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Published in:
Clinical Pharmacology & Therapeutics

DOI:
10.1002/cpt.801

Reverse Translation for Assessment of Confidence in Animal Models of Multiple Sclerosis for Drug Discovery

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The poor predictive quality of currently used animal models in preclinical research is an important cause of the high attrition of promising drug candidates for human autoimmune disease in clinical trials. Examples from own work in a primate multiple sclerosis (MS) model illustrate that important lessons can be learned from a critical reassessment of failed drugs in the animal model, which can help improve the animal model and better understand the targeted disease.

FORWARD TRANSLATION PROBLEMS IN DRUG DEVELOPMENT

Aging Western societies are facing an increasing prevalence of chronic inflammatory and degenerative disorders for which no adequate treatment exists. High investments by the drug development industry into preclinical research have produced a stream of new targets and sophisticated new therapies. However, only for a disappointingly few, estimated at less than 10% in some disease areas, have promising effects been observed in animal models that could be reproduced in the clinic.[1] A main cause of the high attrition is the poor **predictive validity** (see **Box 1**) for clinical success of the animal models that are currently used in the pipeline selection of drug candidates. Frustration about the high frequency of costly failures has stimulated the search for novel human-based *in vitro* technologies for preclinical research, including cell and organ culture systems (induced pluripotent stem cells, organ-on-a-chip, 3D cultures) derived from patients or even direct research in patients.[2]

We believe that moving away from animal disease models will not be the solution, as most diseases are too complex for *in vitro* modeling. Moreover, pathological processes in vulnerable tissues, such as the human brain, cannot be directly examined in patients. A wiser approach may be to invest in the improvement of the **predictive validity** (see **Box 1**) of animal models by a critical analysis of the reasons why **forward translation** (see **Box 1**) of promising therapies from the animal model to the clinic failed (**Figure 1**). We posit here that because of their close proximity to humans, disease models in nonhuman primates (NHPs) are especially useful in such a **reverse translation** (see **Box 1**) strategy, as the drug that failed in the clinic can be retested in the animal model. We will use examples from our own research, autoimmune-mediated inflammatory disease (AIMID), to illustrate how important information gained from reverse translation analysis of failed and successful treatments can guide the improvement of an NHP model for MS.

MS, A PROTOTYPE AIMID

AIMID represents a category of autoimmune diseases driven by auto-aggressive T cells, which cause inflammation and injury in affected organs. MS and its elected animal model, experimental autoimmune encephalomyelitis (EAE), are representative AIMID types that have been intensively investigated by our group. MS specifically affects the human central nervous system (CNS) by a complex pathogenic process. The pathological hallmark of MS and the most likely cause of the neurological symptoms is the lesion. Lesions are usually well-defined abnormalities within the white and gray matter of brain and spinal cord where the protective myelin sheaths around axons, and after some time the axons themselves, are damaged. The abundance of immune cells and molecules in inflammatory active lesions underlies the concept that CNS injury in MS is caused by an immunological process.

The clinical course of MS as it occurs in the majority (±85%) of patients can be divided into three phases (**Figure 2**): 1) a presymptomatic phase where foci of inflammatory activity in the CNS can be visualized with magnetic resonance imaging (MRI), but where clinical signs are absent; 2) a relapsing-remitting (RR) phase where lesions are formed in the white matter disseminated in time and space and where episodes of disease activity (relapse) alternate with recovery (remission); 3) a secondary progressive (SP) phase where MRI-detectable lesion formation occurs also in

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Received 2 June 2017; accepted 17 July 2017; advance online publication 29 August 2017. doi:10.1002/cpt.801

Box 1. *Glossary of terms*

Forward translation: the transformation of a scientific discovery in an animal disease model into an effective therapy for the patient (lab to clinic).
Reverse translation: when a promising new treatment fails to show efficacy in clinical trials, the reason(s) for failure are investigated by retesting in a relevant animal model (clinic to lab).
Face validity: represents the phenomenological and pathological similarities between animal model and human disease;
Construct validity: represents the degree of similarity with regard to pathophysiological mechanisms and symptoms between animal model and human disease;
Predictive validity: represents the ability of a model to correctly predict the efficacy of a treatment;
External validity: represents the extent to which the observed effect of a treatment in an animal model can be generalized to the total MS patient population.

the cortical gray matter and symptoms worsen progressively without intermittent recovery. In a minority of the patients (±15%) the MS course is progressive from the onset; this disease presentation is indicated as primary progressive (PP) MS.

Others[3] and we[4] have proposed that at the pathological level RR and SP disease do not develop sequentially. The recurrent relapses in RR disease may rather be due to episodic immune activation against antigens released from an underlying degenerative process. In this concept (**Figure 2**) recurrent immune-driven clinical exacerbations in the RR phase are superimposed on the progressively accumulating degeneration of oligodendrocytes and neurons, which affects white as well as gray matter from disease onset, but becomes clinically manifest only late in the disease.[3,5]

The autoimmune concept of MS is supported by genome-wide association (GWAS) studies, which have identified polymorphisms in >100 genes associated with enhanced risk to develop the disease.[6] The vast majority of these genes have a function in the immune system. The strongest influence on MS risk is encoded by the major histocompatibility complex (MHC) class II region, a highly polymorphic genomic region that encodes heterodimeric molecular complexes expressed on professional antigen presenting cells (APC), such as dendritic cells (DC) or B cells, which are involved in antigen presentation to CD4 + (helper) T (Th) cells. Less influential are polymorphisms in genes encoding inflammatory mediators, such as cytokines and chemokines. A second argument for the immunological basis of MS is the strong clinical and pathological similarity with autoimmune animal models of MS, in particular the already mentioned EAE model. The important role of this model in the preclinical research of MS will be discussed in further detail below. A third argument is the beneficial effect of disease-modifying drugs (DMD) that work via the modulation or suppression of immune functions.

Several (immuno)therapies have been approved for the treatment of RRMS, including chemical drugs, such as dimethylfumarate (DMF) and fingolimod (FTY), biological factors, such as interferon-β and glatiramer acetate, and several monoclonal antibodies (mAbs), a class of biological molecules with which pathological processes can be influenced with high precision.[7] However, many agents unexpectedly failed to reproduce the promising effects observed in the EAE model when they were tested in the clinic or they even exerted detrimental effects.[8] Overall, a picture emerges that inflammatory lesion activity in presymptomatic MS responds reasonably well to treatment with relatively mild DMD such as with interferon-β; that stronger DMD with higher risk of adverse effects need to be applied in RRMS, such as the chemical drugs DMF and fingolimod or mAbs such as alemtuzumab and natalizumab; whereas none of the available treatments exert a relevant clinical effect in PMS. The only exception may be ocrelizumab, a B lymphocyte-depleting mAb, which displayed, besides a similar beneficial effect in RRMS as other CD20 + B cell targeting mAbs (rituximab and ofatumumab), an unexpected beneficial effect in PPMS.[9]

These observations strongly indicate that RRMS and PMS are driven by distinct pathological processes and emphasize the change of pathogenic events at the transition from RRMS to SPMS as a priority issue in translational MS research. We have used a special type of EAE model in an NHP, which is reviewed here, to unravel immunopathogenic mechanisms underlying RRMS and SPMS.

EXPERIMENTALLY INDUCED AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

According to an immunological dogma, T cells reactive with tissue components from their own body (called self-antigens) are deleted in the thymic medulla by negative selection through

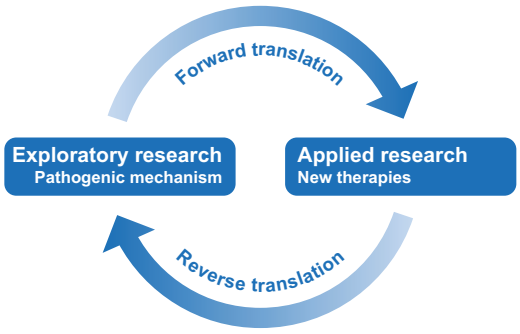

Figure 1 Translational research as an iterative process. A central aim of translational research into pathogenic mechanisms of human disease is to convert discoveries in the laboratory into effective treatments for the patients. This forward translation fails in an unacceptably high number of cases. We posit that much can be learned from a detailed analysis of the reasons why forward translation (lab to clinic) failed. The results from such reverse translational research (clinic to lab) can be used to adjust the animal model in such a way that its predictive validity for therapeutic success of a new treatment developed in the model can be increased.

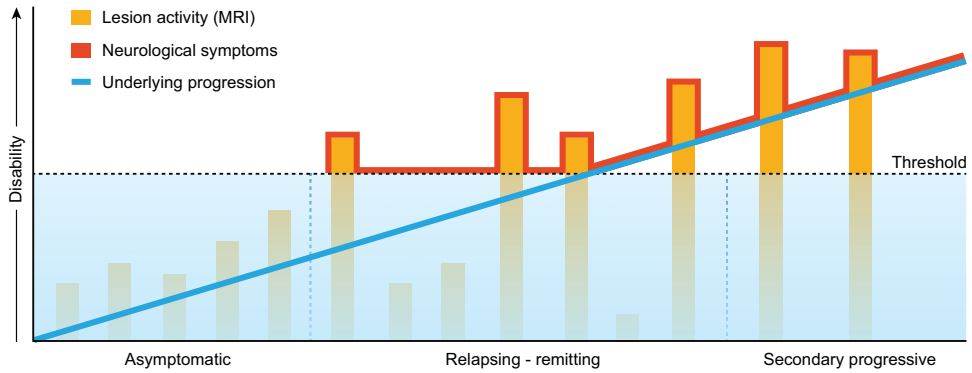

Figure 2 The clinical course of MS. In the vast majority of MS patients three disease phases can be distinguished. In asymptomatic disease abnormalities in the brain can be detected with MRI, but these do not cause neurological symptoms. This is followed by a period of relapsing-remitting disease where episodes of disease activity surpassing a theoretical clinical threshold (relapse) alternate with recovery (remission). After a variable period of time remissions disappear and neurological functions worsen progressively; this phase is indicated as secondary progressive disease. In primary progressive MS, the disease is progressive from the onset.

interaction with APCs on the basis of the affinity of T-cell receptors (TCR) for the antigen.[10] APCs involved in negative selection include thymic epithelial cells, which express tissue-restricted self-antigens under the control of the autoimmune regulator AIRE,[11] as well as thymus-infiltrating B cells and DC, which import antigen molecules sampled in the periphery.[12] However, negative selection is imperfect, as T cells reactive with self-antigens can be found in the normal healthy human immune repertoire; apparently these have somehow escaped thymic selection.[13] This error occurs also in animals and enables the creation of experimental autoimmune disease models in common laboratory species, such as mice, rats, and NHPs, which do not develop the disease spontaneously.

One of probably multiple escape mechanisms is based upon the cleavage of potentially pathogenic epitopes during proteolysis of self-antigens within thymic APC.[14] In line with this finding, we showed that autoreactive and pathogenic T cells present in the immune repertoire of healthy marmoset monkeys are specific for a proteolysis-sensitive epitope of the immunodominant myelin oligodendrocyte glycoprotein (MOG).[15] There is ample evidence in the literature that the activation of such escaped autoreactive T cells is prevented by T regulatory cells, which are either induced in the thymus ($CD4^+CD25^+FOXP3^+$ natural Treg), or in the periphery, as is the case for T regulatory 1 (Tr1) or T helper 3 cells (Th3).[16]

For many years the mouse has been the preferred animal model in human immunology research, as many similarities exist both in the architecture as well as the functioning of the innate and adaptive arms of the immune system.[17] As, by far, the greatest majority of fundamental discoveries in immunology were done in mice, it would be unjust to underestimate the relevance of the mouse for our current understanding of the human immune system. The majority of the currently approved treatments for MS patients with RR disease, such as the anti-VLA4 mAb antibody natalizumab or the synthetic polymer glatiramer acetate, have been developed in the mouse EAE model.[18,19] However, despite the many similarities, there are also essential differences between the immune systems of mice and man, such as complement functions and the ratio between neutrophils and lymphocytes in the blood, to give a few examples.[20] Moreover, recent studies showed that, due to the specific pathogen-free (SPF) breeding conditions, the immune systems of standard laboratory mice are essentially immature and lack effector memory cells.[21,22] As discussed elsewhere, these T cells are important mediators of disease progression in primates.[23]

The most frequently used EAE models in the translational research of MS are those induced in genetically susceptible inbred/SPF mouse strains—such as C57BL/6, SJL/J, or Biozzi ABH.[24] EAE induction is based on the activation of naïve autoreactive T cells present in the mouse immune repertoire via a rather nonphysiological procedure, being the inoculation of a formulation of crude myelin or purified myelin proteins (e.g., MBP, PLP, OSP, MOG) mixed with a strong adjuvant, such as complete Freund's adjuvant (CFA). CFA is an emulsion of heat-inactivated mycobacteria (*M. tuberculosis* or *M. butyricum*) in a mineral oil that contains the emulgator mannide monooleate (called incomplete FA; IFA). The role of the oil is to form a fine water-in-oil emulsion from which the antigen is slowly released, increasing immunogenicity by prolonged exposure.[25] The mycobacteria have a crucial role as they relay essential "danger" signals to APC through conserved pathogen-recognition receptors, such as Toll-like receptors (TLR) and Nod-like receptors (NLR), which boost immune reactions of T and B cells against the antigen. The interaction induces the expression of indispensable activation signals, which withdraw the autoreactive T cells from Treg control and skew their differentiation towards a proinflammatory activity profile.[26]

Even the simplest mouse EAE model presents a multifactorial process that develops as a cascade of pathophysiological reactions, leading to patterns of CNS inflammation and demyelination, which represent various pathological aspects of MS.[24] The activation of only CD4 + T cells induces inflammation, which is expressed clinically as a short self-limiting episode of impaired sensory or motoric functions. For the induction of demyelination, the pathological hallmark of MS and EAE, B cells need to be activated as well. When properly activated, involving besides APC also CD4 + T helper cells, B cells produce antibodies.

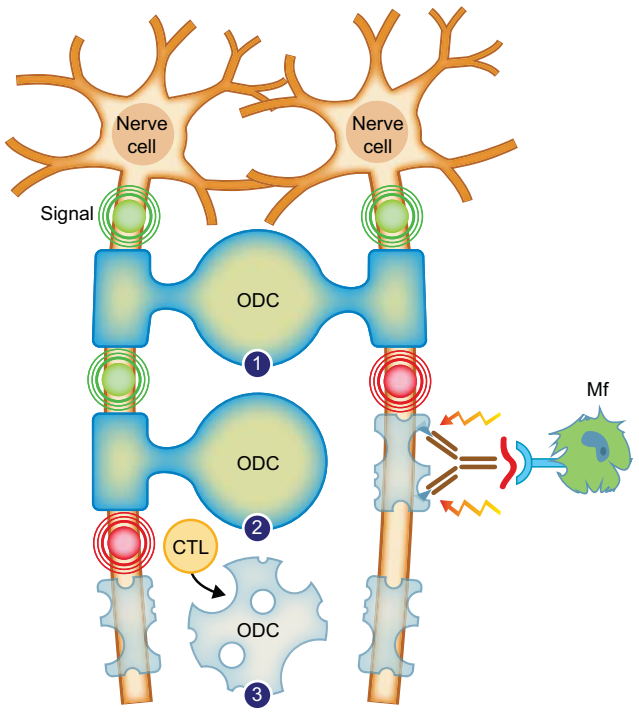

Figure 3 Mechanisms of demyelination in MS. Depicted are two neurons, which send electrical signals along axons to organs on which they project. The axons are wrapped in protective myelin sheaths, which are produced by oligodendrocytes. 1. Healthy myelinated axons; 2. One myelin sheath is attacked by binding of an antibody, which recruits inflammatory cells, such as a macrophage (Mf). The Mf releases myelinotoxic factors that cause demyelination. New myelin formation (remyelination) is possible as oligodendrocytes are spared. 3. The myelin-forming oligodendrocyte is attacked by cytotoxic T lymphocyte. This leads to permanent loss of myelination.

Upon binding to myelin sheaths the antibodies mobilize macrophages and complement factors, which exert destructive attacks on myelin (**Figure 3**). Experiments in Biozzi ABH mice with MOG-deficient myelin point to the surface-expressed glycoprotein MOG as the dominant target of autoantibody binding.[27]

An important aim of preclinical research is to identify the rate-limiting steps in the pathogenic process and to translate this information into therapies for the MS patient. Here a sharp discrepancy has been noticed between EAE and MS, as disease-modifying treatments (DMD) targeting CD4 + T cells appeared much less effective in MS than in EAE.[28] Remarkably, the effects of DMD targeting B cells seem to correspond much better between the animal model and the human disease.[28] The fact that a high proportion of promising therapies is lost in translation warrants the conclusion that the immunopathogenic pathways leading to CNS pathology differ profoundly between EAE and MS.

Intuitively one could imagine several causes of failure, such as:

- fundamental differences between mouse and human biology and immunology, reflecting the wide evolutionary gap between mice and humans[20,29];
- immunological differences between a 12-week-old immunologically naïve laboratory mouse and an adult MS patient;
- different microbial influence on the developing immune system between SPF-bred mice and humans living in a conventional habitat[21];
- the possibility that the artificially induced autoimmune mechanisms in EAE models do not adequately represent MS.[30]

The first three options can be tested by setting up EAE models in adult NHP, which more closely represent the human genetic, immunological, and microbial condition. The fourth option can be tested by comparing the response of NHP-EAE models and MS patients to the same treatments, with therapeutic mAbs, for example.

In this line of research, an advantage is the fact that mAbs raised against human immune cells or molecules often crossreact with NHPs. This means that preclinical research can be performed with the same mAbs as tested at a later stage in patients. The models are a particularly useful test system for reverse translational analysis (=from clinic to laboratory) of the reasons why forward translation (=from laboratory to clinic) failed (**Figure 1**). When forward translation fails, the conclusion is warranted that the targeted process is more relevant in the EAE model than in the MS patient. A critical analysis of the causes of failure provides strategically relevant information that can be used for adjustment of the animal model.

EAE MODELS IN NHP

The outbred nature and close genetic and immunological proximity of NHP to humans makes them valuable models for translational research into the pathogenesis and treatment of AIMID, including MS. Despite ethical limitations and high costs, they are indispensable for bridging the translational gap between mouse models and the patient.[31]

NHP species used for the creation of EAE models include rhesus and cynomolgus macaques (*Macaca mulatta, M. fascicularis)* and the common marmoset (*Callithrix jacchus*). An intriguing discrepancy between these models is that the same immunizing formulation elicits different clinical responses in the three species, which seem to cover the wide spectrum of inflammatory-demyelinating diseases in the human population.[32] As an example, immunization of rhesus monkeys with human myelin oligodendrocyte glycoprotein (MOG; residues 1–125) as nonglycosylated recombinant protein formulated with CFA induced acute onset of a highly destructive pathological process, reminiscent of acute disseminated encephalomyelitis (ADEM). Inoculation of marmosets with the same rhMOG/CFA formulation elicited a chronic disease with MS-like pathology. The reasons for these divergent response types are not completely understood and are the subject of our current research.

For the development and testing of new MS therapies in NHP, the EAE model in marmosets is the first choice. As marmosets do not develop MS-like disease spontaneously, the autoimmune process must be artificially activated. A central question of our research has been to find the minimal stimulus needed for the induction of MS-like disease.

In brief, we achieved stepwise refinement of the original marmoset EAE model that was induced by immunization with myelin isolated from the brain of an MS patient formulated with

the bacterial adjuvant CFA to pepper the immunogenicity of the antigens in myelin.[33] We observed that EAE initiation is not restricted to immunity against a single antigen, whereas induction of chronic disease critically depended on immunity against MOG. Moreover, we found that autoimmunity against recombinant human MOG follows two different pathogenic pathways, which seem to unfold sequentially.[34]

Pathway 1, *the EAE initiation pathway*, essentially replicates the pathogenic mechanism of mouse EAE models. This pathway involves the concerted activity of MHC class II (Caja-DRB*W1201) restricted proinflammatory CD4 + T cells specific for a N-terminal epitope (residues 24–36) with antibodies binding a conformational epitope located at the membrane-distant apical part of the molecule. This implies that CD4 + T cells and CD20 + B cells are both needed for the induction of MS-like lesions in the CNS white matter. Immunization with MOG peptide 14–36/CFA[35] or transfer of a T cell clone specific for human MOG21-40 peptide[36] elicited only mild clinical EAE, with perivascular inflammation as the pathological hallmark. For the induction of demyelination, antibody molecules binding a conformational epitope of MOG are indispensable.[37,38] These gain access to areas of CNS inflammation via permeable sites in the blood–brain barrier.

Pathway 2, *the EAE progression pathway*, has not been found in any mouse EAE model. This unconventional pathway involves MHC class Ib (Caja-E) restricted CD8+CD56 + cytotoxic T cells (CTL) directed against an epitope juxtapositioned to the CD4 + T cell epitope, namely, residues 40–48. We observed that this pathway could be directly activated by inoculating a synthetic peptide of only 23 amino acids length representing residues 34–56 of human MOG (MOG34-56) formulated with IFA.[39] Also in this pathway, B cells have an essential pathogenic role, although not via the production of autoantibodies, but as APCs.[15] Control experiments reported in the same publication show that the MOG34-56/IFA formulation is completely inert in MOG-EAE susceptible but immunologically inexperienced SPF-bred mice (Biozzi ABH, C57BL/6), suggesting that a novel pathogenic mechanism was elicited in the marmosets that is absent in the mice. Of note, autoaggressive T cells specific for MOG peptide 34–56 are present in the mouse immune repertoire, as fulminant EAE can be induced in mice with the same peptide in the bacterial adjuvant CFA.

The epitopes of the T cells that drive the two pathways (residues 24–36 and 40–48) colocalize in a highly conserved region (20–50) located in the extracellular domain of MOG. The region contains at position 32 an asparagine residue to which in healthy human myelin an N-linked fucosylated glycan has been attached, which mediates tolerogenic/antiinflammatory functions of myelin on myeloid APC (microglia, DC).[40]

Another central dogma in immunology implies that immunity against antigens belonging to the host (self) is induced when the confrontation with such antigens occurs in conjunction with molecules alarming danger.[41] Such danger signals are present in pathogens, such as the mycobacteria in CFA, as conserved pathogen-associated molecular patterns (PAMP; e.g., LPS, dsRNA), or are expressed in injured cells in the form of damage-associated molecular patterns (DAMP; e.g., DNA or HMGB1). PAMPS and DAMPS both relay activation signals to APC via conserved pattern recognition receptors (PRR) on APC. Intensively investigated PRR are the already mentioned TLR and NLR.[26]

The danger dogma is challenged by recently developed atypical NHP EAE models, in which the two core pathogenic pathways could be elicited *in vivo* by immunizing monkeys with rhMOG or MOG34-56 in IFA.[39,42] These remarkable models raise the question whether the pathogenic T cells present in the pathogen-educated primate immune repertoire might be antigen-experienced and thus already committed to certain effector functions. This fundamental discrepancy may in part explain the translation failures from the immunologically immature SPF mouse immune system to the mature pathogen-educated human immune system.

VALIDATION OF THE EAE MODELS FOR MS

The relevance of a given animal disease model can be assessed by a number of validity criteria (see **Box 1**). The appreciation of EAE as a relevant preclinical model of MS is in large part based on **face validity**, i.e., similarities in clinical and pathological presentation with the human disease. Obviously, more relevant for usage of the models in therapy development are **construct validity** and **predictive validity**, which can be deduced from a critical comparison of the response to treatment. A systematic review on the **predictive validity** of the (mouse) EAE model showed that for some drugs currently used in the clinic the EAE concept has been a useful basis, but for others it was definitely not.[8]

There seems to be no other rational explanation for the failures in translation than that essential elements of the MS pathogenesis are missing in the mouse EAE model. Whether these are present in the marmoset EAE model has been tested by assessing the clinical effects of treatments that were or were not effective in RRMS clinical trials. The huge advantage of the marmoset model is that often the mAb that will be or has been tested in patients exerts comparable effects in the marmoset immune system, whereas it often fails to bind its target molecule in rodents.

INSIGHTS FROM REVERSE VALIDATION

Example 1

The concept that CD4 + proinflammatory T cells (Th1/Th17) have a central pathogenic role in MS and are thus preferential targets of therapy is in large part based on rodent EAE models. There is ample evidence from mouse EAE models that the differentiation of nonpathogenic Th0 progenitor cells into pathogenic Th1 and Th17 effector cells is directed by the IL-12/IL-23 axis.[43] IL-12 (p35/p40) and IL-23 (p19/p40) are both heterodimeric cytokines produced by macrophages and dendritic cells that signal through a heterodimeric receptor. Binding of IL-12 to its IL-12Rβ1/IL-12Rβ2 dimer receptor promotes antigen-activated Th0 cell differentiation towards a proinflammatory Th1 function. Binding of IL-23 to its heterodimeric IL-12Rβ1/IL-23R dimer receptor promotes antigen-activated Th0 cell differentiation towards a proinflammatory Th17 function. Ustekinumab is a fully human mAb raised against the shared p40 subunit of IL-12 and IL-23. The mAb was tested in the marmoset EAE model

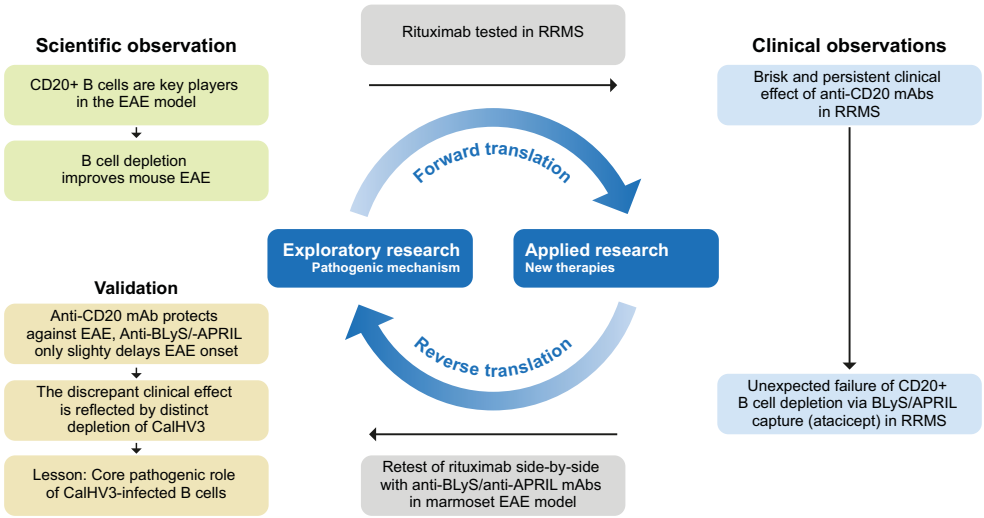

Figure 4 Reverse translation as a learning principle. The important pathogenic contribution of B cells to mouse EAE led to a phase II study of rituximab, an anti-CD20 mAb approved for B cell lymphoma treatment, in RRMS. The remarkable clinical efficacy of this mAb sparked other strategies, such as atacicept, a soluble version of the joint receptor of BlyS and APRIL on B cells, which unexpectedly worsened lesion activity in RRMS. To gain insight into the cause of failure a parallel comparison of anti-CD20 and anti-BlyS/APRIL mAbs was set up in the marmoset EAE model. This reverse translation analysis revealed that with anti-CD20 mAb, but not with anti-BlyS/APRIL mAb, the γ1-herpesvirus of marmosets CalHV3, the marmoset counterpart of EBV, was depleted from the marmoset's immune system.

prior to clinical evaluation in RRMS. Ustekinumab exerted a convincing clinical effect when treatment was started just before EAE induction.[44] Next, we set up a pseudo-clinical trial to test the effect of ustekinumab during ongoing disease. This led to the remarkable observation that the onset of clinical signs was only delayed, although the MRI-detectable activity and enlargement of brain lesions were completely suppressed.[45] When tested in an RRMS clinical trial, ustekinumab was found to be well tolerated but failed to show relevant clinical efficacy.[46] Of note, similar disappointing clinical effects were obtained with an mAb raised against human IL-17A.[47,48] Interestingly, treatment of marmoset EAE with interferon-γ modulated immune functions attributable to Th1 cell function, but exerted no relevant clinical effect.[49]

Conclusion

The marmoset EAE experience shows that antagonizing the IL-12/Th1 and IL-23/Th17 axes may be an effective way to block the mouse EAE-like initiation pathway in marmosets. However, once the disease had been initiated the treatment lost most of its activity. These data yielded the valuable insight that early and late pathogenic processes in the marmoset EAE model involve distinct immunological mechanisms. Of note, ustekinumab is successfully used for the treatment of psoriasis and Crohn's disease.

Example 2 (graphically represented in Figure 4)

The unexpected clinical effects in RRMS of rituximab, an mAb against the B cell marker CD20 that was developed for the treatment of B cell malignancies, has refocused translational research in MS from T cells towards B cells.[50] It was observed that injection of the anti-CD20 mAb exerted a brisk and long-lasting clinical effect in RRMS, although the anticipated suppression of autoantibody production was not observed.[51] Another tested strategy for depletion of B cells clinically tested in RRMS patients was by capturing two cytokines that B cells need for growth and differentiation, B lymphocyte stimulator (BlyS, a.k.a. BAFF) and a proliferation-inducing ligand (APRIL). Atacicept is a chimeric protein composed of a human IgG constant fragment and the dual TACI receptor through which BlyS and APRIL relay activation signals to B cells. Other developed drugs were belimumab, an mAb directed against human BlyS, and an anti-APRIL mAb. Despite the depletion of B cells, atacicept unexpectedly failed to show relevant efficacy in RRMS clinical trials; one trial even had to be stopped because an increase of inflammatory lesion activity was observed.[52,53]

We chose to investigate the mechanisms underlying the discrepant clinical effects of anti-CD20 mAb and atacicept in a marmoset model induced with rhMOG/CFA in which both T cell and B cell pathogenic mechanisms were activated. As an mAb crossreactive with marmosets, we used HuMab7D8, which is a clonal variant of ofatumumab, an antihuman CD20 mAb showing clinical efficacy in RRMS.[54] Instead of atacicept, we used mAbs against human BlyS (belimumab) and APRIL. It was observed that late-stage treatment (21 days after EAE induction) with the anti-CD20 mAb induced brisk and long-lasting depletion of B cells from the circulation as well as lymphoid organs, and exerted robust suppression of EAE symptoms and pathology.[55,56] We found as a likely mode of action of the mAb that the release of activated T cells from secondary lymphoid organs into the circulation was disturbed.[57] In a similar treatment design in the same model the mAbs against BlyS and APRIL only induced a moderate delay of EAE onset.[58] Moreover, the mAbs exerted no clinical effect on T cell release from lymph nodes.[57]

In search of an explanation, we asked whether only a subset of B cells might be pathologically relevant, namely, those capable of cross-presenting autoantigen to the autoaggressive CTL that drive EAE progression. Documented conditions where human B

cells acquire this capacity include stimulation with CpG oligodinucleotides or infection with Epstein Barr virus (EBV). This consideration prompted us to determine the expression of the EBV-related lymphocryptovirus of marmosets, CalHV3, in lymphoid organs. These experiments revealed a sharp reduction of CalHV3 DNA copy numbers in marmosets treated with the anti-CD20 mAb, while CalHV3 DNA copy numbers were even increased in marmosets treated with the mAbs against BlyS and APRIL.[59]

Conclusion

The marmoset EAE experience led to the proposal that the pathogenic role of CD20 + B cells in MS may be exerted by a small subset of B cells that contain EBV, which is the case for 1–50 per million CD20 + cells.[60] This implies that it might not be necessary to deplete all CD20 + B cells at the risk of detrimental side effects, but that depletion of only the fraction that contains the virus might be sufficient.[61] Of note, both atacicept and belimumab show promising clinical effects in systemic lupus erythematosus.[62]

A NEW EAE MODEL

The experiences reviewed above led to the development of a highly refined EAE model in which MS-like disease is elicited by immunization with MOG34-56/IFA. This model is based on the EAE progression pathway in which the crosstalk of CalHV3-infected B cells with effector memory CTL has the core pathogenic role.[63,64] Intriguingly, the model displays pathological features of MS which are usually not seen in mouse EAE models, such as lesion formation in cortical gray matter via a mechanism that includes oxidative injury and iron redistribution.[65]

Recent work shows that in the rendezvous between CalHV3-infected B cells with effector memory CTL, multiple activation signals are exchanged,[66] which are all potential targets of therapy. These include the interaction of PD1 with PD-L1, CD70 with CD27, IL-7 with its receptor, etc. An issue of particular conceptual interest has been the role of EBV infection. Recent work shows that EBV infection of B cells activates the autophagy pathway via which the proteolysis sensitive MOG40-48 epitope of the autoaggressive CTL is protected against degradation by the endolysosomal serine protease cathepsin G and is shuttled into the cross-presentation pathway.[67,68] This mechanism sheds a new light on the association between infection and autoimmunity.[69]

CONCLUDING REMARKS AND TAKE-HOME MESSAGES

GWAS support a core pathogenic role of CD4 + T cells in MS. The reason why, nevertheless, so many, albeit not all, therapies targeting this subset failed to reproduce promising effects observed in the EAE model, when they were tested in the clinic, is a central question in this article. There may be different explanations. First, it is well possible that CD4 + T cells exert their most important pathogenic effects in MS before the disease is diagnosed, while ongoing disease might be sustained by CD8 + T cells, as shown in marmoset EAE. Second, if the inside-out concept for MS is correct, it can be assumed that the immune response to injury takes place in the cervical and lumbar lymph nodes that drain the brain and spinal cord.[70] It is well possible that drugs administered in the periphery may not reach sufficient levels in these draining compartments to exert a therapeutic effect. Third, the T cells that drive disease progression may be antigen-experienced and already functionally committed. It may be very difficult to influence the fate of these T cells via immunomodulatory treatments.

What have we learned from the NHP EAE model? The work reviewed here shows that reverse translation analysis of therapies tested in clinical trials can provide several valuable new insights for therapy development:

1. The reason why the development of an effective treatment of MS often fails may be that these do not target the most relevant pathogenic mechanism for ongoing disease. The concerted autoimmune attack of CD4 + T cells and autoantibodies that is modeled in mouse EAE probably represents an early pathogenic process that may exert its activity before MS is diagnosed.
2. Critical evaluation of the reasons why certain DMD are lost in the translation from the EAE model to the MS patient where others succeed has provided new insights into operational mechanisms during chronic MS-like disease in a primate. This knowledge underlies the development of a new EAE model with high construct validity for MS.
3. One of the key players in the pathogenic process is the CalHV3-infected B cell, which functions as crucial APC presenting the MOG40-48 epitope via Caja-E.[15] The mode of action involves a mechanism that might provide a mechanistic explanation for the elusive association of EBV infection with MS.[71]
4. The other key player is a CD8+CD56 + effector memory CTL which is activated via Caja-E restricted presentation of the MOG40-48 epitope by B cells. Intriguingly, a similar type of T cell is engaged in chronic cytomegalovirus infection[72] and has been found in MS lesions attacking HLA-E expressing oligodendrocytes.[73] These findings led us to propose that the autoaggressive CTL that we identified in the marmoset EAE model might be recruited from a preexisting repertoire of T cells engaged in the control of CMV latency.[74]

ACKNOWLEDGMENTS

The authors thank Mrs. van Hassel for the artwork.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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