MRI-guided immunotherapy development for multiple sclerosis in a primate

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Multiple sclerosis is a serious neurological disease that affects 1 in 1000 young adults in Europe and the USA. The development of an effective therapy for this enigmatic disease is plagued by the failure of many treatments to reproduce in patients the promising effects observed in animal models. This review describes a new preclinical model in a non-human primate that might help to bridge the gap between currently used animal models and the patients.

The populations of Western societies are facing a steadily increasing incidence of chronic immune-mediated inflammatory disorders (IMID) that cause serious damage to affected organs, such as the brain (multiple sclerosis, MS), joints (rheumatoid arthritis), pancreas (type I diabetes) or kidneys (systemic lupus erythematosus). The underlying reason is not clear. Of the variety of environmental factors proposed to explain the enhanced disease incidence, the reduction of infectious diseases in these countries (hygiene hypothesis) has raised considerable interest [1]. The increased prevalence of these disorders has created a request for safe and effective drugs. In reply, the drug development industry has invested heavily in biotechnology with the aim of identifying the best therapy targets and the optimal therapeutic agents, and selection techniques are continuously improved [2]. It is of great concern that, despite the substantial intellectual and financial investments, remarkably few new candidate immunotherapies (NCIs) survive the clinical trials, as already been discussed in several contributions to this journal [2–4].

Once a promising compound has been selected, after extensive laboratory research, proof of therapeutic principle is usually sought in animal models. This is a delicate process because it should identify the most promising NCI for further clinical development. To ascertain that only compounds with the best clinical perspectives are selected from the development pipeline, the animal experiments must be well designed and the animal model(s) should be well chosen. In addition, animal experiments should reveal whether safety concerns are attached to the NCI.

The high failure rate of new therapies for IMID in clinical trials, despite promising effects in animal models, shows that the preclinical selection process of NCI is not without problems and might actually be the Achilles' heel of the drug development process. In many cases, efficacy tests in animal models do not account for the clinical situation. Treatments are often tested before and/or at the induction of the experimental disease, whereas IMID patients will obviously have to be treated only during ongoing disease.

In this review we discuss a new preclinical model for the still enigmatic neurological disease MS in which many shortcomings of the classical disease models have been eliminated.

Current therapeutic approaches for MS

MS is a chronic disabling disease of the human central nervous system (CNS) that affects ~1 in 1000 young adults [5,6]. MS patients develop various deficits of motoric, sensory, optic and cognitive functions. The assessment used by neurologists for quantification of the neurological deficit in MS patients is the extended disability status scale, which records mainly impairment of motoric functions, largely reflecting spinal cord pathology [7]. Detection of CNS abnormalities by magnetic resonance imaging (MRI) is often used to confirm MS diagnosis. In ~80% of MS patients, episodes of

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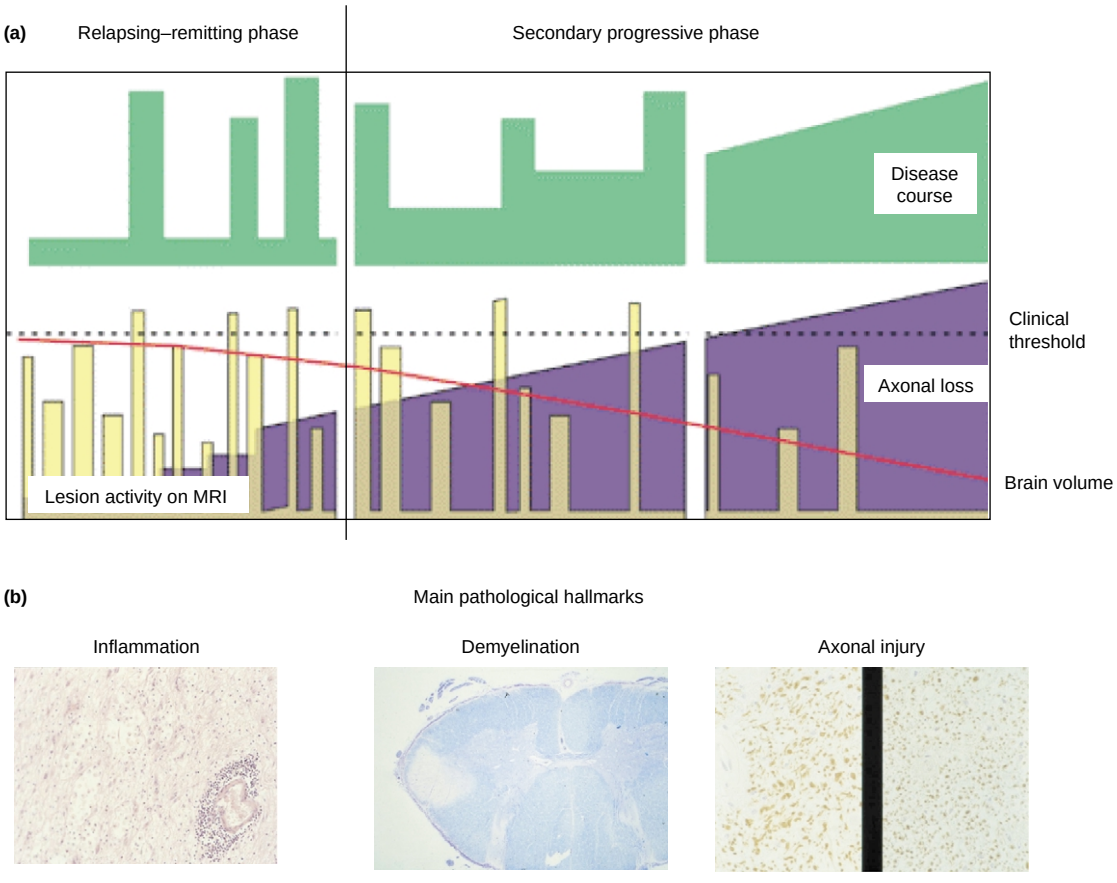

FIGURE 1

Clinical and pathological features of MS. (a) The diagram shows the disease course in a typical case of relapsing–remitting secondary progressive MS case. MRI activity, axonal loss and progressive loss of brain volume (atrophy) are indicated. The figure is modified, with permission, from Ref. [6]. **(b)** Photos illustrating the main pathological features of MS, kind gift from Prof. Dr. Paul van der Valk (VU medical center, Amsterdam). The inflammation picture shows a blood vessel surrounded by a cuff of lymphocytes and monocytes. The demyelination picture shows at the left hand a pale area where the blue myelin staining (Klüverr-Barrera) has disappeared. The right-hand panel of the axonal pathology figure shows the staining pattern of normal axons (anti-neurofilament antibody NE14). The left-hand panel shows the swollen axons present in an MS lesion.

neurological dysfunction alternate with complete recovery. This relapsing–remitting phase can last up to 25 years or more and is in the vast majority of patients followed by a secondary progressive phase where neurological deficits accumulate (Figure 1). In a minority of patients the disease worsens from the onset without intermittent recovery. The pathological hallmark of MS and the most likely cause of the neurological deficits is the lesion. Lesions are focal areas of prominent inflammation and demyelination, with a variable degree of axonal pathology, astrogliosis and remyelination. The lesions in MS are confined to the CNS within the white and gray matter. This pathological heterogeneity should be taken into account during the development of a therapy [8].

Although evidence is lacking, MS is usually regarded as an autoimmune disease that is driven by specific immune reactions occurring in unique components of the CNS. The underlying immunological mechanisms are highly complex and only partly understood [9]. A few essential but unanswered questions in MS are: (i) which genetic and environmental factors initiate the disease and determine its course; (ii) which pathological features cause the various forms of neurological deficit and (iii) what is the exact contribution of the immune system to the disease. The major cause for the lack of effective therapy is that very little is known about the crucial pathogenic mechanisms in MS. An acute disease episode is usually treated with intravenous glucocorticoids and followed by an immuno-modulatory treatment. To date, biotechnology has yielded three registered biological drugs for MS. Two of these, glatiramer acetate and interferon-β, have a moderate effect, mainly in the relapsing–remitting phase of the disease [10]. The third is a blocking antibody directed against the adhesion molecule VLA-4 (natalizumab). However, shortly after approval, the sales of the antibody were voluntarily halted because of two unanticipated MS cases showing progressive multifocal encephalopathy, a fatal disease caused by recrudescence of latent JC virus [11]. Additionally, another therapy for MS with alemtuzumab (Campath®) was stopped because of serious adverse side effects. Clearly, there is much room for improvement in the currently available treatment repertoire of this disease.

Selection of a valid animal model for MS

Spontaneous cases of MS-like disease rarely occur in common laboratory animal species. Therefore, animal models used in NCI tests are

artificially induced, for example, by the inoculation of CNS homogenate or CNS antigens in a suitable formulation or by genetic manipulation [12–15]. The advantage of a disease model is that through a clever choice of experimental conditions and animal strain, specific aspects of MS can be mimicked at will. However, the disadvantage of these experimental MS models is that none of them reproduces the complete clinical and pathological spectrum of the disease.

The most intensively studied MS model is experimental autoimmune encephalomyelitis (EAE). Besides EAE, virus-induced models of encephalomyelitis have been developed in mice, such as with Semliki Forest virus and Theiler's murine encephalomyelitis virus [13,16,17]. Disease models in genetically engineered mice are becoming increasingly important to prove therapeutic principle [14]. It is pertinent to emphasize here that EAE has crucial widespread importance not just as animal model of MS. Many principles of tolerance and autoimmunity have been developed in EAE models. Also, for the study of glia cells, neurodegeneration and neuroinflammation, EAE appears a useful model [13].

Remarkably, there is no clear consensus on the best preclinical MS model for the effectivity testing of NCI. Frequently used EAE models are the myelin basis protein (MBP)-induced model in Lewis rats, the proteolipid protein peptide ($PLP_{139–151}$)-induced model in SJL/J mice and the myelin oligodendrocyte glycoprotein peptide ($MOG_{35–55}$)-induced model in C57Bl/6 mice. The chronic relapsing–remitting EAE model in Biozzi ABH mice is still rarely used [17]. It is important to note that these models were originally developed to study the pathogenic role of T cells and macrophages and not to model the multifaceted pathology of MS. The fact that T-cell-mediated inflammation is the most prominent feature of these models probably explains why most NCIs for MS have been selected on the basis of anti-inflammatory activity.

We propose that, similarly to the situation in toxicology, the optimal animal model for a particular therapy should be selected on the basis of several well-defined criteria. In the following, we describe important factors that should be considered for the selection of a suitable animal model in preclinical tests.

First of all, the chosen animal model should be sensitive to the test compound and reveal possible safety problems. NCIs with a high specificity for humans, such as many biological molecules (monoclonal antibodies, cytokines), are usually ineffective in rodents and should therefore be tested in transgenic mice or in animals that are closely related to humans (e.g. non-human primates). As an example, an important safety concern in the early days of antibody therapy was anaphylactic shock, which was either caused by massive activation of complement or by sudden release of cytokines by the targeted cells [18]. This complication was rarely observed in rodents but emerged during preclinical tests in primates. A more recent example is the already mentioned recrudescence of latent JC virus infection in a small number of MS patients treated with the anti-VLA4 antibody natalizumab in combination with interferon-β [11]. The activation of latent virus by immunosuppressive therapy has been rarely observed in specified pathogen-free (SPF) rodents, unless the animals had been deliberately infected, but can be observed in primates [19].

Second, the biodistribution as well as physiological and pharmacological properties of the therapeutic targets should be comparable in the animal model and the patient. A matter of increasing concern is that the immune systems of commonly used SPF laboratory mice, such as BALB/c, SJL/J or C57BL/6, not only differ substantially from the human immune system [20,21], but are also much more sensitive to manipulation. This is partly caused by the different history of microbial infections [22] and might explain why many NCIs that have a profound effect in animal models lack activity on the more robust human immune system or can even have detrimental effects [10,23]. We think that, because of the evolutionary proximity to humans, non-human primates can help to bridge this gap [4,24–26].

A third aspect is the lack of a chronic disease course in many rodent EAE models. Animal models should account for the possibility that different stages of MS are governed by distinct immunopathogenic processes [27,28]. A similar situation is observed in EAE. Whereas inflammation and axonal suffering are prominent feature in early EAE, chronic EAE models, such as the Biozzi ABH mouse or the common marmoset, show that demyelination and axonal destruction become more prominent at a later stage [17,29,30].

A fourth crucial aspect is the read-out parameters that are used to detect whether the investigated NCI has a therapeutic effect. An effective therapy for MS should ideally have a prompt effect in an ongoing pathogenic process. In many EAE models, where the neurological deficit is only caused by inflammation, this can be achieved relatively easily because suppression of the inflammation stops the disease. However, because in these models complete recovery usually occurs spontaneously within a few days after disease onset, the effect of a NCI is often tested during the induction phase. In more complex models, where the clinical signs are caused by destruction of the myelin sheaths that form an electrical insulation layer around axons (demyelination) or, worse, the nerves themselves, a direct effect is much less likely to be observed because repair of the damage and restoration of function usually takes considerable time. In such cases, imaging techniques can help assess whether an NCI is effective or not.

Brain lesion imaging with MRI

MRI is the imaging modality of choice for the visualization of pathological changes within the brain [31]. We have implemented in the marmoset EAE model essentially the same MRI techniques as used in the diagnosis of MS to facilitate the translation of promising treatment effects to the patient. (A very useful explanation of the fundamentals of MRI can be found at: http://www.cis.rit.edu/htbooks/mri/inside.htm.) Routine techniques used for the detection of lesions in the brain of MS patients are proton density (PD) imaging and T_2 weighted (T_2W) imaging. PD images visualize the presence of protons in a given area. T_2W images are sensitized for changes in T_2 relaxation time, a factor that is mainly determined by altered water content of a tissue, caused for example by edema. T_2W imaging is a sensitive tool to diagnose abnormalities in the CNS white matter of MS patients. However, T_2W images are rather unspecific because the dominant strength of the water signal masks other pathological aspects in the examined tissue. Additional techniques have been developed, such as gadolinium-DTPA (Gd-DTPA) contrast-enhanced T_1-weighted imaging, magnetization transfer ratio (MTR) imaging and magnetic resonance spectroscopy (MRS). With these techniques, important information on the activity and structural aspects of affected brain parts can be obtained (see below).

It is much more challenging to record high-contrast images from the spinal cord than from the brain. The spinal cord is a relatively

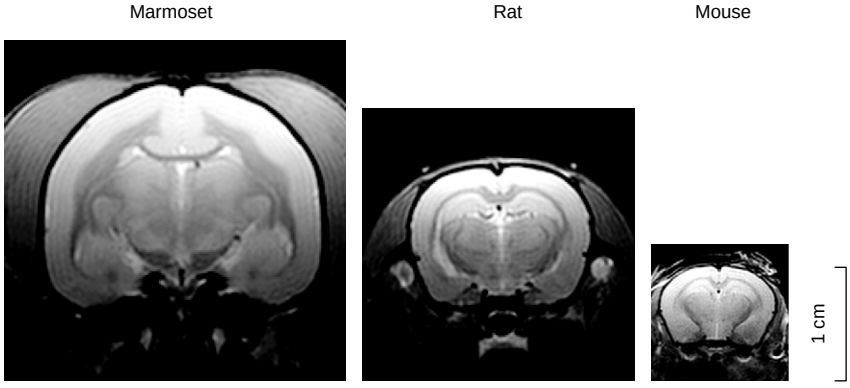

FIGURE 2

Proton density brain images recorded at 4.7 Tesla of an healthy adult marmoset, rat and mouse. The amount of protons in a voxel is converted into signal intensity on a grey scale. Water-rich areas such as the ventricles are white, whereas compact myelin is dark gray.

TABLE 1

Immunological and physiological aspects where the marmoset EAE model resembles MS

General features
Neuroanatomical aspects of the brain
White matter:gray matter ratio
Lymphocyte and monocyte differentiation (CD) markers
Antigen presentation molecules
Antigen receptor of T cells
Antigen receptor of B cells and antibodies
Effector molecules of the immune response
Disease-related features
Chronic progressive clinical course
Histo-morphology of the lesion
Immunological aspect of the lesion
MRI aspect of the lesion
Myelin-reactive T cells in naïve repertoire
Specificity of T cell response to myelin
Specificity of the antibody responses to myelin antigens

small area, which makes it difficult to obtain enough resolution to clearly visualize the different structures and pathological abnormalities in this tissue. An extra problem is that the quality of the images is substantially reduced because of breathing movements of the thorax, although this can be technically overcome by triggering the data acquisition on heart and breathing rate. For these reasons we normally record only brain images. Figure 2 shows proton density scans of a mouse, a rat and a marmoset brain. It is immediately clear that, because of its small size, the mouse is the least preferred model, although, in principle, high-contrast images can be produced. Moreover, compared with the rodent models, the marmoset brain contains a much higher white-to-grey matter ratio, which is an obvious advantage for the study of a white matter disease such as EAE and more closely resembles the situation in human.

We have summarized the advantages of the marmoset EAE model as a useful preclinical model of MS in Table 1.

EAE in the common marmoset is a valid preclinical model of MS

In the past decade, a new EAE model has been developed in the New World primate species, the common marmoset [15,30,32–34]. This model offers an alternative to the acute EAE model in macaques, which has been used as the only available non-human primate MS model for several decades. The marmoset EAE model shares many more clinical and pathological similarities with MS than the rhesus monkey EAE model, which rather resembles the acute forms of MS, such as acute disseminated encephalomyelitis [35]. The marmoset EAE model has several attractive aspects for basic and preclinical research [30].

The marmoset is a common laboratory species, which has been intensively used in neuroscience and behavioral research [36]. The attractiveness of the marmoset for research into IMID is its outbred nature, which implies that the sets of alleles that determine the expression of clinical and neuropathological signs differ between individual animals. Our data show that, analogous to the situation in MS [37,38], major histocompatibility complex (MHC) class II genes have a strong influence on the disease susceptibility [39]. Sequence data in the primate MHC database (www.ebi.ac.uk/ipd/mhc/nhp/align.html) show that the marmoset's MHC (indicated with Caja) encodes equivalents of human MHC (indicated with HLA) class II molecules. The Caja class II region was found to encode the equivalents of *HLA-DQ* and *HLA-DR*, but expression of *HLA-DP*-like alleles has not been found [40]. The genetic heterogeneity of the marmoset can be advantageous when the EAE model is used for the mapping of genetic susceptibility and resistance elements.

The organization of the marmoset's immune system resembles the human immune system to a high extent. This is illustrated by the similarity of many leukocyte surface molecules, as defined by crossreactivity with monoclonal antibodies developed for detection of human CD (cluster of differentiation) markers [41], of the genes that encode the elements assembling immunoglobulins [42] or T cell receptors [43] and of cytokine genes [44]. This high immunological similarity is of obvious importance for research on pathogenic mechanisms and when the model is used for testing reagents intervening into immunopathogenic pathways.

The CNS lesions that develop in the brain white matter [45] and cortex [46], as well as the spinal cord of EAE-affected marmosets show

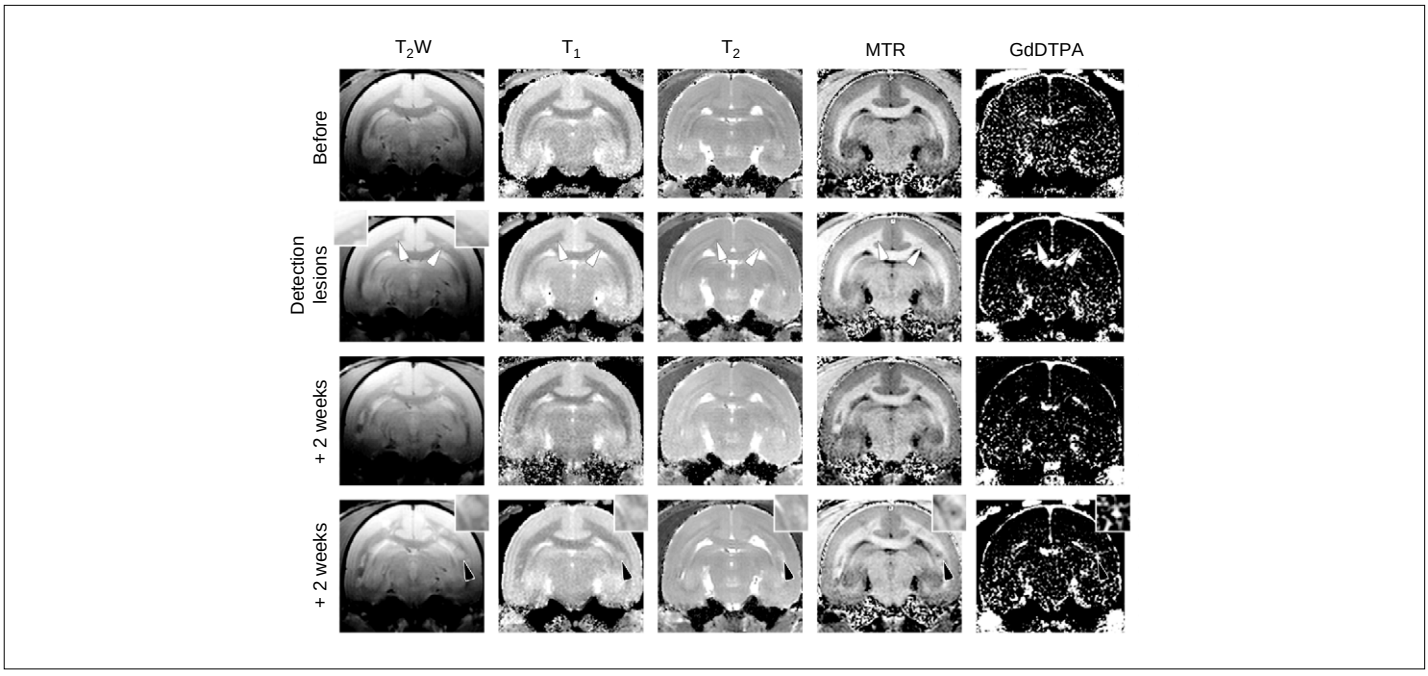

FIGURE 3

Lesion progression as observed with quantitative MRI. An rhMOG-immunized marmoset was scanned every two weeks until it was sacrificed because of the severity of EAE. The figure shows the same slice at consecutive time points (rows). Pictures in the first row were taken two weeks before the first lesion was detected. No abnormalities are visible in the white matter. In the second row, several hyper-intense abnormalities are visible on the T_2W images (white arrowheads and insets), which are invisible on the T_1 and T_2 relaxation time images. MTR values are slightly reduced compared with the surrounding normal-appearing white matter. There is no visible leakage of Gd-DTPA on the Gd-DTPA-enhanced T_1 difference images. The third row of pictures shows that two weeks after the first detection of lesions new lesions were formed in both hemispheres. These newly developed lesions are hardly visible on the relaxation time and Gd-DTPA enhanced images. However, they are clearly hypo-intense on the MTR images. In the fourth row, images taken after two more weeks show that new lesions were formed, which are also visible in the relaxation time images. In particular, one lesion shows increase in relaxation times, clear reduction in MTR and substantial leakage of Gd-DTPA (black arrowheads and insets).

a great histological [45,47–49] and immunological [50] similarity with MS.

Two-phase efficacy analysis of NCI in the marmoset EAE model

The two most important outcome measures of NCI in preclinical trials are safety and efficacy. The marmoset EAE model has been primarily designed to detect efficacy of an NCI but, during a study, a range of hematological and serological parameters are periodically determined. These parameters primarily serve the health screening of the monkeys but can also be used to assess side effects of the NCI. The efficacy of a tested NCI is usually deduced from a cross-sectional analysis of various *in vivo* and *ex vivo* quantifiable parameters. *In vivo* parameters include expression of clinical signs [45] and MRI parameters, such as T_2W lesion load or the proportion of lesions with demonstrable leakage of the blood–brain barrier (BBB) [51]. Similar to the situation in MS, scoring of clinical signs in the marmoset EAE model is predominantly based on disturbance of motor functions. More subtle neurophysiological and behavioral tests are now being developed. The major *ex vivo* parameters are various humoral and cellular immune functions, and the histological and immunological characterization of lesions [45,50].

A preclinical test in marmosets of a NCI developed in rodent EAE models can consist of two phases. Phase 1 has a similar design as many studies in rodent EAE models and aims to test whether beneficial effects observed in rodents can be reproduced in the equivalent marmoset model. Test groups normally consist of 5–6 monkeys, which are randomly assigned to each group before or at the day of immunization. The dosing of the NCI is started around the time of immunization and disease parameters are analyzed in a cross-sectional fashion. MRI scans are planned at predetermined time points, because there is no one-to-one relation between MRI and clinical signs [30]. Examples of typical phase 1 preclinical trials with therapeutic antibodies specific for the human co-stimulatory molecule CD40 or the pro-inflammatory interleukins (IL)-12 and -23 are referred to in previous publications [52–54].

NCIs that show a promising effect in phase 1 can be further tested in phase 2. The aim of this new experimental design is to test the efficacy of promising NCIs in ongoing disease, being an experimental setting that more closely resembles the future treatment situation in patients.

Efficacy evaluation of NCI in ongoing disease

For a phase 2 preclinical trial of a NCI, EAE is induced by a single inoculation of recombinant human myelin oligodendrocyte glycoprotein (rhMOG). We have documented elsewhere [15] that 100% of the monkeys develop EAE because of the ubiquitous presence of the EAE susceptibility marker Caja-DRB*W1201, although the time of onset can vary between 2 and 25 weeks. The advantage of the model, however, is that large lesions are formed that remain inflammatory active for a prolonged time and can be well evaluated with quantitative MRI techniques [55]. The lesions typically resemble the pattern II lesions of MS, which are characterized by mononuclear cell infiltrates, deposition of antibody and complement and various degrees of axonal pathology [30].

BOX 1

Technical description of an MRI procedure in a marmoset

The hardware:
High-resolution MR images are recorded in a 4.7 T horizontal bore NMR spectrometer (Varian, Palo Alto, USA), equipped with a high-performance gradient insert (12 cm inner diameter, maximum gradient strength 500 mT/m). A Helmholtz volume coil (85 mm diameter) and an inductively coupled surface coil (35 mm diameter) are used for radiofrequency transmission and signal reception, respectively. Pictures of the hardware can be found in Ref. [68].

Preparation of the animal:
Anesthesia for MRI recording is induced by intramuscular injection of ketamine and maintained by mechanical ventilation with isofluorane (1.5–2%) in N_2O:O_2 (70:30) via an endotracheal tube. Expiratory CO_2 is monitored and the body temperature is maintained at 37°C with a heated water pad. An infrared sensor (Nonin Medical, USA) is attached to the animal hind paw to monitor heart rate and blood-oxygen saturation. The tail vein is cannulated for injection of the contrast agent Gd-DTPA to probe the BBB permeability. The head is immobilized in a metal-free device, based on a stereotactic frame for rats, which is placed in an animal cradle. The construction with the animal is then inserted into the NMR spectrometer.

MRI procedure:
On a sagittal scout image, 35 contiguous coronal slices of 1 mm are defined, covering the complete brain. From these 35 slices, MRI datasets are collected (field of view 4 × 4 cm^2, matrix: 128 × 128; in-plane resolution: 312.5 μm^2, two transitions):

- T_1 *relaxation time images*. These images are the result of a mono-exponential fit of six inversion recovery multiple snapshot FLASH images [69]. Repetition time (TR) = 920 ms for each image and 7 s for the whole cycle; α = 7°; echo time (TE) = 3.6 ms; inversion time = 230, 1150, 2070, 2990, 3910 and 4830 ms.
- T_2 *relaxation time images*. These images are obtained by a mono-exponential fit of five multi-echo images. TR = 5000 ms; TE = 17.5, 35, 52.5, 70 and 87.5 ms. T_2W images are also obtained from this experiment (TE=35 ms).
- *MTR maps*. These maps are calculated from two T_1W spin echo images with and without a MT-saturation pulse [70], with $MTR = 100 \times [(M_{unsaturated} - M_{saturated}) / M_{unsaturated}]$. TR = 1500 ms; TE = 12.5 ms; MT-pulse = 12 ms Gaussian shaped pulse; nominal flip angle = 1125°; offset = – 4.7 kHz.
- *Gd-DTPA enhanced T_1 relaxation time difference maps (GE-T_1)*. These maps are calculated from two quantitative T_1 relaxation time images, before and after a bolus of 0.3 mmol/kg Gd-DTPA (intravenous, 12.5 min in circulation) with $GE\text{-}T_1 = 100 \times \{[(T1 \text{ (pre Gd-DTPA)} - T_1 \text{ (post GDTPA)}] / T_1 \text{ (pre GDTPA)}\}$. Pixel intensities display the percentage of T_1 decrease caused by Gd-DTPA leakage.

To characterize the lesions *in vivo* we have implemented a set of semiquantitative MRI parameters (Figure 3; Box 1). In a phase 2 preclinical experiment, scans of each animal are made before disease induction to determine the baseline value of each parameter. After EAE induction, the same MRI parameters are recorded at two-week intervals, until at least one brain lesion of sufficient size for evaluation is observed. At this time point a monkey will be randomly assigned to the test group treated either with the NCI or with placebo. Follow-up scans are made every two weeks until the end of the observation period. The two-week time interval between scans is not absolute, but we prefer less-intensive scanning frequency to limit stress to the animals. Two examples of phase 2 preclinical trials have been published recently [56,57].

Phase 2 type preclinical trials provide a wealth of experimental data, including:

(i) The time interval between the appearance of the first lesion and the diagnosis of a particular disease score. This parameter provides information on the effect of the treatment versus placebo on disease progression.

(ii) Volume measurement of the total T_2W lesion load determines the effect of the NCI on the overall progression of brain pathology. Measuring T_2W lesion load is the most straightforward MRI technique to record lesion development and to assess therapeutic effects in MS patients.

(iii) Volume measurement of single T_2W lesions reveals whether the NCI has a different effect on pre-existing and newly formed T_2W lesions.

(iv) T_2 (spin–spin) relaxation time images quantify the amount of free water in single lesions. The free-water content and, thus, T_2 relaxation time increases because of the presence of vasogenic edema associated with pathogenic processes, such as inflammation and BBB leakage.

(v) T_1 (spin–lattice) relaxation time images provide information on tissue compactness. T_1W images are also frequently used to study the leakage of the intravenously administered paramagnetic probe Gd-DTPA into the brain as a measure of increased BBB permeability. The amount of Gd-DTPA leakage into a lesion can be quantified by comparing T_1 relaxation time images recorded before and after injection of the contrast agent.

(vi) MTR images. MTR images are based on the interaction between protons of mobile water and those associated with macromolecular structures, such as cell membranes. Therefore, MTR can reflect the amount of myelin in tissue, although it has been suggested that during inflammation MTR is more sensitive to physiological changes of myelin. MTR reduction can also result from dilution of macromolecules caused by edema. By combining information from MTR and T_2 images, an indirect measure of tissue destruction can be obtained.

(vii) Biodistribution and target organ penetration of the test substance. An important question in the development of a treatment for MS is whether the therapeutic agent penetrates the brain and whether all or only a subgroup of lesions is targeted. To investigate this, a scan should be planned just before an EAE-affected monkey is sacrificed. The injected agent, which should be labeled for easy detection (with biotin, for example), is injected one or two hours before the scan. At necropsy, the brain and other organs of interest are removed *in toto* and mildly fixed. Usually, a T_2W scan is made of the fixed brain for the precise localization of lesions. After that, the tissue is sectioned and processed for immuno-histochemical detection of the therapeutic agent. Using this method we have shown that intravenously injected antibody against a central regulatory factor in CNS inflammation (IL-12 and/or -23) gains access to lesions and possibly exerts locally anti-inflammatory effect [56,57].

Future perspectives

Although for a long time MS has been regarded as a disease of the CNS white matter, more recent data show that the cortex can be severely affected as well [58]. The reason that cortical lesions have received less attention in MS is that they are poorly visible on MRI, most probably because a strong inflammatory component is lacking in these lesions.

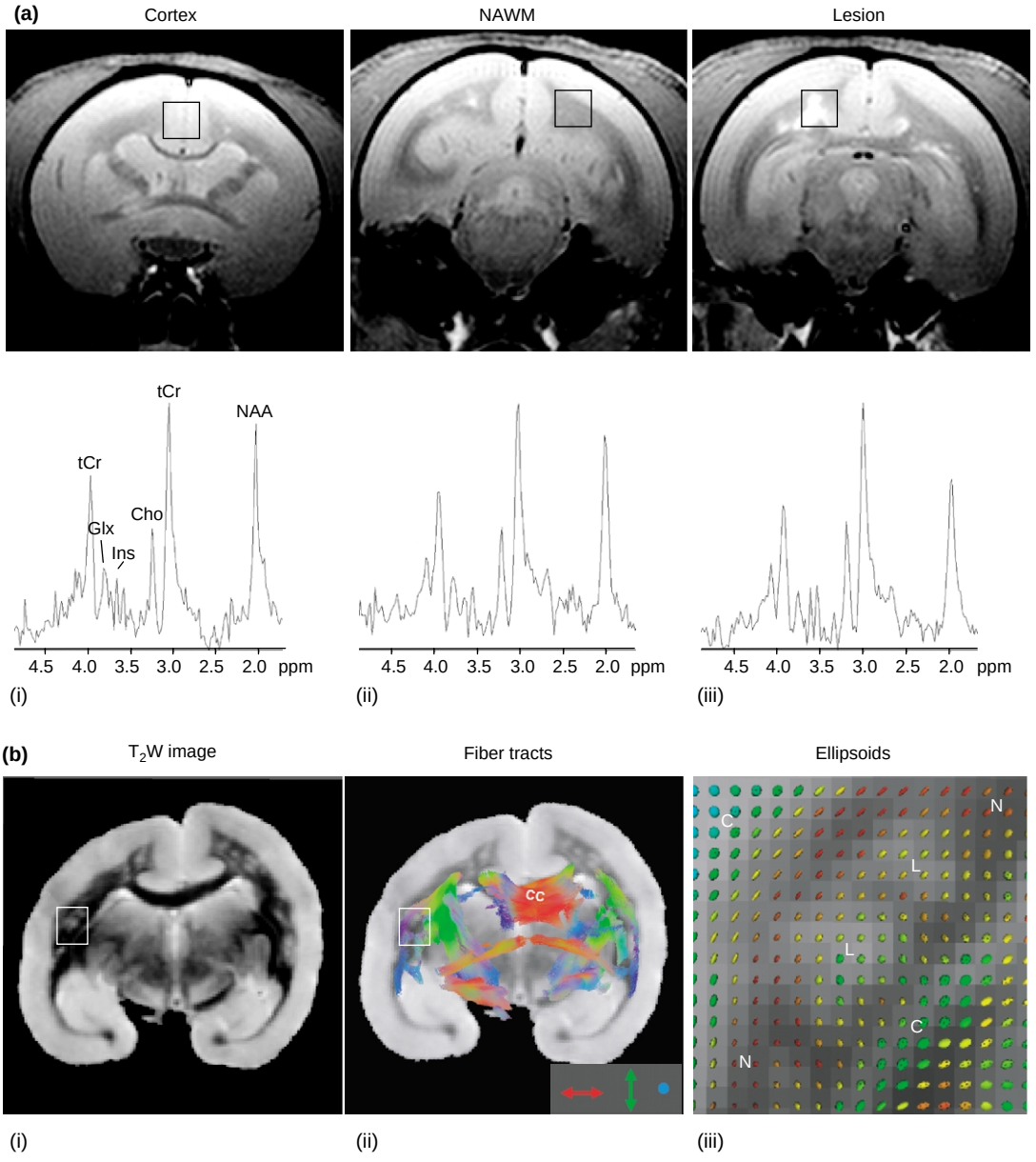

FIGURE 4

Newly implemented MRI techniques in the marmoset EAE model. (a) Localized ^{1}H-NMR spectra from selected areas in an EAE-affected marmoset brain. A box (4 × 4 × 4 mm) is placed either in the (i) cortical area, (ii) normal-appearing white matter and (iii) lesion. The localization of the boxes is depicted in the T_2W images of the top row (black squares). The ratio NAA:tCr (the tCr peak at 3 ppm) can be used as an indicator of neuronal damage. NAA is a marker believed to be present only in neurons. tCr is often used as a suitable stable *in vivo* concentration reference because the sum of creatine and phosphocreatine is constant (except in the chronic phase of many pathologies). Noteworthy, the ratio of NAA:tCr of the lesion area is clearly lower than that of the cortical and normal-appearing white matter area. Abbreviations: Cho, choline containing compounds; Glx, glutamate and glutamine; Ins, myo-inositol; NAA, *N*-acetyl aspartate; tCr, total creatine. **(b)** Diffusion tensor images from an EAE-affected marmoset brain. (i) T_2W image with hyper-intense areas representing lesions. (ii) Fiber track overlaid over a T_2W image, as calculated with DTI. Color codes indicate the directional vectors (left-right: red; up-down: green; perpendicular to the plan: blue). The potential of the technique is shown at the level of the corpus callosum (cc), where the fiber direction is clear. It is not possible to calculate fiber tracks in the lesion area because of loss of myelin organization. (iii) Ellipsoid representation of fiber-track orientation in an area with many lesions [depicted in boxes (i) and (ii)]. The ellipsoids have a more-round shape in areas in the cortex (C) and lesions (L), whereas they are more unidirectional in the normal-appearing white matter (W).

We have recently re-examined archived tissues from previous experiments and noticed that cortical lesions are also prominent in the marmoset EAE model. Notably, leukocortical lesions, which involve the white matter and cortex, can be readily observed in the T_2W images of the marmoset EAE brain [30]. Future research will aim at the visualization and detailed characterization of these lesions with MRI.

There is increasing evidence that the inflammatory demyelination in the MS brain might be superimposed on a progressive neurodegenerative process that starts early in the disease [59,60]. Whether the immune system has a similar impact in neurodegeneration as in the inflammatory demyelination of the white matter is not known and is subject of our current research. Our

studies in the marmoset EAE model have shown that depending on the experimental conditions different grades of axonal and neuronal pathology are induced [30,35]. The next step will be to develop MRI tools for the visualization of neurodegeneration, such as MRS.

In the experimental and clinical setting measurement, MRS is used to determine neurodegeneration. During an MRS measurement, a small box (voxel) is placed over a region of interest, for example over a lesion or normal-appearing white matter (Figure 4). The ^{1}H-NMR spectrum of the tissue enclosed within the box is recorded. With MRS the local concentration of metabolic markers (such as *N*-acetyl-aspartate, a molecule specifically present in neurons) for some pathological processes can be determined and the technique has shown its value in patients with MS [61]. It is also possible to apply this technique in an imaging mode, but these are lengthy experiments and resolution of the images is compromised. Pilot experiments show that this technique is applicable to the marmoset EAE model, but more research has to be done before MRS can be fully implemented (see Figure 4a).

A second attractive technique is diffusion weighted imaging. This is a well-established MR method that shows the diffusivity of water and is increasingly used in MS studies. Recently, improvements in the imaging of water diffusion have been made through the development of diffusion tensor imaging (DTI). This method measures the directional diffusivity of water and allows the visualization of the directional integrity of white matter tracts. It has been suggested that DTI is more sensitive in detecting white matter abnormalities in patients with MS than conventional diffusion weighted MRI [62]. As an illustration, DT images of a fixed marmoset brain are shown in Figure 4b.

A third development in MRI, which has not yet been tested in primate EAE models, is the usage of MRI detectable probes that are targeted to cells or molecules of interest. The fate of the targeted structures can then be visualized *in vivo*. For example, the expression of intercellular adhesion molecule-1 (ICAM-1) and E-selectin, which are involved in leukocyte adherence and extravasation during inflammatory processes, has been visualized with MRI [63,64]. The intracerebral infiltration of leukocytes can also be visualized with MRI. For this purpose, leukocytes are labeled with paramagnetic compounds and the migration of monocytes, macrophages [65] and T cells [66] is shown in animal models of MS. The technique has also been used to visualize the faith of stem cells after intracerebral transplantation in EAE. Stem cell transplantation is being explored as a new strategy for the treatment of demyelinating diseases such as MS [67].

Hurdles and limitations

Preclinical research in non-human primates is generally thought to be very expensive, difficult to interpret because of the low number of animals and associated with many ethical problems. This is only partly true. Although a therapy test in non-human primates is far more expensive than similar studies in rodents, it can nevertheless turn out to be cost-effective. As an example, the efficacy assessment of a new therapeutic antibody directed against human CD40 cost less than 15,000 per monkey [52]. These costs are negligible compared with the investment that is lost when an NCI fails late in the development, for example in Phase II or III clinical trials. Preclinical tests in a valid primate disease model can help select the NCI with the best chance of success in clinical trials and in this way save high investments in clinical trials. An efficacy study in non-human primates is normally planned at the end of the preclinical evaluation. Usually, a test in non-human primates will only be done with the most promising NCIs that have survived rigorous efficacy and safety testing in lower species or that are already used in patients for other indications. The main aim is to obtain proof of a therapeutic concept in a model that more closely resembles the patient than an inbred SPF laboratory mouse or rat.

Studies in non-human primates usually comprise fewer animals per test group than similar studies in mice and rats. Power analysis usually helps to determine the minimal group size needed for a meaningful result that can be statistically evaluated. As an example, the results of a phase 1 preclinical trial that compares the lesion volume in EAE monkeys treated with an NCI or placebo will be evaluated with Student's *t* test. A group size of four animals will be sufficient to evaluate treatment effects exceeding two times the standard deviation, with a power >80% ($\alpha < 0.05$). A typical phase 2 preclinical trial compares the variation of a parameter during the disease course in animals receiving placebo with animals under an experimental treatment. In this type of experiment each monkey serves as its own control, with measurement variation within an individual animal being more important than the variation among different animals. Fischer's exact test can be used for statistical evaluation. At 100% treatment effect, in a group of four animals the power is 80% and in a group of five is 88.5%. To stay on the safe side, we usually recommend including five monkeys per group. Should one animal in an experiment with five animals per group respond different than expected, the power is still 73%. This assumes a 100% disease incidence in the model, which has always been the case thus far.

Conclusion

The marmoset provides an exciting new model for MS that bridges the gap between rodent models and the MS patient. The model shares clinical and neuropathological similarities with rodent EAE models, as well as with the human disease. A particularly important aspect is the possibility to visualize lesions *in vivo* with MRI. In this way, the marmoset EAE model might help to reduce the high-failure rate of new therapies developed for MS. Although the costs and ethical constraints of a disease model in primates are higher than in rodents, the investments that are lost when NCI fails late in the development, such as in Phase I and II clinical trials, are many orders higher.

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