Non-human primates are essential models in the translational research of multiple sclerosis

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Ageing Western societies are facing an increasing prevalence of chronic inflammatory and degenerative diseases for which no effective treatments exist. The pressure on the drug development industry to develop such treatments creates a need for translationally relevant animal models, which faithfully replicate essential pathogenic mechanisms of the human disease. In this Short Review, we discuss the essential role of the non-human primate (NHP) in the translational research into the pathogenesis and treatment of the autoimmune neurological disease multiple sclerosis (MS).

Introduction

Multiple sclerosis (MS) is an autoimmune neurological disease characterized clinically by the accumulation of neurological deficits, including sensory and motor functions, and pathologically by the presence of inflammatory/demyelinated lesions in the brain and spinal cord [1]. Lesions are usually well-defined areas of inflammation and tissue injury, which can be visualized with magnetic resonance imaging (MRI). Fig. 1a shows the clinical presentation of MS in the majority of patients (±85%) with relapse-onset disease: Pre-symptomatic MS is rarely diagnosed as it remains largely subclinical, but lesions can be detected as hyper-intense foci on brain MRI scans, such as depicted in Fig. 1b. This is followed by a period of variable length, ranging from a few to many years, where episodes of neurological disability (relapses) alternate with intermittent recovery (remission). Commonly observed focal neurological deficits include loss of sensation, visual symptoms, motor paralysis, as well as bowel and bladder dysfunction. Fatigue, cognitive disturbance, and neuropathic pain are also very common. In approximately two thirds of patients, relapsing remitting (RR) disease evolves into secondary progressive (SP) disease, in which there is progressive worsening of symptoms that become independent of relapses. By contrast, approximately 15% of the patients experience primary progressive disease, in which symptoms are slowly progressive from the start (primary progressive MS, PPMS).

Fig. 1b shows the main pathological hallmarks of MS, namely infiltration of blood-borne immune cells (inflammation), destruction of myelin sheaths around axons (demyelination) and degeneration of neurons and axons (neurodegeneration), which culminates in substantial reduction of brain mass (atrophy) (for review: Ref. [2]). In
presymptomatic MS, lesions are thought to be mainly inflammatory with limited demyelination, while lesions in RRMS display self-limiting inflammation and demyelination with potential repair by new myelin formation (remyelination) as oligodendrocytes are spared. In progressive MS damage becomes permanent as degeneration of neurons and oligodendrocytes becomes more irreversible.

For the treatment of RRMS a number of reasonably effective disease modifying immunotherapies, ranging from low to medium and high efficacy, are available for clinical use with a tendency for more potent disease-modifying drug (DMD) to be associated with more significant side effects and risks of opportunistic infections. With regards to progressive MS, only one DMD has most recently been approved by the American Food and Drug Administration (ocrelizumab, for PPMS), while the European Medicines Agency and other agencies are still assessing it [3]. Despite the successes in experimental medicine, a considerable number of innovative treatments obtained in animal models, failed to reproduce promising effects when tested in patients and sometimes exerted detrimental effects. Such experiences indicate that the disease models currently used in preclinical research, do not always include essential aspects of the human disease or adequately represent pathological aspects of MS. Accumulating evidence reviewed in this publication indicates that the non-human primate (NHP) models of MS may help bridge the translational gap between currently used rodent disease models and the patient.

**Modeling MS in animals**

Although the cause of MS is unknown, genomic and epidemiological studies indicate that the initiation and progression of MS involves autoimmune reactions elicited by the interaction of genetic and environmental factors. Of the ≥200 genes now found associated with the risk of developing MS, the vast majority has a function in the immune system [4]. Moreover, the strongest environmental risk factors – late infection with Epstein Barr Virus, vitamin D insufficiency and smoking – have been associated with modulation of immunological functions [5]. These observations have identified the immune system as the main culprit in MS and therefore the most relevant target of intervention therapy.

The possibility to directly investigate the disease process in patients is limited by ethical reasons and by the inaccessibility of the target organs, being brain and spinal cord, collectively indicated as CNS. Hence, animal models are indispensable for the translational research of pathogenic...
mechanisms and therapy development. Nowadays, MS researchers can choose from many animal models, ranging from Caenorhabditis elegans worms and Drosophila flies to vertebrate animals, such as zebrafish, mice, rats and monkeys. The cumulative contribution of each of these models to our current understanding of immunological and neurodegenerative processes has been immense. Nevertheless, translation of scientific discoveries into effective therapies for the MS patient has been notoriously difficult. The two main causes of this attrition, lack of efficacy and unforeseen toxicity, indicate that a promising clinical effect of a new treatment in currently used animal models has insufficient predictive value for clinical success [6].

The validity of an animal model for the understanding of a human disease is based on at least 4 criteria: 1. Face validity, representing the phenomenological and pathophysiological similarity; 2. Predictive validity, representing the ability of a model to correctly predict the efficacy of a treatment; 3. Construct validity, representing the degree of similarity in the pathophysiological mechanisms and symptoms; 4. External validity, representing the extent to which the observed effect of a treatment can be generalized to the diverse MS patient population [7]. In addition to these basic validity criteria, several more practical criteria are used such as reproducibility, background knowledge, amenability to experimental manipulation, ethics and costs.

The lowest laboratory animal species with a human-like immune system with regard to basic design and function is the mouse. By far the largest proportion of current preclinical research into MS is based on a limited number of genetically homogeneous (inbred) mouse strains, which are bred and raised under very clean, specific pathogen-free (SPF) conditions. Experimental manipulations eliciting relevant clinical and/or pathological aspects of MS in these mice include: 1. Genetic modification, 2. Active disease induction by the injection of CNS homogenate or purified CNS proteins formulated with (an) immune potentiating adjuvant(s) and 3. Passive disease induction by transfer of immune cells or molecules from a diseased animal to a suitable healthy recipient. The current discussion will be limited to the actively induced model, called Experimental Autoimmune Encephalomyelitis (EAE).

**Experimental autoimmune encephalomyelitis (EAE)**

EAE is by far the most frequently used MS animal model. However, it is pertinent to emphasize here that many differences exist between EAE and MS (Refs. [8–10] and Table 1), which may explain the high failure of new treatments in the translation from EAE to MS. Nevertheless, we believe that despite shortcomings of the model, a well-designed study in an optimal EAE model can provide relevant information on the clinical relevance of a new treatment [11].

**Mouse EAE**

Active EAE is induced by the combined activation of adaptive and innate immune mechanisms in genetically susceptible mouse strains, such as C57BL/6, SJL/J and Biozzi ABH, via inoculation of antigen/adjuvant emulsion [9]. The antigen required for the reproducible induction of robust EAE depends on the genetic background of the mice [11]. The most frequently used and best characterized mouse models in studies on MS pathogenesis and screening of drug candidates are RR EAE induced with proteolipid protein peptide (PLP) 139–151 in SJL/J mice and progressive EAE induced with myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 in C57BL/6 mice. Immunization of Biozzi ABH mice with MOG peptide 8–21 elicits an elegant, albeit less frequently used, RR/SP MS like-disease model [12]. There are also several important EAE models in rats, but with the current focus on the mouse these are much less used than until a decade ago.

Mouse EAE is initiated by CNS infiltration of CD4+ T cells, which upon transmigration of the blood brain barrier (BBB) and interaction with local antigen presenting cells (APC), such as perivascular macrophages and microglia cells, release pro-inflammatory factors within the CNS parenchyma. These enhance permeability of the BBB for macrophages and B cells and for serum factors such as antibodies and complement. Collectively, these factors undertake the autoimmune attack on the myelinated axons (Fig. 2). Debris from the injured myelin sheaths are removed from the CNS by myeloid cells, which drain to cervical and lumbar lymph nodes [13]. The observation that surgical removal of these draining lymph nodes abrogates the characteristic chronic relapsing EAE course in Biozzi ABH mice suggests that new T cell specificities are activated there, which drive EAE chronicity [14].

This cascade of pathophysiological reactions has been the template for therapy development in MS. However, the failure of almost all therapies targeting CD4+ T cells in the translation from EAE model to MS patient has shed doubt on the construct validity of the mouse-EAE based CD4-dominated disease concept [15]. The question is therefore warranted whether EAE is an adequate model for MS, or whether inbred/SPF mice are imperfect models for the human autoimmune disease.

**Non-human primates (NHP) EAE**

The close evolutionary proximity of human and non-human primates is reflected by their close genetic and immunological proximity. For the NHP species used for EAE modeling, Macaca mulatta (rhesus monkey), Macaca fascicularis (cynomolgus monkey) and Callithrix jacchus (common marmoset), the evolutionary distances have been estimated at 25, 25 and 35 million years. Different from SPF-bred mice but just like humans, NHP are genetically outbred and have been freely exposed from birth to environmental microbes, which shape
Table 1. Clinical, pathological and immunological aspects of mouse and primate EAE models, compared with MS

<table>
<thead>
<tr>
<th></th>
<th>Mouse EAE</th>
<th>Old World primate EAE</th>
<th>New World primate EAE</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td>Max lifespan ±2 years</td>
<td>Max lifespan ±20 years</td>
<td>Max lifespan ±15 years</td>
<td>Max lifespan &gt;90 years</td>
</tr>
<tr>
<td></td>
<td>Closed/clean environment</td>
<td>Open/dirty environment</td>
<td>Open/dirty environment</td>
<td>Open/dirty environment</td>
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<td></td>
<td>Standard diet</td>
<td>Varied diet</td>
<td>Varied diet</td>
<td>Varied diet</td>
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<tr>
<td><strong>Induction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active immunization</td>
<td>Myelin/MBP/PLP/MOG + CFA + B. pertussis (toxin)</td>
<td>Myelin/MBP/MOG + CFA or IFA</td>
<td>Myelin/MBP/PLP/MOG + CFA or IFA</td>
<td>N.A. (spontaneous)</td>
</tr>
<tr>
<td>Passive transfer</td>
<td>Yes, within inbred strain</td>
<td>Only autologous</td>
<td>Autologous + between twins</td>
<td>N.A.</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>Inbred</td>
<td>Outbred</td>
<td>Outbred</td>
<td>Outbred</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>MHC II</td>
<td>MHC II</td>
<td>MHC I and II</td>
<td>MHC I and II + &gt;200 genes</td>
</tr>
<tr>
<td><strong>Disease course</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing-remitting</td>
<td>Model-dependent (SJL/PLP)</td>
<td>Uncommon</td>
<td>Common</td>
<td>Rare (ADEM)</td>
</tr>
<tr>
<td>Progressive</td>
<td>Model-dependent (B6/129)</td>
<td>Never</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
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<td>White matter</td>
<td></td>
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<tr>
<td>Inflammation</td>
<td>CD4+ T cell/macrophage</td>
<td>T cell/neutrophil</td>
<td>T cells/macrophage/μglia</td>
<td>Macrophage/μglia/T&amp;B cells</td>
</tr>
<tr>
<td>Demyelination</td>
<td>Primary demyel</td>
<td>Primary demyel + necrosis</td>
<td>Primary demyel</td>
<td>Primary demyel</td>
</tr>
<tr>
<td>Remyelination</td>
<td>Rare</td>
<td>Rare</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Neurodegeneration</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>Grey matter</td>
<td></td>
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<tr>
<td>Inflammation</td>
<td>Rare</td>
<td>Absent</td>
<td>Meningeal</td>
<td>Meningeal</td>
</tr>
<tr>
<td>Demyelination</td>
<td>Rare</td>
<td>Absent</td>
<td>Subpial/intracort/leukocort</td>
<td>Subpial/intracort/leukocort</td>
</tr>
<tr>
<td>Remyelination</td>
<td>NA</td>
<td>NA</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Neurodegeneration</td>
<td>NA</td>
<td>NA</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
<td></td>
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<tr>
<td>CD4+ T cell</td>
<td>Proven pathogenic role Th1/17</td>
<td>Proven pathogenic role Th1</td>
<td>Proven pathogenic role Th1</td>
<td>Early-stage pathogenic role?</td>
</tr>
<tr>
<td>CD8+ T cell</td>
<td>Pathogenic role uncertain</td>
<td>Pathogenic role uncertain</td>
<td>Proven pathogenic role</td>
<td>Late-stage pathogenic role?</td>
</tr>
<tr>
<td>B cell</td>
<td>Depletion improves disease</td>
<td>Not tested</td>
<td>Depletion improves disease</td>
<td>Depletion improves disease</td>
</tr>
<tr>
<td>Antibody</td>
<td>Facilitates ADCC/CDC</td>
<td>Not tested</td>
<td>Facilitates ADCC/CDC</td>
<td>Involvement in type II lesions</td>
</tr>
<tr>
<td>T reg cells</td>
<td>Protective role</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Unclear role</td>
</tr>
</tbody>
</table>

Their immune system and underlie the remarkable susceptibility to EAE [16]. During the past 20 years, an in-depth analysis has been carried out in marmosets of the immune reactions elicited by injection with human CNS myelin. As the details of these studies have been reviewed elsewhere [17–19], we will only summarize the most salient findings here.

- Immunization of marmosets with myelin isolated from an MS patient brain elicited a chronic progressive neurological disease that approximates MS in clinical and pathological presentation [20]. A combined radiological (MRI) and neuropathological analysis showed that formation of MS-like lesions occurs disseminated in time and space, just like in the human disease. Subsequent analyses showed that lesions are present in the white as well as grey matter of brain and spinal cord [21].

- Disease progression in mouse EAE models is associated with diversificiation of the T cell and antibody response, a phenomenon known as epitope spreading, but the nature of the response does not essentially change. This is different in marmoset EAE. After the observation that autoimmunity against MOG is essential for chronic EAE development [22], two pathogenically relevant autoimmune pathways were identified triggered by (recombinant) human MOG. These pathways have been extensively reviewed elsewhere [11,18,23]. In brief, one pathway replicates autoimmune mechanisms and pathology observed in mouse EAE models and involves a synergistic attack on CNS myelin of pro-inflammatory T cells and myelin binding autoantibodies. A second pathway, which has no known correlate in mouse EAE models, involves autoaggressive cytotoxic T cells (CTL), which seem to drive EAE progression and have the capacity to kill myelin forming oligodendrocytes.

- The marmoset EAE model displays a clear pathogenic role of simian herpesviruses related to those associated with MS, namely cytomegalovirus (CMV) [24] and Epstein Barr Virus (EBV) [19]. The available evidence indicates that the auto-
aggressive CTL may originate from a repertoire of effector memory T cells, generated for keeping chronic CMV expression quiescent. In addition, infection with EBV endows B lymphocytes with the capacity to activate these T cells. This concept has been highlighted as a novel association between infection and autoimmunity [25].

- Autoreactive T cells present in the pathogen-educated marmoset immune system can be directly activated by injection of a synthetic peptide, representing residues 34–56 of human MOG, adjuvated with the mineral oil IFA [26]. The absence of danger signals in this formulation may explain why SPF-bred Biozzi ABH and C57BL/6 mice fail to develop a reaction against this formulation [26]. Recent data show that this novel pathogenic mechanism elicits neuropathological aspects of progressive MS, including cortical grey matter demyelination, activation of oxidative injury mechanisms, redistribution of iron and damage to mitochondria [27]. We can therefore speculate that the transition of the mouse EAE-like pathogenic pathway 1 to the more MS-like pathogenic pathway 2 represents the transition from RRMS to SPMS.

**Corroborating the validity of marmoset EAE for MS**

The remarkable neuropathological similarities between the marmoset EAE model and MS (face validity) does not necessarily imply that the underlying pathogenic mechanisms are relevant for MS (construct validity). To assess
the **construct and predictive validities** of marmoset EAE as preclinical MS model, the effects of therapeutic mAbs that survived or failed in the translation from mouse EAE to MS were assessed.

**Pathway 1-CD4+ Th cells**  
In mouse EAE models, two pathogenic Th subsets were defined, namely Th1 and Th17, which differentiate from Th0 progenitor cells under the influence of the IL-12/IL-23 cytokine axis [28]. Experiments with a mAb generated against the shared p40 subunit of both cytokines (ustekinumab; IL-12p40) showed protection of marmosets against EAE when treatment was started at the time of EAE induction [29]. In contrast, late treatment exerted only a moderate clinical effect, although the MRI-detectable activity and enlargement of brain lesions were suppressed [30]. The same antibody exerted no significant beneficial effect in a RRMS clinical trial [31]. We also tested an anti-IL-17A mAb in the marmoset model; this mAb also failed to show a beneficial clinical effect [32]. Of note, the anti-IL-17A mAb secukinumab exerted only a moderate beneficial effect in RRMS on MRI-detectable lesion activity [33]. Interestingly, both ustekinumab and secukinumab show satisfactory clinical effects in psoriasis patients.

An explanation for the discrepancies between EAE and RRMS may be that the pathogenic pathway 1 mechanism may represent only the biological onset of MS, which probably occurs long before the diagnosis RRMS is made.

**B cells**  
A recent publication describes the remarkable history of B cell depletion as exciting new treatment for MS [34]. The original thought behind this treatment was to get rid of autoantibodies that upon binding myelin activate myelin destruction mechanisms. Contrary to expectations, treatment of RRMS patients with a mAb directed against CD20, a broadly expressed surface marker in the B cell lineage, exerted a dramatic and long-lasting clinical effect, associated with dramatic and almost immediate reduction of inflammatory lesion activity [35]. The observation that antibody levels were not altered was remarkable, although may be explained by the lack of CD20 expression on plasma cells. Another type of treatment aiming at the depletion of B cells works by capturing factors that B cells need for survival and differentiation, such as ‘B lymphocyte stimulator’ (BlyS) and ‘a proliferation inducing ligand’ (APRIL) [36]. This was achieved with atacicept, a soluble fusion protein combining the joint receptor of BlyS and APRIL on B cells (TACI) with the Fc part of human IgG. This construct showed promising clinical effects in SLE patients [37], but worsened RRMS [38]. Replication of these two treatment concepts in marmoset EAE showed that in both scenarios circulating B cells were depleted, but that the anti-CD20 mAb exerted a superior clinical effect. The explanation found was the differential depletion of CalHV3, the EBV-related lymphocryptovirus (LCV) of marmosets, from the immune repertoire paralleling the discrepant clinical effect. These findings led us to posit a core pathogenic role of LCV-infected B cells in the pathogenic process [39].

**CD8+ T cells in pathway 2**  
It is well recognized that the T cell infiltrate in the MS lesion is dominated by CD8+ T cells [40]. This dominance is not commonly reflected in EAE models, although it was found in virus-induced models of MS [41]. However, the latter models are beyond the scope of this article as equivalents have not been established in NHP. We are not aware of ongoing clinical trials testing the efficacy of CD8+ T cell antagonists in RRMS. However, we observed a profound clinical effect of anti-human CD20 mAb in the MOG34-56/IFA induced marmoset EAE model, which is driven by autoimmune CD8+ CTL.

**Concluding remarks**  
Despite the dramatic progress made in the molecular analysis of MS pathogenesis, translation of data from biomedical research into clinical applications remains a challenge. The problems encountered with technology transfer from bench to bedside are not new and are not confined to MS, but affects almost all clinical disciplines. It is also not a European science problem, but a world-wide concern. Within the European Union, research programs such as Horizon 2020 and Innovative Medicine Initiative have been set up to fund the building of bridges across this “valley of death”.

The necessity to improve the predictive validity of the animal models used in preclinical research is clear [42]. We believe that lessons should be learned from a detailed analysis of the reasons why translation failed and this knowledge can be used for adjustment of the used animal model. As explained elsewhere, advantage can be taken from the two dimensions of the NHP EAE model for this reverse translation exercise as these create a useful bridge between the rodent EAE model and the MS patient [43]. The relevance of the NHP in drug development exceeds the advantage of immunological cross-reactivity for therapeutic mAb, as the disease models provide also important information on the MS pathogenesis that cannot be obtained in mouse EAE. In this respect, the NHP is an equally important pillar under the bridge that connects preclinical and clinical research, as molecular cell biology and mouse disease models (Fig. 3).

Scientists using NHP for their preclinical research need to take the concerns in society and politics seriously and invest where possible in alternatives for research in the living primate as defined in the 3R principles: Replacement, Reduction and Refinement. However, it is pertinent to emphasize here that these principles were formulated with the discomfort to the animals in mind, not the clinical relevance of a disease
model for drug development. This is illustrated by the experience that an investment in Clinical Relevance and Refinement can create conflicts with the Reduction principle, which hampers their application [44].

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Drug Discovery Today: Disease Models | Use of non-human primate disease models

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