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INVITED REVIEW

Experimental Autoimmune Encephalomyelitis in the Common Marmoset, a Bridge Between Rodent EAE and Multiple Sclerosis for Immunotherapy Development

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Abstract The attrition rate of new drugs for central nervous system diseases including multiple sclerosis (MS) is very high. A widely recognized bottleneck in the selection of promising central nervous system drug candidates from the development pipeline is the lack of sufficiently predictive animal models. Here, we review how the experimental autoimmune encephalomyelitis (EAE) model in the Neotropical primate "common marmoset" can help to bridge the gap between rodent EAE models and MS. The EAE model in the marmoset closely resembles MS in the clinical as well as pathological presentation and can be used for fundamental research into immunopathogenic mechanisms and for therapy development. We discuss recent insights arising from this model, both on novel therapeutics and immunopathogenesis.

Keywords non-human primate · CD40 · IL-23 · IL-12 · B-cells · NK-CTL

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Introduction

Multiple sclerosis (MS) is a chronic neurological disease characterized by inflammation, demyelination, axonal injury, and atrophy in the human central nervous system (CNS). Although the etiology of MS is unknown, it is well accepted that once initiated, autoimmune reactions against CNS components play a critical role in the disease progression. The disease begins in most patients with a relapsing-remitting (RR) MS course caused by transient inflammation and remyelination. Over time, irreversible pathological changes lead to the secondary progressive phase of the disease (Compston and Coles 2008).

The availability of new therapeutic agents for MS has tremendously improved in the past decade (Lopez-Diego and Weiner 2008). Approved drugs include interferon-β, glatiramer acetate, mitoxantrone, and natalizumab. Interferon-β and glatiramer acetate (copaxone) modulate the immune response, e.g., by skewing the T-cell response towards Th2 cells. Mitoxantrone inhibits DNA and RNA synthesis and is, because of its toxic effects, only approved for aggressive forms of MS. Natalizumab is a monoclonal antibody directed against α4β1 integrin (VLA-4) that reduces leukocyte infiltration into the CNS (Polman et al. 2006). Drugs that are currently in clinical trials include simvastatin (Vollmer et al. 2004), the small molecule FTY720 (Kappos et al. 2006), and monoclonal antibodies targeting CD52 (Alemtuzumab; Coles et al. 2008), CD20 (Rituximab; Hauser et al. 2008), or the receptor α chain of IL-2 (Daclizumab; Bielekova et al. 2004). FTY720, also known as fingolimod, targets sphingosine-1-phosphate receptors and prevents the lymph node emigration of lymphocytes. Alemtuzumab targets the CD52 antigen that is widely expressed by T- and B-lymphocytes as well as on

a granulocyte subpopulation, Rituximab targets the B-1 antigen of B-cells, and daclizumab is directed against the IL-2 receptor α chain expressed by activated T- and B-cells.

Unfortunately, a large percentage of new drug candidates fail in clinical trials for reasons of insufficient activity or unforeseen toxicity (Kola and Landis 2004). The current success rate of new drugs varies from 20% for cardiovascular disorders to only about 8% for diseases of the CNS and reflects the predictive quality of the available preclinical animal models (Kola and Landis 2004). Increasing the low success rate is one of the highest priorities for the drug development industry. However, one of the major hurdles in drug development remains the difficulty to translate promising effects in animal models to the patient (Schafer and Kolkhof 2008; von Herrath and Nepom 2005).

The choice of the animal model(s) that will be used for the selection of the most promising agents from the development pipeline is a strategically important decision. Promising results obtained in a valid model can considerably accelerate the development of a new therapy, whereas similar results obtained in an invalid model can lead to false hope for a new therapy. Autoimmune aspects of MS are modeled in experimental autoimmune encephalomyelitis (EAE). Which aspects of MS are modeled depends on animal species, strain, antigen, and immunization protocol (Gold et al. 2006; Krishnamoorthy et al. 2007). However, in preclinical MS research, there is no general agreement on the right animal model. The usefulness of rodent EAE models for preclinical studies versus development of immunopathogenic concepts has been intensely debated recently (Sriram and Steiner 2005; Steinman and Zamvil 2006).

A recognized problem in therapy development for MS is that promising effects obtained in rodent EAE models are often not reproduced in clinical trials, underlining the immunological distance between humans and the young, inbred, specific pathogen-free raised laboratory rodents (Mestas and Hughes 2004; Friese et al. 2006). This pleads for the development of preclinical animal models that are immunologically and pathogenetically more closely related to MS.

The aim of this review is to explain how the unique aspects of EAE models in non-human primates can help to bridge the gap between rodent EAE models and the MS patient.

EAE in non-human primates

EAE models have been developed both in monkeys of Old World origin, i.e., the rhesus macaque (*Macaca mulatta*) and the cynomolgus monkey (*Macaca fascicularis*), and of New World origin, i.e., the common marmoset (*Callithrix jacchus*; ‘t Hart et al. 2005a).

Both macaque species are relatively large-sized, weighing, respectively, 6 to 10 kg (rhesus) or 3 to 5 kg (cynomolgus) at adult age (about 4 years). The genetic, physiological, microbiological, immunological, and neuroanatomical proximity to humans have all been well established. For these reasons, both species provide useful models in a wide range of biomedical research disciplines. For MS research, they are less useful, because all tested models are characterized by an acute clinical course and destructive neuropathology, which is more reminiscent to acute neuroinflammatory disorders, such as acute disseminated (leuko)encephalomyelitis. Hence, the value of these models may lie in elucidating interrelations between acute disseminated encephalomyelitis and MS (‘t Hart et al. 2005a).

Common marmosets are much smaller, weighing about 350 g at adult age (2 years). Experiments in this species thus require ten- to 20-fold less test compound than in the larger macaques. A particularly interesting aspect of marmosets is the stable bone marrow chimerism between twin siblings, which is caused by the sharing of the placental blood stream. Consequently, the immune systems of twins are educated in the same thymic environment, making fraternal siblings immunologically more similar than siblings from different births. This principle can be used in therapy trials where one twin sibling is treated with an experimental agent and the other with a placebo. Some of the most obvious advantages and disadvantages of the marmoset model are listed in Table 1. For the research of MS, a set of highly useful EAE models have been developed, which share many similarities with the human disease (Genain and Hauser 2001; ‘t Hart and Massacesi 2009).

Clinical and neuropathological aspects of the marmoset EAE models

The EAE model in the marmoset has first been described almost 15 years ago (Massacesi et al. 1995). Since then, the model has been continuously refined by the identification of the minimal requirements to induce EAE. The first model employed human myelin in complete Freund’s adjuvant (CFA) and additional *Bordetella pertussis*, resulting in an acute model as also observed in the rhesus macaque, which resembles acute neuroinflammatory disorders rather than MS. Fine-tuning of the model has led to a more chronic progressive disease model with the typical neuropathological hallmarks of MS. The clinical and neuropathological aspects of the different EAE marmoset models are discussed below.

Table 1 Advantages and disadvantages of the marmoset experimental autoimmune encephalomyelitis model (key words in italics)

Advantage
(Neuro)anatomical, immunological, physiological, and microbiological *proximity to humans*.
Outbred nature of the marmoset reflects better the genetic heterogeneity of the multiple sclerosis patient population.
Experimental autoimmune encephalomyelitis is induced *at adult age* when the immune system is fully matured.
The *conventional housing* implies free exposure to immune shaping pathogens both from the "milieu exterieur" (environment and gut flora) and the "milieu interieur" (e.g., latent infection with herpesviruses such as cytomegalovirus and Epstein-Barr virus).
Biological therapeutics developed for human diseases, such as monoclonal antibodies or cytokines, often *cross-react* with marmosets. These can thus be tested in marmosets as a preclinical evaluation of efficacy and safety.
Disadvantage
Ethical limitations: when the same information can be obtained in lower species, marmosets cannot be used. Also, experimental manipulations are limited.
Outbred nature: the genetic heterogeneity creates clinical and pathological heterogeneity, which may affect the interpretation of data. This feature can be compensated in part by using twins, which, due to the bone marrow chimerism, are immunologically more comparable than unrelated monkeys.
High costs: not only of the monkeys themselves, but also of the housing and care.
Cross-reactivity of diagnostic reagents, such as FACS antibodies, are limited.
Amount of blood that can be withdrawn is limited.
Adoptive transfer studies are technically difficult.

Human myelin/CFA

To induce EAE, marmosets were initially immunized with whole human myelin emulsified with CFA in combination with intravenous two doses of heat-inactivated *B. pertussis* (Massacesi et al. 1995). In our hands, this led to an experimental model with an acute disease course, i.e., the animals were sacrificed with neurological symptoms as paresis and paralysis within 13 weeks. The CNS pathology was characterized by active lesions and disrupted axons. Furthermore, a destructive inflammatory process was observed rather than selective demyelination, as seen in MS. Cellular infiltrates consisted mainly of lymphocytes and macrophages. Only in one of the five marmosets, the lesions contained also granulocytes. This contrasted with the acute EAE model in rhesus macaques in which granulocytes dominate the lesion infiltrate ('t Hart et al. 1998). To create a model with a milder clinical course and pathology, we subsequently immunized five marmosets with human myelin in commercial CFA that contains a lower dose of mycobacteria (1 mg/ml). In addition, *B. pertussis* was omitted from the EAE induction protocol ('t Hart et al. 1998). The average day of sacrifice was delayed to almost 37 weeks after immunization. Inactive lesions dominated over active lesions, and the cellular infiltrates contained lymphocytes, macrophages, and in one animal also granulocytes.

RhMOG/CFA

The next step was to determine the contribution of specific immune reaction against two myelin proteins, i.e., myelin basic protein (MBP) and myelin/oligodendrocyte glycoprotein (MOG), to the development of EAE. Immunization with MBP in commercial CFA without *B. pertussis* induced only very mild symptoms, such as loss of appetite and altered walking pattern, and small CNS lesions in two of the three marmosets. In contrast, immunization with recombinant human MOG (rhMOG), which represents the N-terminal domain of MOG from amino acid 1–125 produced as non-glycosylated protein in *Escherichia coli*, formulated with commercial in CFA-induced neurological symptoms as paresis and paralysis. As in the previous models with human myelin, a single immunization with rhMOG in CFA led to a 100% disease incidence with a variable disease onset caused by the outbred nature of the marmosets. The average day of sacrifice in this model is about 70 days (Brok et al. 2000; Kap et al. 2008).

Lesions in this model can be found in different stages as in MS. At the same time, early active lesions, characterized by primary demyelination, active inflammation, and reversible axonal injury, can be found side-by-side with late inactive demyelinated lesions, characterized the absence of inflammation and irreversible axonal destruction (Mancardi et al. 2001; 't Hart et al. 2004).

Five to 39 days after immunization, the first lesion could be detected by magnetic resonance imaging (MRI). Lesion formation is disseminated in time and space, as is also seen in MS. The majority of the animals showed an increase in lesion load before neurological symptoms are detectable (Blezer et al. 2007). Most lesions remained in the active phase (Blezer et al. 2007) and consisted of macrophages and lymphocytes, but no granulocytes were detected. Furthermore, axonal density was lower in the lesions

compared with normal appearing white matter. The lesions in this model resemble the MS pattern II lesions with complement/antibody-mediated damage (Merkler et al. 2006b).

Three types of grey matter lesions were found in this EAE model, which were comparable with cortical lesions in MS (Bo et al. 2006; Merkler et al. 2006a; Pomeroy et al. 2005). Leukocortical lesions, which often engaged both the grey and adjacent white matter, were found in all six marmosets brain examined and accounted for 57% of the total number of cortical lesions. Intracortical lesions, which lie only in the cortex, were found in two of the six marmoset brains. Subpial lesions, which extend from the pial surface, were found in five of the six marmosets and accounted for 88% of the total demyelinated cortical area. Activated macrophages and microglia were found in leukocortical and intracortical lesions, but the density was lower than in white matter lesions, as has also been described for MS. Subpial lesions expressed only little signs of inflammation (Pomeroy et al. 2005). Furthermore, the cortical thickness was reduced in marmosets with EAE compared to controls, but not differences were observed between demyelinated and myelinated areas (Pomeroy et al. 2008). Intracortical lesions, but not subpial lesions, displayed immunoglobulin leakage and complement deposition (Merkler et al. 2006a).

The crucial role of MOG for EAE was also found in another experiment in which marmosets were immunized with myelin obtained from wild type or MOG-deficient mice. Marmosets immunized with wild type myelin all developed chronic progressive EAE. In contrast, only one of the five marmosets immunized with MOG-deficient myelin developed EAE suggesting that MOG is essential for the development of EAE (Jagessar et al. 2008).

$MOG_{34–56}$/CFA

Further refinement has led to a model in which EAE was induced with the single MOG peptide 34-56 ($MOG_{34–56}$) in CFA followed by one to three boosts with $MOG_{34–56}$ in incomplete Freund's adjuvant (IFA), which were given with 28 days interval (Kap et al. 2008). This model is also characterized by a very high disease incidence with a variable day of onset. Figure 1a shows the clinical course of 16 marmosets. The clinical scoring system is explained elsewhere ('t Hart et al. 2008). Two marmosets were sacrificed at day 168 and 203 after immunization with a clinical score of 0.5 and 0, respectively. The other 14 marmosets were sacrificed with paresis or paralysis of one or more limbs. The average day of sacrifice of these 14 marmosets was 103 days after immunization.

Although two marmosets were sacrificed without neurological symptoms, pathology was observed in the CNS of all monkeys. Figure 1 shows a white matter lesion of the marmoset that was sacrificed at day 168 with score 0.5. This lesion is comparable with the lesions found in marmosets that were sacrificed with neurological symptoms. Demyelinated lesions contained proteolipid protein (PLP)-positive macrophages in the lesion and myeloid-related protein-14 positive cells (Fig. 1e–f). Furthermore, the lesion contained IgM and complement deposits as well as a few T-cells and B-cells (Fig. 1g–j). Axonal pathology and subpial demyelination have also been found in this model (not shown; Kap et al. 2008). Whether the lesions in this model also resemble the MS pattern II lesions as in the rhMOG/CFA models remains to be determined.

The potency of the $MOG_{34–56}$ peptide was also shown in another experiment in which marmosets were immunized with $MOG_{74–96}$ peptide in CFA. This only induced mild clinical symptoms and pathological changes. However, when these marmosets were boosted once with $MOG_{34–56}$ in IFA, two of the three marmosets developed full-blown EAE. Whether immunization with $MOG_{34–56}$ in IFA is sufficient to induce EAE is currently under investigation. This would be a major improvement for ethical, practical, and conceptual reasons. First, IFA induces less granulomatous reaction at the injection sites and thus reduces the discomfort to the animals. Second, CFA can stimulate the production of neutralizing antibodies against novel biologicals, which influences the results of therapy experiments. The use of IFA may reduce the occurrence of both false-positive and false-negative studies. Third, if IFA is sufficient to induce EAE, this will change the ideas about the need of microbial ligands for autoimmunity.

Immunopathogenic mechanisms

The classical concept of the MS immunopathogenesis is that activated autoreactive T-cells directed against CNS antigens enter the CNS via the blood-brain barrier or the choroid plexus. In the CNS, the autoreactive T-cells encounter CNS antigens presented by local antigen presenting cells (APC) and are reactivated causing a cascade of events including microglia activation. Autoantibodies directed against myelin or neuronal proteins enhance the immune response by activating the complement system. Many questions regarding the immunopathogenesis still remain, such as: How do the autoreactive T-cells in our repertoire arise? By which mechanism is these autoreactive T-cells activated? How do they pass the blood-brain barrier? Are autoantibodies essential for the pathology? In the last decade, the EAE model in the common marmosets has been used to

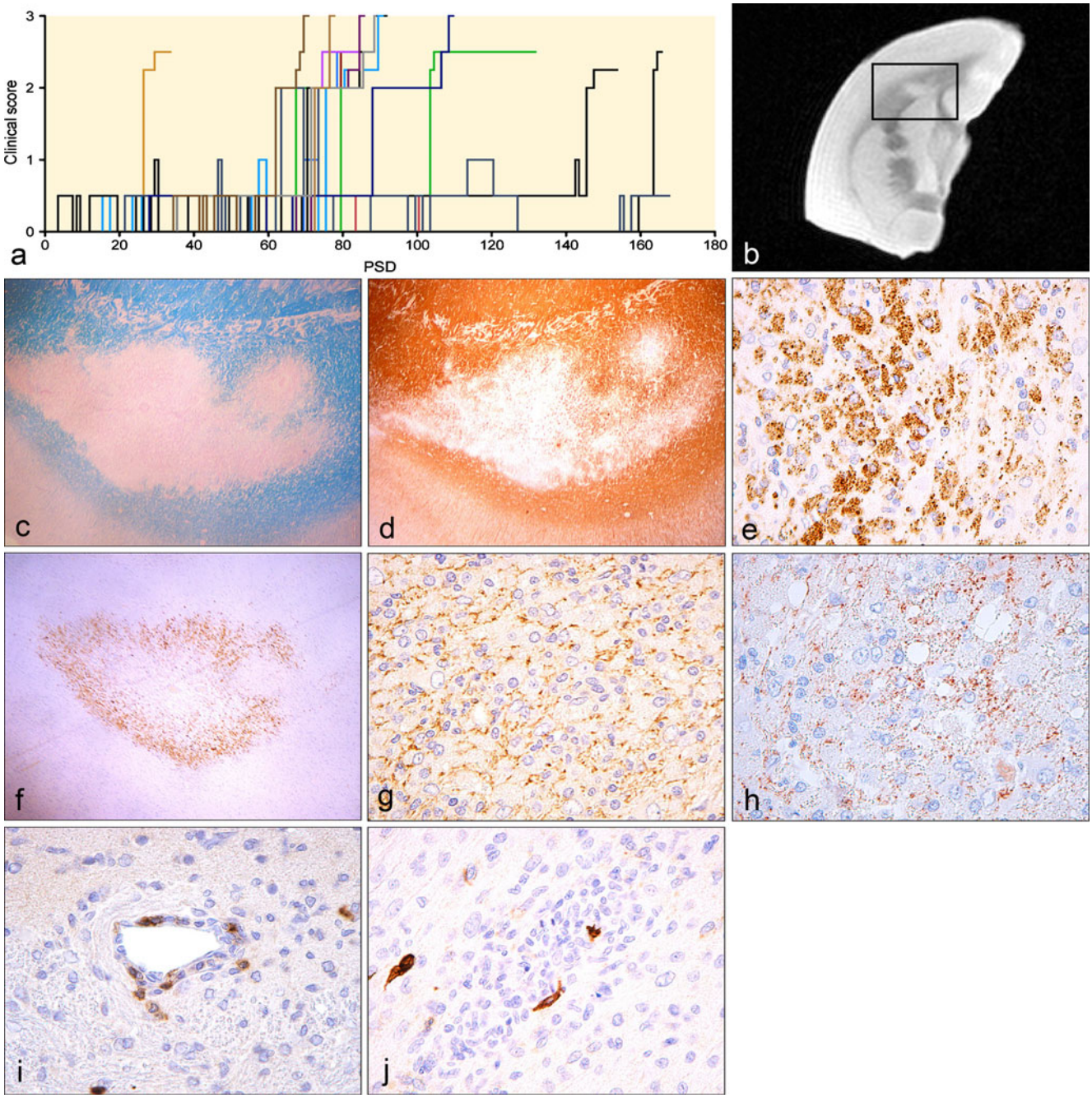

Fig. 1 Clinical and pathological hallmarks of $MOG_{34–56}$/complete Freund's adjuvant (CFA) model in marmosets. Clinical scores of 16 marmosets immunized with $MOG_{34–56}$ in CFA are shown (**a**). Clinical scoring system described in 't Hart et al. (2008). T2-weighted magnetic resonance imaging of a 4% buffered formalin-fixed brain of one of the marmosets shows a characteristic hyperintense brain lesion (**b**). The pathology of the lesion in the *rectangle* is further characterized (**c**–**j**). Luxol fast blue staining shows myelin in *blue*. A large demyelinated lesion is observed (**c**). Demyelination was confirmed by staining the myelin component proteolipid protein (PLP; **d**). PLP-positive macrophages could be observed in the lesion (**e**). Myeloid-related protein-14 positive macrophages are found at the rim of the lesion (**f**). IgM (**g**) and c9neo (**h**) deposits are also found in the lesion. Some CD3 (**i**)- and CD20 (**j**)-positive cells were observed (**c**, **d**, and **f** ×2; **e**, **g**–**j** ×40)

investigate the immunopathogenic mechanisms, aiming to answer the questions, dissect the mechanisms of action, and develop new therapies. Below, we will discuss the pathogenic role of T-cells, B-cells, and antibodies in the marmoset EAE model.

The role of T-cells in the marmoset EAE model

The concept that MS is a T-cell driven disease has mainly been based on passive or actively induced forms of the EAE model. However, despite years of intensive research,

consistent differences in the specificity, quality, or quantity of T-cell autoimmune reactions between MS patients and healthy controls have not been found. Evidence for activation of Th1 and Th17 cells in MS has been reported (Hedegaard et al. 2008), but their exact role is less obvious than in rodent EAE (Kroenke and Segal 2007).

The immune profiling of marmoset EAE models has revealed two pathogenetically relevant MOG epitopes, i.e., $MOG_{14–36}$ and $MOG_{34–56}$. Immunization of marmosets with $MOG_{24–36}$ in CFA induces Th1 responses against the epitope $MOG_{24–36}$ in all animals (Brok et al. 2000). The first peptide, $MOG_{14–36}$, is associated with the 100% EAE prevalence in the rhMOG-induced EAE model. In all monkeys, activation of Th1-cells specific for MOG24-36 can be detected, as the restriction element, *Caja*-DRB*W1201 is shared by all marmosets (Brok et al. 2000). The second peptide is associated with the characteristic variable clinical course between individual monkeys. Immunization with this peptide in CFA or IFA induces activation of cytolytic T-cells, which, without the support of autoantibodies, induces fulminant demyelination in the white and the grey matter.

Immunization of marmosets with $MOG_{34–56}$ in CFA activates $MOG_{34–56}$-specific T-cells. These T-cells have been characterized phenotypically and functionally (Kap et al. 2008). The $CD3^+$ T-cells are CD4 or CD8 positive, and the majority express CD56. In humans, CD56 is a marker for natural killer (NK) cells, NK-T lymphocytes, and NK-cytotoxic T lymphocytes (NK-CTL). However, markers to distinguish NK cells and NK-CTLs in marmosets are not available yet. Since the $MOG_{34–56}$-specific T-cells are also able to lyse Epstein-Barr virus (EBV)-transformed B lymphoblastoid cell-lines that present $MOG_{34–56}$, we suggest that the $MOG_{34–56}$ specific T-cells resemble NK-CTL. In MS patients, a comparable population has been found, i.e., cytotoxic T-cells directed against the MOG epitopes 1–22, 34–56, and 74–96 (Van der Aa et al. 2003). Whether $MOG_{34–56}$ T-cells are able to kill oligodendrocytes is currently under investigation. In addition to the NK-CTL phenotype, $MOG_{34–56}$ specific T-cells produced IL-17A in eight of the ten marmosets and IFNγ in two of the ten marmosets (own unpublished observation). The production of IL-17A may explain how these T-cells enter the brain. According to a recent report, Th17 cells can enter a non-inflamed brain via the choroid plexus through CCR6-CCL20 interaction (Reboldi et al. 2009). In line with this, we have observed that the first lesions often appear around the ventricles (own unpublished observations).

The immune system of humans and non-human primates is continuously exposed to pathogens from the milieu interieur, e.g., latent herpesvirus infections or gut flora, and exterieur. These pathogens play an important role in the shaping and maturation of the T-cell repertoire. In rhesus macaques, $MOG_{34–56}$-specific T-cells were found to cross-react with an 8-mer mimicry peptide present in the major capsid protein of human cytomegalovirus (CMV) $MOG_{34–56}$. Although sensitization against the mimicry peptide did not induce neurological symptoms, CNS infiltration by $CD3^+$ T-cells could be observed (Brok et al. 2007). This may suggests that the large repertoire of anti-CMV memory T-cells may be a source of potentially encephalitogenic T-cells. This discrepancy may also contribute to the immunological difference of between primates (humans and monkeys) and laboratory rodents, which, due to their specific pathogen-free background, have not experienced the immune shaping influence of such pathogens.

The pathogenic role of B-cells and antibodies

The concept that MS is a T-cell driven disease is changing since increasing evidence suggests that B-cells contribute to the disease as well. Recent clinical trials provide indisputable evidence that B-cell depletion with the anti-CD20 antibody Rituximab has a beneficial effect on relapsing-remitting MS patients (Hauser et al. 2008). The depletion did not affect circulating antibody levels, as plasma cells are $CD20^{-ve}$, suggesting that Rituximab exerts its beneficial effect via other B-cell functions, such as antigen presentation, cytokine production, or the formation of ectopic lymphoid structures within the CNS. As these studies have changed the ideas about B-cells in MS, we will review the role of B-cells in marmoset EAE.

The results of the Rituximab trials suggest that antibodies are less important in MS than has been thought. Also, in the marmoset EAE model, antibodies are not essential for the development of clinical signs and pathology. We have clearly established in marmosets sensitized against $MOG_{34–56}$ that demyelination and neurological deficits can be induced without a detectable influence of autoantibodies (Kap et al. 2008). Although deposits of IgM molecules bound to myelin were observed in the lesions of MOG peptide immunized marmosets, it is unclear whether these had a role in lesion formation. It is also possible that antibodies may passively leak into the CNS without a direct pathogenic relevance, as was suggested by Barnett et al. (2009). These results are in line with results obtained several MOG peptide-induced mouse EAE models in which demyelination can occur in the absence of antibodies (Cross et al. 1999; Hjelmstrom et al. 1998; Sekiguchi et al. 2009).

The conclusion that antibodies directed against rhMOG are not essential for lesion formation in EAE does not imply that they are irrelevant. It has been well established that anti-MOG antibodies can amplify demyelination in experimental conditions where demyelination is not or only marginally induced. As an example, marmosets immunized with MBP, PLP, or a chimeric MBP-PLP protein only

develop demyelination when anti-MOG antibodies are present (Genain et al. 1995; McFarland et al. 1999).

Whether B-cells have functions other than autoantibody production in the marmoset EAE model is currently under investigation. Studies in B-cell-deficient mice suggest that B-cells are important in myelin protein-induced EAE, but not in peptide-induced EAE (Gausas et al. 1982). Furthermore, the possibility that B-cells infected with EBV, a pathogen associated with a high risk to MS (Lunemann and Munz 2009), exert essential pathogenic effects on T-cell mediated autoimmune processes should be considered. This can be well investigated in non-human primates, as they are naturally infected with closely related herpesviruses. EBV-positive B-cells have been found in tertiary lymphoid structures in the meninges of MS brains (Serafini et al. 2007). However, this exciting finding could not be reproduced by others (Willis et al. 2009).

Non-invasive imaging of CNS inflammation and injury using nuclear magnetic resonance

The preferred imaging tool for the visualization and characterization of lesions in the brain white matter is MRI. In MRI, the magnetic properties of protons, which occur at different concentrations in different regions of a tissue or organ, are used to generate contrast. The most frequently monitored MRI parameters in MS diagnosis are T2-weighted (T_2W) and contrast-enhanced T1-weighted (T_1W) images. T_2W images are the most sensitive for increased water content of a tissue associated with inflammation (edema) and are routinely used to determine the spatial distribution and volumes of affected brain white matter regions. When applied to formalin-fixed brains, T_2W images are also useful to visualize demyelination, as shown in Fig. 1b. T_1W images are less sensitive for water and can be used to detect the disappearance of tissue, e.g., due to demyelination or axonal injury. T_1W images are also used to visualize the diffusion of intravenously injected paramagnetic probe (gadolinium-diethylene triamine pentaacetic acid) through a leaky blood-brain barrier (Fig. 2). Another useful imaging parameter is the magnetization transfer ratio (MTR) that is calculated from the ratio of lattice-bound and free protons (in tissue water). Reduction of MTR of CNS white matter in an EAE model can occur when tissue is affected by demyelination as well as when the tissue water content is increased due to inflammation (Fig. 2). The closest histological correlate was found to be the intensity of macrophage infiltrate (Blezer et al. 2007). The three discussed imaging parameters can also be quantified by plotting the actual T1 (before and after contrast enhancement) and T2 relaxation times and the absolute MTR values. As will be discussed later in this publication, the MRI technique provides a convenient set of tools to determine the effect of a treatment on already existing CNS pathology, a situation that closely approximates a clinical trial (‘t Hart et al. 2006).

Therapy trials in the marmoset EAE model

The marmoset EAE model can be used as a bridge between rodent EAE models and human MS for therapy trials (‘t Hart et al. 2007; ‘t Hart and Amor 2003). Therapy trials in marmosets often consist of two parts as described elsewhere (‘t Hart et al. 2006). Basically, therapy trials in this model are divided in a prophylactic part, to test whether a therapeutic principle developed in a rodent EAE model hold in the more complex EAE model in marmosets, and a therapeutic part, where the situation in a phase III clinical trial is imitated, i.e., treatment is started after significant CNS pathology can be observed with MRI or after neurological symptoms appear. Two published preclinical trials that illustrate this approach are discussed briefly; a more extensive review can be found in ‘t Hart et al. (2008).

Targeting CD40

CD40 is a surface-expressed marker of APC that belongs to the tumor necrosis factor receptor family. Via its ligand CD154, CD40 provides co-stimulatory signals to T-cells that are engaged in the formation of a tri-molecular complex of T-cell receptor with major histocompatibility complex molecules that present antigenic peptides in the cleft. CD40 is constitutively expressed on B-cells and induced on activated APC from the myeloid lineage, such as macrophages and dendritic cells (DC). Stimulation signals relayed to B-cells via CD40 mediate a plethora of functions, including B-cell activation, expression of other co-stimulatory molecules, and antibody isotype switching (Laman et al. 1996; van Kooten and Banchereau 1997). CD154 interaction with CD40 on DC induces DC maturation and production of pro-inflammatory cytokines, such as IL-12, which induces T-cell differentiation towards Th1. Proof of principle that interruption of CD40-CD154 interaction has a beneficial effect on EAE was obtained in mice with a monoclonal antibody (mAb) targeting CD154 (Gerritse et al. 1996). This was confirmed in subsequent studies in the marmoset EAE model using 5D12, a blocking mAb against CD40 on APC, which, because of the high specificity for human CD40, could not be tested in rodents. A first study demonstrated a strong protective effect of a mouse-anti-human CD40 mAb (original 5D12; PanGenetics BV) in the myelin-induced marmoset EAE model. The treatment was once every 2 days for 28 days, starting well after the

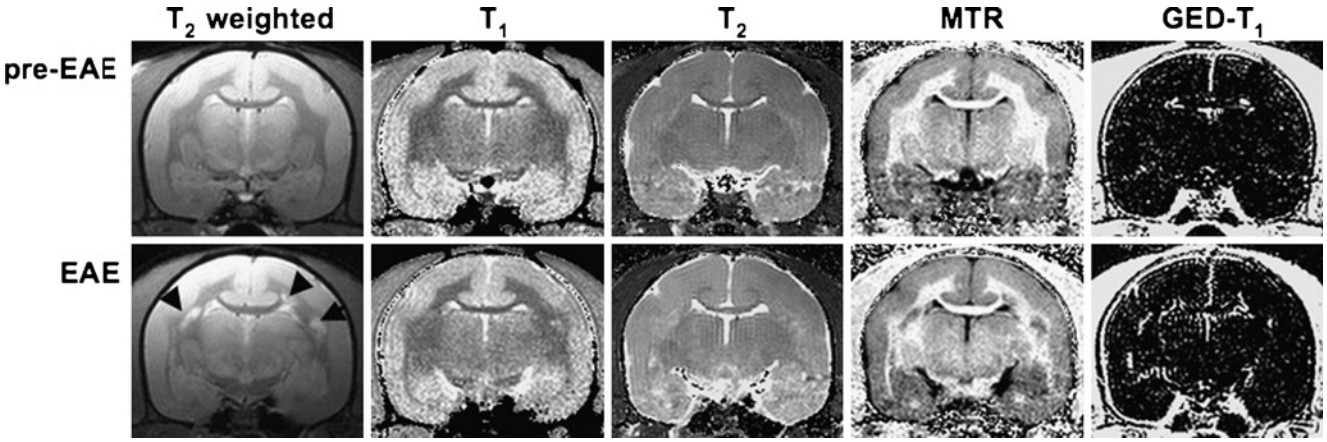

Fig. 2 Magnetic resonance imaging (MRI) sequences used for visualization of brain lesions. A marmoset monkey in which experimental autoimmune encephalomyelitis (EAE) was induced by immunization with rhMOG/complete Freund's adjuvant was subjected at 14 days interval to brain MRI at 4,7 Tesla. Scans of the same monkey before brain lesions that were detected (pre-EAE) and during clinical EAE are shown. The five depicted MRI parameters are (1) high contrast T2-weighted scans, visualizing the spatial distribution and shape of lesions; (2–4) semiquantitative scans in which the T1 and T2 relaxation times and the magnetization transfer ratio ratios are plotted; and (5) a differential scan created by subtracting T1-weighted images recorded before and after intravenous injection of the paramagnetic contrast probe gadolinium-diethylene triamine pentaacetic acid (300 nM; GED-T_1)

immunization (i.e., from post sensitization day 14 to 42 or from psd 25–53). However, shortly after the treatment was stopped, neurological deficits appeared (Laman et al. 2002). On the basis of these promising data, a chimeric version of the antibody was generated, which was further evaluated in the rhMOG model. To obtain proof of concept, treatment with the chimeric anti-CD40 antibody was started 1 day prior to disease induction and continued at a frequency of one injection every 2 to 3 days until the end of a 50-day observation period. Also, in this experiment, the monkeys remained devoid of neurological deficits during the mAb treatment (Boon et al. 2001). In a next experiment, we tested the chimeric anti-CD40 mAb in a therapeutic fashion in the rhMOG-induced EAE model, i.e., starting once brain white matter lesions were detected with MRI. To be able to test the effect of the mAb on the lesion activity, several MRI parameters were quantified. The antibody had a beneficial effect on MRI-detectable CNS pathology, but no clear clinical effect ('t Hart et al. 2005b). The antibody is currently under further clinical development for, e.g., Crohn's disease (Kasran et al. 2005). A first clinical trial with the anti-CD40 antibody in MS is planned in 2011 (http://www.pangenetics.com/pipeline.html).

Targeting IL-12p40

Several groups have documented an altered balance of anti- and pro-inflammatory cytokines in MS, for instance, reflected by the ratio of IL-10 and IL-12 (van Boxel-Dezaire et al. 1999). IL-12 and the related cytokine IL-23 are produced by activated APC upon CD40 engagement with CD154 for example (Laman et al. 2002). IL-12 and IL-23 are both heterodimeric molecules that share the p40 subunit. In collaboration with Centocor scientists, we have set up two studies aimed at the efficacy testing of a fully human IgG1κ mAb directed against the p40 subunit in the marmoset myelin-induced EAE model. The first experiment tested the preventive effect of the antibody that was administered as weekly intravenous injections between day 14 and 86 after immunization. The antibody had a strong clinical effect, i.e., four out of five monkeys remained devoid of neurological deficits, and the degree of inflammation and demyelination in brain and spinal cord were suppressed (Brok et al. 2002).

When tested in a therapeutic setting in the rhMOG-induced EAE model, i.e., the antibody was weekly administered once lesions were detected with MRI; the antibody also showed a beneficial effect albeit less complete than in the protective study design. Again, we observed only a delayed onset of clinical signs, i.e., from 13±5 to 46±23 days after the start of the treatment ('t Hart et al. 2005b).

Unfortunately, a monoclonal antibody of the same specificity directed against p40 (Ustekinumab) failed to show efficacy in reducing the cumulative number of gadolinium-enhancing T1-weighted lesions in MS (Segal et al. 2008). However, criticism has been raised against the study design, in particular, the inclusion of patients with advanced disease (Longbrake and Racke 2009).

Concluding remarks/discussion

EAE models in rodents and non-human primates have virtues and pitfalls as has been described several times (Gold et al. 2006; Sriram and Steiner 2005; Steinman and

Zamvil 2006). Although the EAE model does not exactly resemble MS, the models have proven usefulness by generating plethora of data on immunopathogenesis and new therapies.

The obvious key question in drug development is whether animal models are essential. Concerning this issue, the following key questions have been formulated by Ransohoff (2006):

Is it necessary to conduct EAE-testing for a promising new compound that appears safe in humans, has favorable pharmacokinetics, and targets a molecule likely to be important in MS, before proceeding to clinical trials?

The CNS is a highly vulnerable organ, and injury to the brain can have a much stronger impact on vital functions than when the same degree of injury would take place in a less vulnerable organ. Moreover, a specific feature of MS is that the protective function of the blood-brain barrier is disrupted. It is well possible that certain drugs do not reveal their neurotoxicity in non-MS disorders as they are excluded from the CNS by a fully functional blood-brain barrier, whereas would prove to be highly toxic in MS. It is also pertinent to emphasize that a vast number of molecules are induced or upregulated in MS which are not or differently expressed in a non-affected CNS (Robinson et al. 2003). Also, CNS-infiltrating cells can bring pathogens, viruses in particular, into the CNS that can exert detrimental effects under conditions when immunosurveillance mechanisms are disturbed by immunosuppressive therapies. In conclusion, a treatment that is safe in non-neurological disorders is not necessarily safe in MS.

Which models should be used?

In preclinical MS research, there is no general agreement on the right animal model. Clearly, the choice of the animal model(s) that will be used for the selection of the most promising agents from the development pipeline is a strategically important decision; one harvests what one seeds. While selecting an animal model for preclinical safety and efficacy evaluation of a new therapy, the following selection criteria should be considered:

1. The biodistribution and pharmacological properties of targeted molecule should be comparable between the animal model as in the human disease,
2. The binding kinetics of the therapeutic agent to the target should be comparable between the animal model and the human disease,
3. The model should preferably show unwanted side effects of the treatment that could also occur in humans, such as cytokine release syndrome, complement activation, or the exacerbation of latent infections.

For agents with a broad specificity, the standard models of EAE in laboratory rodent strains can be used (Steinman and Zamvil 2006). However, for the test of biological products, monoclonal antibodies, or recombinant cytokines for example, the standard models often cannot be used as the high species specificity of such molecules precludes cross-reactivity with lower species.

The first three considerations plead for the development of relevant preclinical animal models that are pathogenetically more closely related to MS than the classical models in which new therapeutic agents can be tested, which, due to the high species specificity, cannot be tested in lower species. The data discussed in review illustrate that the marmoset provide a useful set of models that can help the development of safe and effective therapies for MS.

If the EAE results are unpromising, should the drug program be abandoned?

According to a famous phrase by George Box "Essentially, all models are wrong, but some are useful". This also applies to the EAE model, which is established by artificial manipulation of healthy animals. It has been well established that immunopathogenic mechanisms in many EAE models induced using CFA are dominated by Th1 responses, while in MS, CD8+ T-cells have a more prominent role (Lassmann and Ransohoff 2004). The long list of treatments, which have failed to reproduce beneficial effects in EAE models when tested in MS patients, illustrates the discrepancy between EAE and MS (Kleinschnitz et al. 2008). We strongly believe that more lessons can be learned from a meta-analysis of failed clinical trials. These lessons should guide the improvement of animal models.

Conflicts of interest The authors of this review do not report conflict of interest.

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