice developed a runting phenotype, tremor, ataxia, and seizure. Histopathologically, these GFAP-IL-6 mice exhibited a progressive inflammatory encephalopathy associated with loss of BBB integrity. By 3–6 months leptomeningal inflammatory infiltrates develop in the cerebellum. This proceeds to parenchymal inflammation, astrocytosis and microgliosis, and axonal degeneration [211–214]. These results strongly suggest that localized expression of IL-6 can breach immune tolerance by disrupting BBB development and initiating local inflammatory tissue damage.

IL-3 detection has been reported in neurodegenerative diseases such as Alzheimer's disease [215]. Astrocytes, oligodendrocytes and microglia can produce, as well as respond to, IL-3 [216]. Furthermore, encephalitogenic $MOG_{35–55}$-specific $Th_1$ cells produce copious mounts of IL-3 [217]. Transgenic mice selectively expressing IL-3 in astrocytes using the GFAP promoter, were generated [218]. Starting at 4–5 months of age, the GFAP-IL-3 mice exhibited progressive motor dysfunction characterized by head tilting, ataxia, and muscle weakness that progressed to quadriplegia and premature death. The onset and severity of the disease was directly correlated with the level of IL-3 production. Interestingly, in these IL-3-transgenic mice, inflammatory demyelinating lesions were reminiscent of the multifocal plaques found in MS [5, 219]. In the late stage of the disease, the GFAP-IL3 mice also exhibited cerebellar damage with disorganization of the granular layer structure, as well as loss of Purkinje cells and demyelination. CNS-production of IL-3 promoted the recruitment, proliferation and activation of microglia/macrophages in the white matter area where demyelination was detected. Moreover, compared to wild-type littermates, MHC class II molecule level increased progressively from 2 months of age. A similar CNS pathology was observed in transgenic mice with widespread IL-3 expression driven by the CMV promoter [220]. These mice develop a neuronal pathology and motor disorder with similar kinetics and severity. These models indicate that IL-3 expression in the CNS can initiate a severe neuropathology that includes inflammatory demyelination in the CNS white matter.

## Targeting chemokine expression to the CNS

Chemokines direct the migration and homing of leukocytes to tissues. Therefore, these molecules play an important role in a number of inflammatory diseases, including MS [181, 221]. $Th_1$ cells preferentially express the CCR5 and CXCR3 chemokine receptors permitting their recruitment into the CNS parenchyma in response to the CCR5 ligands CCL3 (MIP-1$\alpha$) and CCL5 (RANTES) and the CXCR3 ligand CXCL10 (IP-10) [222–226]. In human, $Th_{17}$ cells have been shown to express CCR6, the receptor for CCL20 (Exodus-1/LARC/MIP-3$\alpha$) [227]. Furthermore, CCL20 (Exodus-1/LARC/MIP-3$\alpha$) is constitutively expressed in epithelial cells of the choroid plexus in both mice and men [228]. This is functionally relevant, as Reboldi and colleagues demonstrated that in EAE, autoreactive $Th_{17}$ cells infiltrate the CNS in a CCR6-dependent manner via the choroid plexus [228]. Macrophage recruitment relies on the release of CCL2 (MCP-1) that binds CCR2, CCL3 (MIP-1$\alpha$) and CCL5 (RANTES) that both bind CCR1 and CCR5 [229–232]. Chemokines are released by the infiltrating inflammatory cells, as well as by activated CNS resident

cells. Astrocytes, microglia, and endothelial cells have been shown to produce a wide range of chemokines [233–241]. Various transgenic mice have been developed to assess whether the local expression of chemokines can infringe the homeostasis of the CNS (Table 2).

CCL2 (MCP-1) has been identified in the CNS during several inflammatory diseases [242–246]. Its transgenic expression in oligodendrocytes, driven by the MBP promoter, resulted in a mononuclear cell infiltration in CNS white matter that was discrete, focal, and almost exclusively perivascular [247]. No demyelination or parenchymal inflammation occurred. Upon expression of CCL2 (MCP-1) in astrocytes using the GFAP promoter, a more diffuse CNS monocyte/macrophage infiltration in line with the broader dispersion of astrocytes in the grey and white matter appeared [248]. Neither study observed any clinical phenotype. These data indicate that the selective expression of CCL2 (MCP-1) in the CNS drives local monocyte/macrophage accumulation, without inducing CNS disease.

CXCL10 (IP-10) is an IFN-inducible chemokine increased in the CSF of MS patients [221] and expressed by activated astrocytes within active demyelinating lesions [222]. Transgenic mice, in which CXCL10 (IP-10) expression was targeted to astrocytes using the GFAP promoter, spontaneously exhibited leukocyte infiltration into the CNS [249]. The inflammatory infiltrate contained monocytes/macrophages and numerous neutrophils. No clinical or histopathological phenotype was observed.

These mouse models, each locally expressing either CCL2 (MCP-1) or CXCL10 (IP-10), provide new insights in the ability of these two cytokines to drive inflammatory cells to the CNS. Nevertheless, the lack of a related clinical phenotype indicates that additional factors are needed for the development of CNS tissue damage.

CCL21 (6Ckine/exodus-2/SLC) is a CC-chemokine, which has been shown to be chemotactic in vitro for naive T cells, mature DCs and naive B cells but not for macrophages or neutrophils [250, 251]. This chemokine is normally not expressed in the CNS. Transgenic mice constitutively expressing CCL21 (6Ckine/exodus-2/SLC) selectively in oligodendrocytes, using the MBP promoter, frequently develop a neurological disease characterized by loss of righting reflex, tonic tremor and ataxia in most animals [252]. These clinical signs were observed as early as postnatal day 9 and were associated with weight loss and premature death. Microscopic examination of the brain and spinal cord revealed scattered leukocyte infiltrates composed of neutrophils and eosinophils. The expression of CCL21 (6Ckine/exodus-2/SLC) induced a significant inflammatory response in the CNS, but no recruitment of lymphocytes was detected.

Rather similar observations were made in transgenic mice in which the MBP promoter drives an oligodendrocyte-specific expression of CXCL1 (MIP-2/GROα/NAP3/KC) (N51 in mice), a chemokine related to the human IL-8 [253]. Thirty percent of transgenic animals exhibited a progressive neurological dysfunction starting with slowing reflexes evolving to rigidity of hind limbs and tail with profound truncal instability leading to premature death. The mortality rate was 37% by the age of 1 year. Histological analysis revealed BBB breakdown and microglia as well as astrocyte activation. A diffuse infiltration mainly consisting of neutrophils was detected by 3 weeks of age. However, neither demyelination nor neuronophagia were detected at this stage. Because mice are asymptomatic at the peak of CXCL1 (MIP-2/GROα/NAP3/KC) expression, the hypothesis of direct toxicity of CXCL1 (MIP-2/GROα/NAP3/KC) and of neutrophils could be excluded. In contrast, microglia, and astrocytes activation increased as the neurological symptoms progressed, suggesting their involvement in the altered neuronal function.

These data indicate that CCL21 (6Ckine/exodus-2/SLC) and CXCL1 (MIP-2/GROα/NAP3/KC) can cause a lethal neurological disease, independent of lymphocyte infiltration and demyelination. The observed accumulation of neutrophils in these mice could suggest a role for these cells in aggravating neuropathologies. This could include inflammatory demyelinating diseases as neutrophils have been found in the CNS of mice with EAE [254]. However, neutrophils are almost absent from the CNS in classical forms of MS [255].

To investigate in depth the molecular mechanisms implicated in the generation of inflammatory processes in CNS disorders such as MS, transgenic mouse lines overexpressing immune mediators directly within the CNS have been generated. Even if such tools provide information on the potential role of the studied molecules in CNS inflammation, complex phenotypes associating demyelination, inflammation, astrogliosis, and axonal damage as seen in EAE and in MS could not be reduced to the effects of a single effector molecule. Moreover, the use of such transgenic animals presents some disadvantages. Indeed, the number of transgene copies inserted in the mouse genome and their integration site are largely unpredictable. Level and kinetics of transgene expression cannot be controlled and largely depends on the promoter. Using a cell-specific promoter restricts the transgene expression to a unique cell type, omitting the fact that other cells could produce this molecule in the context of EAE or MS.

## Perspectives

How can transgenesis be of further use to advance our understanding of the chronic inflammatory processes underlying MS? Many aspects of the pathophysiology of

MS remain ill understood. Notably, the inflammatory dynamics within the diseased CNS, the interplay and evolution of the pathogenic T and B cell repertoires during chronic inflammation, the impact of environmental factors on these pathogenic T/B cell responses and the functional significance of the genetic polymorphisms associated with MS remain to be explored.

Recent technical advances have generated unique tools to tackle the immunopathology of immune-mediated diseases. Intravital imaging using time-lapse two-photon laser scanning microscopy is providing access to the dynamics of pathogenic T cells that enter the CNS and within parenchymal lesions [256, 257]. The generation of 'retrotransgenic' mice by Dario Vignali permits the creation of TCR transgenic mice that harbor multiple T cell clones with individual transgenic TCRs [258]. To model aspects of the MS pathogenesis, mice can be reconstituted with oligoclonal TCR repertoires carrying specificities for different CNS antigens. Using models of viral infection or myelin immunization to induce EAE, the dynamics of the repertoire can be studied at the clonal level to reveal the cellular contributions to epitope spreading, the dialogue between $CD4^+$ and $CD8^+$ T cells, or the interplay between pathogenic and regulatory lymphocyte populations. Furthermore, the differentiation and plasticity of functionally distinct T cell subsets can be monitored using knock-in mice in which genes encoding for fluorescent markers are integrated into the genomic sequence encoding for FoxP3, Ror$\gamma$t, GATA3, T-bet, or other lineage-specific genes. This technique has become even more powerful when the locus drives the expression of both a fluorescence marker and Cre-recombinase (Cre) [259]. Removal of a floxed stop-cassette by Cre in a second transgene knocked into a locus of interest permits the conditional expression of a fluorescent marker that will be maintained in all daughter cells. This combination permits to map the fate of autoreactive T cells using one fixed marker reflecting previous lineage commitment, and one marker indicating current subset adherence. As such, the potential for plasticity between the different effector T cell subsets can be assessed in vivo during chronic CNS inflammation. Lastly, to complement studies on biological samples from MS patients, approaches are developed permitting the functional analysis of MS-associated genetic polymorphisms in vivo. Knock-in approaches in which disease associated alleles and non-associated alleles are introduced into orthologous rodent loci should provide novel humanized models in which the impact of allelic variants on spontaneous or induced EAE can be studied. Consequently, despite the already significant contribution of transgenesis, its future applications will provide an unprecedented resolution of the cellular and molecular mechanisms driving chronic inflammatory diseases, including MS.

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