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Mechanistic underpinning of an inside–out concept for autoimmunity in multiple sclerosis

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Abstract

The neuroinflammatory disease multiple sclerosis is driven by autoimmune pathology in the central nervous system. However, the trigger of the autoimmune pathogenic process is unknown. MS models in immunologically naïve, specific-pathogen-free bred rodents support an exogenous trigger, such as an infection. The validity of this outside–in pathogenic concept for MS has been frequently challenged by the difficulty to translate pathogenic concepts developed in these models into effective therapies for the MS patient. Studies in well-validated non-human primate multiple sclerosis models where, just like in humans, the autoimmune pathogenic process develops from an experienced immune system trained by prior infections, rather support an endogenous trigger. Data reviewed here corroborate the validity of this inside–out pathogenic concept for multiple sclerosis. They also provide a plausible sequence of events reminiscent of Wilkin's primary lesion theory: (i) that autoimmunity is a physiological response of the immune system against excess antigen turnover in diseased tissue (the primary lesion) and (ii) that individuals developing autoimmune disease are (genetically predisposed) high responders against critical antigens. Data obtained in multiple sclerosis brains reveal the presence in normally appearing white matter of myelinated axons where myelin sheaths have locally dissociated from their enwrapped axon (i.e., blistering). The ensuing disintegration of axon–myelin units potentially causes the excess systemic release of post-translationally modified myelin. Data obtained in a unique primate multiple sclerosis model revealed a core pathogenic role of T cells present in the normal repertoire, which hyper-react to post-translationally modified (citrullinated) myelin–oligodendrocyte glycoprotein and evoke clinical and pathological aspects of multiple sclerosis.

Introduction

During fetal development of vertebrates, the immune system is programmed to distinguish the own body (self) from foreign intruders (non-self), such as infectious microbes or a transplanted organ. During an ingenious selection process in bone marrow and thymus, lymphocytes equipped with receptors recognizing self are deleted, while specificities capable of responding to non-self are positively selected.¹ However, some anti-self specificities escape negative selection, for example when their specific antigen is not recognized (cryptic)² or destroyed by proteases of antigen-presenting cells (APC).³ Such escaped

specificities are part of the normal repertoire and can be isolated from blood and lymphoid organs.⁴ Experiments in rodents and non-human primates revealed that, when appropriately activated, escaped T lymphocytes can evoke pathology and symptoms of multiple sclerosis (MS).⁵ However, despite years of intensive research, it is still unclear how autoimmune pathogenic processes are elicited in human autoimmune disease.

In 1989 Terence Wilkin posited in his primary lesion theory that (quote): “autoimmunity is not itself an entity, but a physiological response to sustained excess antigen turnover in diseased tissues (the primary lesion) and fundamentally no different from the response to a foreign

antigen. Those who develop the clinical disease are viewed as high responders to critical antigens. High responder status is determined by immune response (HLA-linked) genotype, not immune dysregulation.”⁶

Data reviewed here corroborate this insightful theory that challenges current dogmas. This Opinion discusses recently reported histological abnormalities in the normally appearing white matter (NAWM) of MS brains that are substantially less prevalent in cerebral NAWM of healthy controls, or in patients with encephalitis or neurodegenerative disease.⁷ Evidence suggests that these primary lesions release post-translationally modified myelin constituents against which the immune system reacts. Moreover, studies in a non-human primate MS model will be discussed that reveal naturally occurring hyper-reactive T cells against the core-pathogenic myelin constituent myelin oligodendrocyte glycoprotein (MOG), modified by citrullination. Significantly contributing to the immune hyper-reactivity in this model is the infection with herpesviruses implicated as MS risk factors, namely cytomegalovirus (CMV)⁸ and CalHV3, a close relative of Epstein Barr Virus (EBV).⁹

MS, a Prototypical Autoimmune Disease (or Not?)

MS is a chronic neuroinflammatory disease, causing neurological impairments and complex pathology in the human brain and spinal cord. The pathological hallmark of MS is the lesion, a focal area of demyelination with a variable degree of inflammation, remyelination, astrogliosis, and neurodegeneration.¹⁰

Although the cause of MS is unknown, evidence indicates that during ongoing disease the immune system has a crucial pathogenic role.^{10,11} This immune-centered concept of MS pathogenesis is supported by:

- Genome-wide association studies, which identified >200 MS risk alleles that almost all are associated with the immune system.¹²
- The remarkable clinical effects of several drugs developed for modulating or suppressing immune functions, although the efficacy is mainly confined to the relapsing phase.¹³
- Presence inside inflammatory-demyelinated lesions in the white matter of the brain and spinal cord of various activated immune cells (T and B lymphocytes, macrophages) and immune molecules (antibodies, complement).¹³

The MS pathogenic process can be divided into three phases (Fig. 1A).¹¹ In a pre-symptomatic phase of unknown duration, inflammatory lesions can be visualized using magnetic resonance imaging (MRI), but overt neurological deficits are not diagnosed. Formation and activity of

lesions can be suppressed with therapies designed to modulate or suppress immune functions, such as the relatively weak anti-inflammatory cytokine interferon- β .¹⁴ The subsequent relapsing phase is characterized by alternating episodes of evident neurological symptoms (relapse) and recovery (remission). Patients in this intermediate disease phase also benefit from immune-modulatory/immunosuppressive therapies.¹⁵

The progressive phase is characterized by gradual worsening of neurological symptoms and decrement of intermittent remissions, while relapses can sometimes still be observed (i.e., relapsing-progressive MS). The transition from relapsing-remitting MS (RRMS) to (secondary) progressive MS (SPMS) occurs after a period of 5–20 years. In the small group of patients with primary progressive MS (PPMS) ($\pm 15\%$) the relapsing phase seems to be skipped; the disease is progressive from the onset. Intriguingly, immune-modulatory/immunosuppressive therapies are not effective in PPMS or SPMS, with the possible exception of the B cell depleting monoclonal antibody (mAb) ocrelizumab.¹⁶ Of note, the efficacy of ocrelizumab in PPMS required active CNS inflammation, which might be needed to open the blood–brain barrier for providing CNS access to the antibody.

Insights into the complex clinical picture of MS emerged from detailed research into the underlying pathology, revealing that the progressive degeneration of neurons and oligodendrocytes starts already at the disease onset; the autoimmune-driven relapses seem to be superimposed thereupon. The reason why relapses disappear after the transition from relapsing to progressive MS, while neurodegeneration continues unrestrained is not known.

Disappointingly, despite years of intensive research, the events evoking the autoimmune process are still unknown. The conventional view is that autoreactive T and B cells present in the immune repertoire of genetically susceptible hosts are activated by infection with a still unidentified microbe. Mechanisms linking microbial infection to the activation of autoreactive T and B cells include molecular mimicry, that is, the presence of immunologically similar structures in the microbe and host tissue, and bystander activation, that is, T/B cell activation by factors produced in the immune response against a microbe.¹⁷

The putative sequence of events in this outside-in pathogenic process is modeled in specific-pathogen-free (SPF)-bred mouse and rat models of experimental autoimmune encephalitis (EAE).¹⁸ Briefly, the first CNS intruders are peripherally activated CD4⁺ T cells (by the injection of an antigen/adjuvant cocktail). Upon interaction with resident APC presenting locally sampled antigens, the T cells trigger a cascade of pathophysiological

reactions that culminates into an attack by cellular immune factors, such as CD8⁺ T cells and macrophages, as well as humoral immune factors (antibody, complement) on oligodendrocytes and myelinated axons (Fig. 1B).¹⁹

A plethora of viruses and bacteria has been implicated as potential MS trigger, but only a few survived rigorous testing, including two human herpesviruses, HHV-4/Epstein Barr Virus (EBV)²⁰ and HHV-6/roseolavirus²¹ and two human endogenous retroviruses, MS-associated retrovirus (MSRV) and Human Endogenous Retrovirus W EnvC7-1 (ERVWE1).²² Indeed, the incidence of EBV infection in MS patients (100%) exceeds the general population (90%) and MS occurs rarely (if at all) in non-infected individuals.²³ However, the notion that only a few EBV seropositive individuals develop MS (<0.1%) seems to preclude EBV as a specific trigger of MS autoimmunity.

The SPF rodent EAE model has been instrumental for the development of immunomodulatory drugs that are now successfully used for the treatment of relapsing MS (e.g., Natalizumab).²⁴ However, the few successes are contrasted by a long list of treatments where the translation of concepts developed in the SPF rodent EAE model into effective therapies for the human disease failed. This high attrition raised the question of whether the (mouse) EAE model adequately replicates pathogenic events in MS.²⁵ A plausible explanation for the failure in translation may be that auto-aggressive immune cells in MS patients are not in a naïve resting state as in immunologically naïve SPF-bred laboratory mice but rather in an activated and committed state due to their role in the defense against the lifelong microbial pressure as in conventionally bred non-human primates (NHP).

The Outside–In Paradigm is Challenged by a Primate MS Model

The common marmoset, a small-bodied Neotropical primate, is an increasingly popular model in the translational research of human biology and disease.²⁶ The validity of marmoset EAE as a preclinical MS model is determined by several factors:

- The model displays the same three disease phases as depicted for MS as well as MS-like lesion pathology in CNS white and grey matter²⁷ (face validity).
- The evolutionary proximity of marmosets to humans (35 million years) is reflected by high immunological similarity, both at the protein and the genetic level.²⁶ Just like in human MS, autoimmune pathogenic mechanisms develop from a human-alike antigen-experienced immune system that has been trained by the lifelong exposure to environmental and internal microbes^{26,28} (construct validity).

- Marmosets are naturally infected with CalHV3, a γ 1-herpesvirus closely related to EBV,²⁹ the principal environmental MS risk factor.²⁰ In the context of a dietary intervention experiment, we determined the percentage of CalHV3 positive monkeys in the captive-bred colony from which we purchased our experimental animals at \pm 80%.³⁰ Intriguingly, mitigation of the EAE incidence and severity by a dietary modification was associated with a sharp reduction of the CalHV3 load in the immune system. It is tempting to speculate on a causal relation.

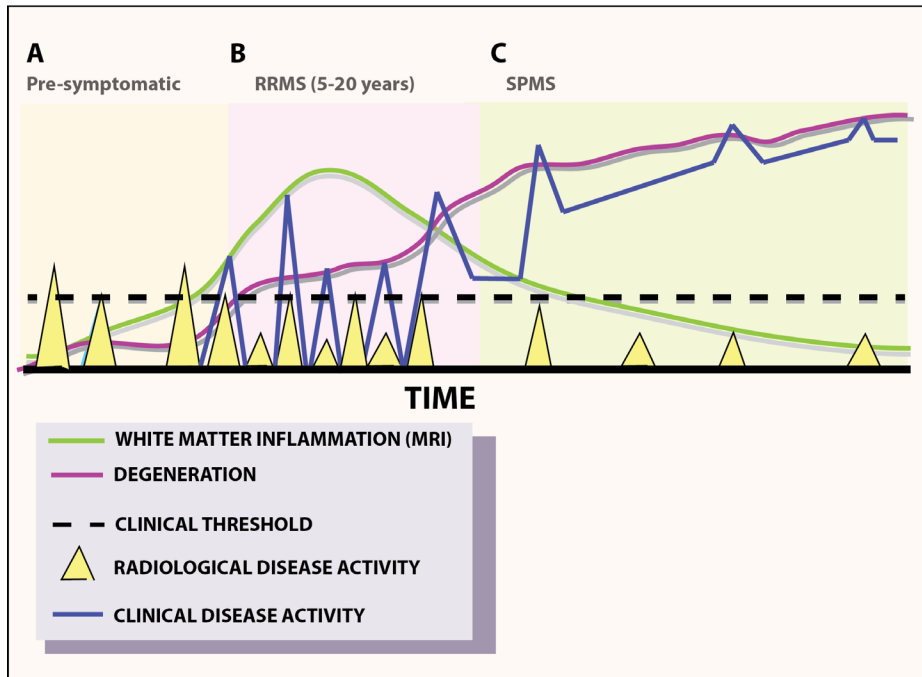
The antigen-experienced immune status of marmosets has significant consequences for the activation requirements of autoreactive T cells in the repertoire, which differ fundamentally from those in SPF mouse EAE models. MS-like pathology in the white and grey matter of the brain and spinal cord could be elicited by the injection of a small (23 amino acids length) synthetic MOG peptide formulated with the mineral oil incomplete Freund's adjuvant. This formulation lacks the normally requisite danger signals and is therefore inactive in SPF-bred laboratory mice.³¹ These observations clearly challenge the SPF mouse-based concept that the trigger of autoimmunity in MS is associated with microbial infection. This warrants the question of whether the alternative concept that the autoimmune pathogenic process is triggered by an internal event (inside–out paradigm) more closely represents the situation in MS.³²

The Primary Lesion: Evidence for Pre-Immune Abnormalities in MS Brain

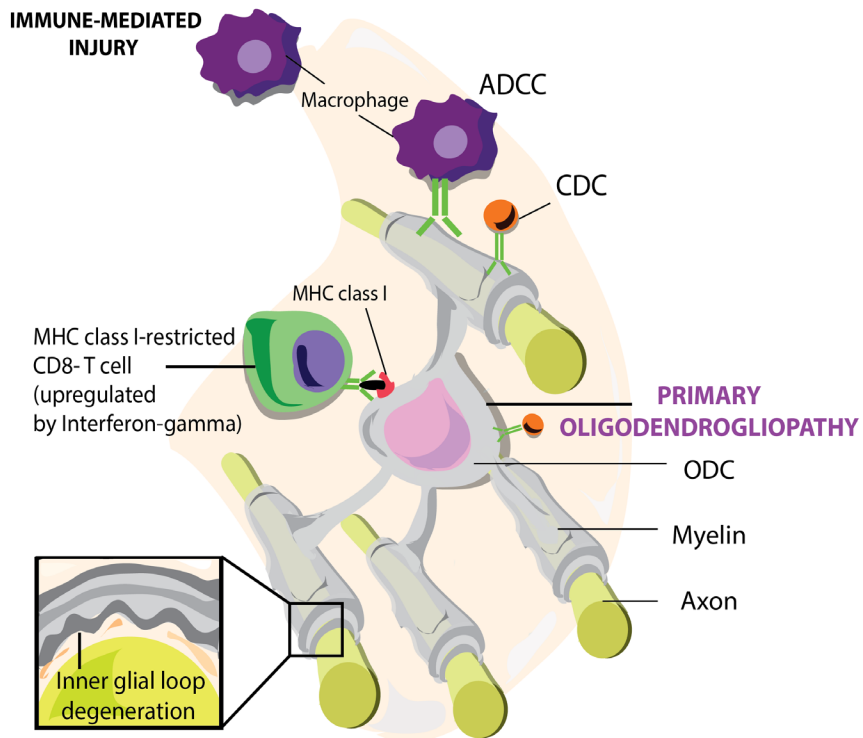
Mounting evidence from microscopy studies shows structural abnormalities in normal-appearing parts of MS brain white matter (NAWM) that are not found in a healthy brain. Recent studies revealed typical swellings of axon–myelin units.⁷ Although these are rather commonly found in the aging brain as well as in neuro-inflammatory (MS, encephalitis) and neurodegenerative (Alzheimer) disorders, we found one swelling type to be prevalent in MS, namely “blisters” formed by focal detachments of myelin sheaths from normal-appearing axons⁷ (Fig. 2). The cause of myelin blistering is unknown and is the subject of our current research. It is tempting to speculate that swelling of axon–myelin units may be causally related to recently documented disturbances in neural development and energy metabolism.³³

Around and within the myelin blisters, strong tissue immunoreactivity with a mAb detecting citrullinated proteins was detected, indicating the post-translational modification of myelin proteins. These changes occur in the

A



B



absence of infiltrated immune cells or HLA-DR expressing microglia, suggesting that myelin blisters are due to an endogenous pathological process preceding the autoimmune attack. These findings seem to corroborate the observation that the disintegration of axon-enwrapping myelin sheaths in MS does not start at the surface of the sheath, as would be expected in case of an autoimmune attack by CNS invading immune factors, but at the inner lamellae, hinting at defective axon–myelin interaction.³⁴

At the molecular level, the blister-like swellings are formed in NAWM displaying besides other morphologically relevant alterations (i.e., axon swelling), altered expression of adhesion and tethering proteins, such as contactins, which mediate stability of axon–myelin units, and of the sialic acid-binding lectin myelin-associated glycoprotein (MAG/Siglec4), which mediates compact binding of inner myelin lamellae to gangliosides on the axon surface.³⁵ Evidence also indicates altered myelin polarity associated with the swellings.^{7,36}

We posit that myelin blisters are an early feature of myelin degeneration from which fragments carrying citrullinated antigens are released. Free myelin fragments were, indeed, found in meninges and draining fluids of the MS brain.³⁷ Intriguingly, leptomeninges in MS brains show pronounced staining for citrulline.⁷ Of note, post-translational modification of self-antigen by citrullination (altered self) is an acceptable explanation for their immunogenicity.³⁸

Another intriguing feature is the presence of small microglia clusters (nodules), centering on a degenerating axon.³⁹ The cause of axon degeneration is still unclear. Studies in a non-human primate EAE model, where such structures have also been found, revealed the presence of IL-1 β in a subset of microglia clusters, indicating the activation of inflammasomes.⁴⁰ This feature enables microglia to sustain inflammatory activity of autoreactive T cells against myelin.⁴¹ Conceptually, the combination of free myelin antigen and activated APC, both present preceding an autoimmune attack, provides fertile

ground for the reactivation of peripherally activated infiltrating T cells.

The (Hyper)Immune Reactivity of Primates to Post-Translationally Modified Myelin

Removal of myelin debris from the CNS occurs via lymphatic draining pathways that end in peripheral lymphoid organs.⁴² To model the ensuing immune response in an accessible human-like experimental system, we immunized marmosets with myelin isolated from an MS donor brain, formulated with a strong bacterial adjuvant (CFA). The marmosets developed a chronic neurological disease (EAE) displaying striking clinical, radiological, and neuropathological similarities with MS.⁴³ Autoimmunity against the quantitatively minor myelin constituent MOG (0.5%–1% of the protein fraction) was found to be pathogenically highly relevant as the immunization of marmosets with MOG-deficient myelin elicited only a mild form of acute EAE.⁴⁴ The observation that marmosets immunized with a chimeric protein of the major myelin proteins myelin basic protein (MBP) and proteolipid protein (PLP) developed clinical EAE only after anti-MOG autoimmunity emerged, provides additional evidence for MOG's crucial role in the autoimmune pathogenic process.⁴⁵

The complex autoimmune mechanisms of the marmoset EAE model were reviewed elsewhere.²⁷ In brief, injection of recombinant human (rh) MOG/CFA evoked the activation of CD4⁺ T cells specific for rhMOG residues 24–36. Specific activation of these T cells with synthetic MOG14-36 peptide elicits mild inflammation in the CNS white matter and weak neurological symptoms. When co-transferred with an antibody binding conformationally intact MOG into a naïve marmoset, CD4⁺ T cells of this specificity evoke large lesions with MS-like inflammation and demyelination. Intriguingly, manifestation of severe neurological symptoms appears closely associated with the activation of another autoreactive T cell type,

Figure 1. Clinical course of MS (A) and the targets of the autoimmune attack (B). (A) In the majority of MS patients (>80%) their disease starts with alternating episodes of neurological disability (relapse) and recovery (remission); this phase is indicated as relapsing-remitting MS (RRMS). However, prior to symptom diagnosis, focal inflammation of white matter can be visualized with MRI; this is, therefore, indicated as pre-symptomatic MS. In about 60% of patients with RRMS, conversion to progressive worsening of symptoms with decreasing remission occurs; this is secondary progressive (SP) MS. Patients with primary progressive MS (\pm 15%) seem to have skipped the RR phase; their disease is progressive from the onset. Intriguingly, MRI-detectable white matter inflammation is substantially lower in PMS than in RRMS. (B) Depicted is one oligodendrocyte that forms myelin sheaths around four different axons. The autoimmune attack on myelin sheaths (outside–in) comprises binding of autoantibody to a surface-exposed antigen (MOG?). This complex is bound by a macrophage, eliciting antibody-dependent cytotoxicity (ADCC), or by complement factors, eliciting complement-dependent cytotoxicity (CDC). Oligodendrocytes can be directly attacked by CD8⁺ T cells, which react to MHC-mediated presentation of myelin antigens. Injury by primary oligodendroglialopathy occurs independent of an immune attack and may lead to myelin sheath disintegration starting at the inner myelin lamellae (panel B has been reproduced from: 't Hart *et al.* eBiomedicine 2021, in press).

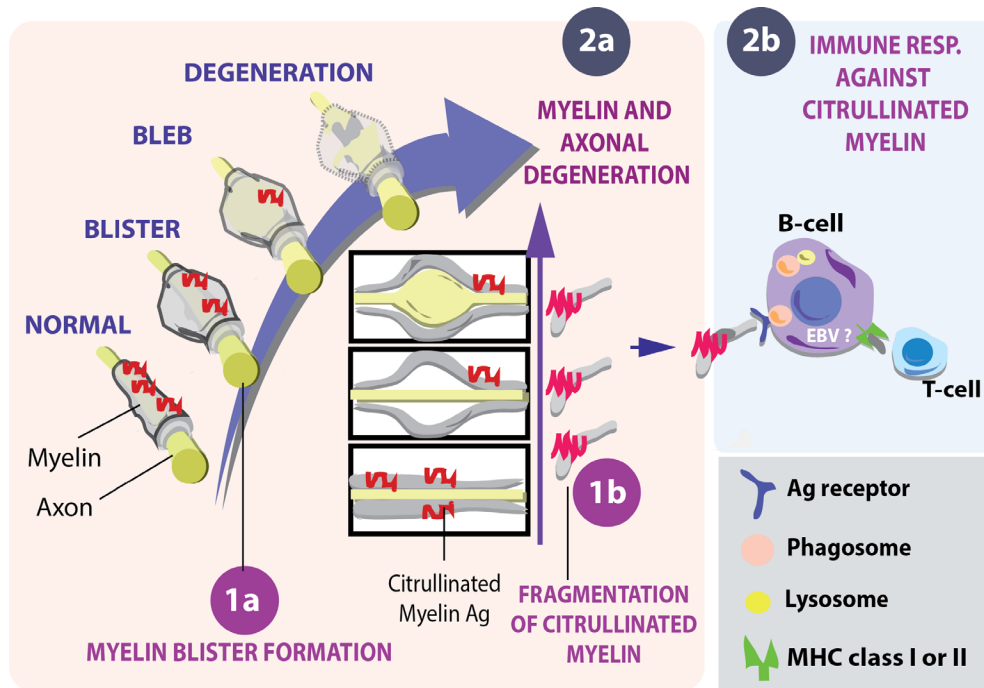


Figure 2. Swelling types of the axon–myelin unit. Depicted in 1a are the three types of swellings of myelinated axons identified in MS normal-appearing white matter, placed in a putative sequence: normal axon–myelin unit => myelin detachment from axon (blister) => swelling of the demyelinated axon segment (bleb) => degeneration of axon and myelin resulting in the release of myelin debris (1b). In areas enriched with such swellings, myelin proteins are post-translationally modified by citrullination, that is, the enzymatic substitution of positively charged arginine for neutrally charged citrulline. 2b. Myelin debris containing citrullinated MOG are taken up by EBV-infected B cells and processed intracellularly in phagolysosomal compartments. Presentation of strongly immunogenic citrullinated MOG results in autoreactive T cell activation.

namely CD8⁺CD56⁺ T cells specific for rhMOG residues 34–56. Conceptually, these models represent autoimmune events at the transition of pre-symptomatic to RRMS.

More important for the current discussion is the observation that immunization with a synthetic peptide representing rhMOG residues 34–56 (MOG34-56) formulated with the mineral oil IFA suffices to induce MS-like CNS pathology and neurological symptoms.³¹ This formulation lacks danger signals and (therefore?) proved completely inert in immunologically naïve EAE susceptible C57Bl/6 and Biozzi ABH mice.³¹ Analysis of the underlying autoimmune process in marmosets revealed the activation of MHC class I/Caja-E restricted CD8⁺CD28⁻CD56⁺ effector memory cytotoxic T cells (EM-CTL) specific for the epitope MOG40-48 by B cells infected with the EBV-related γ 1-herpesvirus CalHV3 as APC.⁴⁶ Intriguingly, the specific epitope of these T cells (MOG40-48) shares 90% molecular mimicry with an immunodominant epitope of the cytomegalovirus major capsid protein (CMV; ORF UL86).⁴⁷ As CMV is both a risk factor in MS⁸ and a crucial driver of immunosenescence,⁴⁸ we postulated that chronic autoimmune inflammation in MS essentially reflects a senescent immune response to CNS injury.⁴⁹ This novel concept is supported by the observation that

leukocyte telomere lengths (LTL; at baseline) are significantly shorter in RRMS patients than in healthy control individuals and that shorter LTLs at baseline are associated with higher conversion rate from RRMS to SPMS.⁵⁰ Intriguingly CMV exacerbates neuroinflammation in MS via a comparable mechanism.⁵¹

We reported that citrullination protects the critical MOG40-48 epitope against instantaneous degradation by the serine protease cathepsin G in EBV-infected marmoset B cells.^{52,53} Cathepsin G of marmosets has chymotryptic activity⁵⁴ and theoretically cleaves MOG34-56 peptide (MEV**G**WY**R**⁴¹**S**P**F**S**R**⁴⁶**V**V**H**L**Y**R⁵²**N**G**K**) at multiple positions (indicated in bold). However, a proportion of peptide fed to EBV B cells escapes degradation and is excreted in the form of spherical structures (Fig. 3). Escape can be achieved via substitution of the arginine residue at position 46 (**R**⁴⁶) for citrulline, which suffices to protect the complete peptide against fast proteolytic degradation by association with autophagosomes,⁵³ where it can be loaded on MHC-E molecules for presentation to the pathogenic CD8⁺ EM-CTL.⁵⁵

EBV-infected B cells themselves have the capacity to citrullinate ingested proteins.⁵⁶ Intriguingly, additional substitution of the **R**⁴¹ or **R**⁵² residues by citrulline

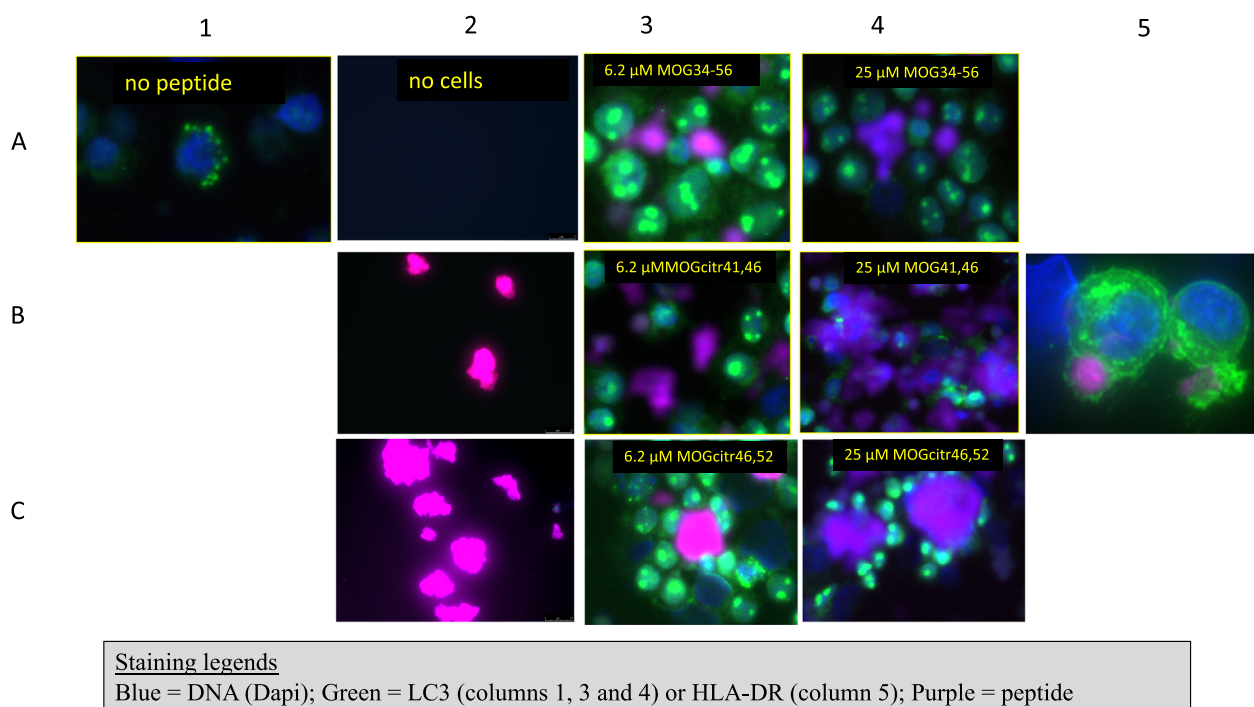


Figure 3. Formation of self-aggregating structures of MOG34-56 and citrullinated derivatives in overnight incubation with or without human EBV-infected B cell (EBV-BC) line. Native MOG34-56 (row A) and the citrullinated versions MOGcitr41,46 (row B) and MOGcitr46,52 (row C) were fed to EBV-BC. The peptides were labeled with purple fluorochrome via click chemistry. The cells were stained with DAPI (blue) to visualize nuclei and with anti-LC3 (autophagosomes) or anti-HLA-DR (B cell membrane antigen) mAb (both green). The figure shows low background LC3 staining of EBV-BCL incubated without peptide (A1). Feeding MOG34-56 increases LC3 staining (A3,4) indicating upregulation of autophagy. The peptide appears in spherical structures (A3,4 purple) of varying size. Citrullinated MOGcitr41,46 peptide incubated without cells spontaneously forms medium-sized aggregates (B2). When incubated with EBV-BC at a low dose similar spherical structures as the native peptide (B3). Containment in an MHC class II+ staining capsule (B5) suggests that the spheres originate from the EBV-BC. Incubates of EBV-BC with high dose MOGcitr41,46 (B4) contain large peptide aggregates and virtually no B cells, indicating that these may have been killed, as confirmed in reference.⁵⁷ MOGcitr46,52 peptide incubated without EBV-BC (C2) or with EBV-BC (C3,4) spontaneously forms much larger aggregates than in B2 and at a much higher rate (see Ref. [57]), but spherical structures were not found.

abolished T cell recognition of the MOG34-56 peptide. Addition of peptides citrullinated at positions 41 and 46 (*MOGcitr41,46*) or at positions 46 and 52 (*MOGcitr46,52*) to co-cultures of marmoset EBV-B cells and T cells did not evoke T cell proliferation, but instead induced the apoptotic degeneration of both cell types.⁵⁷ EBV-B cells were found to secrete the fed peptides as amyloid-type aggregates (Fig. 3). Preliminary data show that these aggregates may be toxic for oligodendrocytes and/or neurons (own unpublished data).

Conceptually, the latter models may represent (auto) immune events at the transition of relapsing to progressive MS.

Discussion

The overlap between the two opposing paradigms of MS autoimmunity (outside–in vs. inside–out) is that both

concepts implicate the elicitation of an autoimmune attack on the CNS by the synergistic action of genetic (HLA) and environmental (e.g., infection) risk factors. A conceptual difference, however, is that in the outside–in paradigm microbes directly trigger the autoimmune pathogenic process, whereas in the inside–out paradigm their role is indirect, namely induction of immune hyper-reactivity against certain antigens released by CNS injury. It could be argued that autoimmunity in MS may result from a combination of outside–in and inside–out pathogenic events. However, the data discussed in this publication tilt the balance toward the notion that the core pathogenic process in MS essentially comprises an inside–out sequence of events. Obviously, this does not preclude that relapses may be triggered by exogenous factors, such as infections or stressful life events, as proposed in Ref. [58, 59].

When the autoimmune process in MS is elicited by an infection, one would expect a short time interval (weeks)

between the triggering event and the onset of disease symptoms. Moreover, when the autoimmune process involves the reactivation of virus-induced effector memory T cells following exposure to antigens released from CNS damage, one can envisage that the time interval between infection and disease onset can be substantially longer. These two opposite conditions are reflected in rodent and primate MS models.

In mouse EAE models, the time interval between exogenous activation of naïve pathogenic precursor T cells and disease onset is usually relatively short (<14 days). The situation in the marmoset EAE model is more complex.²⁷ Formation of brain white matter lesions, involving the concerted action of CD4⁺ T cells and autoantibodies, starts within a few weeks after antigen injection.⁶⁰ However, the onset of neurological symptoms requires secondary activation of pathogenically more relevant autoaggressive EM-CTL, which can occur several months later.⁶¹

Based on demographic studies it has been estimated that encounter with the elusive MS trigger occurs at young adolescent age (around age 15),⁶² whereas the onset of MS symptoms usually occurs between age 25 and 40. The long time interval between MS trigger and disease onset seems to comply better with an inside-out than an outside-in pathogenic concept, although the argument that it may take substantial time to build up a sufficient repertoire of autoaggressive T and B cells cannot be denied.

We are well aware of studies refuting an endogenous trigger of MS autoimmunity. One study argued that autoreactive precursor T cells are part of the peripheral autoimmune repertoire and that T cells acquire the capacity to infiltrate the CNS by activation in peripheral lymphoid organs through a combination of antigen-specific and antigen non-specific (danger) stimulatory signals.⁶³ Observations in mouse and marmoset EAE models revealed co-localization of autoreactive T and B cells with myelin antigens in the cervical and lumbar lymph nodes that drain the brain and spinal cord.⁶⁴ Surgical removal of these CNS draining lymph nodes in a mouse EAE model impaired chronic EAE development,⁶⁵ indicating that T cell activation at these locations has a crucial role in the pathogenic process.

Another study used SPF-bred female C57BL/6 mice expressing a transgenic receptor of diphtheria toxin (DT) in oligodendrocytes.⁶⁶ Oligodendrocyte death induced via intraperitoneal DT injection failed to elicit autoreactive lymphocyte infiltration into the brain or spinal cord. The authors, therefore, concluded that primary oligodendrocyte death is an unlikely trigger of anti-CNS autoimmunity. However, with the marmoset EAE data in mind, one would not expect EAE induction by brain injury to occur in SPF-bred mice lacking pathogen-educated T cells and γ 1-herpesvirus-infected B cells capable of activating them.

Moreover, in these naïve mice healthy autologous myelin nor its dominant antigen MOG are immunogenic without the support of danger signals from adjuvant. Conceptually, by post-translational modification of its autoantigens under the neuroinflammatory conditions in MS, myelin acquires the capacity to evoke an autoimmune process without adjuvant support. Examples of potentially relevant post-translational modifications of MOG are alteration of its N-glycosylation⁴¹ and citrullination.⁷ It has been well documented that the citrullination of CNS myelin is prominent in MS; citrullination of the major myelin protein MBP can be as high as 45% in MS versus 18% in age-matched controls. Citrullination has profound consequences for the structural integrity of compact myelin sheets.⁶⁷

The here presented inside-out MS pathogenic concept has implications for the treatment of MS. A critical feature of the conceptual primary lesion is that myelin antigens are post-translationally modified by citrullination. Of the responsible enzyme peptidyl-arginine deiminase (PAD) 5 isoforms are distinguished, which have different tissue distributions: PAD2 is active in the CNS, while PAD4 is active in immune cells.⁶⁷ Inhibition of PADs as MS therapy will be challenging regarding their role in normal skin physiology and the immune system. A safer approach may be to test which of the drugs currently used in MS treatment can be safely applied to inhibit PAD activity.

It is a challenging perspective to mitigate the hyper-reactive state of the immune system. Based on the central pathogenic role of CalHV3-infected B cells in the marmoset EAE model, we proposed that EBV-infected B cells may be the Achilles heel of the MS pathogenic process and may for that reason be a prime target of immunotherapy.⁶⁸ Recently published data by Pender and coworkers illustrate the remarkable clinical effects that can be achieved in MS by the physical depletion of EBV-infected B cells.⁶⁹

Conclusion

The data discussed here underscore the relevance of Wilkin's primary lesion theory for understanding autoimmunity in MS. Prime candidate for the conceptual "primary lesion" is the injured axon-myelin unit (blisters and blebs) that can be ubiquitously found in the NAWM. Myelin associated with this early CNS injury is post-translationally modified by citrullination. We posit that citrullinated myelin fragments released from the primary lesion elicit a secondary CNS attack by hyper-reactive auto-aggressive EM T cells present in the immune repertoire. The marmoset EAE model corroborates the profound consequences of citrullination for the pathogenicity of the immunodominant myelin antigen MOG. The model shows that the progressive poly-citrullination of MOG may facilitate MS progression from pre-

symptomatic disease to autoimmune-driven relapsing disease to non-autoimmune-driven progressive disease.

In the marmoset EAE model the immunodominant myelin antigen is MOG but in MS other myelin antigens may have the core pathogenic role.

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Author Contribution

Bert A. 't Hart, PhD: Literature search, data analysis, work draft; Figures 1 and 3. Antonio Luchicchi, PhD: Literature search, data analysis, work draft; Figure 2. Peter Stys, M.D., PhD: Critically revised the work. Geert Schenk, PhD: Critically revised the work. Jeroen J. G. Geurts, PhD: Critically revised the work.

Conflict of Interest

No disclosures relevant to the publication are reported.

References

1. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu Rev Immunol* 2003;21:139–176.
2. Sercarz EE, Lehmann PV, Ametani A, et al. Dominance and crypticity of T cell antigenic determinants. *Annu Rev Immunol* 1993;11:729–766.
3. Serre L, Girard M, Ramadan A, et al. Thymic-specific serine protease limits central tolerance and exacerbates experimental autoimmune encephalomyelitis. *J Immunol* 2017;199:3748–3756.
4. Meinel E, Weber F, Drexler K, et al. Myelin basic protein-specific T lymphocyte repertoire in multiple sclerosis. Complexity of the response and dominance of nested epitopes due to recruitment of multiple T cell clones. *J Clin Invest* 1993;92:2633–2643.
5. 't Hart BA, Gran B, Weissert R. EAE: imperfect but useful models of multiple sclerosis. *Trends Mol Med* 2011;17:119–125.
6. Wilkin T. Autoimmunity: attack, or defence? (The case for a primary lesion theory). *Autoimmunity* 1989;3:57–73.
7. Luchicchi A, Hart B, Frigerio I, et al. Axon-myelin unit blistering as early event in MS normal appearing white matter. *Ann Neurol* 2021;89:711–725.
8. Vanheusden M, Stinissen P, 't Hart BA, Hellings N. Cytomegalovirus: a culprit or protector in multiple sclerosis? *Trends Mol Med* 2015;21:16–23.
9. 't Hart BA, Kap YS, Morandi E, et al. EBV infection and multiple sclerosis: lessons from a marmoset model. *Trends Mol Med* 2016;22:1012–1024.
10. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* 2015;15:545–558.
11. Massey JC, Sutton IJ, Ma DDF, Moore JJ. Regenerating immunotolerance in multiple sclerosis with autologous hematopoietic stem cell transplant. *Front Immunol* 2018;9:410.
12. Sawcer S, Franklin RJ, Ban M. Multiple sclerosis genetics. *Lancet Neurol* 2014;13:700–709.
13. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple sclerosis: mechanisms and immunotherapy. *Neuron* 2018;97:742–768.
14. Kieseier BC. The mechanism of action of interferon-beta in relapsing multiple sclerosis. *CNS Drugs* 2011;25:491–502.
15. Winkelmann A, Loebermann M, Reisinger EC, et al. Disease-modifying therapies and infectious risks in multiple sclerosis. *Nat Rev Neurol* 2016;12:217–233.
16. Montalban X, Hauser SL, Kappos L, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N Engl J Med* 2017;376:209–220.
17. Fujinami RS, von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev* 2006;19:80–94.
18. Baxter AG. The origin and application of experimental autoimmune encephalomyelitis. *Nat Rev Immunol* 2007;7:904–912.
19. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med* 2000;28:938–952.
20. Bar-Or A, Pender MP, Khanna R, et al. Epstein-Barr virus in multiple sclerosis: theory and emerging immunotherapies. *Trends Mol Med* 2020;26:296–310.
21. Leibovitch EC, Jacobson S. Evidence linking HHV-6 with multiple sclerosis: an update. *Curr Opin Virol* 2014;9:127–133.
22. Dolei A. The aliens inside us: HERV-W endogenous retroviruses and multiple sclerosis. *Mult Scler* 2018;24:42–47.
23. Pakpoor J, Disanto G, Gerber JE, et al. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. *Mult Scler* 2013;19:162–166.
24. Steinman L. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. *Nat Rev Drug Discov* 2005;4:510–518.
25. Sriram S, Steiner I. Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann Neurol* 2005;58:939–945.
26. 't Hart BA, Abbott DH, Nakamura K, Fuchs E. The marmoset monkey: a multi-purpose preclinical and translational model of human biology and disease. *Drug Discov Today* 2012;17:1160–1165.
27. 't Hart BA, van Kooyk Y, Geurts JJG, Gran B. The primate autoimmune encephalomyelitis model; a bridge

- between mouse and man. *Ann Clin Transl Neurol* 2015;2:581–593.
28. Pulendran B, Davis MM. The science and medicine of human immunology. *Science* 2020;369:eaay4014.
 29. Cho Y-G, Ramer J, Rivaille P, et al. An Epstein-Barr-related herpesvirus from marmoset lymphomas. *Proc Natl Acad Sci USA* 2001;98:1224–1229.
 30. Kap YS, Bus-Spoor C, van Driel N, et al. Targeted diet modification reduces multiple sclerosis-like disease in adult marmoset monkeys from an outbred colony. *J Immunol* 2018;201:3229–3243.
 31. Jagessar SA, Kap YS, Heijmans N, et al. Induction of progressive demyelinating autoimmune encephalomyelitis in common marmoset monkeys using MOG34–56 peptide in incomplete Freund adjuvant. *J Neuropathol Exp Neurol* 2010;69:372–385.
 32. Stys PK, Zamponi GW, van Minnen J, Geurts JJ. Will the real multiple sclerosis please stand up? *Nat Rev Neurosci* 2012;13:507–514.
 33. Mosleth EF, Vedeler CA, Liland KH, et al. Cerebrospinal fluid proteome shows disrupted neuronal development in multiple sclerosis. *Sci Rep* 2021;11:4087.
 34. Rodriguez M, Scheithauer B. Ultrastructure of multiple sclerosis. *Ultrastruct Pathol* 1994;18:3–13.
 35. Schnaar RL, Lopez PH. Myelin-associated glycoprotein and its axonal receptors. *J Neurosci Res* 2009;87:3267–3276.
 36. Teo W, Caprariello AV, Morgan ML, et al. Nile Red fluorescence spectroscopy reports early physicochemical changes in myelin with high sensitivity. *Proc Natl Acad Sci USA* 2021;118:e2016897118.
 37. Kooi E-J, van Horssen J, Witte ME, et al. Abundant extracellular myelin in the meninges of patients with multiple sclerosis. *Neuropathol Appl Neurobiol* 2009;35:283–295.
 38. Rosen A, Casciola-Rosen L. Autoantigens as partners in initiation and propagation of autoimmune rheumatic diseases. *Annu Rev Immunol* 2016;34:395–420.
 39. Singh S, Metz I, Amor S, et al. Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons. *Acta Neuropathol* 2013;125:595–608.
 40. Burm SM, Peferoen LA, Zuiderwijk-Sick EA, et al. Expression of IL-1beta in rhesus EAE and MS lesions is mainly induced in the CNS itself. *J Neuroinflammation* 2016;13:138.
 41. García-Vallejo JJ, Ilarregui JM, Kalay H, et al. CNS myelin induces regulatory functions of DC-SIGN-expressing, antigen-presenting cells via cognate interaction with MOG. *J Exp Med* 2014;211:1465–1483.
 42. Laman JD, Weller RO. Drainage of cells and soluble antigen from the CNS to regional lymph nodes. *J Neuroimmune Pharmacol* 2013;8:840–856.
 43. 't Hart BA, Bauer J, Muller H-J, et al. 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* 1998;153:649–663.
 44. Jagessar SA, Smith PA, Blezer E, et al. 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* 2008;67:326–340.
 45. McFarland HI, Lobito AA, Johnson MM, et al. Determinant spreading associated with demyelination in a nonhuman primate model of multiple sclerosis. *J Immunol* 1999;162:2384–2390.
 46. 't Hart BA, Dunham J, Faber BW, et al. A B cell-driven autoimmune pathway leading to pathological hallmarks of progressive multiple sclerosis in the marmoset experimental autoimmune encephalomyelitis model. *Front Immunol* 2017;8:804.
 47. Brok HPM, Boven L, van Meurs M, et al. 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* 2007;182:135–152.
 48. Koch S, Larbi A, Ozcelik D, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. *Ann N Y Acad Sci* 2007;1114:23–35.
 49. 't Hart BA, Chalan P, Koopman G, Boots AMH. Chronic autoimmune-mediated inflammation: a senescent immune response to injury. *Drug Discov Today* 2013;18:372–379.
 50. Hecker M, Fitzner B, Jager K, et al. Leukocyte telomere length in patients with multiple sclerosis and its association with clinical phenotypes. *Mol Neurobiol* 2021;58:2886–2896.
 51. Vanheusden M, Broux B, Welten SPM, et al. Cytomegalovirus infection exacerbates autoimmune mediated neuroinflammation. *Sci Rep* 2017;7:663.
 52. Jagessar SA, Holtman IR, Hofman S, et al. Lymphocryptovirus infection of nonhuman primate B cells converts destructive into productive processing of the pathogenic CD8 T cell epitope in myelin oligodendrocyte glycoprotein. *J Immunol* 2016;197:1074–1088.
 53. Morandi E, Jagessar SA, 't Hart BA, Gran B. EBV infection empowers human B cells for autoimmunity: role of autophagy and relevance to multiple sclerosis. *J Immunol* 2017;199:435–448.
 54. Raymond WW, Trivedi NN, Makarova A, et al. How immune peptidases change specificity: cathepsin G gained tryptic function but lost efficiency during primate evolution. *J Immunol* 2010;185:5360–5368.
 55. Camilli G, Cassotta A, Battella S, et al. Regulation and trafficking of the HLA-E molecules during monocyte-macrophage differentiation. *J Leukoc Biol* 2016;99:121–130.
 56. Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J Exp Med* 2011;208:2625–2632.

57. Araman C, van Gent ME, Meeuwenoord NJ, et al. Amyloid-like behavior of site-specifically citrullinated myelin oligodendrocyte protein (MOG) peptide fragments inside EBV-infected B-cells influences their cytotoxicity and autoimmunogenicity. *Biochemistry* 2019;58:763–775.
58. Buljevac D, Flach HZ, Hop WCJ, et al. Prospective study on the relationship between infections and multiple sclerosis exacerbations. *Brain* 2002;125(Pt 5):952–960.
59. Buljevac D, Hop WC, Reedeker W, et al. Self reported stressful life events and exacerbations in multiple sclerosis: prospective study. *BMJ* 2003;327:646.
60. Brok HPM, Uccelli A, Kerlero de Rosbo N, et al. Myelin/oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis in common marmosets: the encephalitogenic T cell epitope pMOG24-36 is presented by a monomorphic MHC class II molecule. *J Immunol* 2000;165:1093–1101.
61. Kap YS, Smith P, Jagessar SA, et al. 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* 2008;180:1326–1337.
62. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* 1993;6:382–427.
63. Wekerle H, Lassmann H. Contra: evidence against a primary lesion in the target organ in autoimmune disease. *Int Arch Allergy Immunol* 1994;103:328–331.
64. de Vos AF, van Meurs M, Brok HP, et al. Transfer of central nervous system autoantigens and presentation in secondary lymphoid organs. *J Immunol* 2002;169:5415–5423.
65. van Zwam M, Huizinga R, Heijmans N, et al. Surgical excision of CNS-draining lymph nodes reduces relapse severity in chronic-relapsing experimental autoimmune encephalomyelitis. *J Pathol* 2009;217:543–551.
66. Locatelli G, Wörtge S, Buch T, et al. Primary oligodendrocyte death does not elicit anti-CNS immunity. *Nat Neurosci* 2012;15:543–550.
67. Chirivi RGS, van Rosmalen JWG, Jenniskens GJ, et al. Citrullination: a target for disease intervention in multiple sclerosis and other inflammatory diseases? *J Clin Cell Immunol* 2013;4:146–153.
68. 't Hart BA, Jagessar SA, Haanstra K, et al. The primate EAE model points at EBV-infected B cells as a preferential therapy target in multiple sclerosis. *Front Immunol* 2013;4:145.
69. Pender MP, Csurhes PA, Smith C, et al. Epstein-Barr virus-specific T cell therapy for progressive multiple sclerosis. *JCI Insight* 2018;3:e124714.