University of Groningen

An unexpected symbiosis of animal welfare and clinical relevance in a refined nonhuman primate model of human autoimmune disease

't Hart, Bert A.; Laman, Jon D.; Kap, Yolanda S.

Published in:
Nonhuman Primate Welfare

DOI:
10.1007/978-3-030-82708-3_25

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
't Hart, B. A., Laman, J. D., & Kap, Y. S. (2023). An unexpected symbiosis of animal welfare and clinical relevance in a refined nonhuman primate model of human autoimmune disease. In L. M. Robinson, & A. Weiss (Eds.), *Nonhuman Primate Welfare: From History, Science, and Ethics to Practice* (pp. 605-626). Springer. https://doi.org/10.1007/978-3-030-82708-3_25

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

An Unexpected Symbiosis of Animal Welfare and Clinical Relevance in a Refined Nonhuman Primate Model of Human Autoimmune Disease

Bert A. 't Hart, Jon D. Laman, and Yolanda S. Kap

Abstract

Aging Western populations are confronted with an increasing prevalence of chronic inflammatory and degenerative diseases for which adequate treatments are lacking. One of the major hurdles in therapy development is the poor translation of disease concepts, often developed in rodent disease models, into effective treatments for the patient. Reasons for the high failure rate of promising drug candidates are unforeseen toxicity and lack of efficacy. Essential elements of human disease are apparently lacking in the current preclinically used animal models. Results obtained in a generic nonhuman primate model of human autoimmunity, the marmoset experimental autoimmune encephalomyelitis (EAE) model, are discussed to emphasize the claim that primates are essential complementary models that can help to bridge the wide translational gap between mouse and man.

Keywords

Animal model · Neuroinflammation · Multiple sclerosis · 3R's · Validity

B. A. 't Hart (✉)
Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, The Netherlands

Department of Neuroscience, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Department of Anatomy and Neuroscience, Amsterdam University Medical Center, Amsterdam, The Netherlands

J. D. Laman
Department of Neuroscience, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Y. S. Kap
Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, The Netherlands

© Springer Nature Switzerland AG 2023

L. M. Robinson, A. Weiss (eds.), *Nonhuman Primate Welfare*,
https://doi.org/10.1007/978-3-030-82708-3_25

1 Introduction

Animal models have an important role in the translational research of human disease. Although many aspects of the disease process can be investigated in cell or tissue cultures, most scientists are convinced that research into the complex connections and interactions of these processes requires live animals (Barre-Sinoussi and Montagutelli 2015). Nevertheless, the use of animal models in preclinical research of human disease is the subject of increasing debate. Some opponents in the public debate even claim that animal models are completely irrelevant and therefore are unethical.

When considering the relevance of animal models for translational research into the pathogenesis and treatment of human disease, a classical aphorism by the statistician George Box is worth mentioning: *Essentially all models are wrong, but some are useful* (Box and Draper 1987). In the context of a discussion on the relevance of a certain animal model for human disease, the aphorism can be interpreted as: *the relevance of the model depends to a large extent on its intended use*. In our field of expertise, which is neuroimmunology in general and multiple sclerosis (MS) in particular, a plethora of potentially useful animal models exists, including *Caenorhabditis elegans* worms, *Drosophila* flies, *Brachydanio rerio* fish, mice, rats, and primates. Each of these models has provided important information on pathogenic mechanisms in MS, but none of them faithfully replicates all pathological and clinical aspects of the human disease. It is therefore not surprising that the translation of the accumulated scientific knowledge into safe and effective treatments for the patient has been notoriously difficult. Apparently, essential aspects of the human disease are lacking in each of the available animal models.

The subject of this chapter is the (essential) role of primates in preclinical research. The discussion will be focused on a subgroup of diseases caused by the immune system, namely those in which the own body is attacked causing autoimmune disease. Experimental autoimmune encephalomyelitis (EAE) is one of the most intensively investigated autoimmune animal models and is used both as a specific model of the autoimmune neuroinflammatory disease MS and as a model of human autoimmune disease in general.

We will discuss that although the specific pathogen-free (SPF)-bred laboratory mouse is the gold standard in this research, unique aspects of primate EAE make it an essential complementary model that can help bridge the wide gap between the laboratory mouse and the patient. Specific attention will also be paid to welfare aspects of the primate EAE model, in particular the compliance with the 3R principles (Russell and Burch 1959).

2 Concise Phylogeny of Animal Models Used in Preclinical Immunology Research

The basic role of the human immune system is to protect the organism against infections and cancer (nonself), without causing harm to the organism (self), and to promote repair. This vital task involves a complex interplay of innate and adaptive immune functions, which are activated upon exposure to hostile intruders, while at the same time self is ignored (Nossal 1991). A fundamental modification of this dogma has been the discovery that the adaptive arm of the immune system is only activated when the innate arm recognizes danger (Matzinger 2002).

The nematode worm, *Caenorhabditis elegans* has an ancestral immune system via which it can recognize and combat viral, bacterial, and fungal infections (Ermolaeva and Schumacher 2014). The template of the worm immune system shows similarities with the innate arm of the human immune system. Consequently, *C. elegans* has been used to unravel principles of human innate immunity. The worm is a powerful model as its whole genome has been sequenced and annotated, and loss of function mutants of almost all genes are available. This, added to the neurological (only 320 neurons) and immunological (only innate immunity) simplicity of the worms creates a strong research tool for developing a deep understanding of the neural regulation of innate immunity and the innate immune regulation of neurological functions.

Drosophila is well equipped for the recognition and combating of infection by microorganisms as they have a capable innate immune system. The *Drosophila* system uses a set of germ-line encoded receptors together with effector cells and molecules, which have evolved into the essential factors of the human innate immune system (Hoffmann et al. 1999; Janeway and Medzhitov 2002). However, just like *C. elegans, Drosophila* lacks adaptive immune functions executed by T- and B-lymphocytes (Langenau and Zon 2005) and is therefore incomplete models of human immunity.

Zebra fish have both innate and adaptive immunity, which enable them not only to recognize and combat infections, but also to store information on previous pathogen exposures in memory cells. The latter capacity enables a faster and more effective response upon subsequent exposures to the same microorganisms. The basic templates of the fish and human immune system are remarkably similar (Langenau and Zon 2005).

For many years, the mouse has been the elected animal model of human immunology as many similarities exist both in the architecture as well as the functioning of the innate and adaptive immune systems (Davis 2008). As, by far, the greatest majority of fundamental discoveries in immunology were done in mice, it would be ridiculous to downscale the relevance of the mouse for our current understanding of the human immune system. 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 blood, to give a few examples (Mestas and Hughes 2004). Moreover, recent studies showed that, due to the SPF breeding conditions, the immune systems of standard

laboratory mice are essentially immature and lack effector memory cells (Beura et al. 2016; Abolins et al. 2017).

Nonhuman primates are the closest living relatives of man. This evolutionary proximity is reflected in the high immunological similarity between humans and nonhuman primates, as expressed in the highly polymorphic genes that encode molecules involved in antigen presentation and recognition (Bontrop et al. 1995). Moreover, captive colonies of nonhuman primates in research centers such as the BPRC (Rijswijk, Netherlands) are bred and raised under conventional conditions, where they are exposed to similar and often the same types of pathogens as humans are exposed to (www.bprc.nl). Work from our group shows that the pathogen-educated nonhuman primate immune system harbors potentially auto-aggressive effector memory T cells, which, upon in vivo activation, can turn on pathogenic mechanisms leading to features of MS pathology that are not seen in other animal models ('t Hart et al. 2011; 't Hart 2016) (Fig. 1). The observation that in vivo activation of these autoaggressive effector memory T cells can be achieved with relatively mild adjuvants, such as incomplete Freund's adjuvant (IFA), has formed the basis for a set of atypical EAE models which are not only more animal friendly than the classical models based on strong bacterial adjuvants, but also clinically more relevant ('t Hart et al. 2011).

3 Multiple Sclerosis (MS)

Multiple sclerosis (MS) is an autoimmune neuroinflammatory disease that selectively affects the human central nervous system (CNS). The cause of the disease is not known. Genome-wide association studies and the beneficial effects of therapies targeting immune functions indicate an important role of the immune system in the initiation and/or perpetuation of the disease (Sospedra and Martin 2005; Sawcer et al. 2011). Indeed, once established, chronic disease development is driven by the synergy of autoreactive T and B cells specific for components of the myelin sheaths that wrap around axons (Sospedra and Martin 2005). Also, the trigger of the pathogenic autoimmune reactions is not known, but it could be an interplay of genetic and microbial factors or a dysregulated response to autoantigens released from an idiopathic lesion within the CNS (Stys et al. 2012).

Mouse EAE models have shaped our current understanding of immunopathogenic mechanisms (Steinman 2014). However, despite the vast body of accumulated knowledge, there remain open questions for which we have no satisfactory answer yet. Accumulating evidence indicates that nonhuman primate EAE models can help bridging the gap between mouse EAE and MS.

A poorly understood phenomenon in MS is the heterogeneous clinical course. In the majority of MS patients (±85%), the disease initially follows a relapsing-remitting course, where episodes of neurological dysfunction (relapse) alternate with recovery (remission). In most patients, the relapsing-remitting course of the disease transits after a variable time into a secondary progressive course. During the latter course, recovery no longer occurs and neurological functions worsen

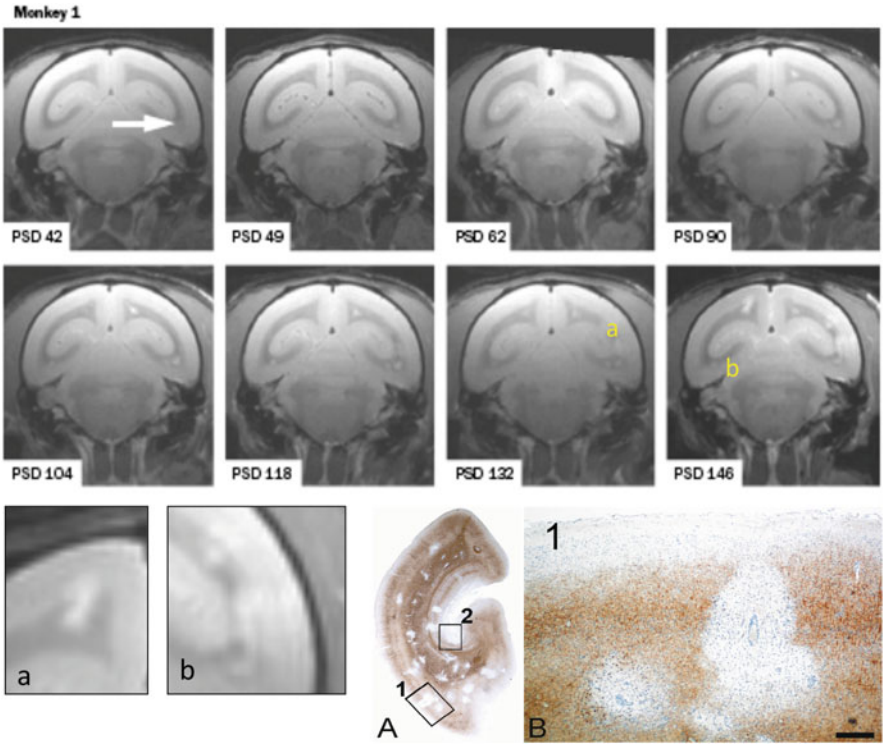

Fig. 1 Pathological characterization of marmoset EAE induced with rhMOG/CFA. Following a single inoculation with recombinant human MOG (residues 1–125) emulsified with complete Freund's adjuvant (CFA), T2-weighted brain MR images were made (psd = postsensitization day). Lesion development, visible as hyperintense spots, is disseminated in time and space and initially confined to the white matter. The white arrow in the image at psd 42 points to the first formed lesion. In late stage disease (psd 132 and 146), lesions seem to colonize also the cortical gray matter. Inserts **a** and **b** show late lesions at higher magnification. The histological pictures (A, B) show PLP staining of an EAE brain from the same model albeit another monkey. Figure composed of parts of figures published in 't Hart et al. (2004a) and Jagessar et al. (2015)

progressively. In a minority of patients (± 15%), the disease is progressive from the onset, and is referred to as primary progressive disease. The factor(s) that underlie the transition of relapsing-remitting to secondary progressive disease are unknown; the cause of primary progressive MS is not known either (Steinman and Zamvil 2016). The available therapies for relapsing-remitting MS do not show a relevant beneficial effect in progressive disease, indicating that relapsing-remitting and secondary progressive MS may be driven by different pathogenic mechanisms.

According to the prevailing concept, MS is an autoimmune disease which is elicited when a genetically predisposed individual encounters an environmental trigger. However, despite decades of intensive research in patients and animal models, an environmental trigger of MS has not been identified. Demographic studies indicate that the thus far elusive trigger may be infection with a virus or

bacterium, which is encountered around the age of 15. The infection more frequently leads to MS in moderate climate areas than around the equator. Moreover, people migrating from a high-risk to a low-risk region before the age of 15 adopt the risk of their new environment, whereas people migrating after age 15 keep the risk of their country of origin.

The genetic risk is dominated by the major histocompatibility complex (MHC) class II genomic region, which is a cluster of highly polymorphic genes encoding molecules expressed on professional antigen-presenting cells (APC), via which antigens are presented to CD4+ T cells. However, the dominant subset of T cells present in established MS lesions is not CD4+ but CD8+, and depletion of CD4+ T cells with anti-CD4 monoclonal antibody (mAb) did not reduce disease activity (van Oosten et al. 1997). Moreover, treatment of RRMS patients with ustekinumab (another mAb) against the shared p40 subunit of interleukin (IL)-12 and -23, two sister cytokines engaged in the skewing of CD4+ T cells toward a proinflammatory profile (Th1 and Th17), was clinically ineffective (Segal et al. 2008). This does not preclude, however, a pathogenic role of CD4+ T cells early in the disease, i.e., before the diagnosis MS has been made. The question which pathogenic roles autoaggressive CD4+ and CD8+ T cells subsets exert is subject of intensive research.

Epstein Barr Virus (EBV) is the most important infectious risk factor for MS. Overall, a conservative estimate indicates that the relative risk of developing MS is 15 for people with evidence of asymptomatic EBV infection at adolescent age and even 30 for those having experienced symptomatic infection, i.e., infectious mononucleosis (Thacker et al. 2006). By contrast, a negative risk factor for developing MS has been linked to a minority of the adult population (<10%) who have not encountered EBV infection (Pakpoor et al. 2013). These are striking ratios for a disease in which the strongest genetic factors (the presence of the HLA-DRB1*1501, –DRB5*0101, -DQB1*0602 alleles) confer a relative risk of 3–4 (Hoppenbrouwers and Hintzen 2011). However, the mechanisms underlying the association between EBV infection and enhanced MS risk are poorly understood. An explanation for the paradox between the high EBV infection prevalence in the healthy population (90%) and the low prevalence of MS (0.1%) eludes us as well.

The poor translation of scientific concepts into effective treatments for MS patients is probably the best illustration that essential elements of MS are lacking in currently used animal models. Accumulating evidence presented in the next paragraph indicates that several of these elements are present in the well-established EAE model in common marmosets (*Callithrix jacchus*). As argued elsewhere, we believe that more investment should be made in a (reverse translational) analysis of the reasons why promising treatments failed in clinical trials ('t Hart et al. 2014). With this information in hand, the translational relevance of the currently used rodent and nonhuman primate EAE models can then be improved.

4 Translational Relevance of the Marmoset EAE Model

Nonhuman primate species used for the modeling of human autoimmune disease includes the larger rhesus and cynomolgus macaques (*Macaca mulatta* and *M. fascicularis*) and the small-bodied common marmoset. In our hands, the EAE models in both macaque species are rather acute, more closely resembling acute postinfectious demyelinating diseases, such as acute disseminated encephalomyelitis, while the model in marmosets more closely resembles chronic MS (Brok et al. 2001; 't Hart et al. 2005a). Marmoset EAE is therefore often the model of choice, while the macaque EAE models are used to test the efficacy of drugs that are inactive in marmosets, or for experiments requiring larger volumes of blood.

Marmosets provide translationally relevant models for a variety of clinical conditions, including (age-associated) autoimmune-mediated inflammatory diseases (AIMID) ('t Hart et al. 2012, 2013). Marmosets are nonprotected, small-bodied nonhuman primates (weighing 300–400 g at adult age), which have as their natural habitat the Amazon rainforest. They breed well in captivity, giving birth to one or two pairs of nonidentical twin pairs or triplets per year. Twin siblings often develop as bone marrow chimeras due to the sharing of the placental bloodstream (Haig 1999). As the immune systems of twin siblings are educated in the same thymic and bone marrow compartments, they are not only allotolerant, but also immunologically highly comparable. This is an important advantage for preclinical therapy studies as these can be set up in twins, where one sibling receives an experimental treatment and the other a relevant control preparation. Despite common marmosets' small body-size, it is nevertheless possible to perform immunological studies by using methods specially designed for working with small blood volumes (Jagessar et al. 2013b).

The outbred, pedigreed, purpose-bred marmoset colony at the BPRC is housed under conventional conditions in partly outdoor enclosures (Bakker et al. 2015). The monkeys are thus exposed to similar environmental factors as humans are exposed to. Marmosets also harbor chronic latent infections with herpesviruses related to the ones that humans are infected with and which have been implicated in the pathogenesis of MS (see below). Thus, just like humans, marmosets have a "*pathogen-educated*" immune system, which contains auto-aggressive effector memory T cells that mediate the high immune reactivity of marmosets against human CNS myelin ('t Hart et al. 2015).

The original EAE model was established by sensitization of marmosets against CNS myelin from an MS patient formulated with a suitable adjuvant (CFA), which elicited a neuroinflammatory disease that approximates MS in clinical and pathological presentation ('t Hart et al. 1998). A noticeable difference between marmoset and mouse EAE models is that in the former demyelinated lesions are present in the white and gray matter of the brain and spinal cord, while in the latter lesions are confined to the white matter of the spinal cord. This aspect of marmoset EAE has enabled an in-depth analysis of the histological correlates of brain lesions detectable with magnetic resonance imaging (MRI), the most frequently used imaging method in MS. We observed that essentially all MS white matter lesion types are also present

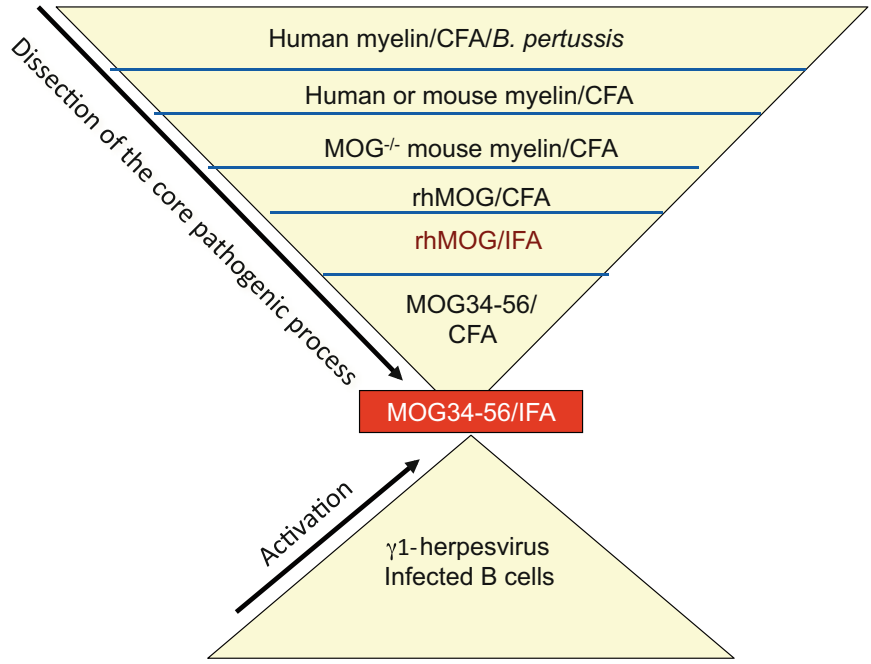

Fig. 2 Dissection of the core pathogenic mechanism and its mode of activation. Step-wise refinement of the marmoset EAE model induced by sensitization against MS myelin in CFA was performed on the guidance of clinical and pathological characteristics. The minimal induction requirement is a peptide of 23 residues emulsified with mineral oil. The activation of this core-pathogenic process appears to involve B cells infected with the EBV-related γ1-herpesvirus CalHV3

in the marmoset EAE model ('t Hart et al. 1998; Blezer et al. 2007). Later research demonstrated that this was also the case for the lesions in the cortical gray matter (Merkler et al. 2006; Kap et al. 2011; Dunham et al. 2017b). Moreover, brain MRI could be used as a clinically relevant read-out in immunotherapy studies in the model ('t Hart et al. 2006).

One focus of our research has been the dissection of the core pathogenic mechanism, as this should be the optimal target of therapy. To achieve this, we used the stepwise refinement procedure depicted in Fig. 2, as reviewed in 't Hart et al. (2009).

As a first step, we showed that autoimmunity against CNS myelin glycoprotein MOG is dispensable for EAE initiation, but essential for the evolution to progressive disease (Jagessar et al. 2008). A similar critical role of MOG was found in the Biozzi mouse EAE model (Smith et al. 2005). MOG has an essential role in the regulation of tolerance and autoimmunity against myelin (Garcia-Vallejo et al. 2014). As a normally glycosylated protein, MOG is tolerogenic as it binds the C-type lectin receptor DC-SIGN, which relays inhibitory signals for DC maturation to dendritic cells. Alteration of the normal glycosylation, for example under the inflammatory

conditions present in MS lesions, makes MOG strongly immunogenic ('t Hart and Weissert 2016).

Next, we showed that sensitization of marmosets against a recombinant protein that encompasses the extracellular domain MOG (residues 1–125) of human MOG activates two nonoverlapping pathogenic mechanisms, which respectively mediate the initiation and the progression phase of the EAE model ('t Hart et al. 2011). The **initiation mechanism** involves T-helper 1 (Th1) cells recognizing a specific fragment (epitope) of the immunizing MOG protein, namely residues 24–36, which is presented via an invariant MHC class II allele, Caja-DRB*W1201. Moreover, antibodies binding a conformational epitope of the MOG molecule are induced. Both factors seem to act synergistically the Th1 cells induce inflammation and the antibodies elicit demyelination. This synergistic mechanism essentially replicates mouse EAE models. The clinical relevance of the initiation pathway for the EAE model was confirmed by the beneficial effect of therapeutic antibodies targeting the formation of proinflammatory Th1/Th17 cells (Brok et al. 2002; 't Hart et al. 2005b) or B cells (Boon et al. 2001; Kap et al. 2010; Kap et al. 2014).

We then found that the EAE **progression mechanism** involves the activation of autoaggressive CD8+CD56+ cytotoxic effector memory T cells specific for the epitope MOG40-48, which is presented by the invariant MHC class Ib allele Caja-E (Kap et al. 2008; Jagessar et al. 2012). This pathway has no known correlate in mouse EAE models. T cells driving the progression pathway require B cells infected with the EBV-related lymphocryptovirus CalHV3 for their activation. This was deduced from the discrepant clinical effects between mAbs against the pan B-cell marker CD20 and the B-cell growth and differentiation factors BlyS and APRIL (Jagessar et al. 2013a). It is noteworthy that this distinction has not been found in SPF mouse EAE models, while a similar paradoxical effect has been observed in MS clinical trials (Barun and Bar-Or 2012; Kappos et al. 2014).

5 Mechanistic Basis of MS Risk Factors: Lessons from the Marmoset EAE Model

5.1 Predisposing Genes

The strongest genetic effect on the risk to develop MS is exerted by the HLA-DR2 locus. Strong candidate risk alleles are HLA-DRB1*1501/HLA-DRB5*0101/HLA-DQB1*0602 (Hoppenbrouwers and Hintzen 2011). Studies in mice expressing HLA-DRB1*1501 transgene show that this MHC class II specificity binds the immunodominant MOG34-56 peptide and activates proinflammatory CD4+ T cells capable of inducing CNS inflammation (Rich et al. 2004). A direct equivalent of this allele has not been found in the MHC of marmosets, which is indicated with the acronym Caja (from *Callithrix jacchus*); hence the finding could not be confirmed. Nevertheless, the marmoset model shows a similar pathogenic role of Caja-DRB*1201 restricted Th1 cells specific for another epitope, MOG24-36, in the initiation of EAE. Blockade of this EAE initiation mechanism with therapeutic

mAbs, such as anti-CD20, anti-CD40 or ustekinumab (anti-IL-12p40), abrogated EAE development (Boon et al. 2001; Brok et al. 2002; Kap et al. 2010). As the results from immunotherapies targeting CD4+ T cells in RRMS have been disappointing thus far, the relevance of this subset in MS has been disputed (Lassmann and Ransohoff 2004). However, the negative results obtained during ongoing MS do not preclude that Th1 cells exert a pathogenic function early in the disease process, possibly even before the disease is diagnosed.

Genome-wide association studies (GWAS) are designed for the identification of genes that are differentially expressed between MS patients and healthy controls. Ubiquitously expressed invariant genes are usually not detected. This may explain why HLA-E, which comprises only two alleles (HLA-E*0101/E^R and HLA-E*0103/E^G), did not emerge as a dominant risk factor in MS. Studies in the marmoset demonstrated that the direct equivalent of HLA-E, called Caja-E, functions as the restriction element of core pathogenic autoaggressive cytotoxic T lymphocytes (CTL) specific for the epitope MOG40-48. Upon in vivo activation, these CTL were found to be capable of inducing essential pathological elements of RRMS and SPMS (Jagessar et al. 2010; Dunham et al. 2017a).

A non-MHC gene associated with enhanced MS risk is the receptor of IL-7 (CD127). A mAb against this receptor was found to exert a beneficial effect on marmoset EAE, but only in monkeys that developed fast-progressing EAE (Dunham et al. 2016).

In summary, the marmoset EAE model revealed that distinct pathogenic mechanisms are involved in the induction of brain pathology and the induction of neurological symptoms. Therapies targeting the former mechanism, which accurately replicates pathogenic mechanisms in rodent EAE models, frequently failed to reproduce promising effects observed in mouse EAE when they were tested in the clinic. Data obtained thus far show that the latter mechanism, which is novel and has no equivalent in rodents, better represents the situation in MS. The new atypical EAE model in which this mechanism has a central pathogenic role ('t Hart et al. 2017) offers new unmet opportunities for therapy development.

5.2 Infections

The family herpesviridae comprises eight members (indicated HHV) that are known to cause disease in humans. A role of three of these HHV in MS pathogenesis is supported by marmoset EAE models.

HHV5/cytomegalovirus (CMV) is a β-herpes virus that causes usually asymptomatic infections in at least 60% of the adult human population; the infection prevalence can be >90% in high-risk groups, such as AIDS patients and offspring of mothers infected during pregnancy. The virus is viewed as a driving factor behind the aging of the immune system, in particular via the induction of oligoclonal expansion of potentially pathogenic T cells ('t Hart et al. 2013). Latent CMV infection is controlled by HLA-E restricted CD8+ CTL, which also expresses markers of natural killer (NK) cells (Moretta et al. 2003). Marmosets are naturally

infected with a simian CMV or can be experimentally infected with human CMV. (Nigida et al. 1975, 1979), whether CMV has a pathogenic role in MS is debated (Vanheusden et al. 2015). Based on the specificity for a mimicry epitope shared between MOG and the UL86 antigen of CMV (Brok et al. 2007), the restriction by Caja-E, and the expression of the NK cell marker CD56 (Jagessar et al. 2012), we tentatively placed the CTL that drives the EAE progression pathway in the repertoire of anti-CMV effector memory T cells. Although a high proportion of mice in nature are normally infected with mouse CMV, SPF laboratory mice are not infected by the virus.

HHV4/Epstein Barr Virus (EBV) is a γ1-herpesvirus that causes usually asymptomatic infections in about 90% of the healthy adult population (Bar-Or et al. 2020). However, only a small minority of B cells actually contain the virus (Khan et al. 1996). The geographical latitude effect on MS has been attributed to the age at which children are infected with EBV. Around the equator, children are infected before the age of two, usually without clear clinical consequences. Exposure to the virus in adolescence can induce infectious mononucleosis, which is characterized by oligoclonal expansion of B cells, strong activation of antiviral T cells, and flu-like symptoms. It has been difficult to prove a causal relation between EBV infection and MS as the difference in infection prevalence between MS patients (100%) and the healthy population (>90%) is small. Nevertheless, seronegativity for EBV has been associated with a low-to-absent risk of developing MS (Pakpoor et al. 2013). On the other hand, a history of infectious mononucleosis (IM) has been reported to increase the risk of developing MS by at least twofold, when compared to individuals infected with EBV earlier in life (Ascherio and Munger 2015). Finally, in a case study in one secondary progressive MS patient, remission could be achieved by the infusion of cytotoxic T cells designed to clear the host of EBV-infected B cells (Pender et al. 2014). This is the first clear indication that EBV-infected B cells may have a core pathogenic role in progressive MS.

Mice infected with the murine gammaherpesvirus-68 are used as a model of human EBV infection for therapy development (Marquez and Horwitz 2015). However, this virus belongs to the group of γ2-herpesviruses, which have no known pathogenic role in MS. The model should therefore be deemed as suboptimal.

Marmosets are naturally infected with the EBV-related lymphocryptovirus callithrichine herpesvirus 3 (CalHV3) (Cho et al. 2001), but marmoset B cells can also be infected ex vivo with an EBV laboratory strain (95-8). Immunotherapy studies support a crucial pathogenic role of CalHV3-infected B cells most likely in the recruitment of the autoaggressive CTL from the anti-CMV repertoire (Jagessar et al. 2013a). The role of EBV/CalHV3 infection seems to be protection of the proteolysis sensitive MOG40–48 epitope against fast degradation by the serine protease cathepsin G in the endolysosomal compartment of B cells so that it can be cross-presented via Caja-E to the autoaggressive CTL. The protection mechanism involves citrullination of essential arginine residues and association of the peptide with autophagosomes ('t Hart et al. 2016).

HHV6A is a neurotropic β-herpesvirus that infects >90% of the human population (Clark 2004). Primary infections in immunocompetent individuals can result in

neurological problems, such as meningitis and meningoencephalitis. The virus infects cells involved in MS, including CD4+ T cells and precursors of oligodendrocytes, the myelin-forming glial cells. The virus has been detected in brain tissue and CSF of MS patients. Marmosets infected with HHV6A develop signs of neuroinflammation and neurological problems (Leibovitch et al. 2013). Humanized SCID mice can also be infected with HHV6A, but obviously provide a highly artificial system (Reynaud and Horvat 2013).

HHV8/Kaposi's sarcoma-associated herpesvirus (KSHV) is a γ2-herpesvirus/rhadinovirus which has no known role in MS. Nevertheless, two animal studies hint at a possible pathogenic role in the disease. The murine herpesvirus 68 (MHV-68) infects mouse B cells, and for this reason, MHV-68 has been proposed as mouse model of EBV infection (Marquez and Horwitz 2015). However, MHV-68 is more closely related to HHV-8/KSHV than to EBV. A publication from the Oregon National Primate Center reported a spontaneous outbreak of MS-like disease in a colony of Japanese macaques, which was found to be associated with a thus far unknown simian rhadinovirus (Axthelm et al. 2011).

In conclusion, marmosets are susceptible to infections with three human herpesviruses, which all have been implicated in the initiation and/or course of MS. Marmosets are therefore a highly useful model for studies on the separate and interactive roles of these viruses in MS. The fact that these marmosets naturally infected with the EBV-related CalHV3, offers unique opportunities for translational research into the still poorly understood relation between EBV and MS.

6 Welfare Aspects

Aging societies are facing an increasing prevalence of chronic invalidating disorders of the central nervous system, such as Alzheimer's and Parkinson's, and MS. Despite substantially increased investments by the pharmaceutical industry, the output of successful new drugs for these disease remains disappointingly low (Kola and Landis 2004; Schafer and Kolkhof 2008). A main reason is the wide gap between animal models used in the pipeline selection of candidate drugs and the human disease.

The lack of valid preclinical animal models added to the increasing costs of animal research has stimulated the development of nonanimal models based on human-derived cells ranging from single cell cultures to complex multicellular systems, such as organs on a chip ('t Hart and Bajramovic 2008; Balls et al. 2019). Although the developments are promising and these models can be useful for the study of isolated pathological processes, we believe that the high complexity of neurological disorders such as MS cannot be adequately modeled without animals.

Important criteria in the selection of a valid animal model are: (i) whether the clinical and pathological presentations adequately replicate the human disease (face validity), (ii) whether disease mechanisms adequately replicate the human disease (construct validity), and (iii) whether pharmacological effects of a new drug are

comparable between the model and the human disease. The data presented above illustrate the validity of the marmoset in the translational research and treatment of MS. Especially for the new generation of highly human-specific biological drugs, monoclonal antibodies for example, replacement by other species is often not an option (Chapman et al. 2007).

However, the two other Rs of the Russell and Burch triplet (1959) require special consideration. Marmosets are an outbred nonendangered species, which adapt well to captive conditions in moderate climate areas. The marmosets that we use for our research come from the purpose-bred and pedigreed colony that has been held for at least 30 years at the BPRC. Large investments have enabled the creation of optimal housing conditions of marmoset families (see www.bprc.nl). For a detailed description of our animal welfare policy in general and more specific information on housing, enrichment, and animal training, we refer readers to the institute's website: http://www.bprc.nl/en/welfare/. Marmosets selected for EAE experiments are moved to the experimental facility where in agreement with international standards, they are pair-housed in spacious indoor cages ($0.75 \times 0.70 \times 1.90$ m^3), enriched with sticks, branches, toys, and boxes that can be used for shelter.

Inevitably, the welfare of marmosets participating in EAE experiments is affected at different levels, including the procedures used for disease induction, stress or physical damage caused by the impairment or loss of neurological functions, and the procedures for collection of body fluids for immune monitoring. A large part of our research has been dedicated at achieving compliance of the marmoset EAE model with the 3R principles (Russell and Burch 1959), while keeping an eye on the clinical relevance of the model. Our work revealed a potential conflict among the 4R's (Relevance, Replacement, Reduction, and Refinement), which cannot easily be solved ('t Hart 2016).

The **Relevance** of an animal model for the preclinical efficacy screening of potential therapies, depends on whether essential clinical and pathological aspects of MS are reproduced in the EAE model (face validity) and whether the pathogenic mechanisms resemble those in the human disease (construct validity). The close similarity of the marmoset EAE model with MS implies that a certain amount of discomfort due to the loss of sensory and motor functions is inevitable. A potentially problematic factor is that marmosets in the experimental facility are pair housed in tall cages. To protect a motorically affected EAE marmoset against falling from a high altitude, separators are placed in the cage. Moreover, padded shelter is provided in the cage where a sick monkey can rest. Another important measure to minimize suffering is that the duration of the different levels of discomfort is maximized in a cumulative fashion (Jagessar et al. 2013b).

Considering the **Replacement** paradigm, it is important to stress that according to European legislation (EU directive 2010/63/EU; European Commission 2010), experiments in live nonhuman primates are only allowed when there is no other way to obtain the same information. Typically, the marmoset EAE model is used for the preclinical efficacy testing of new biological therapeutics, which, due to their high specificity cannot be tested in other animals. Importantly, usage of the model

Table 1 The effect of response variation on group size

Response to EAE	Response to treatment	Group size	Response to treatment	Group size
10/10	100%	4	80%	6
9/10	100%	5	80%	8
8/10	100%	6	80%	11
7/10	100%	8	80%	16
6/10	100%	10	80%	24
5/10	100%	12	80%	40

Shown is a power calculation of group size for a hypothetical experiment in the marmoset EAE model. The depicted example shows that the occurrence of nonresponders to EAE induction has a dramatic effect on group size even when 80% of the monkeys respond to the experimental treatment

for therapy evaluation requires deep understanding of the pathogenic mechanisms, which necessitates exploratory research in the model.

Compliance with the **Reduction** principle is achieved by using power analysis for calculating the minimum size of experimental groups needed for obtaining results that can be tested statistically (Cohen 1992). Moreover, techniques for the collection of more information from fewer animals have been developed, including live imaging, longitudinal immune monitoring, and biomarker analysis in body fluids ('t Hart et al. 2004b; Jagessar et al. 2013b). In addition, tissues collected at necropsy, including lymphoid organs, brain, and spinal cord, are intensively used for further analysis by histological and molecular biological techniques. As marmosets are an outbred species, heterogeneity in the response to EAE induction and to an experimental treatment should always be anticipated. Fortunately, the MHC class II (Caja-DRB*W1201) and class I (Caja-E) susceptibility alleles are invariant, but non-MHC genes, such as those encoding the IL-7 receptor, also appeared to exert a variable influence (Dunham et al. 2016). This variation can be dealt with to some extent by using bone marrow chimeric twins, which, as discussed above, are immunologically more comparable than nonrelated monkeys.

Considering **Refinement**, it is a central dogma in immunology that autoreactive T cells that have escaped negative selection in the thymus, and are therefore present in the healthy immune repertoire, are kept under strict control by potent regulatory cells (Bluestone and Abbas 2003; Peterson et al. 2008). Adjuvants are used for breaking such tolerance mechanisms and for the awakening of autoreactive T cells (Baxter 2007). The frequently used adjuvant CFA, which in rodent EAE models is combined with systemic administration of another adjuvant (*Bordetella pertussis*), is notorious for its serious adverse effects, of which the formation of necrotic granulomas at the injection sites is the most visible, albeit not the only, damage. The observation that the T cells that mediate EAE initiation and progression in marmosets can be activated in vivo by immunization with antigen in the much milder adjuvant IFA (discussed above) implies a major reduction of discomfort. However, these atypical EAE models are sensitive to variation in individual characteristics of the monkeys, such as their genetic background and history of infections. The inevitable consequence for these models is higher variation in the response to immunization and to

the experimental treatment than observed in the more robust CFA-based models. Table 1 illustrates the impact of higher variation on the group size. The given example illustrates that an investment in one R (i.e., Refinement) can create a conflict with other Rs (i.e., Reduction).

A possible way out of this dilemma would be a different view on the design of studies involving precious animals, such as nonhuman primates. It has been argued by Bacchetti et al. that underpowered studies are not by definition irrelevant and can provide innovative data (Bacchetti et al. 2011, 2012). Above a certain sample size, the scientific or clinical value of each extra animal decreases, while the potential discomfort is the same for each added animal. In a recent marmoset EAE experiment comprising seven marmoset twins of which six developed EAE, we observed that only three twins responded to the experimental treatment, which was a novel mAb against the human IL-7 receptor. As the EAE course in these three responder twins clearly evolved faster than in the three nonresponder twins, we concluded that the treatment may have intervened in the process that accelerated EAE development (Dunham et al. 2016).

Of note, it is commonly observed in clinical trials that less than 100% of the participants respond to a tested treatment, which is usually attributed to heterogeneity of the pathogenic process. Even for a highly successful antirheumatic drug, the anti-TNFα mAb infliximab, which has been a trendsetting treatment for autoimmune inflammatory diseases, a response rate of 70 to 80% has been recorded (Maini and Taylor 2000).

7 Perspectives and Concluding Remarks

For many years, research in immunology has been concentrated on the adaptive arm of the immune system, i.e., the mechanism(s) used by T and B lymphocytes to distinguish infectious nonself from a species' self. The SPF-bred mouse has been at the center of all discoveries that shaped our current understanding of the system. Just two decades ago, interest in the role of the innate immune system was sparked by the discovery of evolutionary conserved pattern recognition receptors (PRR), such as Toll- and NOD-like receptors, with which immune cells recognize equally conserved pathogen-associated and cell damage-associated molecular patters (PAMPS and DAMPS) (Janeway and Medzhitov 2002). In addition, lectin-type receptors were identified on antigen-presenting cells that recognize carbohydrate structures, via which, self can be distinguished from nonself or altered self (e.g., on infected cells or cancer cells) ('t Hart and van Kooyk 2004; Geijtenbeek et al. 2004; Rabinovich et al. 2012). Research into the basic principles ofinnate immunity has involved, besides mice, other species, including invertebrates.

The current impressive body of immunological knowledge has enabled the development of treatments with satisfactory efficacy in RRMS. However, the list of failures, where the promising effects of new drugs in animal models could not be reproduced in patients, is much longer than the list of successes. There is growing awareness that the over-reliance of immunologists on a few well-defined SPF-bred

and genetically homogeneous laboratory mouse strains hinders the development of better therapies for autoimmune diseases, cancer, and neurological diseases (Davis 2008).

As the causes of failure are usually not investigated, the predictive quality of the animal models currently used in preclinical research has not really changed. We have proposed elsewhere that lessons should be learned from failed clinical trials and that this knowledge should be used for elucidating why a given animal model has failed to predict efficacy of a promising treatment in the clinic ('t Hart et al. 2014). Unfortunately, this is rarely done.

We have used such a reverse translation approach for therapeutic biologicals that failed unexpectedly in RRMS clinical trials, namely the anti-IL-12p40 antibody ustekinumab (Segal et al. 2008) and atacicept, a chimeric construct combining IgG-Fc with the soluble TACI receptor of the B-cell cytokines BlyS and APRIL (Kappos et al. 2014).

Regarding ustekinumab, we discovered that the mAb is much more effective during EAE onset (Brok et al. 2002) than during established disease, although late stage treatment inhibited the activity and enlargement of lesions ('t Hart et al. 2005b). The explanation for this phenomenon could be that after a variable period of time, the autoimmune attack on the CNS transits from a mouse EAE like pathogenic mechanism, driven by the synergistic action of MHC class II-restricted Th1 cells and autoantibody, to an MS-like pathogenic mechanism, driven by MHC class Ib-restricted CD8+ CD56+ CTL, which seem to be absent in SPF mice (Kap et al. 2008).

Regarding atacicept, we discovered that capture of growth and differentiation factors, such as BlyS and APRIL, did induce depletion of B cells, but not of a small γ1-herpesvirus-infected fraction, which could be achieved with an anti-CD20 mAb that was effective in the clinic (ofatumumab). Indeed, survival of EBV-infected marmoset B lymphoblastoid cell lines in culture was not affected by the depletion of BlyS and APRIL (own unpublished data). This unexpected finding led us to the novel insight that immunotherapies targeting the small fraction of EBV-infected B cells (<0.01%) may not only be effective, but also safe as nearly the entire B-cell compartment is left intact. In subsequent studies we analyzed why the virus-infected B-cell fraction is especially pathogenically relevant in MS ('t Hart et al. 2016). We discovered that EBV infection converts the destructive processing of the core pathogenic MOG34-56 peptide, which is a potential tolerance mechanism ('t Hart et al. 2016), into a productive processing and cross-presentation of the epitope, which is a potential autoimmune mechanism. This novel concept was recognized in an editorial in Science Translational Medicine as "*a new pathway by which infection triggers autoimmunity*" (Moore 2016).

In conclusion, we believe that the nonhuman primate is certainly not the translationally most relevant or the preclinically most useful model of human autoimmune disease. We also recognize that high costs and ethical constraints limit their use. This publication argues, however, that nonhuman primates are essential complementary models where the gap between mouse and man hinders progress in translational research. Moreover, when it comes to the development of

innovative treatments, such as gene therapy (Goossens et al. 1999; Poliani et al. 2001; Bevaart et al. 2015) or stem cell therapy (Pluchino et al. 2009; Thiruvalluvan et al. 2016), nonhuman primate disease models have proven their usefulness.

Acknowledgments We would like to thank Mrs. Francisca van Hasselt (BPRC) for the artwork. The authors do not report conflicts of interest.

References

Abolins S, King EC, Lazarou L, Weldon L, Hughes L, Drescher P et al (2017) The comparative immunology of wild and laboratory mice, *Mus musculus* domesticus. Nat Commun 8:14811. https://doi.org/10.1038/ncomms14811

Ascherio A, Munger KL (2015) EBV and autoimmunity. Curr Top Microbiol Immunol 390 (Pt 1):365–385. https://doi.org/10.1007/978-3-319-22822-8_15

Axthelm MK, Bourdette DN, Marracci GH, Su W, Mullaney ET, Manoharan M et al (2011) Japanese macaque encephalomyelitis: a spontaneous multiple sclerosis-like disease in a nonhuman primate. Ann Neurol 70(3):362–373. https://doi.org/10.1002/ana.22449

Bacchetti P, Deeks SG, McCune JM (2011) Breaking free of sample size dogma to perform innovative translational research. Sci Transl Med 3(87):87ps24. https://doi.org/10.1126/scitranslmed.3001628

Bacchetti P, McCulloch C, Segal MR (2012) Being 'underpowered' does not make a study unethical (comment letter). *Stat Med* 31(29). https://doi.org/10.1002/sim.5451

Bakker J, Ouewerling B, Heidt PJ, Kondova I, Langermans JAM (2015) Advantages and risks of husbandry and housing changes to improve animal wellbeing in a breeding colony of common marmosets (*Callithrix jacchus*). J Am Assoc Lab Anim Sci 54(3):273–279

Balls M, Bailey J, Combes RD (2019) How viable are alternatives to animal testing in determining the toxicities of therapeutic drugs? Expert Opin Drug Metab Toxicol 15(12):985–987. https://doi.org/10.1080/17425255.2019.1694662

Bar-Or A, Pender MP, Khanna R, Steinman L, Hartung HP, Maniar T et al (2020) Epstein-Barr virus in multiple sclerosis: Theory and emerging immunotherapies. Trends Mol Med 26 (3):296–310. https://doi.org/10.1016/j.molmed.2019.11.003

Barre-Sinoussi F, Montagutelli X (2015) Animal models are essential to biological research: issues and perspectives. Future Sci OA 1(4):FSO63. https://doi.org/10.4155/fso.15.63

Barun B, Bar-Or A (2012) Treatment of multiple sclerosis with anti-CD20 antibodies. Clin Immunol 142(1):31–37. https://doi.org/10.1016/j.clim.2011.04.005

Baxter AG (2007) The origin and application of experimental autoimmune encephalomyelitis. Nat Rev Immunol 7(11):904–912. https://doi.org/10.1038/nri2190

Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA et al (2016) Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature 532 (7600):512–516. https://doi.org/10.1038/nature17655

Bevaart L, Aalbers CJ, Vierboom MP, Broekstra N, Kondova I, Breedveld E et al (2015) Safety, biodistribution, and efficacy of an AAV-5 vector encoding human interferon-Beta (ART-I02) delivered via intra-articular injection in rhesus monkeys with collagen-induced arthritis. Hum Gene Ther Clin Dev 26(2):103–112. https://doi.org/10.1089/humc.2015.009

Blezer EL, Bauer J, Brok HP, Nicolay K, 't Hart BA (2007) Quantitative MRI-pathology correlations of brain white matter lesions developing in a non-human primate model of multiple sclerosis. NMR Biomed 20(2):90–103. https://doi.org/10.1002/nbm.1085

Bluestone JA, Abbas AK (2003) Natural versus adaptive regulatory T cells. Nat Rev Immunol 3 (3):253–257. https://doi.org/10.1038/nri1032

Bontrop RE, Otting N, Slierendregt BL, Lanchbury JS (1995) Evolution of major histocompatibility complex polymorphisms and T-cell receptor diversity in primates. Immunol Rev 143:33–62. https://doi.org/10.1111/j.1600-065x.1995.tb00669.x

Boon L, Brok HP, Bauer J, Ortiz-Buijsse A, Schellekens MM, Ramdien-Murli S et al (2001) Prevention of experimental autoimmune encephalomyelitis in the common marmoset (*Callithrix jacchus*) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B-cell responses. J Immunol 167(5):2942–2949. https://doi.org/10.4049/jimmunol.167.5.2942

Box GEP, Draper NR (1987) Empirical model-building and response surfaces. Wiley, Boca Raton, FL

Brok HP, Bauer J, Jonker M, Blezer E, Amor S, Bontrop RE et al (2001) Non-human primate models of multiple sclerosis. Immunol Rev 183:173–185. https://doi.org/10.1034/j.1600-065x.2001.1830114.x

Brok HPM, van Meurs M, Blezer E, Schantz A, Peritt D, Treacy G et al (2002) Prevention of experimental autoimmune encephalomyelitis in common marmosets using an anti-IL-12p40 monoclonal antibody. J Immunol 169(11):6554–6563. https://doi.org/10.4049/jimmunol.169.11.6554

Brok HPM, Boven L, van Meurs M, Kerlero de Rosbo N, Celebi-Paul L, Kap YS et al (2007) The human CMV-UL86 peptide 981-1003 shares a crossreactive T-cell epitope with the encephalitogenic MOG peptide 34-56, but lacks the capacity to induce EAE in rhesus monkeys. J Neuroimmunol 182(1–2):135–152. https://doi.org/10.1016/j.jneuroim.2006.10.010

Chapman K, Pullen N, Graham M, Ragan I (2007) Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nat Rev Drug Discov 6(2):120–126. https://doi.org/10.1038/nrd2242

Cho Y, Ramer J, Rivailler P, Quink C, Garber RL, Beier DR et al (2001) An Epstein-Barr-related herpesvirus from marmoset lymphomas. Proc Natl Acad Sci U S A 98(3):1224–1229. https://doi.org/10.1073/pnas.98.3.1224

Clark D (2004) Human herpesvirus type 6 and multiple sclerosis. Herpes 11(Suppl 2):112A–119A

Cohen J (1992) A power primer. Psychol Bull 112(1):155–159. https://doi.org/10.1037//0033-2909.112.1.155

Davis MM (2008) A prescription for human immunology. Immunity 29(6):835–838. https://doi.org/10.1016/j.immuni.2008.12.003

Dunham J, Lee LF, van Driel N, Laman JD, Ni I, Zhai W et al (2016) Blockade of CD127 exerts a dichotomous clinical effect in marmoset experimental autoimmune encephalomyelitis. J Neuroimmune Pharmacol 11(1):73–83. https://doi.org/10.1007/s11481-015-9629-6

Dunham J, van Driel N, Eggen BJ, Paul C, 't Hart BA, Laman JD et al (2017a) Analysis of the cross-talk of Epstein-Barr virus-infected B cells with T cells in the marmoset. Clin Transl Immunol 6(2):e127. https://doi.org/10.1038/cti.2017.1

Dunham J, Bauer J, Campbell GR, Mahad DJ, van Driel N, van der Pol SMA et al (2017b) Oxidative injury and iron redistribution are pathological hallmarks of marmoset experimental autoimmune encephalomyelitis. J Neuropathol Exp Neurol 76(6):467–478. https://doi.org/10.1093/jnen/nlx034

Ermolaeva MA, Schumacher B (2014) Insights from the worm: the *C. elegans* model for innate immunity. Semin Immunol 26(4):303–309. https://doi.org/10.1016/j.smim.2014.04.005

EU Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Available via https://eur-lex.europa.eu/649 legal-content/EN/TXT/?uricelex%3A32010L0063

Garcia-Vallejo JJ, Ilarregui JM, Kalay H, Chamorro S, Koning N, Unger WW et al (2014) CNS myelin induces regulatory functions of DC-SIGN-expressing, antigen-presenting cells via cognate interaction with MOG. J Exp Med 211(7):1465–1483. https://doi.org/10.1084/jem.20122192

Geijtenbeek TBH, van Vliet SJ, Engering A, 't Hart BA, van Kooyk Y (2004) Self- and nonself-recognition by C-type lectins on dendritic cells. Annu Rev Immunol 22:33–54. https://doi.org/10.1146/annurev.immunol.22.012703.104558

Goossens PH, Schouten GJ, 't Hart BA, Bout A, Brok HP, Kluin PM et al (1999) Feasibility of adenovirus-mediated nonsurgical synovectomy in collagen-induced arthritis-affected rhesus monkeys. Hum Gene Ther 10(7):1139–1149. https://doi.org/10.1089/10430349950018139

Haig D (1999) What is a marmoset? Am J Primatol 49(4):285–296. https://doi.org/10.1002/(SICI)1098-2345(199912)49:4<285::AID-AJP1>3.0.CO;2-X

Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA (1999) Phylogenetic perspectives in innate immunity. Science 284(5418):1313–1318. https://doi.org/10.1126/science.284.5418.1313

Hoppenbrouwers IA, Hintzen RQ (2011) Genetics of multiple sclerosis. Biochim Biophys Acta 1812(2):194–201. https://doi.org/10.1016/j.bbadis.2010.09.017

Jagessar SA, Smith PA, Blezer E, Delarasse C, Pham-Dinh D, Laman JD et al (2008) Autoimmunity against myelin oligodendrocyte glycoprotein is dispensable for the initiation although essential for the progression of chronic encephalomyelitis in common marmosets. J Neuropathol Exp Neurol 67(4):326–340. https://doi.org/10.1097/nen.0b013e31816a6851

Jagessar SA, Kap YS, Heijmans N, van Driel N, van Straalen L, Bajramovic JJ et al (2010) Induction of progressive demyelinating autoimmune encephalomyelitis in common marmoset monkeys using MOG34-56 peptide in incomplete freund adjuvant. J Neuropathol Exp Neurol 69(4):372–385. https://doi.org/10.1097/NEN.0b013e3181d5d053

Jagessar SA, Heijmans N, Blezer EL, Bauer J, Blokhuis JH, Wubben JA et al (2012) Unravelling the T-cell-mediated autoimmune attack on CNS myelin in a new primate EAE model induced with MOG34-56 peptide in incomplete adjuvant. Eur J Immunol 42(1):217–227. https://doi.org/10.1002/eji.201141863

Jagessar SA, Fagrouch Z, Heijmans N, Bauer J, Laman JD, Oh L et al (2013a) The different clinical effects of anti-BLyS, anti-APRIL and anti-CD20 antibodies point at a critical pathogenic role of gamma-herpesvirus infected B cells in the marmoset EAE model. J Neuroimmune Pharmacol 8(3):727–738. https://doi.org/10.1007/s11481-013-9448-6

Jagessar SA, Vierboom M, Blezer EL, Bauer J, 't Hart BA, Kap YS (2013b) Overview of models, methods, and reagents developed for translational autoimmunity research in the common marmoset (*Callithrix jacchus*). Exp Anim 62(3):159–171. https://doi.org/10.1538/expanim.62.159

Jagessar SA et al (2015) Immune profile of an atypical EAE model in marmoset monkeys immunized with recombinant human myelin oligodendrocyte glycoprotein in incomplete Freund's adjuvant. J Neuroinflammation 12:169. https://doi.org/10.1186/s12974-015-0378-5

Janeway CA Jr, Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20:197–216. https://doi.org/10.1146/annurev.immunol.20.083001.084359

Kap YS, Smith P, Jagessar SA, Remarque E, Blezer E, Strijkers GJ et al (2008) Fast progression of recombinant human myelin/oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis in marmosets is associated with the activation of MOG34-56-specific cytotoxic T cells. J Immunol 180(3):1326–1337. https://doi.org/10.4049/jimmunol.180.3.1326

Kap YS, van Driel N, Blezer E, Parren PW, Bleeker WK, Laman JD et al (2010) Late B-cell depletion with a human anti-human CD20 IgG1kappa monoclonal antibody halts the development of experimental autoimmune encephalomyelitis in marmosets. J Immunol 185(7):3990–4003. https://doi.org/10.4049/jimmunol.1001393

Kap YS, Bauer J, van Driel N, Bleeker WK, Parren PW, Kooi EJ et al (2011) B-cell depletion attenuates white and gray matter pathology in marmoset experimental autoimmune encephalomyelitis. J Neuropathol Exp Neurol 70(11):992–1005. https://doi.org/10.1097/NEN.0b013e318234d421

Kap YS, van Driel N, Laman JD, Tak PP, 't Hart BA (2014) CD20+ B-cell depletion alters T-cell homing. J Immunol 192(9):4242–4253. https://doi.org/10.4049/jimmunol.1303125

Kappos L, Hartung H-P, Freedman MS, Boyko A, Radu EW, Mikol DD et al (2014) Atacicept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Neurol 13(4):353–363. https://doi.org/10.1016/S1474-4422(14)70028-6

Khan G, Miyashita EM, Yang B, Babcock GJ, Thorley-Lawson DA (1996) Is EBV persistence in vivo a model for B-cell homeostasis? Immunity 5(2):173–179. https://doi.org/10.1016/s1074-7613(00)80493-8

Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 3(8):711–715. https://doi.org/10.1038/nrd1470

Langenau DM, Zon LI (2005) The zebrafish: a new model of T-cell and thymic development. Nat Rev Immunol 5(4):307–317. https://doi.org/10.1038/nri1590

Lassmann H, Ransohoff RM (2004) The CD4-Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. Trends Immunol 25(3):132–137. https://doi.org/10.1016/j.it.2004.01.007

Leibovitch E, Wohler JE, Cummings Macri SM, Motanic K, Harberts E, Gaitan MI et al (2013) Novel marmoset (*Callithrix jacchus*) model of human Herpesvirus 6A and 6B infections: immunologic, virologic and radiologic characterization. PLoS Pathog 9(1):e1003138. https://doi.org/10.1371/journal.ppat.1003138

Maini RN, Taylor PC (2000) Anti-cytokine therapy for rheumatoid arthritis. Annu Rev Med 51:207–229. https://doi.org/10.1146/annurev.med.51.1.207

Marquez AC, Horwitz MS (2015) The role of latently infected B cells in CNS autoimmunity. Front Immunol 6:544. https://doi.org/10.3389/fimmu.2015.00544

Matzinger P (2002) The danger model: a renewed sense of self. Science 296(5566):301–305. https://doi.org/10.1126/science.1071059

Merkler D, Böscke R, Schmelting B, Czéh B, Fuchs E, Brück W et al (2006) Differential macrophage/microglia activation in neocortical EAE lesions in the marmoset monkey. Brain Pathol 16(2):117–123. https://doi.org/10.1111/j.1750-3639.2006.00004.x

Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172(5):2731–2738. https://doi.org/10.4049/jimmunol.172.5.2731

Moore DJ (2016) Viral infection crosses up antigen presentation to drive autoimmunity. Sci Transl Med 8(349):349ec120. https://doi.org/10.1126/scitranslmed.aah4507

Moretta L, Romagnani C, Pietra G, Moretta A, Mingari MC (2003) NK-CTLs, a novel HLA-E-restricted T-cell subset. Trends Immunol 24(3):136–143. https://doi.org/10.1016/s1471-4906(03)00031-0

Nigida SM Jr, Falk LA, Wolfe LG, Deinhardt F, Lakeman A, Alford CA (1975) Experimental infection of marmosets with a cytomegalovirus of human origin. J Infect Dis 132(5):582–586. https://doi.org/10.1093/infdis/132.5.582

Nigida SM, Falk LA, Wolfe LG, Deinhardt F (1979) Isolation of a cytomegalovirus from salivary glands of white-lipped marmosets (*Saguinus fuscicollis*). Lab Anim Sci 29(1):53–60

Nossal GJ (1991) Molecular and cellular aspects of immunologic tolerance. Eur J Biochem 202(3):729–737. https://doi.org/10.1111/j.1432-1033.1991.tb16427.x

Pakpoor J, Disanto G, Gerber JE, Dobson R, Meier UC, Giovannoni G et al (2013) The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. Mult Scler 19(2):162–166. https://doi.org/10.1177/1352458512449682

Pender MP, Csurhes PA, Smith C, Beagley L, Hooper KD, Raj M et al (2014) Epstein-Barr virus-specific adoptive immunotherapy for progressive multiple sclerosis. Mult Scler 20(11):1541–1544. https://doi.org/10.1177/1352458514521888

Peterson P, Org T, Rebane A (2008) Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. Nat Rev Immunol 8(12):948–957. https://doi.org/10.1038/nri2450

Pluchino S, Gritti A, Blezer E, Amadio S, Brambilla E, Borsellino G et al (2009) Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. Ann Neurol 66(3):343–354. https://doi.org/10.1002/ana.21745

Poliani PL, Brok H, Furlan R, Ruffini F, Bergami A, Desina G et al (2001) Delivery to the central nervous system of a nonreplicative herpes simplex type 1 vector engineered with the interleukin 4 gene protects rhesus monkeys from hyperacute autoimmune encephalomyelitis. Hum Gene Ther 12(8):905–920. https://doi.org/10.1089/104303401750195872

Rabinovich GA, van Kooyk Y, Cobb BA (2012) Glycobiology of immune responses. Ann N Y Acad Sci 1253:1–15. https://doi.org/10.1111/j.1749-6632.2012.06492.x

Reynaud JM, Horvat B (2013) Animal models for human herpesvirus 6 infection. Front Microbiol 4:174. https://doi.org/10.3389/fmicb.2013.00174

Rich C, Link JM, Zamora A, Jacobsen H, Meza-Romero R, Offner H et al (2004) Myelin oligodendrocyte glycoprotein-35-55 peptide induces severe chronic experimental autoimmune encephalomyelitis in HLA-DR2-transgenic mice. Eur J Immunol 34(5):1251–1261. https://doi.org/10.1002/eji.200324354

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London

Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L et al (2011) Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 476(7359):214–219. https://doi.org/10.1038/nature10251

Schafer S, Kolkhof P (2008) Failure is an option: learning from unsuccessful proof-of-concept trials. Drug Discov Today 13(21–22):913–916. https://doi.org/10.1016/j.drudis.2008.03.026

Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH et al (2008) Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol 7(9):796–804. https://doi.org/10.1016/s1474-4422(08)70173-x

Smith PA, Heijmans N, Ouwerling B, Breij EC, Evans N, van Noort JM et al (2005) Native myelin oligodendrocyte glycoprotein promotes severe chronic neurological disease and demyelination in Biozzi ABH mice. Eur J Immunol 35(4):1311–1319. https://doi.org/10.1002/eji.200425842

Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23:683–747. https://doi.org/10.1146/annurev.immunol.23.021704.115707

Steinman L (2014) Immunology of relapse and remission in multiple sclerosis. Annu Rev Immunol 32:257–281. https://doi.org/10.1146/annurev-immunol-032713-120227

Steinman L, Zamvil SS (2016) Beginning of the end of two-stage theory purporting that inflammation then degeneration explains pathogenesis of progressive multiple sclerosis. Curr Opin Neurol 29(3):340–344. https://doi.org/10.1097/WCO.0000000000000317

Stys PK, Zamponi GW, van Minnen J, Geurts JJ (2012) Will the real multiple sclerosis please stand up? Nat Rev Neurosci 13(7):507–514. https://doi.org/10.1038/nrn3275

't Hart BA (2016) Primate autoimmune disease models; lost for translation? Clin Transl Immunol 5(12):e122. https://doi.org/10.1038/cti.2016.82

't Hart BA, Bajramovic J (2008) Non-human primate models of multiple sclerosis. Drug Discov Today Dis Model 5(2):97–104. https://doi.org/10.1016/j.ddmod.2008.06.001

't Hart BA, van Kooyk Y (2004) Yin-Yang regulation of autoimmunity by DCs. Trends Immunol 25(7):353–359. https://doi.org/10.1016/j.it.2004.04.006

't Hart BA, Weissert R (2016) Myelin oligodendrocyte glycoprotein has a dual role in T-cell autoimmunity against central nervous system myelin. MS J Exp Transl Clin 2:1–5. https://doi.org/10.1177/2055217316630999

't Hart BA, Bauer J, Muller HJ, Melchers B, Nicolay K, Brok H et al (1998) Histopathological characterization of magnetic resonance imaging-detectable brain white matter lesions in a primate model of multiple sclerosis: a correlative study in the experimental autoimmune encephalomyelitis model in common marmosets (*Callithrix jacchus*). Am J Pathol 153(2):P649–P663. https://doi.org/10.1016/S0002-9440(10)65606-4

't Hart BA, Vogels JT, Bauer J, Brok HPM, Blezer E (2004a) Non-invasive measurement of brain damage in a primate model of multiple sclerosis. Trends Mol Med 10(2):85–91. https://doi.org/10.1016/j.molmed.2003.12.008

't Hart BA, Laman JD, Bauer J, Blezer ED, van Kooyk Y, Hintzen RQ (2004b) Modelling of multiple sclerosis: lessons learned in a non-human primate. Lancet Neurol 3(10):589–597. https://doi.org/10.1016/s1474-4422(04)00879-8

't Hart BA, Bauer J, Brok HPM, Amor S (2005a) Non-human primate models of experimental autoimmune encephalomyelitis: variations on a theme. J Neuroimmunol 168(1–2):1–12. https://doi.org/10.1016/j.jneuroim.2005.05.017

't Hart BA, Brok HP, Remarque E, Benson J, Treacy G, Amor S et al (2005b) Suppression of ongoing disease in a nonhuman primate model of multiple sclerosis by a human-anti-human IL-12p40 antibody. J Immunol 175(7):4761–4768. https://doi.org/10.4049/jimmunol.175.7.4761

't Hart BA, Smith P, Amor S, Strijkers GJ, Blezer EL (2006) MRI-guided immunotherapy development for multiple sclerosis in a primate. Drug Discov Today 11(1–2):58–66. https://doi.org/10.1016/s1359-6446(05)03673-1

't Hart BA, Hintzen RQ, Laman JD (2009) Multiple sclerosis - a response-to-damage model. Trends Mol Med 15(6):235–244. https://doi.org/10.1016/j.molmed.2009.04.001

't Hart BA, Gran B, Weissert R (2011) EAE: imperfect but useful models of multiple sclerosis. Trends Mol Med 17(3):119–125. https://doi.org/10.1016/j.molmed.2010.11.006

't Hart BA, Abbott DH, Nakamura K, Fuchs E (2012) The marmoset monkey: a multi-purpose preclinical and translational model of human biology and disease. Drug Discov Today 17 (21–22):1160–1165. https://doi.org/10.1016/j.drudis.2012.06.009

't Hart BA, Chalan P, Koopman G, Boots AM (2013) Chronic autoimmune-mediated inflammation: a senescent immune response to injury. Drug Discov Today 18(7–8):372–379. https://doi.org/10.1016/j.drudis.2012.11.010

't Hart BA, Jagessar SA, Kap YS, Haanstra KG, Philippens IHCHM, Serguera C et al (2014) Improvement of preclinical animal models for autoimmune-mediated disorders via reverse translation of failed therapies. Drug Discov Today 19(9):1394–1401. https://doi.org/10.1016/j.drudis.2014.03.023

't Hart BA, van Kooyk Y, Geurts JJ, Gran B (2015) The primate autoimmune encephalomyelitis model; a bridge between mouse and man (review). Ann Clin Transl Neurol 2(5):581–593. https://doi.org/10.1002/acn3.194

't Hart BA, Kap YS, Morandi E, Laman JD, Gran B (2016) EBV infection and multiple sclerosis: lessons from a marmoset model. Trends Mol Med 22(12):1012–1024. https://doi.org/10.1016/j.molmed.2016.10.007

't Hart BA, Dunham J, Faber BW, Laman JD, van Horssen J, Bauer J et al (2017) A B cell-driven autoimmune pathway leading to pathological hallmarks of progressive multiple sclerosis in the marmoset experimental autoimmune encephalomyelitis model. Front Immunol 8:804. https://doi.org/10.3389/fimmu.2017.00804

Thacker EL, Mirzaei F, Ascherio A (2006) Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. Ann Neurol 59(3):499–503. https://doi.org/10.1002/ana.20820

Thiruvalluvan A, Czepiel M, Kap YA, Mantingh-Otter I, Vainchtein I, Kuipers J et al (2016) Survival and functionality of human induced pluripotent stem cell-derived oligodendrocytes in a nonhuman primate model for multiple sclerosis. Stem Cells Transl Med 5(11):1550–1561. https://doi.org/10.5966/sctm.2016-0024

van Oosten BW, Lai M, Hodgkinson S, Barkhof F, Miller DH, Moseley IF et al (1997) Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. Neurology 49(2):351–357. https://doi.org/10.1212/wnl.49.2.351

Vanheusden M, Stinissen P, 't Hart BA, Hellings N (2015) Cytomegalovirus: a culprit or protector in multiple sclerosis? Trends Mol Med 21(1):P16–P23. https://doi.org/10.1016/j.molmed.2014.11.002