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REVIEW ARTICLE

The primate autoimmune encephalomyelitis model; a bridge between mouse and man

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Abstract

Introduction: Multiple sclerosis (MS) is an enigmatic autoimmune-driven inflammatory/demyelinating disease of the human central nervous system (CNS), affecting brain, spinal cord, and optic nerves. The cause of the disease is not known and the number of effective treatments is limited. Despite some clear successes, translation of immunological discoveries in the mouse experimental autoimmune encephalomyelitis (EAE) model into effective therapies for MS patients has been difficult. This translation gap between MS and its elected EAE animal model reflects the phylogenetic distance between humans and their experimental counterpart, the inbred/specific pathogen free (SPF) laboratory mouse. **Objective**: Here, we discuss that important new insights can be obtained into the mechanistic basis of the therapy paradox from the study of nonhuman primate EAE (NHP-EAE) models, the well-validated EAE model in common marmosets (*Callithrix jacchus*) in particular. **Interpretation**: Data presented in this review demonstrate that due to a considerable immunological and pathological overlap with mouse EAE on one side and MS on the other, the NHP EAE model can help us bridge the translation gap.

Introduction

Multiple sclerosis (MS) is an enigmatic disease of the human central nervous system (CNS), affecting brain, spinal cord, and optic nerves. The cause of the disease is not known, but general consensus exists that autoimmune reactions against axon-enwrapping myelin sheaths and myelin-producing oligodendrocytes influence the disease course. The autoimmune concept is supported by genetic association of MS susceptibility with the immune system[1] and the clinical effect of treatments modulating immunological processes.[2] The mouse experimental autoimmune encephalomyelitis (EAE) model has shaped our understanding of immunopathogenic mechanisms. However, translation of scientific discoveries made in EAE into effective treatments for patients has been notoriously difficult. The frequent failure of new treatments to reproduce promising effects observed in the EAE model when they were tested in the clinic, indicate that essential elements of MS are missing in current EAE models. This raises the question which lessons can be learned from EAE in more human-like species, such as nonhuman primates (NHP).

Accumulating evidence demonstrates a decisive role of the environment on the manifestations of EAE in genetically susceptible animals. Mice bred and raised under germ-free conditions develop significantly attenuated EAE, indicating that CNS targeting autoreactive proinflammatory CD4+ T cells receive essential activation signals in the gut.[3,4] In addition, we discovered a new pathogenic mechanism in a NHP model of MS, EAE in the common marmoset (*Callithrix jacchus*; CJ).[5] This mechanism does not exist in mouse EAE and seems closely associated with the pathogen-educated nature of the primate immune system, which is shaped by chronic latent infections with herpesviruses such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV). This new mechanism shows an MS-like response to treatment with clinically relevant monoclonal antibodies (mAb), illustrating its potential relevance for MS.

Results obtained in a new model developed on the new mechanism shed light on the pathogenic role of CD8+ T cells and B cells and the elusive association of MS with EBV.

MS, a Human Disease of Unknown Cause(s)

The two prevailing concepts in the MS literature are that the primary pathogenic event in MS occurs either inside or outside the CNS.[6] According to an "outside-in" paradigm, MS is caused by an exogenous factor, such as an infection, which in genetically susceptible individuals induces the activation of naïve autoreactive T and B cells present in the immune repertoire. The presence of autoreactive lymphocytes in the healthy human immune repertoire has been well established.[7] Activation renders these autoreactive T cells capable of infiltrating the CNS. Interaction with local antigen presenting cells (APC) elicits a cascade of pathophysiological reactions that leads to CNS inflammation. An infectious cause of MS is supported by some epidemiological data.[8]

According to an "inside-out" paradigm, one or more pathogenic event(s) inside the CNS causes instability of myelin and release of myelin antigens,[6] which may induce the activation of hyperreactive T and B cells in CNS draining lymph nodes.[9] The initial pathogenic event is not necessarily unique to MS and may also differ between patients.

At this stage it remains unclear which of the two complementary views most closely approximates reality. It is interesting that spontaneous manifestation of MS pathology and symptoms has only been observed in the human population and is not found in animals, not even in our nearest kin, the hominoid primate (e.g., bonobo, chimpanzee). To our knowledge there is only one report documenting post-infectious inflammatory/demyelinating disease in a captive colony of Japanese macaques (*Macaca fuscata*).[10] This may imply that the pathogenic event that triggers MS is specific for the human primate.

There is no single pathological aspect that is unique to this enigmatic disease. This may imply that there is no single cause of MS, but that multiple triggering factors may converge in causing instability of oligodendrocyte/myelin and neuro/axonal complexes. Whether myelin instability in MS is a direct consequence of autoimmune-driven inflammation, as observed in EAE models,[11] or a secondary consequence of axon degeneration, as observed in Theiler's murine encephalomyelitis,[12] has also not been formally established. It is thus well possible that MS is determined by a unique combination of immune responses to self-antigens released from unstable CNS regions.[6]

The Autoimmune Concept of MS

In an outside-in MS concept autoreactive T and B cells are activated by dynamic interactions between genetic and environmental (risk) factors. In addition, unknown stochastic factors may be involved. With regard to the genetic risk factors, genome-wide association studies have identified more than 100 genes contributing to MS susceptibility, although their individual contribution is usually modest.[1] Although almost all identified genes have a function in cellular immunity, the major histocompatibility complex (MHC) exerts the strongest effect. MHC class II alleles, such as HLA-DRB1*1501/-DRB5*0101 and HLA-DQA1*0102/-DQB2*0602 and the MHC class I allele HLA-B7 were found associated with increased MS susceptibility, whereas other alleles seem to reduce the risk, such as HLA-DR1 and -DR8.[13]

Environmental risk factors can be broadly categorized as infectious, such as with EBV, or noninfectious, such as smoking and a low serum vitamin D level.[14,15] The focus of this review is on infectious triggers of MS. Although a large number of bacteria and viruses have been proposed as candidate trigger, only few have survived rigorous testing. Seroepidemiological studies indicate age-at-risk (age $\pm$15 years) exposure of genetically susceptible individuals to EBV as likely cause of MS (reviewed in [16]).

EBV is a γ1-herpesvirus belonging to the large genus of primate lymphocryptoviruses (LCV). EBV infects about 90% of the adult human population. Although primary infection usually occurs in childhood without marked symptoms, exposure during adolescence can elicit infectious mononucleosis, a condition characterized by significant flu-like symptoms, such as fatigue and malaise, but also lymphadenopathy and often splenomegaly, reflecting the robust systemic immune response to the virus.[17] EBV mainly infects B cells via binding CD21 and HLA-DR. LCV causing chronic latent infection of NHP resemble EBV in molecular and functional aspects.[18] NHP are therefore potentially useful animal models of human EBV infection.

A concept connecting autoimmunity with infection is molecular mimicry,[19] which implies that autoimmunity can be the consequence of an immune response against structurally related (mimicry) antigens shared between an infectious agent and host tissues. T cells and antibodies induced by the infection will therefore cross-react with host tissue. Activated T cells can infiltrate target organs, including the CNS in MS as illustrated by adoptive transfer EAE, which is induced by the transfer of myelin-sensitized T cells obtained from immunized mice to healthy MHC-matched recipient mice.[20] Conceptually, engagement of transferred autoreactive T cells in cognate interactions inside the CNS with APC, such as microglia or

coinfiltrated dendritic cells (DC), elicits EAE pathology. The APC present epitopes from CNS antigens that are recognized by T cells as if they were epitopes of the infecting pathogen. In MS T-cell cross-recognition of EBV and the CNS myelin antigen MBP has been documented.[21] Another explanation for the association of EBV with MS might be that EBV-infected B cells are directly involved in the development of neuroinflammation.[22] However, the validity of this finding was seriously criticized.[23]

Two lines of evidence obtained in NHP-EAE support an active role of EBV in MS (reviewed in [24]). First, adoptive transfer of autologous LCV-infected B cells induced autoreactive T-cell activation and (mild) meningeal inflammation in rhesus monkeys[25] and marmosets.[26] Second, we observed that the clinical effect in the marmoset EAE (Cj-EAE) model of B-cell-depleting mAb was associated with their ability to deplete LCV-infected B cells.[26]

However, the prevalence of the HLA-DR2/-DQ6 haplotype in the human population is 10–25% and that of EBV infection in young adults is ±60%. As MS develops only in 1 per 1000 young adults, it is difficult to envisage how the interaction of two such common factors can be the cause of a relatively low prevalent disease. In addition, translation of pathogenic concepts developed in the EAE model into effective treatments for the MS patients has been notoriously difficult. We postulate that a refined and well-validated NHP model, such as the Cj-EAE model, can be useful in the search of missing links between EAE and MS.

The Common Marmoset EAE (Cj-EAE) Model

Cj is a small-bodied nonprotected Neotropical primate (weighing ±350 g at adult age) that breeds well in captivity. Marmosets share the outbred nature and a high degree of genetic, immunological, and microbiological similarity with humans. Marmosets and tamarins, another genus within the *Callitrichidae* family, are unique among mammalian species as they usually produce nonidentical twins that exchange hemopoietic stem cells in utero via the fused placental bloodstream. The ensuing chimerism not only implies mutual tolerance for alloantigens between fraternal siblings but also a high degree of immunological similarity. The complete Cj genome has now been sequenced and annotated.[27]

Hauser and colleagues first developed the Cj-EAE model using a mouse EAE protocol, that is, human myelin formulated with complete Freund's adjuvant (CFA) in combination with intravenous *Bordetella pertussis* particles.[28,29] In our hands, these immunized marmosets developed acute EAE with severely destructive pathology.[30] Slight modification of the protocol, usage of CFA containing less mycobacteria and omission of *Bordetella* injection, delivered an MS-like chronic disease model resembling relapsing-remitting MS (RRMS) in clinical and neuropathological presentation.[30] The original concept was that, just like in classical rodent EAE models, Cj-EAE is driven by the synergistic action of T helper 1 (Th1) cells inducing inflammation and autoantibodies eliciting demyelination via macrophage- and complement-mediated cytotoxic injury to myelin sheaths as dominant pathogenic process (antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity, respectively).[5] For validation of this paradigm we used a fully human anti-IL-12p40 monoclonal antibody (mAb) ustekinumab, unexpectedly. The IL-12p40 subunit is shared by IL-12 and IL-23,[31] which are the signature cytokines of two important autoimmune inflammatory pathways, respectively, driven by the activation of proinflammatory and encephalitogenic Th1 and Th17 cells.[32] Although a convincing protective effect of the anti-IL-12p40 mAb was observed in the Cj-EAE model,[33,34] the same mAb showed no detectable clinical effect in RRMS patients.[35] The observation in mice that many of the pathogenic effects attributed to IL-12 are mediated by IL-23 via induction of Th17 cells,[32] prompted us to test a novel anti-IL-17A antibody in Cj-EAE. We found only a trend toward delayed onset of clinical signs, suggesting that the treatment may affect events in late-stage disease.[36]

These paradoxical results do not imply that the Th1/Th17 paradigm is an artifact of the EAE model that is irrelevant for MS. It is well possible that the acute pathogenic mechanisms modeled in EAE operate early in the MS pathogenesis and may be extinguished when MS is diagnosed (see also: [37]). The notion that different immunopathogenic processes may drive the initiation and progression of MS is strongly supported by the marmoset EAE model. The existence of distinct pathogenic mechanisms is well-illustrated by the observation in Cj[38] as well as in Biozzi ABH mice[39] that autoimmunity against MOG is dispensable in EAE initiation, but is essential in EAE progression. Fine mapping of the T-cell response against recombinant MOG (rMOG) revealed that initially Th1 cells are activated specific for the epitope $MOG_{24–36}$.[40] Progression of Cj-EAE involved activation of another autoimmune mechanism, mediated by cytotoxic effector memory T cells specific for the epitope $MOG_{40–48}$.[41] These EM-CTL have an important pathogenic role, as they seem to shift the focus of the autoimmune attack to the gray matter, where they induce severe demyelination.[42]

Two Faces of MOG in Autoimmunity

MOG, a unique constituent of the mammalian CNS, is expressed as a homodimeric complex in head-to-tail

orientation on the surface of oligodendrocytes and the outer lamellae of the axon-enwrapping myelin sheaths within the CNS.[43] MOG is encoded in the extended class I region of the MHC located in humans on chromosome 6p21.3-p22 at 60 kb telomeric to HLA-F. MOG transcripts are extensively spliced and splicing profiles are more complex in higher than in lower species.[44] In the human brain at least 15 splice variants were found, but it remains unknown whether these have a different function and whether certain variants are associated with autoimmunity. Crystallization of the mouse[43] and rat[45] MOG_{ED}, elucidated the 3D configuration and provided insights into the antigenicity of the protein (see below). The surface-exposed $Asn^{31}Arg^{32}Tyr^{33}$ motif encodes the N-glycosylation site.[46] A refined analysis of the glycan attached to the Asn^{31} residue has recently been published and identified the C-type lectin DC-SIGN as a potential receptor for MOG within the CNS and draining lymph nodes of human and NHPs.[47] We hypothesized that at the level of the interaction of MOG with DC-SIGN the decision on tolerance or immunity against MOG is made.[48]

Monomeric human MOG consists of an evolutionary conserved Ig-like extracellular domain (ED; residues 1–121) (Fig. 1) that contains the EAE-associated B- and T-cell epitopes, two hydrophobic segments domains (residues 121–151 and 174–201), of which only the first is

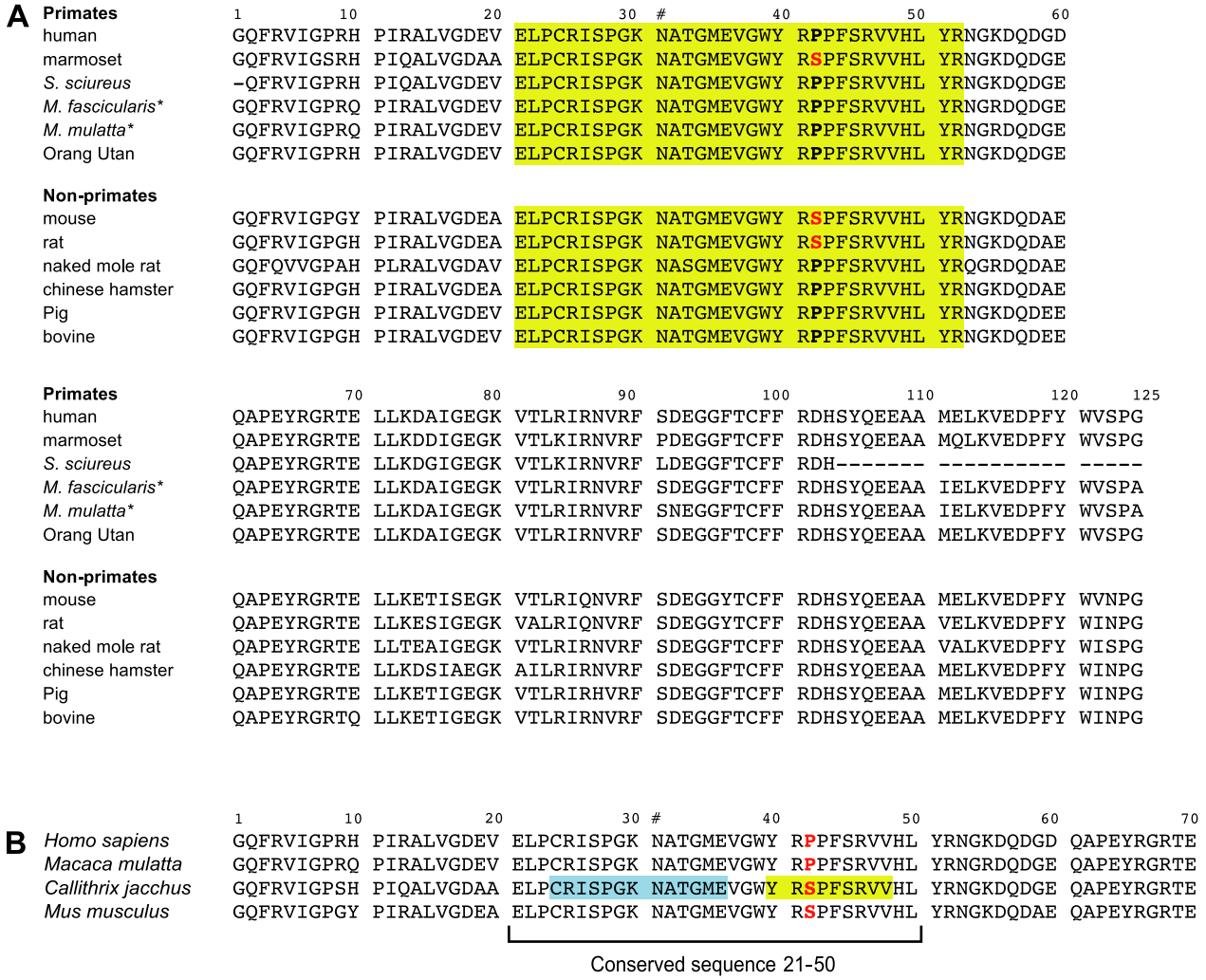

Figure 1. Alignment of mammalian MOG^{ED} sequences. The depicted sequences were downloaded from the Swissprot database (http://www.uniprot.org/uniprot/?query=myelin+oligodendrocyte+glycoprotein&sort=score). (A) Highlighted in yellow is the highly conserved domain MOG21–52, which contains the only N-glycosylation site at Asn^{31} (marked with #). (B) The conserved sequence 21–50 contains the two most relevant epitopes, namely residues 24–36 (Th1 epitope; yellow) and residues 40–48 (NK-CTL epitope; green). **M. fascicularis* = cynomolgus macaque; *M. Mulatta* = rhesus macaque; *S. sciureus* = *Saimiri* sciureus.

transmembrane spanning and the second is partially buried in the lipid bilayer, and a short intracytoplasmic tail that may be connected to the oligodendrocyte cytoskeleton.[49]

Autoimmunity against MOG appears a crucial factor in the development of chronic Cj-EAE, as was demonstrated by the observation that the normal chronic EAE course is impaired when MOG-deficient mouse myelin was used for the immunization.[38] MOG is best known for its capacity to induce in the EAE model[50] autoantibodies that opsonize myelin and elicit demyelination via cytotoxicity of complement (CDC) and macrophages (ADCC).[51]

From an evolutionary point of view it is difficult to understand that expression of a potentially harmful protein without a clear beneficial function is permitted in a vital organ as the brain. We therefore examined whether MOG may have a beneficial role in the healthy brain. A physiological role of MOG in the CNS has not been clearly established thus far, although several functions were proposed. These include an adhesive function in maintaining the structural integrity of CNS myelin, involvement in the microtubule integrity of oligodendrocytes and binding of complement factors.[43,45] The developmental pattern of MOG expression in the CNS coinciding with late stages of myelination[52] hints at a role in the formation of compact CNS myelin. However, mice lacking MOG expression in their CNS develop normally without detectable myelination defects.[53]

Studies in wild type and MOG-deficient mice showed that potentially pathogenic anti-MOG T cells are not completely cleared from the immune repertoire during thymic selection.[54] Interestingly, T cells from MOG-deficient mice displayed higher reactivity to recombinant MOG, measured by proliferation and cytokine production, and were more encephalitogenic, as assessed by adoptive transfer.[55] These observations hint at a dual function of MOG, namely a homeostatic role inside the CNS and an antigenic role outside the CNS in the lymphoid organs.

Innate Immune Recognition of Myelin

Myeloid dendritic cells (mDC) have a central regulatory role in tolerance and immunity.[48] For this task mDC are equipped with pattern-recognition receptors (PRR) binding conserved pathogen-associated molecular patterns (PAMPS), which inform the cell about the presence of friend or foe.[48] Danger-sensing receptors inducing DC activation include toll-like and nod-like receptor families (TLR and NLR).[56,57] C-type lectin receptors (CLR) are PRR with a role in the sensing of nondanger signals, which antagonize DC activation.[58] In contrast to TLR, CLR can mediate antigen internalization. CLR also recruit signaling molecules that modulate activation signals relayed via danger receptors. It can thus be envisaged that via the integration of activation signals received via TLR/NLR and inhibitory signals received via CLR, DC are instructed whether they should adopt an immunogenic or a tolerogenic function[59] (Fig. 2).

DC-SIGN is a transmembrane receptor expressed on immature mDC in peripheral tissues, mature mDC in lymphoid organs and on subsets of macrophages. DC-SIGN has broad physiological functions.[60] DC-SIGN expression on mDC is upregulated by IL-4 and downregulated by inflammatory factors.[61] DC-SIGN contains a Ca^{2+}-dependent mannose-binding carbohydrate recognition domain with dual specificity for high-mannose and fucose-type sugars, such as Lewis X. Various self-antigens are decorated with glycans that mediate binding to DC-SIGN, such as the adhesion molecules ICAM-2 and ICAM-3, the MOG-related milk protein butyrophilin and tumor antigens, such as the carcinoembryonic antigens CEA and CEACAM1. Moreover, a range of pathogens express carbohydrate epitopes that bind DC-SIGN (see references in review [58]). Accumulating evidence indicates that these pathogens use DC-SIGN binding for avoiding a neutralizing immune response.[62]

We analyzed the interaction of myelin purified from healthy human brain white matter with human mDC.[47] Lipid-rich myelin emulsified in aqueous buffers forms micellar structures of variable sizes, which can be separated by flow cytometry. We observed that DC-SIGN binding is confined to larger myelin particles, whereas smaller particles did not bind. A possible explanation is that for binding to DC-SIGN MOG needs to multimerize in lipid rafts, which because of their size can only be formed in large myelin particles.[63] The functional consequence of MOG interaction with DC-SIGN is modulation of the mDC response to TLR triggering. The MOG-mediated binding of DC-SIGN to myelin converted mDC activation signals from LPS-TLR4 interaction into tolerogenic signals. This resulted in inhibition of mDC maturation and NALP3 inflammasome formation as well as the secretion of anti-inflammatory cytokines, such as IL-10. Small myelin particles that lack MOG multimers or myelin produced under inflammatory conditions, inducing altered glycosylation of MOG, did not bind DC-SIGN. In this circumstance TLR4 stimulation by LPS induced strong proinflammatory signals, expressed by NALP3 inflammasome activation and secretion of IL-17A.

DC-SIGN-expressing APC relevant for Cj-EAE are microglia located within the CNS[47] and phagocytic cells within the CNS draining cervical and lumbar lymph nodes.[64] We hypothesize the following scenarios: Injury to myelin without simultaneous inflammation, a situation

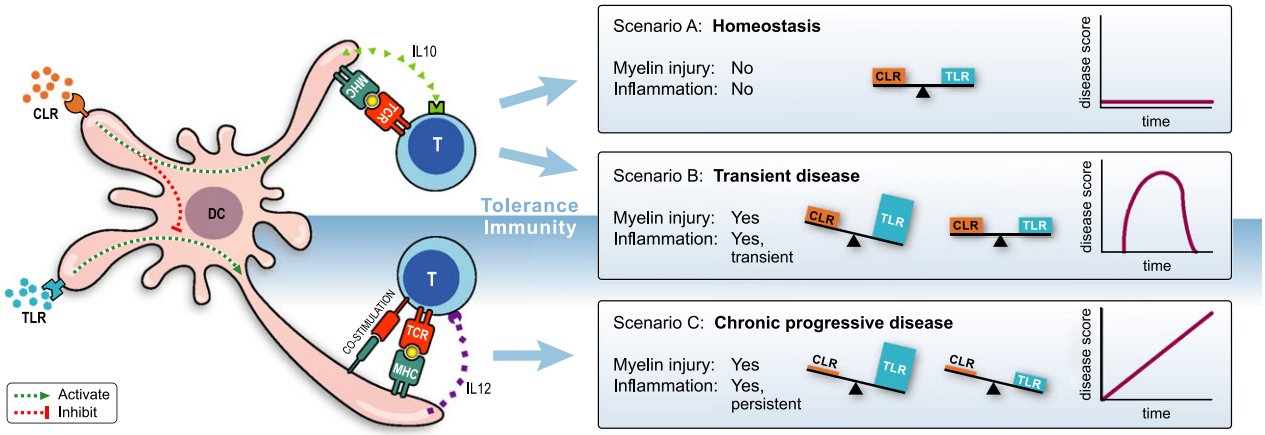

Figure 2. Yin-Yang regulation of myeloid APC. Depicted is a myeloid APC, for example, a microglia or dendritic cell, which expresses C-type lectin (CLR) and toll-like receptors (TLR). Via the integration of inhibitory input signals via CLR and stimulatory input signals via TLR the APC is instructed whether it needs to display tolerogenic or immunogenic activity. In a healthy brain APC are in a tolerogenic state as danger signals received via TLR are counterbalanced by inhibitory signals via CLR, for example, binding of normally glycosylated MOG to DC-SIGN (scenario A). CNS infection or tissue damage induces increase in danger signals, resulting in a, CLR/TLR dysbalance and maturation of APC to an immunogenic state. When the danger signals are cleared, the CLR/TLR balance that maintains homeostasis is restored (scenario B). When scenario B occurs in inflamed tissue where normal glycosylation is disturbed resulting in impaired CLR signaling, return to homeostasis after clearance of the danger does not occur. APS, antigen presenting cell; CNS, central nervous system; DC, dendritic cell.

present in noninflammatory brain injury, does not (necessarily) induce autoimmunity as tolerogenic mechanisms are activated (scenario B in Fig. 2). In contrast, myelin injury together with inflammation, a condition present in inflammatory-demyelinating MS lesions, induces autoimmune mechanisms that amplify injury and perpetuate inflammation (scenario C in Fig. 2).

Adaptive Immune Recognition of MOG

Most EAE studies focused on the MOG_{ED}, which is used as a nonglycosylated recombinant protein. Despite the clear pathogenic role of MOG in EAE, there is controversy about the relevance of anti-MOG T cells and antibodies in MS. Part of the controversy may be due to the assays utilized in different studies. Tests of cells and sera from MS patients for reactivity with recombinant human MOG_{ED}, often gave contradictory results. However, assays based on cells expressing conformationally intact, glycosylated MOG on their surface detected different serum IgG reactivity between healthy controls and pediatric MS patients with clinically isolated syndrome or an acute disseminated encephalomyelitis ADEM-like disease presentation. The same assays only rarely detected differences between adult MS patients and healthy controls.[65]

Figure 1A shows MOG_{ED} amino acid sequences from a variety of mammalian species are aligned. Noteworthy is the remarkable sequence homology (>90%) between two species widely separated in evolution, that is, the naked mole rat (*Heterocephalus glaber*) and man (*Homo sapiens*). The MOG_{ED} contains a domain of 30 amino acids length (21–50) that is almost completely conserved in evolution (Fig. 1B). The only difference is a nonsynonymous substitution of proline and serine at position 42. The $Ser^{42}Pro$ substitution alters the protein structure,[66] the strength of antibody binding[67] and the autoantibody response in B6 mice.[68] The two key pathogenic T-cell epitopes of Cj-EAE model (24–36 and 40–48) are juxta-positioned within this conserved 21–50 region.

B-cell epitopes

Studies with the mAb 8-18C5, which was a potent inducer of CNS demyelination in marmosets[69] showed that the dominant B-cell epitope is conformation-dependent. The mAb binds to an epitope formed by three loops at the membrane-distal end of the molecule.

For the studies in Cj-EAE recombinant proteins were used representing the MOG^{ED} fragment from rat ($rrMOG^{ED}$) or human origin ($rhMOG^{ED}$).[28,70] Marmosets immunized with $rrMOG^{ED}$ (Ser^{42}) or $rhMOG^{ED}$(Pro^{42}) in CFA developed a similar polyclonal IgG response.[40,71] In our hands the reaction of immune sera with ELISA plate-bound $rhMOG^{ED}$ was reduced >90% when the immune sera were preincubated with synthetic $MOG_{54–76}$ peptide.[72] This observation complies with the identification of peptide 65–75 of $rrMOG^{ED}$ as part of a marmoset B-cell epitope.[73]

Marta et al. have reported the remarkable observation that C57/BL6 mice immunized with rhMOG, but not those

immunized with rrMOG, produce IgG antibodies against the N-linked carbohydrate epitope.[68] This finding was attributed to the P^{42} residue as in mice immunized with rhMOG (Ser^{42}), IgG antibody induction against glycosylated myelin was markedly reduced.[68] Theoretically, such glycan-binding antibodies may promote EAE development by blocking the homeostatic interaction of MOG with DC-SIGN on DC and microglia and thus abrogate the maintenance of homeostasis. This remains to be proven.

T-cell epitopes

The human MOG^{ED} domains containing dominant T-cell epitopes in rhMOG-induced Cj-EAE overlap in part with those identified for MS (residues 14–46, 34–56, 64–78).[40] With two peptides Cj-EAE could be elicited, $MOG_{14–36}$ and $MOG_{34–56}$. These two peptides induced two different pathogenic pathways leading both to clinical EAE (Fig. 3).[5] One pathway is mediated by Th1 cells specific for MOG epitope 24–36. Immunization with MOG peptide 14–36 in CFA[40] or adoptive transfer of a Th1 clone specific for MOG peptide 21–40[74] elicited mild clinical EAE with modest inflammatory pathology in the white matter without demyelination.[74] The second pathway is activated by immunization with MOG peptide 34–56 in CFA or even incomplete Freund's adjuvant (IFA) resulting in severe clinical EAE with MS-like inflammation and demyelination within the white and gray matter of the brain (Fig. 4).[50]

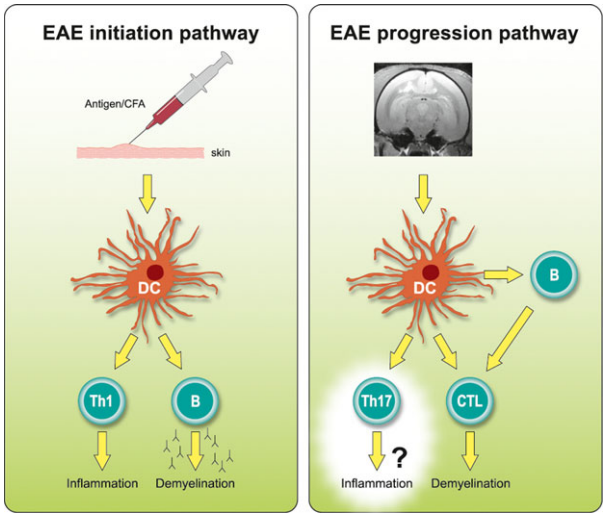

Figure 3. EAE development in marmosets involves two consecutively activated pathways. Injection of rhMOG/CFA emulsion into the skin induces local activation of APC, which induce activation of MHC class II/Caja-DRB1*W1201-restricted Th1 cells specific for MOG24–36 and B cells against conformational epitopes. The combined autoimmune attack initiates the inflammation and demyelination of white matter. Antigens released from such primary lesions can be retrieved within DC-SIGN+ APC within the cervical lymph nodes.[64] Autoimmune factors induced at this location, in particular MHC-E restricted CTL, are critical mediators of chronic EAE. Virus-infected B cells have an important role in the progression pathway as requisite APC of the CTL. EAE, experimental autoimmune encephalomyelitis; CFA, complete Freund's adjuvant; APS, antigen presenting cell; MHC, major histocompatibility complex; DC, dendritic cell.

MOG, a Critical Autoantigen in Chronic EAE

Marmosets given a single immunization with $rhMOG^{ED}$ in the strong bacterial adjuvant CFA, developed a severe neurological disease, characterized by MS-like CNS pathology.[75,76] Thus far, almost all immunized marmosets (>95%; $N > 100$) developed clinically evident MS-like disease irrespective of their MHC differences. In the few exceptions without detectable neurological symptoms MS-like neuropathology was nevertheless found. Serial magnetic resonance imaging showed that the development of brain lesions in this model starts within the white matter and spreads to the cortical gray matter at later stages of the disease (Fig. 5). The frequent detection of MS-like demyelination[75] and diffuse atrophy[77] in the cerebral gray matter is an important, albeit not unique, aspect of the Cj-EAE model.

The 100% incidence of induced EAE is higher than expected for an outbred species but could be explained by the observation that EAE susceptibility maps to the ubiquitously expressed monomorphic MHC class II allele, *Caja-DRB1*W1201*.[78] Presentation of $MOG_{24–36}$ by Caja-DR12 molecules induced Th1 cells[40] and marmosets immunized with $MOG_{14–36}$ peptide in CFA developed only mild clinical EAE with small-sized inflammatory lesions.[40] With the data reported by Genain et al.[69] and Menge et al.[79] in mind, one might speculate that the absence of demyelination is due to a lack of autoantibody production against conformational epitopes.

In a significant proportion of the cases, those with a rapid disease progression in particular, we found activation of T cells specific for other parts of the rhMOG molecule, including the peptides 4–11, 11–21, 34–56, 64–86, 74–96, and 94–110.[41] The pathogenic potential of the N-terminal 4–21 peptide or the C-terminal 94–110 peptide, which is encephalitogenic in DA rats,[80] still needs to be confirmed for marmosets. Cj sensitized against $MOG_{74–96}$ peptide, being encompassed within the dominant $MOG_{64–96}$ T-cell epitope in MS,[81] failed to develop EAE but developed severe neurological defects following booster-immunization with $MOG_{34–56}$ in IFA. It was already observed in rhesus monkeys that T cells sensitized against $MOG_{34–56}$ cross-react with a peptide derived from the UL86 ORF encoded major capsid protein of human CMV and its macaque equivalent in rhesus monkeys.[82]

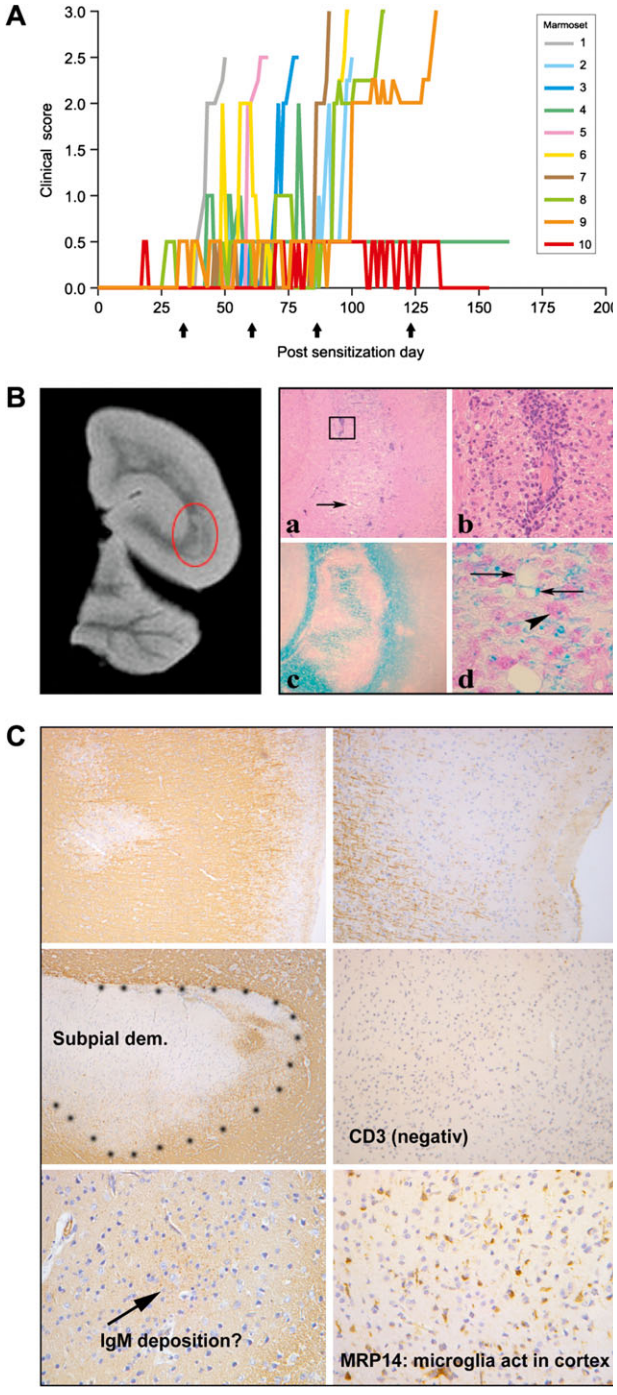

Figure 4. Clinical and pathological presentation of the EAE model induced with $MOG_{34–56}$/IFA. A total of 10 marmosets were immunized with $MOG_{34–56}$/IFA on days 0, 28, 56, 84, and 112 (arrows). (A) Nine monkeys developed clinically evident progressive EAE; one monkey went into remission after short lasting neurological impairment Characteristic pathological changes in cerebral white matter (B) and cortical gray matter (C) are shown. EAE, experimental autoimmune encephalomyelitis; IFA, incomplete Freund's adjuvant.

The synthetic peptide-in-oil formulation ($MOG_{34–56}$/IFA) lacked detectable innate stimulatory activity and therefore did not elicit EAE in Biozzi ABH and C57BL6 mice.[50] Nevertheless, >90% of marmosets immunized with MOG34–56/IFA ($N > 30$) developed full-blown clinical EAE, associated with MS-like pathology in the CNS white and gray matter (Fig. 4).[50] We therefore speculated that the immunization with $MOG_{34–56}$/IFA might induce the activation of effector memory T cells from the preexisting anti-CMV repertoire. However, formal proof awaits purification and molecular characterization of the marmoset CMV, that is, CalHV2.

Anti-B-Cell Antibody Trials Highlight a Pathogenic Role of LCV-Infected B Cells

Anti-CD20 mAbs, such as rituximab, ocrelizumab, or ofatumumab, have a remarkable clinical effect in RRMS that could not be attributed to the abrogation of autoantibody production.[83] We speculated that detailed analysis of the immune reactions in Cj-EAE that are modified by B-cell depletion might help gaining insight into essential pathogenic mechanisms. For the studies in Cj-EAE we used HuMab7D8, a clonal variant of the fully human anti-CD20 mAb ofatumumab with equivalent specificity and efficacy.[84] The mAb was evaluated in the EAE models induced with rhMOG/CFA, in which anti-rhMOG antibodies mediating demyelination are formed, or with $MOG_{34–56}$/IFA, where anti-rhMOG antibodies are not formed. In both Cj-EAE models we observed profound suppression of neurological deficits together with modulation of T- and B-cell functions by anti-CD20 mAb treatment.[76,85,86] Intriguingly, in the rhMOG/CFA model indirect peripheral B-cell depletion via treatment with fully human mAbs against two major stimulatory factors of B-cell development and survival, BLyS/BAFF (belimumab) or APRIL, exerted a disappointing clinical effect although serum levels of anti-MOG antibodies were reduced.[87] The marginal clinical effect of anti-BlyS mAb compared with the robust treatment effect of anti-CD20 mAb the treatment with anti-CD20 mAb reminds to the ineffectivity of atacicept, a soluble version of the joint TACI receptor of BLyS and APRIL, in clinical trials. An unexpected increase in inflammatory disease activity observed in one trial led to suspension of all atacicept trials in MS.[88]

We chose to investigate in the marmoset EAE model why the three mAbs (anti-CD20, anti-BlyS and anti-APRIL), which eventually all induced depletion of peripheral CD20+ B cells albeit with different kinetics, had such a remarkably different clinical effect. We observed that treatment with anti-CD20 mAb induced a sharp reduc-

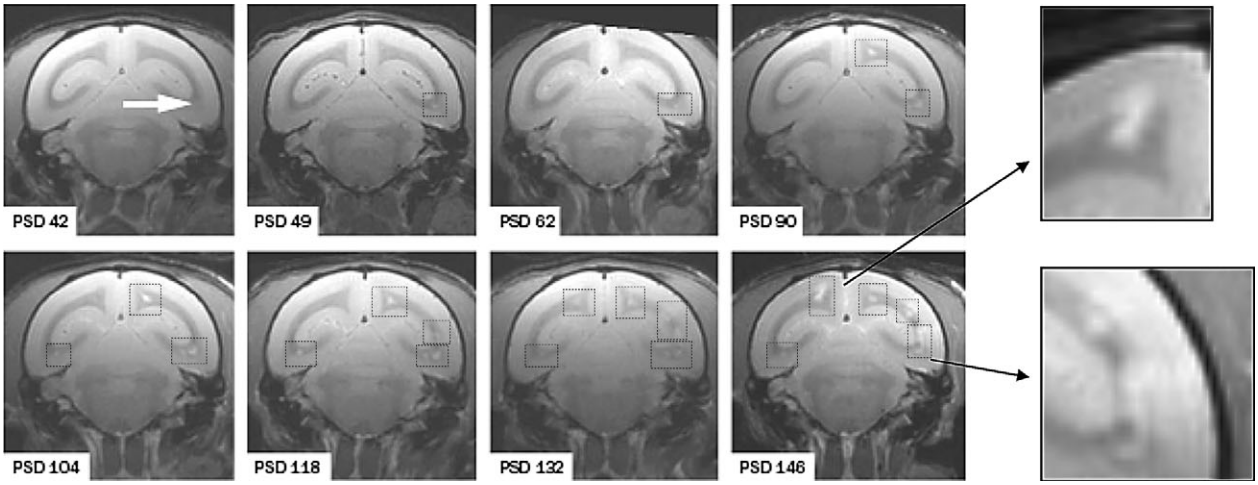

Figure 5. Brain lesion development in the rhMOG/CFA EAE model disseminated in time and space. Depicted is a series of T2-weighted MRI scans of the same coronal brain section recorded at periodic intervals (psd = postsensitization day). The arrow in the first picture points to the first abnormality recorded at 6 weeks after EAE induction. At 7 days later the first lesion has been enlarged and again 40 days later a second lesion can be observed. Squares with dotted lines are placed around lesions for better visualization. The inserts show an enhancement of lesions expanded into the cortical gray matter, which is a late event. CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis.

tion in CalHV3 virus DNA copy numbers in lymphoid organs. CalHV3 is a Cj γ-herpesvirus with comparable B-cell transforming activity as its human counterpart EBV.[89] In monkeys treated with anti-BLyS or anti-APRIL mAbs we did not observe depletion of CalHV3 DNA, rather an increment of CalHV3 DNA copy numbers.[26] On the basis of these findings, we postulated that the subset of CalHV3-infected marmoset B cells may have a central role in Cj-EAE development, namely as APC for the subset of EM-CTL, which can induce demyelination of gray matter independent of autoantibody. The observation that infusion of EBV-induced B lymphoblastoid cells prepulsed with $MOG_{34–56}$ induced the in vivo activation of $MOG_{34–56}$-specific marmoset T cells, albeit without clinical signs, supports this hypothesis.[26]

We examined the secondary lymphoid organs (SLO) of EAE marmosets from the placebo, anti-CD20, anti-BlyS and anti-APRIL treatment groups for histological changes that might explain the discrepant clinical effects of these mAbs. We observed that the anti-CD20 mAb induced profound changes in the immunogenic milieu inside SLO resulting in impairment of T-cell activation, which was not observed in monkeys treated with anti-BlyS or anti-APRIL mAbs.[90] In the anti-CD20-treated Cj-EAE cases the T cells retained high expression of CCR7 and might therefore not be released into the circulation. We thus propose that the brisk and profound beneficial effect of rituximab in RRMS might be explained by retention of pathogenic T cells within SLO. Note that the clinical effect of the S1P receptor antagonist fingolimod in RRMS is based on the same principles.[91]

Concluding Remarks

Despite obvious similarities between the EAE model and MS, there are several discrepancies, which require clarification. The main discrepancy is in the pathogenic role of CD4+ proinflammatory T cells. Th1 and Th17 have an important pathogenic role in mouse and marmoset EAE models, as illustrated by the efficacy of therapies targeting this EAE initiation mechanism. However, difficulties encountered in the translation of these findings to the MS patient have raised questions on the relevance of the EAE initiation pathway for MS. A second discrepancy is the absence of neurodegeneration in the EAE model, indicating that there may be no causal relation between this pathological aspect of MS and the autoimmune process. Advantage can be taken from the availability of well-characterized NHP-EAE models, which can be used to test whether these paradoxes are due to technical shortcomings of the models or to inconsistencies in the current concept of MS pathogenesis.[92]

The most important message of this publications is that NHP-EAE models are unique because, similar to humans, autoimmunity and the ensuing autoimmune neurological disease develop in a mature immune system. Maturity is induced by the daily combat with environmental pathogens and with chronic latent infection by endogenous pathogens. As reviewed elsewhere, the need to suppress exacerbation of the endogenous infections creates a repertoire of highly reactive effector memory, which are potentially autoreactive. Absence of a pathogen-educated immune repertoire in inbred/SPF (specific pathogen free)

rodents may be the missing link between MS and the EAE model. NHP model can therefore provide relevant information on the still poorly understood relation between infection and autoimmunity.

Obviously, there are many outstanding questions waiting for an answer, such as:

1 Which events trigger the formation of primary lesions in MS?
2 How might the marmoset EBV homolog CalHV3 alter the pathogenic function of B cells?
3 How do the NK-CTL induce demyelination of cortical gray matter?
4 Is it possible to selectively deplete the EBV-infected B-cell subset and is this sufficient for the prevention of ongoing disease?

We believe that the unique marmoset EAE model provides an excellent experimental platform to help with finding an answer for these burning questions.

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Conflict of Interest

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