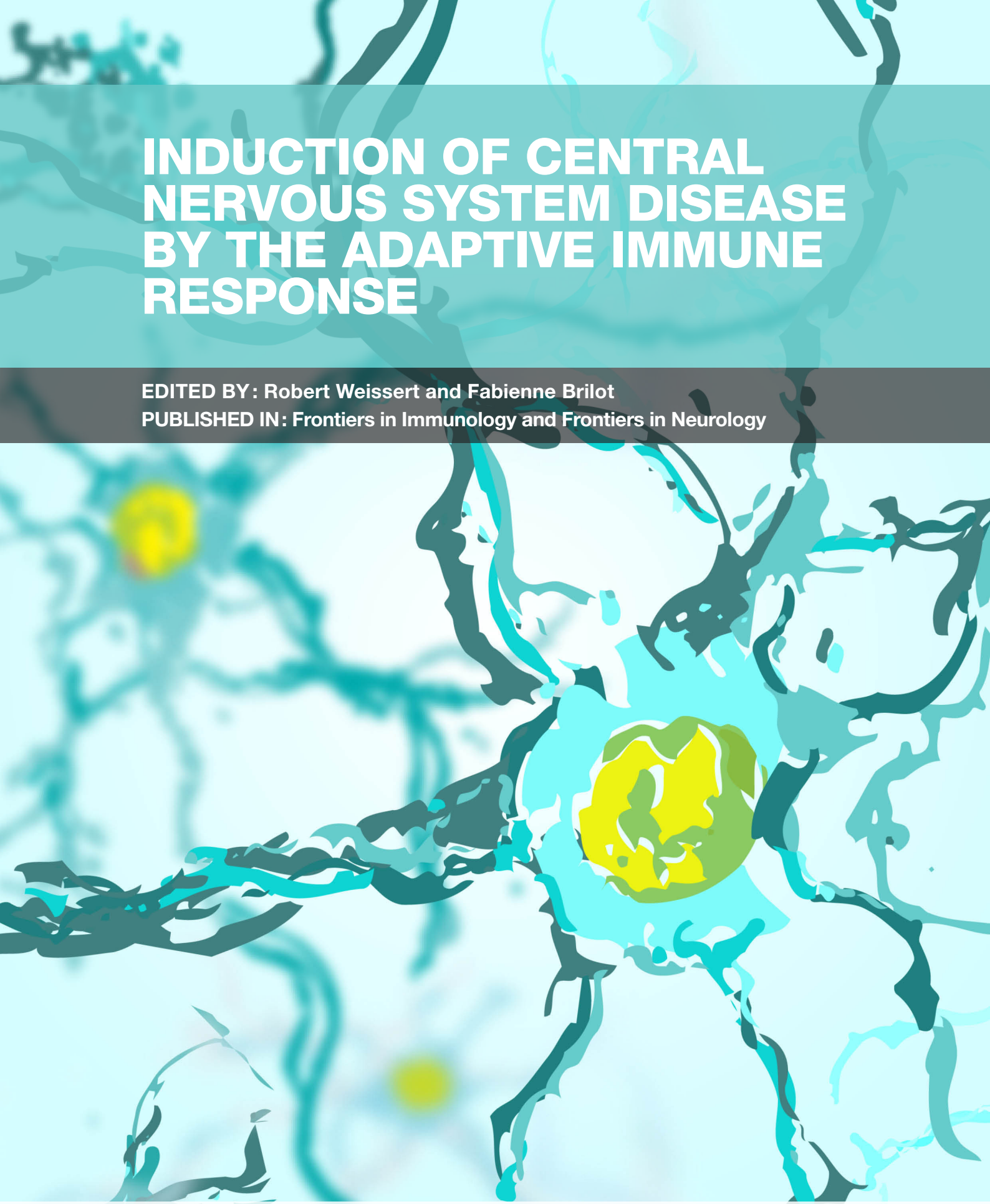
# INDUCTION OF CENTRAL NERVOUS SYSTEM DISEASE BY THE ADAPTIVE IMMUNE RESPONSE

EDITED BY: Robert Weissert and Fabienne Brilot
PUBLISHED IN: Frontiers in Immunology and Frontiers in Neurology



frontiers Research Topics

**ISSN 1664-8714**
**ISBN 978-2-88945-347-4**
**DOI 10.3389/978-2-88945-347-4**

## About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to quality

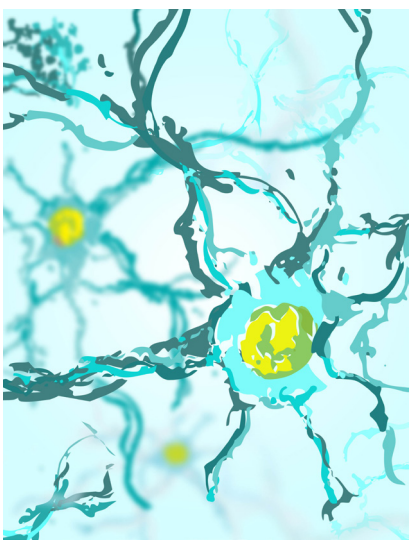
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

# INDUCTION OF CENTRAL NERVOUS SYSTEM DISEASE BY THE ADAPTIVE IMMUNE RESPONSE

Topic Editors:
**Robert Weissert,** University of Regensburg, Germany
**Fabienne Brilot,** The Children's Hospital at Westmead, University of Sydney, Australia



Artistic rendering of primary murine hippocampal neurons at 15 days of in vitro culture. The neurons were fixed and immunolabeled with anti-microtubule-associated protein 2 (MAP2) antibody and nuclei were counterstained with DAPI.

Image by Deepti Pilli of the Brain Autoimmunity Group, INMR, Children's Hospital at Westmead, University of Sydney, Australia.

Over the last years it has become evident that many neurological diseases of the central nervous system (CNS) are induced by a specific adaptive immune response directed against molecules expressed on CNS-resident cells. Well-recognized examples are anti-N-Methyl-D-Aspartate Receptor (NMDAR) encephalitis which is characterized by the presence of antibodies against neuron-expressed NMDAR, or neuromyelitis optica (NMO), induced by antibodies to astrocyte-expressed aquaporin-4. Many more examples exist, and antibodies, and T or/and B cells have increasingly been associated with CNS disease. Often the symptoms of these diseases have not been typically reported to have an immune aetiology. Beside classical neurological symptoms like ataxia, vision disturbance, and motor or sensory symptoms, these can include cognitive disturbances, behavioral abnormalities, or/and epileptic seizures. Although much has been learned regarding the pathophysiology of prototypic examples of these disorders, there are still major gaps in our understanding of their biology. This may be due to the fact that they are rare diseases, and their therapies are still very limited. This research topic includes contributions addressing the analysis of the adaptive immune response driving disease including target antigens, molecular epitope mapping, and factors involved in the disease pathogenesis such as complement activation cascades, genetic

and genomic regulation, as well as environmental triggers. Diagnostic criteria and methods, and treatment are also discussed. The overall aim of the volume is to review progress in our pathophysiological understanding of immune-mediated CNS disorders in order to advance diagnostic and therapeutic approaches, and ultimately improve outcomes for patients.

**Citation:** Weissert, R., Brilot, F., eds. (2017). Induction of Central Nervous System Disease by the Adaptive Immune Response. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-347-4

# Table of Contents

frontiers in Immunology

EDITORIAL
published: 02 October 2017
doi: 10.3389/fimmu.2017.01218

# Editorial: Induction of Central Nervous System Disease by the Adaptive Immune Response

*Robert Weissert*[1]* *and Fabienne Brilot*[2]*

[1] Department of Neurology, University of Regensburg, Regensburg, Germany, [2] Brain Autoimmunity Group, Institute for Neuroscience and Muscle Research, Kids Research Institute at the Children's Hospital at Westmead, Brain and Mind Centre, University of Sydney, Sydney, NSW, Australia

**Keywords: multiple sclerosis, neuromyelitis optica spectrum disorder, autoimmune encephalitis, T cell, B cell, NMDA receptor, myelin oligodendrocyte glycoprotein, aquaporin-4**

**Editorial on the Research Topic**

**Induction of Central Nervous System Disease by the Adaptive Immune Response**

T and B cells are of paramount importance in autoimmune diseases. This has been recognized for a long time and is not called into question. Nevertheless, it is often surprising how much energy it takes to convince colleagues that are not directly working in the field of neuroimmunology that the role of the adaptive immune responses is getting more and more important in many diseases of the central nervous system (CNS). In the past, many of those have not been considered to have an autoimmune origin, namely diseases with behavioral and/or psychiatric phenotypes. A prototype example of such a novel type of disease is anti-N-methyl D-aspartate receptor (anti-NMDAR) encephalitis which often mimics in certain aspects psychiatric diseases, such as schizophrenia or depression (1). In this specific form of autoimmune encephalitis (AE) that can have a paraneoplastic or purely autoimmune origin, antibodies target the NMDAR that is subsequently being internalized (2). This leads to changes in neural functioning with consequences on behavior. So far, the B cell and antibody side of this disease has been investigated in much detail, there is still incomplete knowledge regarding the T cell response. More and more CNS antigens exposed on cells are presently recognized as autoantigens of autoimmune CNS disorders. Based on this scientific progress, we decided that it would be of interest to summarize the current state of the field of autoimmune CNS disorders in which the adaptive immunity is the major disease driver.

Ehrenreich summarizes in her article the current understanding of anti-NMDAR encephalitis. She stresses the importance of the presence of anti-NMDAR antibodies in cerebrospinal fluid for disease precipitation (3). She also provides recommendations for the clinical diagnosis of this novel disease entity. Platt et al. also focus on the role of antibodies in various types of AE and discuss the triggers and induction of CNS autoimmune diseases. Strong emphasis is put on the blood–brain barrier and its role in AE. Zong et al. assess the impact of autoantibodies against additional target structures and consequences on disease phenotypes such as emergence of depression. The authors stress the value of broader assessment of antibody responses against different targets such as receptor complex, as well as and discrete epitopes within targets.

Pilli et al. review the current knowledge regarding T and B cell immunity against various antigens expressed in the CNS in multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSDs), and AE and assessment of immune responses to such CNS antigens using various methodological approaches.

OPEN ACCESS

***Edited and Reviewed by:***
*Hans-Peter Hartung,*
*Heinrich Heine Universität*
*Düsseldorf, Germany*

***Correspondence:**
*Robert Weissert*
*robert.weissert@ukr.de,*
*robert.weissert@googlemail.com;*
*Fabienne Brilot*
*fabienne.brilot@sydney.edu.au*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *03 August 2017*
***Accepted:*** *14 September 2017*
***Published:*** *02 October 2017*

***Citation:***
*Weissert R and Brilot F (2017) Editorial: Induction of Central Nervous System Disease by the Adaptive Immune Response. Front. Immunol. 8:1218. doi: 10.3389/fimmu.2017.01218*

Weissert summarizes the current knowledge regarding presence and localization of such antigens in CNS target cells and the distribution of such antigens within the cell. Differences and similarities between autoimmune and paraneoplastic immune-mediated disorders of the CNS are also discussed. The current understanding of the impact of the major histocompatibility complex (human leukocyte antigens) in MS as well as in NMOSD and AE is presented. Parnell and Booth review the current knowledge regarding genetic regulation in MS. They provide insight into the genetic regulation of function of single cell types by multiple genes, an interesting research area in the context of recent progresses in immunogenetics.

NMOSDs have been in the focus of research for the last years since the discovery of immunity against aquaporin-4, a water channel protein, expressed on astrocytes (4). Long et al. provide novel data that indicates that the disease course in NMOSD can strongly vary dependent on the initial symptom of the presentation of the disease. Zhao et al. provide some additional insight about the strong therapeutic effects of rituximab in patients with NMOSD. They found a reduction of circulating T follicular helper (cTfh) cells after treatment with rituximab in NMOSD patients. They conclude that this reduction in cTfh cell numbers and the reduced function of such cells could contribute to the observed beneficial effects of treatment of rituximab in NMOSD patients. This data could also be of relevance for other autoimmune disease of the CNS like AE.

Myelin oligodendrocyte glycoprotein (MOG) is a protein which is exposed on the outer surface of the myelin sheath. Research regarding this molecule in relation to MS has started in the 80s of the last century (5). Presently, the role of MOG in the pathogenesis of MS is still strongly debated. A subgroup of patients with NMOSD has persistent immunity against MOG (6). Peschl et al. reviewed in their article the current knowledge regarding MOG-directed autoimmunity in humans and experimental models of MS. The knowledge on MOG immunity that has been consolidated over the last decades will be of value also for other autoimmune diseases of the CNS.

Greer et al. demonstrate reactivity in patients with MS against novel thyroid-derived autoantigens. They also show overlapping autoimmunity against CNS and thyroid antigens in patients. These data are possibly of great importance and can explain much of the observed comorbidity of CNS demyelination in MS and thyroid disease. Interestingly, all the affected individuals were females underscoring the impact of genetics or/and hormonal regulation.

Autophagy has a strong impact on development of autoimmunity (7) and is a very important topic that is relevant for the pathogenesis of MS, NMOSD, and AE is. The field is currently fast-growing and much will be learned in the future about the relevance of autophagy in CNS-related autoimmune disorders. Keller and Lünemann et al. provide a current overview about autophagy and potential impact on immune-mediated diseases of the CNS.

Beside the ambitious research themes and stringent data that have been summarized in the collection of articles, there is another great serendipitous achievement. The researchers that contributed articles work in research institutions all over the world namely in Europe, Asia, USA, and Australia. Most of them did not previously know each other but were enthusiastic to be part of this project based on shared knowledge. Possibly this has already lead to novel research projects, collaborations, and funding. Finally, we want to thank the Frontiers Multiple Sclerosis and Neuroimmunology team for their continuing support, and especially the many colleagues who served as critical referees and contributed to the all-important peer-review process of this research topic.

## AUTHOR CONTRIBUTIONS

RW and FB drafted and wrote the Editorial.

## REFERENCES


1. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* (2007) 61(1):25–36. doi:10.1002/ana.21050
2. Moscato EH, Peng X, Jain A, Parsons TD, Dalmau J, Balice-Gordon RJ. Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Ann Neurol* (2014) 76(1):108–19. doi:10.1002/ana.24195
3. Castillo-Gomez E, Kastner A, Steiner J, Schneider A, Hettling B, Poggi G, et al. The brain as immunoprecipitator of serum autoantibodies against N-Methyl-D-aspartate receptor subunit NR1. *Ann Neurol* (2016) 79(1):144–51. doi:10.1002/ana.24545
4. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* (2005) 202(4):473–7. doi:10.1084/jem.20050304
5. Lebar R, Lubetzki C, Vincent C, Lombrail P, Boutry JM. The M2 autoantigen of central nervous system myelin, a glycoprotein present in oligodendrocyte membrane. *Clin Exp Immunol* (1986) 66(2):423–34.
6. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, Takahashi T, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* (2014) 82(6):474–81. doi:10.1212/WNL.0000000000000101
7. Schuster C, Gerold KD, Schober K, Probst L, Boerner K, Kim MJ, et al. The autoimmunity-associated gene CLEC16A modulates thymic epithelial cell autophagy and alters T cell selection. *Immunity* (2015) 42(5):942–52. doi:10.1016/j.immuni.2015.04.011


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 01 March 2017
doi: 10.3389/fimmu.2017.00181

# Autoantibodies against the *N*-Methyl-D-Aspartate Receptor Subunit NR1: Untangling Apparent Inconsistencies for Clinical Practice

*Hannelore Ehrenreich**

*Clinical Neuroscience, Max Planck Institute of Experimental Medicine, DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany*

This viewpoint review provides an integrative picture of seemingly contradictory work published on *N*-methyl-D-aspartate receptor 1 (NMDAR1) autoantibodies (AB). Based on the present state of knowledge, it gives recommendations for the clinical decision process regarding immunosuppressive treatment. Brain antigen-directed AB in general and NMDAR1-AB in particular belong to a preexisting autoimmune repertoire of mammals including humans. Specific autoimmune reactive B cells may get repeatedly (perhaps transiently) boosted by various potential stimulants (e.g., microbiome, infections, or neoplasms) plus less efficiently suppressed over lifespan (gradual loss of tolerance), likely explaining the increasing seroprevalence upon aging (>20% NMDAR1-AB in 80-year-old humans). Pathophysiological significance emerges (I) when AB-specific plasma cells settle in the brain and produce large amounts of brain antigen-directed AB intrathecally and/or (II) in conditions of compromised blood–brain barrier (BBB), for instance, upon injury, infection, inflammation, or genetic predisposition (*APOE4* haplotype), which then allows substantial access of circulating AB to the brain. Regarding NMDAR1-AB, functional effects on neurons *in vitro* and elicitation of brain symptoms *in vivo* have been demonstrated for immunoglobulin (Ig) classes, IgM, IgA, and IgG. Under conditions of brain inflammation, intrathecal production and class switch to IgG may provoke high NMDAR1-AB (and other brain antigen-directed AB) levels in cerebrospinal fluid (CSF) and serum, causing the severe syndrome named "anti-NMDAR encephalitis," which then requires immunosuppressive therapy on top of the causal encephalitis treatment (if available). However, negative CSF NMDAR1-AB results cannot exclude chronic effects of serum NMDAR1-AB on the central nervous system, since the brain acts as "immunoprecipitator," particularly in situations of compromised BBB. In any case of suspected symptomatic consequences of circulating AB directed against brain antigens, leakiness of the BBB should be evaluated by CSF analysis (albumin quotient as proxy) and magnetic resonance imaging before considering immunosuppression.

**Keywords: blood–brain barrier dysfunction, immunoglobulin class, serum, cerebrospinal fluid, inflammation, functionality assays, neuropsychiatric diseases, healthy subjects**

OPEN ACCESS

***Edited by:***
*Robert Weissert,*
*University of Regensburg, Germany*

***Reviewed by:***
*Hans Lassmann,*
*Center for Brain Research, Austria*
*Reinhild Klein,*
*University of Tuebingen, Germany*

***Correspondence:**
*Hannelore Ehrenreich*
*ehrenreich@em.mpg.de*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *03 January 2017*
***Accepted:*** *08 February 2017*
***Published:*** *01 March 2017*

***Citation:***
*Ehrenreich H (2017) Autoantibodies against the N-Methyl-D-Aspartate Receptor Subunit NR1: Untangling Apparent Inconsistencies for Clinical Practice. Front. Immunol. 8:181. doi: 10.3389/fimmu.2017.00181*

This viewpoint review is arranged around two tabulated figures, one summarizing NMDAR1 autoantibody (AB) findings and integrating them into an explanatory model (**Figure 1**) and the other trying to give clear recommendations for the clinical decision process on immunosuppressive treatment based on the present state of knowledge (**Figure 2**).

Please note that the new nomenclature GluN1 for NMDAR1/NR1 is disregarded here for consistency with most of the respective reviewed literature.

## NMDA RECEPTORS IN BRAIN AND PERIPHERY

*N*-methyl-D-aspartate receptors (NMDAR) are glutamate-gated ion channels, abundantly expressed in mammalian brain (1). They form heteromers of NR1, NR2, and NR3 subunits, with NR1 being the only obligatory partner. NMDAR are pivotal for regulating neuronal/synapse function and are also expressed by non-neuronal cell types in the brain like astrocytes, oligodendrocytes, or endothelial cells (2–5). In addition, peripheral expression has been reported, e.g., in the gastrointestinal tract or in immune cells (6).

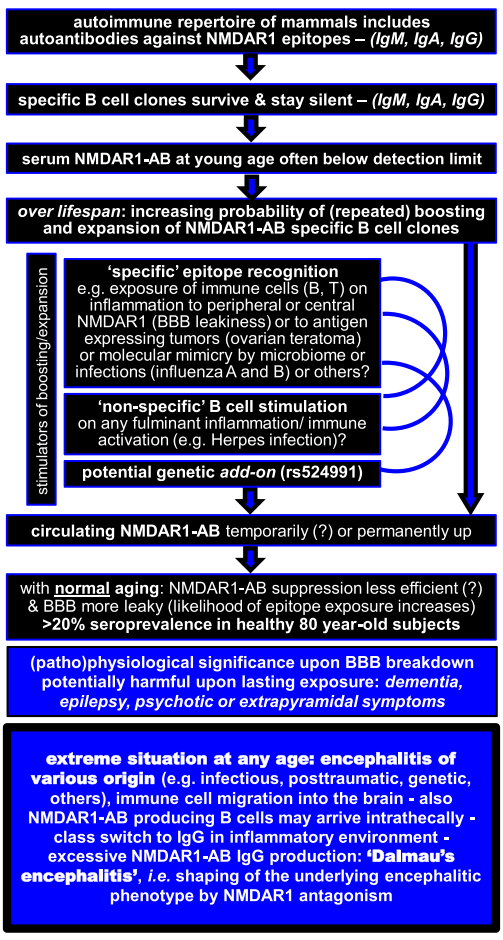



**FIGURE 1 | Integration of NMDAR1 autoantibodies (AB) findings into an explanatory model.**

## ANTI-NMDAR ENCEPHALITIS

Autoantibodies of the immunoglobulin G (IgG) class directed against NMDAR1 have been originally linked with a condition named "anti-NMDAR encephalitis" (7–10). In 2007, Dalmau and colleagues first described a paraneoplastic syndrome, based on 12 women with ovarian teratoma, carrying IgG AB against NMDAR NR1/2 subunits. The syndrome variably consisted of psychosis, cognitive decline, epileptic seizures, dyskinesia, decreased consciousness, and autonomic instability. The authors reported in many subsequent publications, based on increasing numbers of individuals with anti-NMDAR encephalitis, high serum and cerebrospinal fluid (CSF) titers of NMDAR1-AB of the IgG class in this condition as well as frequently favorable response to immunosuppressive therapy (7–10). As syndrome-pertinent pathophysiological mechanism, NMDAR1-AB induced decrease of NMDAR-mediated currents, due to enhanced receptor internalization, and thus reduced surface expression, has been suggested (11). However, over several years, healthy subjects were not even investigated in appreciable numbers for NMDAR1-AB seroprevalence. Nevertheless, the presence of NMDAR1-AB of the IgG class in serum (not only in CSF) was and still is claimed to be disease specific (7–10), causing some confusion in the literature and unfortunately also in clinical practice.

## SYNDROMES REMINISCENT OF NMDAR1 ANTAGONISM

Since NMDAR hypofunction had been hypothesized to be a central mechanism in schizophrenia, due to induction of psychotic symptoms by antagonists (12, 13), the question arose several years ago whether a subpopulation of schizophrenic subjects may be previously overlooked anti-NMDAR encephalitis cases. So far, the literature—mostly based on small sample sizes and following the original "disease-specificity claim of NMDAR1-AB of the IgG class"—yielded discordant results (14–20). Analogously, other pathological conditions, likewise reminiscent of NMDAR antagonism, e.g., epilepsy or dementia, were investigated for the presence of NMDAR1-AB. A flood of publications appeared—many of them case reports—describing associations of NMDAR1-AB with a wide variety of syndromes. Finally, NMDAR1-AB of other immunoglobulin (Ig) classes (IgM and IgA) were also reported to be associated with disease conditions (17, 21–23). An interesting question that has remained totally open up to now is whether NMDAR1-AB can also lead to "peripheral phenotypes," considering the expression of NMDAR in peripheral organs and tissues (6).

## EQUAL DISTRIBUTION OF SERUM NMDAR1-AB ACROSS HEALTH AND DISEASE

Unexpectedly, recent work of us and others on together >5,000 individuals challenged the "disease-specificity claim" of any

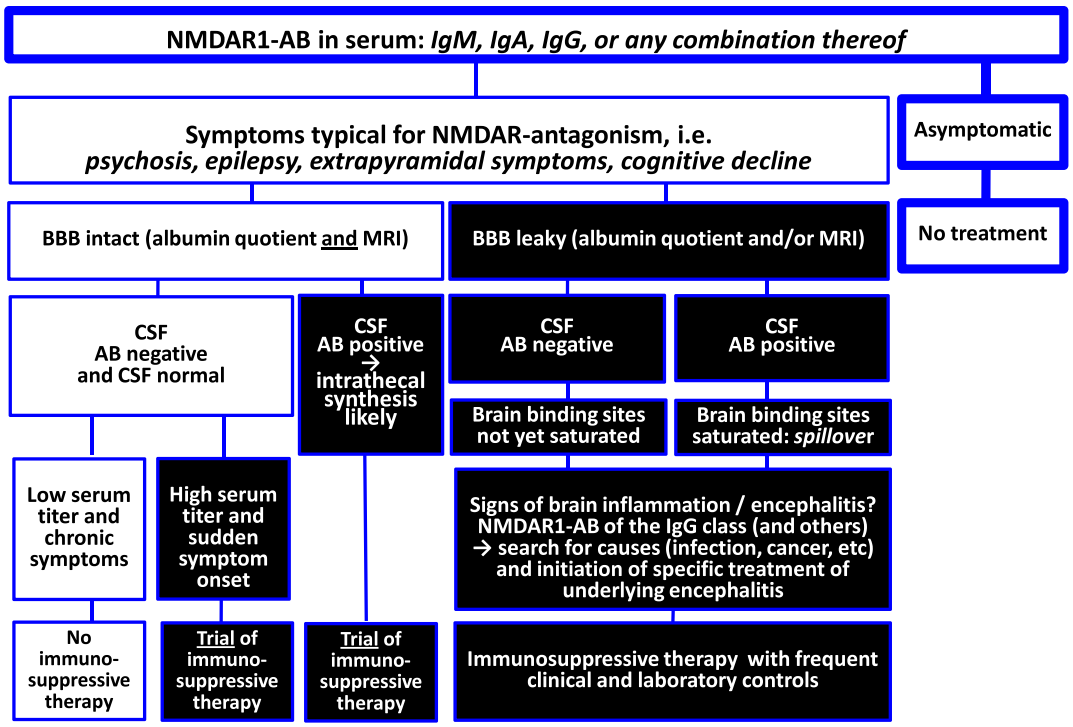



**FIGURE 2** | **Recommendations for the clinical decision process**.

NMDAR1-AB by demonstrating age dependent up to >20% NMDAR1-AB seroprevalence, including IgM, IgA, and IgG, in both healthy and ill subjects. Interestingly, NMDAR1-AB of the IgE class were searched for but never detected (24). Diseases investigated in these studies comprise neuropsychiatric conditions (schizophrenia, affective disorders, Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, stroke, multiple sclerosis, and personality disorders) as well as general medical conditions, e.g., diabetes or hypertension (24–28). Also NMDAR1-AB titer range in serum and the distribution of Ig classes were comparable across all investigated disease groups as well as healthy individuals (24–28). Any 40-year-old person has a ~10% and any 80-year-old person has a ~20% chance of displaying NMDAR1-AB seropositivity (24).

## FUNCTIONALITY OF NMDAR1-AB

This surprising discovery raised the question of whether these AB are all functional. Since biochip mosaics and a cell-based assay, the clinical standard procedure (HEK293T-cells transfected with NMDAR1 and secondary AB against human IgG, IgM, or IgA; Euroimmun, Lübeck, Germany), were used for all of these NMDAR1-AB determinations (see also below), additional assays had to be performed to further consolidate these unanticipated findings by proving AB functionality. These *in vitro* assays (all conducted with sera following ammonium sulfate precipitation of immunoglobulins and dialysis) revealed similar effects of NMDAR1-AB—independent of the Ig class—on receptor internalization in human IPSC-derived neurons as well as in primary mouse neurons. Likewise, NMDAR1-AB of all Ig classes reduced glutamate-evoked currents in NR1-1b/NR2A expressing *Xenopus laevis* oocytes (26, 28, 29). *In vivo* studies in mouse and human suggest comparable effects of serum NMDAR1-AB of all Ig classes regarding modulation of brain functions (see more details below).

## METHODS OF AB DETECTION—STILL ROOM FOR IMPROVEMENT

A still pending problem calling for standardization is the diversity of methods applied for AB determination with different specificity and sensitivity. Regarding NMDAR1-AB (where we have the most solid own experience), cell-based assays are certainly the superior method to detect NMDAR1-AB since epitopes are exposed in a natural way to enable AB to specifically detect them. But even these assays differ, with some authors using transiently transfected live cells accepting their potential variability and batch-to-batch variation problems, versus others using fixed and permeabilized cells expressing the whole NMDAR1 subunit, likely allowing better standardization (Euroimmun). This latter assay is currently being used throughout the world to diagnose NMDAR1-AB encephalitis. Based on our own experience with this assay in association with functionality studies performed in parallel (receptor internalization, electrophysiology, and *in vivo*

studies), it appears to be the most reliable method at this point. It is, however, strongly recommended to use this assay in combination with secondary AB that are highly specific for the various Ig classes (anti-human IgG, anti-human IgA, and anti-human IgM) since cross-reacting AB may lead to wrong conclusions regarding, e.g., the prevalence of IgG AB. The use of rat, mouse, human, or monkey brain sections for immunohistochemical detection of specific AB may be a helpful addition providing supportive evidence. In contrast, the typical ELISA based on peptides cannot be recommended as a detection method for NMDAR1-AB, since many false-positive and/or false-negative results may be obtained due to the unnatural (removed from the position in the cell membrane) epitope exposure. These assays seem only suitable for follow-up analyses, for instance, the determination of the AB titer course using a series of samples from the same donor, previously clearly diagnosed as seropositive by cell-based and functional assays.

## A DECISIVE ROLE OF THE BLOOD–BRAIN BARRIER (BBB) FOR SYNDROMIC RELEVANCE

Wondering why so many serum NMDAR1-AB carriers remain healthy, we hypothesized that a compromised BBB might decide on their pathophysiological significance. Importantly, enhanced BBB permeability may differ regionally, thereby explaining individually variable symptomatic consequences (30). As an animal model, we studied *ApoE*$^{-/-}$ mice with known BBB leakage in comparison to wild-type littermates (31). Intravenous injection of purified Ig fractions from NMDAR-AB seropositive (IgM, IgG, and IgA) human subjects led to alterations in spontaneous open field activity and hypersensitive (psychosis related) response to MK-801 in the open field exclusively in *ApoE*$^{-/-}$ mice (28). Exploring the role of a compromised BBB subsequently also in humans, we saw indeed more severe neurological symptoms in NMDAR1-AB carriers (of any Ig class) with a history of birth complications or neurotrauma, conditions with likely chronically leaky BBB (28). Along the same lines, we investigated *APOE4* carriers since the *APOE4* haplotype has been associated with a permeable BBB (32, 33). We obtained first hints that NMDAR-AB may enhance delusions of grandiosity and mania in neuropsychiatrically ill *APOE4* carriers, which are then more likely diagnosed schizoaffective (29). A modifier role of preexisting circulating NMDAR1-AB (again of all classes) was also seen in human ischemic stroke. In patients with intact BBB before occurrence of the insult, NMDAR1-AB were protective with respect to evolution of lesion size, whereas in *APOE4* carriers, NMDAR1-AB were associated with larger insult volumes (24). These findings emphasize that not only degree but also duration of BBB dysfunction, acute versus chronic, may play a pivotal role in syndrome shaping by NMDAR1-AB.

## THE BRAIN AS IMMUNOPRECIPITATOR OF NMDAR1-AB

Circulating NMDAR1-AB of all Ig isotypes temporarily decreased after stroke (24). This led us hypothesize that brain tissue with its densely expressed NMDAR1 (accessible after BBB breakdown) may act as a trap for circulating NMDAR1-AB (25). We first addressed the question of whether serum NMDAR1-AB would be detectable in the CSF. Of $N = 271$ middle-aged subjects (diagnosed with multiple sclerosis or disease controls) with CSF–serum pairs available, 26 were NMDAR1-AB seropositive (which is in the expected range) but, remarkably, only 1 was CSF positive. In contrast, tetanus-AB (omnipresent due to obligatory vaccination but not binding to brain tissue) were present in serum and CSF of all subjects, with CSF levels higher upon compromised BBB. Translational experiments in mice proved the hypothesis that the brain acts as "immunoprecipitator": simultaneous injection of NMDAR1-AB IgG and a non-brain-binding "nonsense-AB" (anti-GFP IgG) resulted in high detectability of the former only in the brain (distinctly more pronounced upon BBB dysfunction) and the latter only in CSF (25). These data may help explaining potential symptomatic consequences of serum AB directed against brain antigens. Whereas leakiness of the BBB has a major role and should be evaluated in cases where pathological relevance of circulating NMDAR1-AB is suspected, negative results regarding AB titers in CSF cannot automatically exclude brain effects.

## EPITOPES RECOGNIZED BY NMDAR1-AB

The next question was whether these apparently overall functional NMDAR1-AB would recognize the same epitope and whether this could potentially explain their high seroprevalence. Again unexpectedly, epitope mapping using seven different NMDAR1 constructs revealed recognition by NMDAR1-AB-positive sera of different epitopes, located in the extracellular ligand binding and the *N*-terminal domain (NTD) as well as the intracellular *C*-terminal and the extra large pore domain. NMDAR1-AB seropositivity was polyclonal/polyspecific in half of the investigated sera and likely mono or oligoclonal/oligospecific (mainly IgG) in the other half. Overall, no particular disease-related pattern appeared: NMDAR1 epitopes were comparable across disease groups (26). Published work on NMDAR1-AB epitopes has been scarce before this systematic investigation and had focused on IgG recognizing NTD and the NTD-G7 domain (N368/G369), probably because this region and Ig class were first deemed pathognomonic for anti-NMDAR encephalitis (8, 34). Indeed, it seems that factors predisposing young women [including those with ovarian teratoma and with lupus erythematosus (35)] to neuropsychiatric manifestations of NMDAR1-associated autoimmunity are connected with NTD or NTD-G7 epitopes. The accentuated role of IgG in this context is still a matter of speculation but likely related to inflammation-induced class switch in the brain (36).

## PREDISPOSING FACTORS TO CARRY OR BOOST NMDAR1-AB

On the basis of these *in vitro* and *in vivo* findings, we have to assume that basically all naturally occurring NMDAR1-AB

have pathogenic potential irrespective of epitope and Ig class. This does, however, not mean that the type of Ig class cannot initiate distinct cascades of secondary events and thereby further shape the ultimate tissue response. But now even more questions arise: How can we explain the disease-independent high seroprevalence of NMDAR1-AB, increasing with age? Do we know of any predisposing factors, and if so, how can we integrate their role into the full picture? NMDAR1-AB were initially associated with oncological conditions (teratoma) (7). Later on, a predisposition to carry these AB was seen upon influenza A and B seropositivity, a finding replicated in an independent sample (25, 28). Also a genome-wide significant genetic marker, rs524991, even related to NMDAR biology, was found associated with NMDAR1-AB (28). Whether a leaky BBB, causing enhanced exposure of central NMDAR1 to cells of the immune system, can induce NMDAR1-AB formation and/or boost preexisting specific B cell clones is presently unclear and needs to be systematically investigated. Another attractive idea that has not yet been pursued in the NMDAR1-AB field is the potential modulatory influence of the microbiome on boosting of NMDAR1-AB (37).

## OTHER BRAIN ANTIGEN-DIRECTED AB

Why do we see NMDAR1-AB so abundantly in health and disease? Does this also hold true for other AB directed against brain antigens? To address these questions, we analogously studied 24 other brain antigen-directed serum AB, previously connected with pathological conditions. Again to some surprise, this work revealed comparable frequency, titers, and Ig class distribution in healthy and ill subjects. Seroprevalence, however, of all of these 24 AB was distinctly lower (<2%) in contrast to NMDAR1-AB (up to >20%) (27). Strikingly, the predominant Ig class did not depend on health or disease state either, but on antigen location, with intracellular epitopes predisposing to IgG (27). The equal distribution of these 24 other AB in health and disease is less astonishing when considering that multiple brain-directed AB have been reported in serum of healthy humans and of different other mammalian species (38, 39) as well as abundantly in CSF of encephalitis cases (40), even though the respective brain antigens were not specified. To sum up, brain antigen-directed AB in general and NMDAR1-AB in particular seem to be part of a preexisting autoimmune repertoire (37, 41–44) that gains (patho)physiological significance in conditions of intrathecal synthesis or compromised BBB, for instance, upon injury, infection, brain inflammation, or genetic predisposition to BBB leakiness (*APOE4* haplotype).

## CONCLUSIONS AND RECOMMENDATIONS

All naturally occurring serum NMDAR1-AB obviously have pathogenic potential. For still widely unexplored reasons, they are highly frequent (more than other so far identified brain-directed AB), and their prevalence clearly increases with age. NMDAR-AB seropositivity alone definitely does not justify immunosuppressive treatment. Syndromal relevance of serum NMDAR1-AB depends on accessibility to the brain, i.e., BBB permeability. Moreover, brain inflammation likely plays a crucial role in determining syndrome acuteness and severity as contributed by circulating NMDAR1-AB and even more pronounced by respective plasma cells that reside in or potentially migrate to the brain in inflammatory conditions to produce AB intrathecally (40). In the inflammatory milieu, they are boosted upon epitope exposure and experience class switch to IgG (36). Findings in individuals with herpes encephalitis may further support this view (22, 45).

Any underlying encephalitis, be it infectious, lesion induced, genetic, or "idiopathic", may undergo prominent syndrome shaping by the presence of NMDAR1-AB in the sense of "Dalmau's encephalitis" (7–10), which then requires immunosuppressive therapy on top of the causal encephalitis treatment (if available). Whether intrathecally produced NMDAR1-AB alone, without any underlying preexisting inflammation, can cause "Dalmau's encephalitis" remains to be determined. In any case of otherwise suspected symptomatic consequences of serum AB directed against brain antigens in the absence of overt encephalitis, leakiness of the BBB should be evaluated. Since the albumin quotient (employed as clinical approximation to diagnose BBB breakdown) rather indicates blood–CSF barrier disturbance and may not always be pathological in mild cases of BBB leakiness (46–48), additional determination of BBB disruption (global or local) by a novel magnetic resonance imaging (MRI) method (47), which can be established as *add-on* to routine contrast-enhanced MRI, may prove helpful for estimating necessity and benefit especially of extended immunosuppressive therapeutic interventions.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

## FUNDING

This work was supported by the Max Planck Society, the Max Planck Förderstiftung, the DFG (CNMPB), EXTRABRAIN EU-FP7, and the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT).

## REFERENCES

1. Li F, Tsien JZ. Memory and the NMDA receptors. *N Engl J Med* (2009) 361:302–3. doi:10.1056/NEJMcibr0902052
2. Dzamba D, Honsa P, Anderova M. NMDA receptors in glial cells: pending questions. *Curr Neuropharmacol* (2013) 11:250–62. doi:10.2174/1570159X11311030002
3. Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci* (2007) 8:413–26. doi:10.1038/nrn2153
4. Reijerkerk A, Kooij G, van der Pol SM, Leyen T, Lakeman K, van Het Hof B, et al. The NR1 subunit of NMDA receptor regulates monocyte transmigration through the brain endothelial cell barrier. *J Neurochem* (2010) 113:447–53. doi:10.1111/j.1471-4159.2010.06598.x

5. Saab AS, Tzvetavona ID, Trevisiol A, Baltan S, Dibaj P, Kusch K, et al. Oligodendroglial NMDA receptors regulate glucose import and axonal energy metabolism. *Neuron* (2016) 91:119–32. doi:10.1016/j.neuron.2016.05.016
6. Du J, Li XH, Li YJ. Glutamate in peripheral organs: biology and pharmacology. *Eur J Pharmacol* (2016) 784:42–8. doi:10.1016/j.ejphar.2016.05.009
7. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-*N*-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* (2007) 61:25–36. doi:10.1002/ana.21050
8. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* (2008) 7:1091–8. doi:10.1016/S1474-4422(08)70224-2
9. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* (2011) 10:63–74. doi:10.1016/S1474-4422(10)70253-2
10. Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol* (2013) 12:157–65. doi:10.1016/S1474-4422(12)70310-1
11. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* (2010) 30:5866–75. doi:10.1523/JNEUROSCI.0167-10.2010
12. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* (2007) 27:11496–500. doi:10.1523/JNEUROSCI.2213-07.2007
13. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* (1995) 52:998–1007.
14. Coutinho E, Harrison P, Vincent A. Do neuronal autoantibodies cause psychosis? A neuroimmunological perspective. *Biol Psychiatry* (2014) 75:269–75. doi:10.1016/j.biopsych.2013.07.040
15. Crisp SJ, Kullmann DM, Vincent A. Autoimmune synaptopathies. *Nat Rev Neurosci* (2016) 17:103–17. doi:10.1038/nrn.2015.27
16. Masdeu JC, Gonzalez-Pinto A, Matute C, Ruiz De Azua S, Palomino A, De Leon J, et al. Serum IgG antibodies against the NR1 subunit of the NMDA receptor not detected in schizophrenia. *Am J Psychiatry* (2012) 169:1120–1. doi:10.1176/appi.ajp.2012.12050646
17. Steiner J, Walter M, Glanz W, Sarnyai Z, Bernstein HG, Vielhaber S, et al. Increased prevalence of diverse *N*-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from *N*-methyl-D-aspartate glutamate receptor encephalitis. *JAMA Psychiatry* (2013) 70:271–8. doi:10.1001/2013.jamapsychiatry.86
18. Steiner J, Teegen B, Schiltz K, Bernstein HG, Stoecker W, Bogerts B. Prevalence of *N*-methyl-D-aspartate receptor autoantibodies in the peripheral blood: healthy control samples revisited. *JAMA Psychiatry* (2014) 71:838–9. doi:10.1001/jamapsychiatry.2014.469
19. Tsutsui K, Kanbayashi T, Tanaka K, Boku S, Ito W, Tokunaga J, et al. Anti-NMDA-receptor antibody detected in encephalitis, schizophrenia, and narcolepsy with psychotic features. *BMC Psychiatry* (2012) 12:37. doi:10.1186/1471-244X-12-37
20. Zandi MS, Irani SR, Lang B, Waters P, Jones PB, McKenna P, et al. Disease-relevant autoantibodies in first episode schizophrenia. *J Neurol* (2011) 258:686–8. doi:10.1007/s00415-010-5788-9
21. Choe CU, Karamatskos E, Schattling B, Leypoldt F, Liuzzi G, Gerloff C, et al. A clinical and neurobiological case of IgM NMDA receptor antibody associated encephalitis mimicking bipolar disorder. *Psychiatry Res* (2013) 208:194–6. doi:10.1016/j.psychres.2012.09.035
22. Pruss H, Finke C, Holtje M, Hofmann J, Klingbeil C, Probst C, et al. *N*-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. *Ann Neurol* (2012) 72:902–11. doi:10.1002/ana.23689
23. Pruss H, Holtje M, Maier N, Gomez A, Buchert R, Harms L, et al. IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment. *Neurology* (2012) 78:1743–53. doi:10.1212/WNL.0b013e318258300d
24. Zerche M, Weissenborn K, Ott C, Dere E, Asif AR, Worthmann H, et al. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in acute ischemic stroke. *Stroke* (2015) 46:1180–6. doi:10.1161/STROKEAHA.114.008323
25. Castillo-Gomez E, Kastner A, Steiner J, Schneider A, Hettling B, Poggi G, et al. The brain as immunoprecipitator of serum autoantibodies against *N*-methyl-D-aspartate receptor subunit NR1. *Ann Neurol* (2016) 79:144–51. doi:10.1002/ana.24545
26. Castillo-Gomez E, Oliveira B, Tapken D, Bertrand S, Klein-Schmidt C, Pan H, et al. All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. *Mol Psychiatry* (2016). doi:10.1038/mp.2016.125
27. Dahm L, Ott C, Steiner J, Stepniak B, Teegen B, Saschenbrecker S, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann Neurol* (2014) 76:82–94. doi:10.1002/ana.24189
28. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry* (2014) 19:1143–9. doi:10.1038/mp.2013
29. Hammer C, Zerche M, Schneider A, Begemann M, Nave KA, Ehrenreich H. Apolipoprotein E4 carrier status plus circulating anti-NMDAR1 autoantibodies: association with schizoaffective disorder. *Mol Psychiatry* (2014) 19:1054–6. doi:10.1038/mp.2014.52
30. Diamond B, Huerta PT, Mina-Osorio P, Kowal C, Volpe BT. Losing your nerves? Maybe it's the antibodies. *Nat Rev Immunol* (2009) 9:449–56. doi:10.1038/nri2529
31. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc Natl Acad Sci U S A* (1992) 89:4471–5.
32. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* (2012) 485:512–6. doi:10.1038/nature11087
33. Halliday MR, Pomara N, Sagare AP, Mack WJ, Frangione B, Zlokovic BV. Relationship between cyclophilin a levels and matrix metalloproteinase 9 activity in cerebrospinal fluid of cognitively normal apolipoprotein e4 carriers and blood-brain barrier breakdown. *JAMA Neurol* (2013) 70:1198–200. doi:10.1001/jamaneurol.2013.3841
34. Gleichman AJ, Spruce LA, Dalmau J, Seeholzer SH, Lynch DR. Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. *J Neurosci* (2012) 32:11082–94. doi:10.1523/JNEUROSCI.0064-12.2012
35. Ogawa E, Nagai T, Sakuma Y, Arinuma Y, Hirohata S. Association of antibodies to the NR1 subunit of *N*-methyl-D-aspartate receptors with neuropsychiatric systemic lupus erythematosus. *Mod Rheumatol* (2016) 26:377–83. doi:10.3109/14397595.2015.1083163
36. Zhang J, Jacobi AM, Wang T, Berlin R, Volpe BT, Diamond B. Polyreactive autoantibodies in systemic lupus erythematosus have pathogenic potential. *J Autoimmun* (2009) 33:270–4. doi:10.1016/j.jaut.2009.03.011
37. Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. *Immunol Today* (1991) 12:105–10.
38. DeMarshall C, Sarkar A, Nagele EP, Goldwaser E, Godsey G, Acharya NK, et al. Utility of autoantibodies as biomarkers for diagnosis and staging of neurodegenerative diseases. *Int Rev Neurobiol* (2015) 122:1–51. doi:10.1016/bs.irn.2015.05.005
39. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. *PLoS One* (2013) 8:e60726. doi:10.1371/journal.pone.0060726
40. Kreye J, Wenke NK, Chayka M, Leubner J, Murugan R, Maier N, et al. Human cerebrospinal fluid monoclonal *N*-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. *Brain* (2016) 139:2641–52. doi:10.1093/brain/aww208
41. Abramson J, Husebye ES. Autoimmune regulator and self-tolerance – molecular and clinical aspects. *Immunol Rev* (2016) 271:127–40. doi:10.1111/imr.12419
42. Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. *Curr Opin Immunol* (1995) 7:812–8.
43. Lobo PI. Role of natural autoantibodies and natural IgM anti-leucocyte autoantibodies in health and disease. *Front Immunol* (2016) 7:198. doi:10.3389/fimmu.2016.00198

44. Nguyen TT, Baumgarth N. Natural IgM and the development of B cell-mediated autoimmune diseases. *Crit Rev Immunol* (2016) 36:163–77. doi:10.1615/CritRevImmunol.2016018175
45. Westman G, Studahl M, Ahlm C, Eriksson BM, Persson B, Ronnelid J, et al. *N*-methyl-D-aspartate receptor autoimmunity affects cognitive performance in herpes simplex encephalitis. *Clin Microbiol Infect* (2016) 22:934–40. doi:10.1016/j.cmi.2016.07.028
46. Bechter K, Reiber H, Herzog S, Fuchs D, Tumani H, Maxeiner HG. Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders: identification of subgroups with immune responses and blood-CSF barrier dysfunction. *J Psychiatr Res* (2010) 44:321–30. doi:10.1016/j.jpsychires.2009.08.008
47. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* (2015) 85:296–302. doi:10.1016/j.neuron.2014.12.032
48. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci* (2001) 184:101–22. doi:10.1016/S0022-510X(00)00501-3

**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 21 April 2017
doi: 10.3389/fimmu.2017.00442

# Hello from the Other Side: How Autoantibodies Circumvent the Blood–Brain Barrier in Autoimmune Encephalitis

*Maryann P. Platt[1], Dritan Agalliu[1,2,3,4] and Tyler Cutforth[1,4]**

[1] Department of Neurology, Columbia University Medical Center, New York, NY, USA, [2] Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY, USA, [3] Department of Pharmacology, Columbia University Medical Center, New York, NY, USA, [4] Columbia Translational Neuroscience Initiative, Columbia University Medical Center, New York, NY, USA

Antibodies against neuronal receptors and synaptic proteins are associated with autoimmune encephalitides (AE) that produce movement and psychiatric disorders. In order to exert their pathological effects on neural circuits, autoantibodies against central nervous system (CNS) targets must gain access to the brain and spinal cord by crossing the blood–brain barrier (BBB), a tightly regulated gateway formed by endothelial cells lining CNS blood vessels. To date, the pathogenic mechanisms that underlie autoantibody-triggered encephalitic syndromes are poorly understood, and how autoantibodies breach the barrier remains obscure for almost all AE syndromes. The relative importance of cellular versus humoral immune mechanisms for disease pathogenesis also remains largely unexplored. Here, we review the proposed triggers for various autoimmune encephalopathies and their animal models, as well as basic structural features of the BBB and how they differ among various CNS regions, a feature that likely underlies some regional aspects of autoimmune encephalitis pathogenesis. We then discuss the routes that antibodies and immune cells employ to enter the CNS and their implications for AE. Finally, we explore future therapeutic strategies that may either preserve or restore barrier function and thereby limit immune cell and autoantibody infiltration into the CNS. Recent mechanistic insights into CNS autoantibody entry indicate promising future directions for therapeutic intervention beyond current, short-lived therapies that eliminate circulating autoantibodies.

**Keywords: blood–brain barrier, autoimmune encephalitis, basal ganglia encephalitis, NMDA receptor, dopamine receptor, autoantibodies, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, Sydenham's chorea**

OPEN ACCESS

***Edited by:***
*Fabienne Brilot, University of Sydney, Australia*

***Reviewed by:***
*Anna Fogdell-Hahn, Karolinska Institutet, Sweden*
*Takashi Kanda, Yamaguchi University, Japan*

****Correspondence:***
*Tyler Cutforth*
*tc2756@cumc.columbia.edu*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *07 February 2017*
***Accepted:*** *30 March 2017*
***Published:*** *21 April 2017*

***Citation:***
*Platt MP, Agalliu D and Cutforth T (2017) Hello from the Other Side: How Autoantibodies Circumvent the Blood–Brain Barrier in Autoimmune Encephalitis. Front. Immunol. 8:442. doi: 10.3389/fimmu.2017.00442*

## INTRODUCTION

Antibody-mediated central nervous system (CNS) autoimmunity, the hallmark of several autoimmune encephalitis (AE) syndromes that produce movement and psychiatric disorders, is initiated when antibodies recognize neuronal receptors or synaptic proteins as foreign proteins (1–3). Antibodies directed against neuronal antigens can arise in the periphery when target proteins are expressed ectopically on tumor cells. AE has also been linked to various infectious triggers,

including bacteria, viruses, fungi, and other parasites. However, an underlying infection is the causative agent for only a small fraction of all AE cases; in many cases, the triggers are either unknown or unidentified infectious agents (4, 5). While the evidence supporting various microbial triggers is scant, some autoimmune encephalopathies have well-established infectious links. For example, untreated Group A *Streptococcus* (*S. pyogenes*) infections cause autoimmune sequela in many target tissues such as the heart or CNS, manifested as either rheumatic fever or Sydenham's chorea (SC), respectively (6–8). Several recent reviews have highlighted the clinical and mechanistic features for many of these currently accepted autoimmune encephalitides and the aberrant autoimmunity/CNS axis (4, 5, 9–15). Here, we review infectious and non-infectious triggers for AE and discuss findings from animal models of AE related to blood–brain barrier (BBB) integrity, immune cell infiltration into the CNS, and neuronal circuit dysfunction that provide useful avenues to improve diagnosis (e.g., clinical assays and imaging techniques). We will then outline routes for antibody and immune cell entry into the CNS, with a focus on the predominant pathways leading to BBB breakdown that allows entry of autoantibodies into the CNS. Finally, we will briefly review current therapies for selected AEs and propose future treatment options aimed at preventing autoantibody entry. Recent advances point to an underlying autoimmune etiology that may be relevant for many movement and neuropsychiatric diseases (2, 4, 16). Thus, elucidating the mechanisms for autoantibody access to the CNS may provide a wider spectrum of treatment options for patients with these complex and puzzling disorders.

## AUTOIMMUNE ENCEPHALITIS TRIGGERS

Autoimmunity against brain targets is inherently mysterious, because traditional thinking holds that the immune system minimally surveys the CNS compared to other organs. Non-CNS self-antigens are selected against to maintain tolerance; however, CNS antigens have been thought to be excluded from immune monitoring (17). Although this thinking has been recently challenged with the identification of both glymphatic and lymphatic circulation in the CNS (18, 19), re-exposure of the immune system to brain antigens, or the presence of an outside trigger that causes production of cross-reactive autoantibodies, is a crucial step in CNS autoimmune disease.

### Bacteria

Perhaps the earliest identified CNS autoimmune disease is linked to an infectious trigger: untreated Group A *Streptococcus* (GAS or *S. pyogenes*) infections can in some cases give rise to SC, in which antibodies directed against a streptococcal surface protein cross-react with brain antigens (20). While SC is characterized by movement difficulties (chorea) affecting both gait and the tone of large muscle groups, more recently, a group of children within an SC cohort displaying both prominent psychiatric symptoms and fine choreiform movements prompted the recognition of a new syndrome: pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) (21). Both SC and PANDAS are part of a group of basal ganglia autoimmune encephalopathies (BGE) for which the humoral adaptive immune response (i.e., autoantibodies) plays an important role in disease pathogenesis, as described in human patients as well as rodent models (3, 16). In most cases, a recent GAS exposure or infection can be identified in afflicted children and subsequent exposures typically prompt an acute exacerbation of symptoms (22). Anti-GAS titers correlate with symptom severity in many but not all SC and PANDAS cases (23). Anti-neuronal autoantibodies that erroneously recognize dopamine D1R/D2R receptors or other neuronal targets in the basal ganglia have been identified in sera from patients with SC or PANDAS, and they respond positively to immune therapies such as intravenous immunoglobulin (IVIg) or plasmapheresis, consistent with an autoimmune mechanism (7, 16, 21, 24–30). The autoimmune mimicry hypothesis, namely that antibodies generated from an aberrant humoral immune response to *S. pyogenes* infections recognize host-specific proteins due to epitope similarity, has been proposed to underlie the secondary sequela in BGE (20, 31). However, this hypothesis assumes that BBB permeability is impaired to allow antibody entry into the CNS, because BGE occurs in the absence of brain infection.

Infections by *Campylobacter jejuni* and, in rare cases, by other bacteria (32, 33), induce Guillain–Barré syndrome (GBS) and the atypical Guillain–Barré-related diseases [Miller Fisher syndrome (MFS) and Bickerstaff brainstem encephalitis (BBE)], whose symptoms lie on a continuum with traditional GBS including prickling, weaknesses in extremities, motor deficits, and pain (34–36). While these diseases are caused by the same autoantibodies against gangliosides (GD3, GQ1b, GM1, or GT1a), GBS and MFS affect peripheral nerves whereas BBE affects primarily the CNS (37, 38). Blood vessels in peripheral nerves are protected by a blood–nerve barrier (BNB) that has some similarities to the BBB (39–41). Although the BNB can be disrupted by autoantibodies present in sera from patients with multifocal motor neuropathy (42), this review is primarily focused on autoantibody entry into the CNS across the BBB rather than PNS across the BNB.

### Viruses

Viruses have been proposed to initiate some autoimmune encephalopathies. In systemic lupus erythematosus (SLE), autoantibodies cross-reacting with Epstein–Barr nuclear antigen-1 and the 60 kDa Ro protein target a variety of organs, including the CNS (31). Anti-Ro antibodies are frequently generated and detected early in clinical SLE, making them attractive candidates for an initiating autoantigen. Other viruses implicated in neuropsychiatric disease include influenza, herpes virus-1 and -2, Epstein–Barr virus, and bornavirus (43, 44). Herpes simplex encephalitis has been linked to subsequent development of NMDAR encephalitis in some cases (2, 9). Notably, the majority of viral triggers are hypothesized to create a pro-inflammatory state that "primes" the immune system, including CNS-resident immune cells termed microglia, to become overactive leading to an autoimmune response against the CNS (2, 45). This contrasts with the molecular mimicry hypothesis proposed for *S. pyogenes*-induced BGE, in which antibodies directed against bacterial surface antigens cross-react directly with self-antigens (20, 31).

### Tumors

In contrast to molecular mimicry, production of antibodies against a brain antigen can occur in the periphery when a tumor cell expresses surface proteins found in the brain. Tumor masses are known to express a wide variety of non-tissue-specific surface proteins, including neuronal antigens. The original cohort of NMDA receptor encephalitis (NMDARE) patients contained young women bearing ovarian teratomas that express NMDAR, thus initiating peripheral immune activation (46). Subsequent BBB permeability by an unknown mechanism would then allow NMDAR antibodies to target glutamatergic synapses also containing this receptor. However, tumors are not present in all cases of NMDARE, and indeed, the tumor rate is approximately 50% for such patients (47). Anti-GAD65 antibodies, which are the putative culprits underlying cerebellar ataxia, stiff person syndrome, and Batten's disease (3), are also associated with neoplasms that aberrantly express GAD65, albeit at lower frequency than those producing NMDAR antibodies (48, 49). Finally, serum antibodies targeting the voltage-gated potassium channel (VGKC) complex may be also linked to tumors. These include antibodies against leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein 2 (Caspr2), and contactin 2 as well as those recognizing the entire VGKC protein complex. IgGs targeting the VGKC complex have been found in sera (50, 51) and cerebrospinal fluid (CSF) (51) from patients with both limbic encephalitis and Morvan syndrome (VGKC complex encephalitis, peripheral nerve hyperexcitability) (52), and Morvan syndrome has been linked to thymoma in 37% of patients (53). Conversely, anti-VGKC complex antibodies are present in sera from 32% of patients with verified thymoma, the majority of whom develop myasthenia gravis, which targets acetylcholine receptors on muscles that are supplied by blood vessels lacking a tight endothelial barrier (54). Since only a minority of patients with VGKC complex antibodies have co-occurring thymoma, this suggests that other mechanisms underlie development of these autoantibodies, similar to NMDARE. In addition, the clinical features accompanying patients with VGKC antibodies are highly variable (52). VGKC antibodies may, therefore, be useful biomarkers for inflammatory neurological disease in general and are potentially associated with the presence of other autoantibodies or immune cell infiltration into the CNS (52).

## ANIMAL MODELS FOR CNS AUTOIMMUNE DISORDERS

Numerous rodent studies have expanded our understanding of the molecular events involved in antibody generation and pathogenicity for several varieties of AE, shedding light on the mechanisms of immune cell infiltration and barrier breakdown. Rodent models for NMDARE, BGE, GAD65 encephalitis, BBE, and Morvan syndrome vary widely in both their methods of antigen exposure and the disease parameters recapitulated (see **Table 1** for summary). A majority of the studies surveyed here use animal models in which disease is initiated either by exposure to an initial trigger (bacteria or virus) in the periphery or by direct infusion into the CNS of sera or purified autoantibodies isolated from patients, thereby circumventing the BBB.

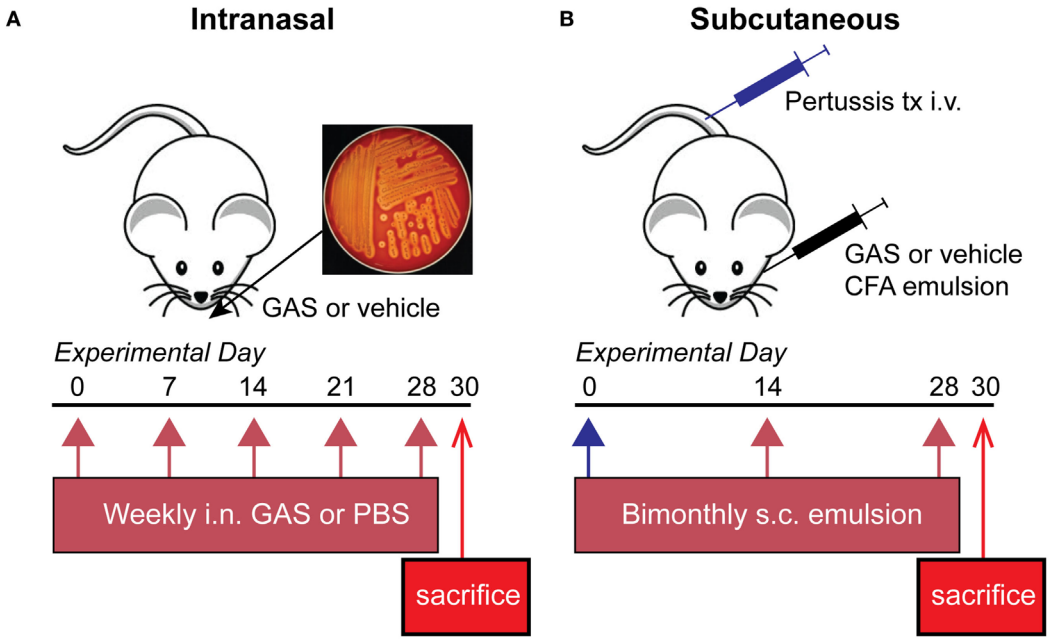
### *S. pyogenes*-Triggered BGE

Animal models for post-streptococcal BGE have been focused on demonstrating the ability of GAS to prime development of an autoimmune reaction by stimulating adaptive cellular and humoral immune responses. In the mouse, intranasal (i.n.) infections with live bacteria polarize T cells located in the nasal-associated lymphoid tissue (NALT, the mouse structural analog of human tonsils and adenoids) toward a Th17 phenotype, a T cell subtype that is both essential for mucosal immune protection against bacteria but also strongly implicated in many autoimmune diseases (**Figure 1A**). Multiple i.n. *S. pyogenes* infections strengthen this Th17 immune response, largely due to induction of IL-6 and TGF-β1, which are two pro-inflammatory cytokines essential for Th17 differentiation (68). IL-6 is essential for clearance of bacteria after i.n. infection, *via* generation of Th17 cells; *IL-6*$^{-/-}$ mice are capable of generating a Th1 immune response to an i.n. bacterial challenge but cannot control infection (69). This model has been used to demonstrate that repeated i.n. infections with *S. pyogenes* induce migration of GAS-specific Th17 cells and other T cell subtypes from the nasal epithelium to the olfactory bulb (OB) (**Figure 2**), where sensory axons make connections with projection interneurons to form the neural circuitry essential for odor discrimination, as well as to other CNS regions (55). The presence of *Streptococcus*-specific Th17 cells in the CNS after repeated i.n. infections increases the permeability of capillaries in several CNS regions, including the OB, amygdala, and hypothalamus, thereby enabling deposition of serum IgGs and potential anti-CNS autoantibodies. This is largely due to disruption in the organization of tight junction (TJ)-associated proteins, which control an essential aspect of BBB function (55) (see below for a more detailed discussion). The intranasal model produces profound changes in olfactory neural circuitry by reducing vGluT2 expression and thus excitatory input at the presynaptic terminals of olfactory sensory axons and perturbing the excitatory/inhibitory balance within the primary olfactory circuit (55). This model of post-*S. pyogenes* autoimmunity demonstrates a central role for the cellular adaptive immune response (e.g., bacterial-specific Th17 cells in the CNS) in disrupting BBB function, thus promoting entry of antibodies into the CNS and inducing changes in synaptic signaling. Although such a cellular adaptive immune response has not been identified to date in the nervous systems of children suffering from BGE, *S. pyogenes*-specific Th17 cells can be found in the tonsils of human patients (55), making Th17 lymphocytes a potential causative agent in either initiation or persistence of BGE disease pathogenesis.

A second group of rodent models for BGE employs subcutaneous immunization with an antigenic target (bacterial homogenate) plus complete Freund's adjuvant to activate the immune system, in conjunction with agents (i.e., *B. pertussis* toxin) that open the BBB to provide access to brain targets (**Figure 1B**). In this model, mice and rats develop a strong humoral immune response toward *S. pyogenes* and show behavioral abnormalities. Specifically, GAS-immunized rodents display increased rearing and decreased locomotion, as well as increased repetitive and

**TABLE 1 | Summary of published rodent models for several autoimmune encephalitides.**

| Disease modeled | Strain/ species (sex) | Autoantibody source | Delivery | Immune response | Neural consequences | Reference |
|---|---|---|---|---|---|---|
| Sydenham's chorea/pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections | SJL mouse (F) | Unknown | Intranasal infection with *S. pyogenes* | Microglial activation and infiltrating $CD4^+$ T cells in olfactory bulb (OB) | Decreased excitatory synapse proteins in OB glomeruli; blood–brain barrier breakdown in OB, lateral hypothalamus, and amygdala | (55) |
| | Lewis rat | Induced in model | Subcutaneous immunization with *S. pyogenes* emulsion | Autoantibodies detected against tubulin | Dopamine D2-dependent compulsive grooming; impaired motor coordination; IgG deposition in striatum, thalamus, and cortex; IgG-induced elevation of CaMKII signaling in cultured neurons | (56) |
| | SJL mouse | Induced in model | Subcutaneous immunization with *S. pyogenes* emulsion | Specific increase in IgG1 subclass; no change in IgG2 nor IgG3 pool | Increased rearing; decreased motor coordination; impaired olfactory discrimination; improved spatial memory performance; IgG deposition in striatum and cerebellum | (57) |
| | SJL mouse (M) | Adoptive transfer from immunized cohort | Intravenous injection paired with intraperitoneal lipopolysaccharide injection | Not analyzed | Increased rearing; IgG deposition in dentate gyrus | (57) |
| | Lewis rat (M) | Induced in model | Subcutaneous immunization with *S. pyogenes* emulsion | Autoantibodies against D1R, D2R, and serotonin receptors | Impaired motor coordination; compulsive grooming | (58) |
| | Lewis rat (M) | Adoptive transfer from immunized cohort | Intra-striatal infusion | Not analyzed | Impaired motor coordination; IgG deposition in striatum | (58) |
| | SJL mouse (M) | Induced in model | Subcutaneous immunization with *S. pyogenes* emulsion | Microglial activation in white matter tracts; infiltrating $CD3^+$ T cells | Impaired motor coordination; repetitive behaviors; increased rearing; excessive lactate; blunted startle response (PPI) | (59) |
| NMDA receptor encephalitis | Lewis rat | Patient cerebrospinal fluid (CSF) | Bath application to cultured neurons | Not analyzed | Autoantibody-mediated internalization of NMDAR from synapses; selective loss of NMDA-mediated currents | (60) |
| | Lewis rat (F) | Patient CSF | Intrahippocampal infusion | Not analyzed | Decreased NMDAR density in hippocampus | (60) |
| | C57BL/6 mouse | Patient sera | Intraventricular injection | Not analyzed | IgG deposition in hippocampus; more seizures and higher seizure scores after pro-convulsant challenge; no change in total NMDAR number | (61) |
| | C57BL/6N ($ApoE^{-/-}$) mouse (M) | Patient sera | Intravenous injection | All autoantibody isotypes affect behavioral assessments and endocytosis | Decreased spontaneous locomotion and increased MK-801-evoked locomotion in $ApoE^{-/-}$ mice, but not WT, treated with autoantibody; increased endocytosis by cultured neurons after autoantibody treatment | (62) |
| | C57BL/6J mouse | Patient CSF | Intraventricular infusion | Not analyzed | Reversible memory deficits, anhedonia, and depressive-like behavior without locomotor impairment; hippocampal IgG deposition; decreased NMDAR densityin hippocampus | (63) |
| Stiff person syndrome/ cerebellar ataxia | Wistar rat (M) | Patient sera | Intracerebellar infusion | Not analyzed | Decreased potentiation from excitatory stimulus trains; decreased NMDA-mediated NO synthesis | (64) |
| | Wistar rat (M) | Patient sera | Lumbar paraspinal injection | Not analyzed | Abnormal high baseline activity; increased excitability of anterior horn neurons | (64) |
| | Lewis rat (F) | Patient sera | Intrathecal infusion | Not analyzed | Recapitulation of paralysis; autoantibody-mediated internalization of amphiphysin on GABAergic neurons; decreased GABA release from cultured neurons; increased IPSC frequency and amplitude recorded *in vivo* from hippocampal granule cells | (65) |
| | Lewis rat (F) | Patient CSF | Intrahippocampal injection | Not analyzed | No changes in evoked and spontaneous GABAergic transmission in CA1 neurons | (66) |
| | Cultured mouse hippocampal neurons | Patient sera | Bath application | Not analyzed | No changes in evoked and spontaneous GABAergic transmission in cultured hippocampal networks | (67) |




C

| | Subcutaneous | Intranasal |
|---|---|---|
| T cell phenotype | Th1 Macri et al., (59) | Th17 Dileepan et al. (55) |
| T cells in CNS | Few Macri et al., (59) | Many Dileepan et al. (55) |
| Microglial activation | Moderate Macri et al., (59) | Robust Dileepan et al. (55) |
| Glomerular synapses | ? | Degraded Dileepan et al. (55) |
| Anti-GAS titer | High, autoreactive Brimberg et al., (56); Lotan et al. (58) | ? |
| BBB permeability | Not assessed | High Dileepan et al. (55) |
| Behavioral outcomes | Perseveration, Motor deficits Yaddanapudi et al. (57); Brimberg et al., (56) | ? |

**FIGURE 1 | Comparison of mouse pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS)/Sydenham's chorea (SC) models. (A)** Schematic representing the initiation of the intranasal model, where mice receive live bacteria intranasally once a week for 5 weeks prior to sacrifice. **(B)** Subcutaneous GAS exposure involves adjuvant and antigen exposure three times, every 2 weeks, following an initial boost with intravenous pertussis toxin. **(C)** Comparison of immune, neural, and behavioral outcomes after each route of GAS exposure. Investigators have used either subcutaneous or intranasal routes to induce an immune response against *S. pyogenes* [Group A *Streptococcus* (GAS)] in efforts to understand the mechanisms underlying the behavioral and motor symptoms characteristic of PANDAS and SC patients. The former route necessitates opening the blood–brain barrier (BBB) artificially using *B. pertussis* toxin, whereas the latter features intranasal inhalation of live bacteria to trigger a Th17 response in nasal tissue that is directly communicated to the brain along the olfactory nerve.

perseverative behaviors, impaired pre-pulse inhibition, and reduced concentrations of serotonin in the prefrontal cortex as compared to controls (56, 57, 59, 70). Moreover, adoptive transfer of serum IgGs from *S. pyogenes*-immunized mice to naive recipient mice, or direct infusion of sera into rat brains, recapitulates some of the behavioral deficits in recipient rodents, whereas no effects were observed after adoptive transfer of IgG-depleted serum (57, 58). Histological examination of brain tissue revealed antibody deposition in the deep cerebellar nuclei and hippocampus in mice and the striatum, cortex, and thalamus in rats (56, 57, 70). Serum IgG isolated from immunized rodents recognizes both cerebellar targets and human D1/D2 dopamine receptors by either western blotting or ELISA (56, 57). There is a high variability among the mouse and rat

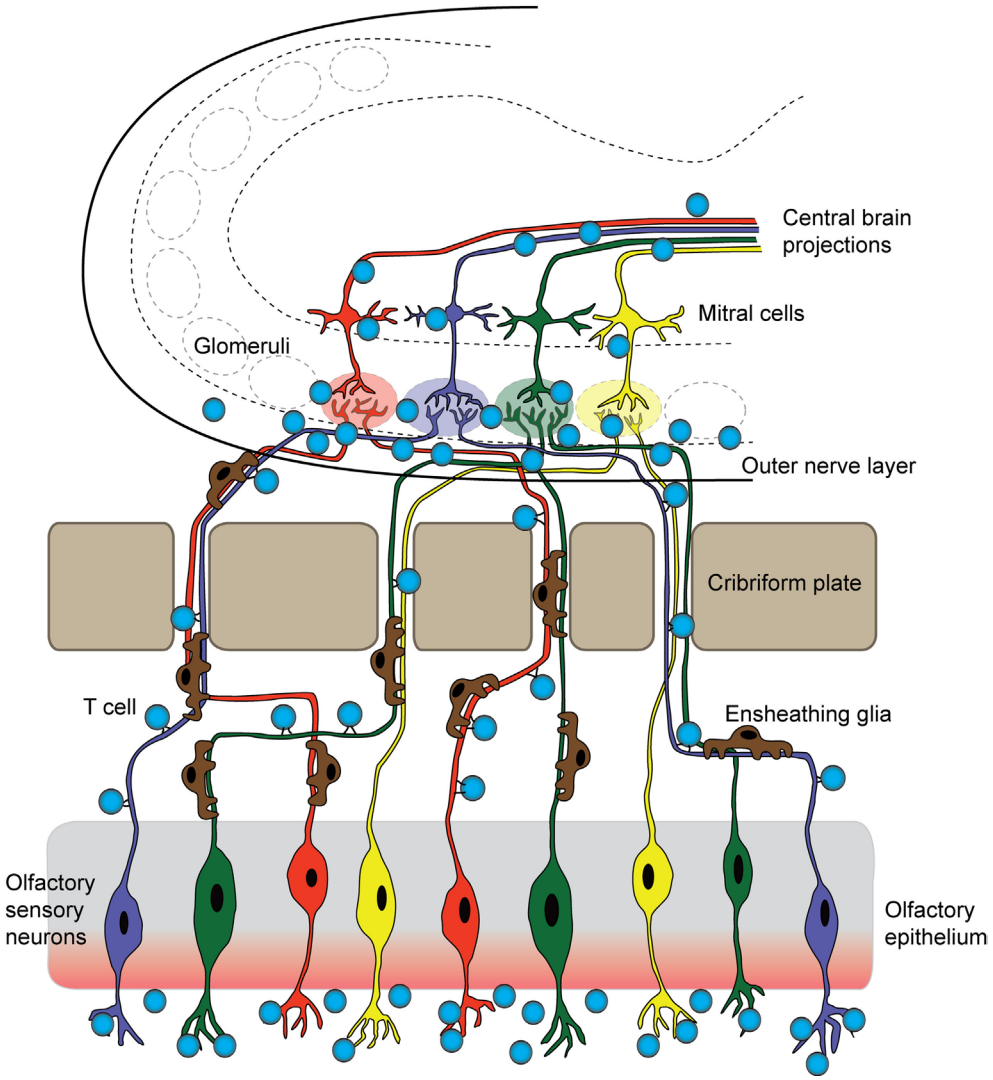



**FIGURE 2 | T cells originating in the nose infiltrate the brain parenchyma**. In a mouse model for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, T cells first arise in the nasal-associated lymphoid tissue and olfactory epithelium at the site of a latent *S. pyogenes* infection. These cells then respond to chemotactic cues release by olfactory ensheathing glia to accompany sensory axons into the brain. Once there, infiltrating T cells release inflammatory cytokines and chemokines, damaging synapses within olfactory glomeruli and breaking down tight junctions of olfactory bulb capillaries. These T cells may then move centrally, against the rostral migratory stream and toward the SVZ, and exit through the ventricles, or continue following the projections of olfactory mitral/tufted neurons.

BGE models. This may reflect differences in key immune-related genes between species, especially those related to T cell function or within the MHC locus for antigen presentation and immune cell stimulation. All studies used inbred animals (Lewis rats and SJL or C57BL/6 mice); therefore, intra-strain variability is low in these highly variable regions; however, differences between strains and species may be more pronounced. It is also possible that differences observed in mouse and rat studies are due to variability in their humoral immune response to GAS or differences in immunization protocols. Taken together, subcutaneous animal models for BGE have provided useful information regarding the humoral immune response after bacterial infection (i.e., the presence of antibodies directed against GAS and CNS) and demonstrate a clear link between *S. pyogenes* exposure and behavioral abnormalities (see **Figure 1C** for comparison of intranasal and subcutaneous models). However, these are somewhat artificial models for immune system activation, because human GAS infections occur primarily by the i.n. route. Moreover, subcutaneous animal models for BGE leave unanswered an important question for disease pathogenesis, namely how these autoantibodies can penetrate the CNS since the BBB is artificially opened (71).

## NMDA Receptor Encephalitis

Most animal models for NMDARE involve infusing serum antibodies from acutely ill patients into the rodent brain in an

attempt to replicate behavioral symptoms of the human disease. Infusion of autoantibodies from NMDARE patients into the lateral ventricles of mice causes impaired recognition memory after 10 days of infusion, which is reversible after antibody washout (63). Histologically, patient IgG binding is strongest in the hippocampus, which has a very high concentration of NMDARs, supporting the conclusion that internalization of NMDAR in this region may underlie memory deficits with minimal effects on aggression, locomotion, and anxiety-like behavior (61, 63). Intraventricular delivery of NMDAR antibodies also decreases seizure thresholds after administration of the convulsant pentylenetetrazol, resulting in more frequent and stronger seizures in NMDAR antibody-recipient mice (61). These data mirror the course of human disease, because NMDARE patients frequently present with seizures in conjunction with memory loss, hallucinations, and anxiety (72). Using the electroencephalograph (EEG), NMDARE patients show unusual delta rhythmic activity with superimposed beta or gamma activity (72). Similar spike events are also detectable using EEG during seizures in mice after NMDAR antibody infusion. Electrophysiological changes are also apparent in hippocampal neurons after intrahippocampal infusion of anti-NMDAR antibodies (73). Patient-derived NMDAR antibodies decrease NMDAR-dependent hippocampal long-term potentiation (LTP) in a manner similar to antibodies directed against extracellular domains of the NR2 or NR1 subunits of the NMDAR. After anti-NMDAR antibody treatment, dentate granule cells show smaller evoked responses to stimulation, increased spike thresholds after EPSP, impaired LTP, and decreased NMDAR densities in postsynaptic areas (60). In summary, there is strong evidence that antibody binding to the NMDAR leads to cross-linking and internalization but not necessarily destruction of the receptor, causing deficits in synaptic transmission that include LTP. NMDAR antibody deposition is strongest in the hippocampus after intraventricular infusion, indicating that deficits in perforant pathway may be the most prominent in this animal model for NMDARE. However, this model bypasses the BBB. In an elegant series of experiments, Hammer and colleagues showed that the BBB efficiently excluded NMDAR antibodies from the hippocampus if delivered intravenously (i.v.) in healthy mice. However, in *ApoE*$^{-/-}$ mice that have a defective BBB, intravenously injected anti-NMDAR antibodies can access brain antigens, and mutant mice injected i.v. with anti-NMDAR antibodies from patient sera show decreased locomotion as compared to those treated with control sera (62). Therefore, there is a clear need to develop animal models for NMDARE that incorporate mechanisms that disrupt BBB integrity to more closely mirror the human syndrome.

## Anti-GAD65 Antibodies

Autoantibodies against proteins involved in GABAergic transmission, including anti-glutamic acid decarboxylase (GAD) 65, anti-GAD67, and anti-amphiphysin, are the putative autoantibodies for cerebellar ataxia, stiff person syndrome, and Batten's disease (3, 74). Animal models using intraventricular infusion of anti-GAD65 antibodies isolated from patients have provided conflicting results. Bath application of such antibodies causes increased IPSP frequencies in cultured hippocampal neurons (75). Cerebellar infusion of anti-GAD65 antibodies in rodents also leads to reduced GABA synthesis in cerebellar basket cell terminals, resulting in disinhibition of Purkinje cells. However, hippocampal infusion of anti-GAD65 antibodies *in vivo* yields no changes in synaptic transmission assessed with physiological measurements in hippocampal slice preparations, whereas anti-NMDAR antibodies are sufficient to induce defective LTP (66). It is possible that anti-GAD65 antibodies do not gain access to their intracellular antigen after infusion in the hippocampus, or that pathogenic antibodies are in fact directed against a different antigen. Antibodies against amphiphysin rather than GAD65 have been shown to induce stiff-person syndrome-like symptoms in rats (65). Methodological differences and debate about pathogenicity of GAD65 antibodies has complicated the interpretation of these studies, because GAD65 antibodies are also prevalent, albeit at lower concentrations, in type 1 diabetic patients who have no neurological abnormalities. Careful screening of patient antibody samples is, therefore, necessary for anti-GAD65 collections, in order to exclude the likely non-pathogenic antibodies that are present in patients with diabetes.

## Anti-GQ1b Antibodies

There are few studies on the CNS effects of anti-GQ1b antibodies, the pathogenic basis for BBE, which hinders our understanding of this disease; however, there have been some reports on their binding location and disease mechanism. Anti-GQ1b antibodies were shown to induce complement deposition; the combination of antibody and complement is necessary to disrupt neuromuscular junction function *in vivo* (76). Binding of the antibody-complement complex induces local $Ca^{2+}$ flux into neurons *in vitro*, which in turn alters mitochondrial function and causes hydrogen peroxide production in addition to neuronal excitation (76). Mechanistically, this provides an interesting perspective on GBS and related syndromes, which lie at the intersection of innate and adaptive immune mechanisms. However, other than lesions identified by MRI in human MFS and BBE patients, there has been no investigation into how anti-GQ1b antibodies enter the CNS to affect brain function (77, 78).

## Anti-VGKC Complex Antibodies

The VGKC complex contains a signaling protein tetramer as well as several scaffolding proteins. Early identification of antigenic targets had attributed antigenicity to the potassium channel $K_v1.1/1.2$ itself, while more recent work argues that the antibody targets are more likely the associated scaffold proteins (LGI1, Caspr2, and contactin-2) that precipitated along with the channel during the original identification (79). Most investigation into VGKC antibodies has used rodent tissue or cultured cells to test for reactivity of patient antibodies that are harvested from CSF or serum. Sera from both AE and neuromyotonia patients react with rat (80) and mouse (81) hippocampal sections, as well as with the potassium channels $K_v1.1$ and $K_v1.2$ transfected into HeLa cells (80), but not with *Caspr2*$^{-/-}$ mouse hippocampal sections (81). One rodent study used adoptive transfer of patient plasma or purified IgG into mice to address how these antibodies alter neuronal transmission in peripheral nerves (82). After repeated injections with patient IgG, mice showed no clinical symptoms but

had increased quantal size and potassium-dependent increases in EPP amplitude in peripheral nerves as compared to control mice (82). While there has been much progress in identifying the target antigen of these antibodies, many questions about their pathogenicity and CNS access remain unanswered.

## POTENTIAL ROUTES OF ANTIBODY ENTRY INTO THE CNS

### Blood–Cerebrospinal Fluid Barrier (BCSFB)

Epithelial cells within the choroid plexus form a tight barrier termed the BCSFB that restricts diffusion of serum proteins and immune cells from the more leaky blood vessels of this tissue into the CSF (83). The molecular composition of TJs within the BSCFB is less well understood than those comprising the BBB. However, several key junctional proteins that regulate paracellular permeability across epithelial cells of the choroid plexus are present at high levels in both embryonic and adult stages, suggesting that this barrier matures early during development (84). The critical function of these junctions is to create a physical barrier to paracellular diffusion, allowing cells to become polarized with distinct luminal and abluminal components. In addition, epithelial cells of the BCSFB also express specialized transporter proteins to allow transit of certain plasma proteins across this barrier (84, 85). The BCSFB is less tightly regulated than its brain counterpart and can become more permeable to immune cells or antibodies during disease states. Th17 lymphocytes cross the BCSFB several days prior to BBB damage, in order to enter the CNS and initiate their immune attack during experimental autoimmune encephalitis (EAE), a mouse model for human multiple sclerosis (86). This entry appears to be an essential step in initiating the CNS immune response, because CCR6 receptor-deficient Th17 cells that cannot cross the BCSFB do not induce EAE (86). In mouse models for SLE, the cell adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) show elevated expression in the choroid epithelium and promote large amounts of cellular infiltrates (T and B cells) in the choroid (87, 88). The role of such infiltrating immune cells is still debated, but the BCSFB epithelium is clearly activated and disrupted by increased cytokine expression (87–90). However, the role of BSCFB during AE remains unexplored. It is possible that either B cells or antibodies may cross this barrier more efficiently than the BBB to enter the CSF, but data from either *in vitro* studies or animal models are currently lacking (**Figure 3A**).

### Olfactory Route

The proximity of the OB to the nasal mucosa makes it both a vulnerable niche to insult and infection of the brain and an attractive option for delivery of therapeutics that cannot otherwise traverse the BBB (91, 92). Many viruses and bacteria co-opt this olfactory route for brain infection, and more than 40 substances have been shown to enter the brain by this means (93, 94). Viruses, such as Venezuelan equine encephalitis virus, initially infiltrate the CNS *via* olfactory axons, then induce BBB permeability throughout the brain to promote a second wave of infection, as virion particles enter the brain from the circulation *via* the damaged barrier (95). The neurotrophic Nipah virus also infects hamster olfactory sensory axons, travels into the OB, and disseminates from olfactory processing centers like the olfactory tubercle before spreading throughout the brain (96). After an initial injury to nasal mucosa, *S. aureus* infects olfactory sensory neurons and spreads to the OB *via* the olfactory axons within 6 h (97). In addition to intranasal pathogens, the olfactory route has been successfully used to deliver drugs, large proteins, and stem cells into the brain (91), largely due to rapid diffusion *via* cerebral perivascular spaces (98). Why is the olfactory route so amenable for CNS delivery? This is due to several factors: (1) diffusion across the heavily vascularized nasal mucosa that also contains lymphatic vessels, (2) direct transport by olfactory sensory axons into the OB, and (3) direct transport by the trigeminal nerve into the brainstem. Although the nasal mucosa is highly vascularized, the mean capillary density and relative permeability to hydrophilic macromolecule tracers is significantly greater in the respiratory epithelium of the nose than in olfactory sensory regions (99). Thus, sensory regions of the nasal mucosa may provide easier access to the brain due to their relatively slower clearance rates into the bloodstream (99). We have recently shown that GAS-specific Th17 lymphocytes and other T cell subtypes generated in the olfactory cavity use the sensory axon route to enter the CNS in an animal model for BGE (**Figures 2** and **3B**). Because T lymphocytes are primarily found in the outer nerve and glomerular layers within the OB, where incoming sensory axons form synaptic connections with projection neurons, we propose that T cells travel along the sensory axon route into the CNS (55). However, it is currently unclear whether GAS-specific T cells passively move into the brain along these sensory axons or are actively recruited into the brain by resident antigen-processing macrophages, microglia, or the olfactory ensheathing glia, the last of which are known to phagocytose bacterial debris. Olfactory ensheathing glia guide olfactory sensory axons toward their targets in the OB and thus may also serve this role for GAS-specific T cells. Once in the brain, Th17 cells likely persist due to high levels of CCR6 and LFA-1 signals, which are required for $CD4^+$ T cells to remain in the CNS in the EAE model. The olfactory route is also used by immune cells to exit the CNS through the OB and drain into the deep cervical lymph nodes, in order to dampen anti-CNS immune responses in the periphery (100, 101). There are, however, no data on whether antibodies use the olfactory route to gain access to the CNS from sites of infection in the olfactory mucosa, especially for BGE associated with i.n. GAS infections.

### The BBB in Healthy and Inflamed CNS

The BBB achieves its selective permeability to proteins and immune cells by the presence of (1) TJs that prevent paracellular diffusion of small molecules and immune cells between endothelial cells, (2) very few endocytotic vesicles that restrict movement of large molecules through the transcellular pathway, and (3) transporters that shuttle select nutrients between the blood and the brain (**Figure 3C**) (102). The junctional transmembrane proteins claudin-3, -5, -12, and occludin are expressed at the barrier and interact in a homotypic manner to form paracellular pores that restrict diffusion between cells (**Figure 3C**) (102). Endocytotic caveolae in the CNS endothelium provide an essential route

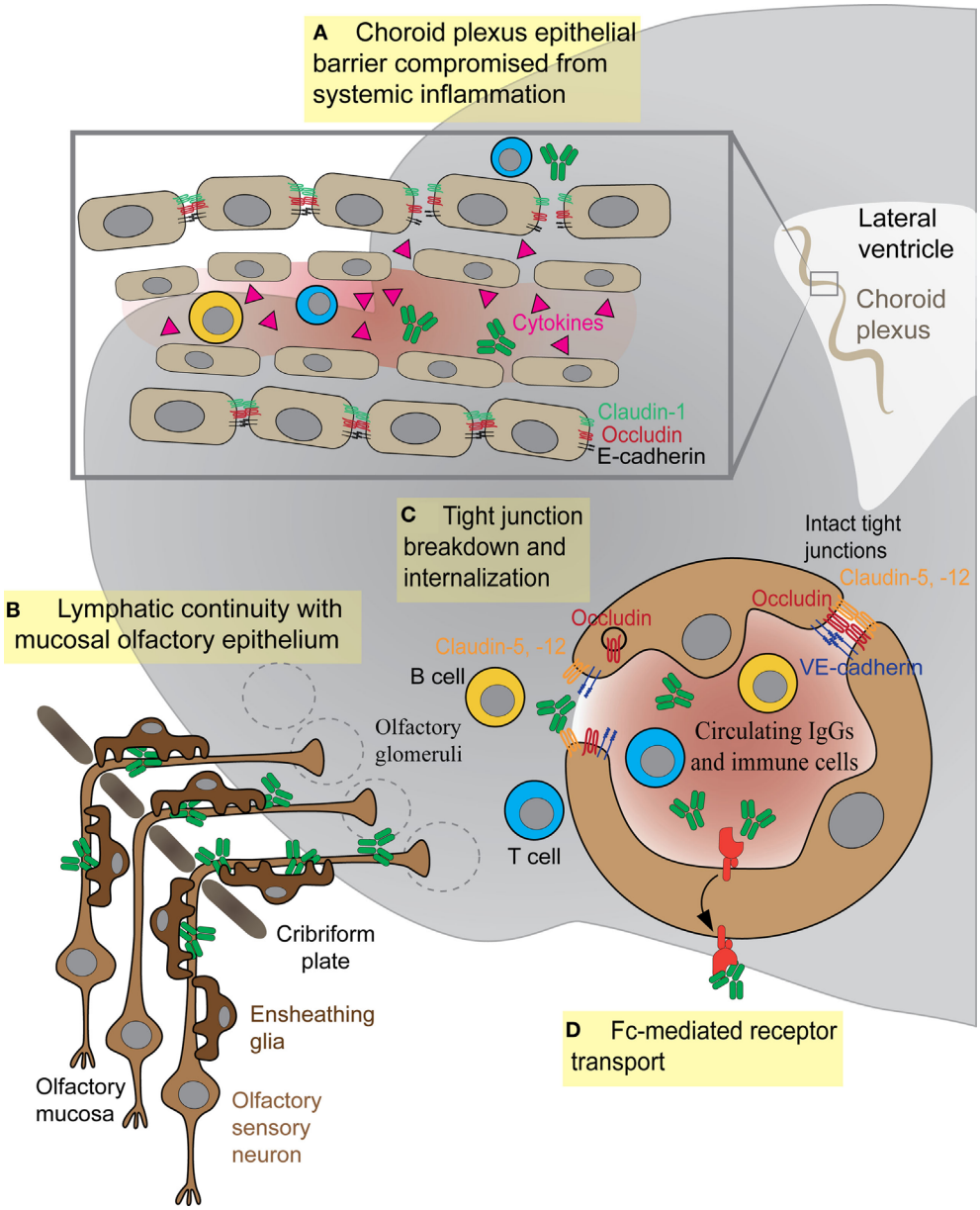



**FIGURE 3 | Antibody and immune cell access to the brain parenchyma *via* four distinct routes**. **(A)** Systemic cytokines break down tight junctions (TJs) within the brain–cerebrospinal fluid barrier to allow central nervous system (CNS) access of antibodies or immune cells. **(B)** Olfactory ensheathing glia facilitate transport of IgGs or immune cells along sensory axons exiting the olfactory mucosa. **(C)** Inflammatory cytokines in the bloodstream damage TJs between endothelial cells, thus allowing antibodies or immune cells (T or B cells) to enter the CNS. **(D)** $F_c$ receptor directionality reverses, shuttling IgG from vessels into brain parenchyma as in systemic lupus erythematosus.

for receptor-mediated transcytosis (102). This process requires caveolin-1 (Cav-1), a transmembrane protein expressed at low levels within CNS blood vessels. Cav-1 levels increase during BBB breakdown following ischemic stroke, when enhanced transcytosis initiates BBB dysfunction (103). Healthy BBB vasculature also has low levels of leukocyte adhesion molecules such as VCAM-1, ICAM-1, and ICAM-2, which are upregulated on CNS vessels during neuroinflammation to promote T lymphocyte trafficking into the parenchyma (104, 105).

Just as capillaries in the CNS possess unique characteristics as compared to systemic capillaries, the BBB may be differentially porous throughout the CNS. Several studies have suggested that endothelial barriers in the brain and spinal cord are functionally different. Blood–brain and blood–spinal cord barriers differ in expression of some BBB-specific proteins and their functional permeability. Endothelial cells in the spinal cord have decreased expression of adherens junction (VE-cadherin; β-catenin) and TJ (occludin, ZO-1) proteins, as well as increased permeability to

small molecular weight tracers, compared to brain endothelium (106). Endothelial cells in these two CNS regions also differ in their affinities for immune cells. Encephalitogenic T cells in the cervical spinal cord rapidly arrest on the vasculature without crawling, whereas they do not display this behavior on brain blood vessels (104). Higher expression of sphingosine 1-phosphate receptors in spinal cord ECs may facilitate preferential immune cell infiltration as compared to other CNS regions during EAE (107). Within the CNS, blood vessels in the OB and neurogenic niches also have a relatively higher permeability as compared to other CNS regions (excepting the circumventricular organs that have leaky blood vessels); blood vessels in these regions become highly permeable, in particular following viral infections (108–110), and may predispose them to infiltration of immune cells or antibodies in addition to viral particles.

Blood–brain barrier function can be impaired by several factors including inflammatory cytokines, hormones (e.g., epinephrine), and substances of abuse (e.g., alcohol, cocaine, and methamphetamine) (31, 102, 111). Several inflammatory cytokines including IL-1β, TNF-α, CCL-2, and IL-17A, which are present in the CNS or blood during neuroinflammation, affect the stability of the BBB by either degrading TJ proteins, modifying their phosphorylation states, or affecting the turnover rate (**Figure 3C**). IL-1β indirectly destabilizes the BBB by inducing expression of matrix metalloproteinase-9 and VEGF, two factors that promote degradation of Claudin-5, Occludin, and ZO-1 (112, 113). TNF-α also induces formation of gaps between cell junctions by upregulating transcription of NF-κB and myosin light chain kinase, a factor known to internalize TJ proteins *via* caveolae (114, 115). The chemokine CCL-2 produced by macrophages also enhances BBB permeability by inducing phosphorylation of occludin and claudin-5 and promoting their endocytosis *via* caveolae (116). IL-17 and IL-22 produced by Th17 cells in EAE/MS are also known to disrupt endothelial cell TJs (117). Although these cytokines have been shown to disrupt barrier function during neuroinflammation, it is unclear whether they are present in AE. Recent cytokine profiling of CSF from patients with viral and autoimmune encephalitis revealed that several Th1 cytokines, such TNF-α, IFN-γ, CXCL9, and CXCL10, are elevated during viral encephalitis, but not in AE samples. In contrast, IL-6, a cytokine essential for development of Th17 cells, is elevated in all CNS autoimmune disorders (11). The predominant mechanism for BBB disruption in our animal model for *S. pyogenes*-induced BGE appears to be disruption of endothelial TJs *via* formation of gaps and protrusions that enhance BBB permeability and promote entry of autoantibodies into the CNS (55). Although we cannot exclude other factors such as cytokines released by activated microglia/macrophages that are present in the CNS after multiple i.n. GAS infections (55), or the ability of GAS antibodies to break down endothelial cell junctions, the high degree of correlation between the presence of bacterial-specific Th17 cells and BBB leakage suggests that IL-17 likely contributes to BBB dysfunction in a similar fashion as in EAE/MS (55, 115). *S. pyogenes*-specific Th17 cells are present in the tonsils of human patients (55); however, it remains to be shown whether they are important for human disease pathogenesis.

B cells can arrest along endothelial cell walls using the same adhesion mechanisms as T cells (VLA-4- and ICAM-1-mediated arrest and adhesion). In addition, B cells can infiltrate into the CNS, by extravasation through disrupted TJs, reminiscent of T cell entry across the BBB (118–120). However, the number of B cells that has been reported to present in the CNS following histological analyses of patients with GBS, BBE, MFS, or any of the animal models of AE discussed above is indeed extremely small compared to the number of T cells (77). B cells can infiltrate the inflamed CNS in MS and SLE patients and form ectopic lymphoid structures, or cellular aggregates outside germinal centers, which reinforce the immune response locally (90, 121–123). In MS, B cells within cellular aggregates present antigens to T cells, contribute to epitope spreading, and produce antibodies that are detectable as oligoclonal bands in the CSF collected from patients (123). However, studies using mouse models of SLE have produced confounding results as to whether B cells that are resident within the CNS secrete autoantibodies (89, 124). In addition, there is scant evidence for B cell infiltration during AE. It is possible that these diseases are mediated by locally infiltrating B or plasma cells (125), but firm evidence for CNS-resident B cell populations is still lacking.

Endothelial cells are also highly vulnerable to endotoxins secreted by infectious agents. Both antigenic surface proteins on Gram-positive bacteria and lipopolysaccharide from Gram-negative bacteria change endothelial barrier properties (126). Environmental toxins and food additives can also increase BBB permeability, as evidenced by serum protein leakage after bis(tributyltin) oxide exposure (127). Finally, the gut microbiome may also affect the function of the barrier (128). Given the presence of many anti-neuronal autoantibodies in circulation, transient BBB permeability from any of these sources, or reactivated $F_c$ receptor-mediated transcytosis through blood vessels (**Figure 3D**), would provide opportunistic access to brain targets. Pathological antibodies may also enhance BBB permeability, promote production of inflammatory cytokines, and stimulate adhesion and migration of immune cells across the CNS. For example, anti-NR2 glutamate receptor antibodies (anti-NR2) derived from patients with SLE promote expression of both VCAM-1 and ICAM-1 and increase the production of IL-6 in brain endothelium that promotes BBB inflammation and changes to its permeability (129). Antibacterial antibodies can also change endothelial barrier permeability; however it is unknown whether this mechanism mediates antibody entry into the CNS during AE. Furthermore, rare cases of GBS with CNS lesions provide insight into how the same bacterial infection may cause central as opposed to peripheral autoimmunity (77, 78). CNS lesions in the brain (77, 78) and spinal cord (77), as well as CSF pleiocytosis or excess protein (130), together with the presence of immune cell infiltration in both CNS regions (77) indicate immune involvement in GBS infections of the CNS. Active forebrain lesions in the patient described by Okumura are compelling evidence for a BBB breach (78). Cytokine-producing $CD4^+$ and $CD8^+$ immune cells in the CNS were mostly confined to dense clusters in the meninges and perivascular cuffs, but a population of $CD4^+$ and $CD8^+$ T cells were found in the brainstem with a very few B cells intermixed in perivascular immune

cell infiltrates (77). Therefore, brain lesions or T cell infiltration into perivascular spaces may be important steps in breaching the BBB and thereby providing antibody access to the brainstem, for certain GBS subtypes.

## TREATMENT OF AE SYNDROMES

The treatments currently employed for BGE focus on immunotherapy to weaken the autoimmune response. Screening for cancer is essential in ruling out a neoplastic syndrome; if a tumor is present, surgical interventions are effective in alleviating symptoms for 75% of NMDAR encephalitis cases. In cases without a tumor, first-line treatment with immunotherapy using high doses of corticosteroids, plasma exchange, and/or IVIg improves patient outcomes in two-thirds of cases. Cases that do not respond to primary immunotherapy receive treatment with Rituximab (a monoclonal antibody that neutralizes B cells, thereby halting antibody production) or cyclophosphamide (a potent immunosuppressant) (72). EEG monitoring and antiepileptic treatments are frequently necessary in AE; seizures are common with encephalitides of autoimmune and infectious origin (13) and may trigger BBB leakage due to release of the potent inflammatory cytokines IL-1 and TNF-α from dying neurons. Controlling further seizures is paramount to stave off permanent damage, especially for pediatric patients.

Encephalitis with an infectious trigger such as *S. pyogenes* warrants primary treatment with antibiotics to eliminate the latent infection (131). First-line treatment for SC or PANDAS, both of which are associated with streptococcal infections, centers on eliminating autoantibodies from circulation by means of plasma exchange or plasma apheresis, coupled with corticosteroid treatment. Plasma exchange shows promise as a treatment option, but IVIg has only a minor effect (29, 30). A recent double-blind clinical study of IVIg for 35 PANDAS patients found no improvement compared to placebo after two rounds of IVIg (132). Plasmapheresis is a beneficial, if invasive, treatment option, with an average improvement of 65%, 6 months after treatment (30). Indeed, many immunotherapies recommended for AE require several weeks or months to show effectiveness.

Outcomes are generally good in younger patients, including children. Young adult and pediatric autoimmune encephalitis patients have a recovery rate of up to 80%, although improvements continue slowly for up to 2 years. In patients who recovered from NMDAR encephalitis, the relapse rate is around 25%, so yearly screening for tumors is recommended after recovery (133). After surgical excision of tumors, if warranted, following up by treatment with immunotherapy leads to generally good outcomes for NMDAR encephalitis. PANDAS patients typically also recover after treatment and learn to manage their exposure to *S. pyogenes*. Some patients continue prophylactic antibiotics to minimize such exposure, sometimes for several years after their most recent relapse.

For any type of AE, studying BBB function during both disease initiation and periods of remission would greatly clarify the role of the barrier over the course of disease and help inform treatment options. MRI with gadolinium enhancement is a standard tool for MS diagnosis and disease monitoring (102, 134) and is a sensitive diagnostic tool for GBS. Based on our studies on the animal model for *S. pyogenes*-induced BGE, we argue that MRI with gadolinium enhancement may reveal latent BBB damage in AE patients or foci of BBB damage (135–137). Our working hypothesis that barrier breakdown is a crucial step during AE pathogenesis would, therefore, be strengthened by evidence from patient MRI with gadolinium enhancement or careful analysis of BBB integrity using intravenous fluorescently labeled low- or high-molecular-weight tracers in animal models for other types of AE (55).

Future treatment avenues may rely on modulating BBB permeability using biological or chemical therapeutics. One exciting option is reactivation of signaling pathways that normally function to form the BBB during development, in order to repair the dysfunctional barrier during disease. The barrier properties of CNS vessels develop prenatally in most CNS regions for both mice and humans with the exception of the retina, which vascularizes postnatally in mice. Wnt/β-catenin signaling promotes both CNS angiogenesis and BBB formation by production of Wnts from neural progenitors (138–142). Wnts also induce expression of some EC-specific proteins, including the glucose transporter Glut-1 and several TJ components (138–142). Following angiogenesis, Hedgehog signaling is required for acquisition of barrier properties in CNS ECs, including expression of TJ proteins occludin and claudin-5 (142, 143). Mature blood vessels continue to respond to Wnt and Shh cues, indicating that these pathways are active in maintaining EC barrier properties (139, 142, 143). Recently, Wnt signaling was reported to be upregulated in both EAE and human MS lesions, correlating with increased neuronal Wnt3 expression and TJ breakdown. Reactivation of Wnt signaling in EAE may serve to repair the damaged BBB in inflammation. Inhibition of Wnt signaling hastened disease progression and resulted in increased numbers of $CD4^+$ T cells in the CNS (105). Chemical modulators of Wnt signaling have been validated *in vitro* and in rodent models of neuroinflammation (144, 145). Translation of such therapies to clinical use could repair the damaged barrier, translating to improved clinical outcomes in AE patients by limiting influx of blood-borne immune cells, cytokines, and/or antibodies.

## CONCLUSION

Autoimmunity in the CNS remains confoundingly complex, in both etiology and treatment. While triggers for AE are defined in some cases, frequently, no clear infectious or cancerous cause can be found. BBB integrity clearly plays a role in disease development, and more research on differential barrier permeability over the course of disease would resolve many questions about autoantibody entry and pathogenesis. Animal models for these diseases have the potential to uncover new routes of antibody entry that are not apparent from patient imaging studies. Indeed, infectious triggers themselves may have the inherent ability to disrupt the BBB to allow access to brain antigens. By approaching AE from several angles, a more complete picture of pathogenesis,

contributions to symptom exacerbations, and recommended treatment options will emerge.

## AUTHOR CONTRIBUTIONS

MP, DA, and TC conceived of the topic and wrote the manuscript, and MP prepared figures and figure legends.

## ACKNOWLEDGMENTS

The authors thank Holly and Mark Kerslake from Newport Equities LLC for their generous financial support that made much of our work on the animal model for *S. pyogenes*-induced BGE possible.

## FUNDING

MP, DA, and TC are supported by grants from the NIH (NHLBI R01 HL116995-01, NIMH R56 MH109987-01A1, and NCATS CTSA CaMPR-BASIC at CUMC) and the International OCD Foundation. DA is partially supported by an unrestricted gift from John. F. Castle to the Stroke Division of the Department of Neurology at Columbia University Medical Center.

## REFERENCES

1. van Coevorden-Hameete MH, de Graaff E, Titulaer MJ, Hoogenraad CC, Sillevis Smitt PA. Molecular and cellular mechanisms underlying anti-neuronal antibody mediated disorders of the central nervous system. *Autoimmun Rev* (2014) 13(3):299–312. doi:10.1016/j.autrev.2013.10.016
2. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann N Y Acad Sci* (2015) 1338:94–114. doi:10.1111/nyas.12553
3. Sinmaz N, Amatoury M, Merheb V, Ramanathan S, Dale RC, Brilot F. Autoantibodies in movement and psychiatric disorders: updated concepts in detection methods, pathogenicity, and CNS entry. *Ann N Y Acad Sci* (2015) 1351(1):22–38. doi:10.1111/nyas.12764
4. Brimberg L, Mader S, Fujieda Y, Arinuma Y, Kowal C, Volpe BT, et al. Antibodies as mediators of brain pathology. *Trends Immunol* (2015) 36(11):709–24. doi:10.1016/j.it.2015.09.008
5. Dubey D, Sawhney A, Greenberg B, Lowden A, Warnack W, Khemani P, et al. The spectrum of autoimmune encephalopathies. *J Neuroimmunol* (2015) 287:93–7. doi:10.1016/j.jneuroim.2015.08.014
6. Swedo SE. Sydenham's chorea. A model for childhood autoimmune neuropsychiatric disorders. *JAMA* (1994) 272(22):1788–91. doi:10.1001/jama.272.22.1788
7. Church AJ, Cardoso F, Dale RC, Lees AJ, Thompson EJ, Giovannoni G. Anti-basal ganglia antibodies in acute and persistent Sydenham's chorea. *Neurology* (2002) 59(2):227–31. doi:10.1212/WNL.59.2.227
8. Cunningham MW. *Streptococcus* and rheumatic fever. *Curr Opin Rheumatol* (2012) 24(4):408–16. doi:10.1097/BOR.0b013e32835461d3
9. Venkatesan A, Benavides DR. Autoimmune encephalitis and its relation to infection. *Curr Neurol Neurosci Rep* (2015) 15(3):3. doi:10.1007/s11910-015-0529-1
10. Balint B, Bhatia KP. Stiff person syndrome and other immune-mediated movement disorders – new insights. *Curr Opin Neurol* (2016) 29(4):496–506. doi:10.1097/WCO.0000000000000351
11. Kothur K, Wienholt L, Brilot F, Dale RC. CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: a systematic review. *Cytokine* (2016) 77:227–37. doi:10.1016/j.cyto.2015.10.001
12. Selmi C, Barin JG, Rose NR. Current trends in autoimmunity and the nervous system. *J Autoimmun* (2016) 75:20–9. doi:10.1016/j.jaut.2016.08.005
13. Vezzani A, Fujinami RS, White HS, Preux PM, Blumcke I, Sander JW, et al. Infections, inflammation and epilepsy. *Acta Neuropathol* (2016) 131(2):211–34. doi:10.1007/s00401-015-1481-5
14. Becher B, Spath S, Goverman J. Cytokine networks in neuroinflammation. *Nat Rev Immunol* (2017) 17(1):49–59. doi:10.1038/nri.2016.123
15. Prinz M, Priller J. The role of peripheral immune cells in the CNS in steady state and disease. *Nat Neurosci* (2017) 20(2):136–44. doi:10.1038/nn.4475
16. Dale RC, Brilot F. Autoimmune basal ganglia disorders. *J Child Neurol* (2012) 27(11):1470–81. doi:10.1177/0883073812451327
17. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat Rev Immunol* (2012) 12(9):623–35. doi:10.1038/nri3265
18. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The glymphatic system: a beginner's guide. *Neurochem Res* (2015) 40(12):2583–99. doi:10.1007/s11064-015-1581-6
19. Louveau A, Harris TH, Kipnis J. Revisiting the mechanisms of CNS immune privilege. *Trends Immunol* (2015) 36(10):569–77. doi:10.1016/j.it.2015.08.006
20. Cunningham MW. Rheumatic fever, autoimmunity, and molecular mimicry: the streptococcal connection. *Int Rev Immunol* (2014) 33(4):314–29. doi:10.3109/08830185.2014.917411
21. Swedo SE. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). *Mol Psychiatry* (2002) 7(Suppl 2):S24–5. doi:10.1038/sj.mp.4001170
22. Williams KA, Swedo SE. Post-infectious autoimmune disorders: Sydenham's chorea, PANDAS and beyond. *Brain Res* (2015) 1617:144–54. doi:10.1016/j.brainres.2014.09.071
23. Murphy TK, Snider LA, Mutch PJ, Harden E, Zaytoun A, Edge PJ, et al. Relationship of movements and behaviors to Group A *Streptococcus* infections in elementary school children. *Biol Psychiatry* (2007) 61(3):279–84. doi:10.1016/j.biopsych.2006.08.031
24. Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nat Med* (2003) 9(7):914–20. doi:10.1038/nm892
25. Kirvan CA, Swedo SE, Snider LA, Cunningham MW. Antibody-mediated neuronal cell signaling in behavior and movement disorders. *J Neuroimmunol* (2006) 179(1–2):173–9. doi:10.1016/j.jneuroim.2006.06.017
26. Murphy TK, Kurlan R, Leckman J. The immunobiology of Tourette's disorder, pediatric autoimmune neuropsychiatric disorders associated with *Streptococcus*, and related disorders: a way forward. *J Child Adolesc Psychopharmacol* (2010) 20(4):317–31. doi:10.1089/cap.2010.0043
27. Ben-Pazi H, Stoner JA, Cunningham MW. Dopamine receptor autoantibodies correlate with symptoms in Sydenham's chorea. *PLoS One* (2013) 8(9):e73516. doi:10.1371/journal.pone.0073516
28. Cox CJ, Zuccolo AJ, Edwards EV, Mascaro-Blanco A, Alvarez K, Stoner J, et al. Antineuronal antibodies in a heterogeneous group of youth and young adults with tics and obsessive-compulsive disorder. *J Child Adolesc Psychopharmacol* (2015) 25(1):76–85. doi:10.1089/cap.2014.0048
29. Kovacevic M, Grant P, Swedo SE. Use of intravenous immunoglobulin in the treatment of twelve youths with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *J Child Adolesc Psychopharmacol* (2015) 25(1):65–9. doi:10.1089/cap.2014.0067
30. Latimer ME, L'Etoile N, Seidlitz J, Swedo SE. Therapeutic plasma apheresis as a treatment for 35 severely ill children and adolescents with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *J Child Adolesc Psychopharmacol* (2015) 25(1):70–5. doi:10.1089/cap.2014.0080
31. Diamond B, Honig G, Mader S, Brimberg L, Volpe BT. Brain-reactive antibodies and disease. *Annu Rev Immunol* (2013) 31:345–85. doi:10.1146/annurev-immunol-020711-075041
32. Sauteur PM, Hackenberg A, Tio-Gillen AP, van Rossum AM, Berger C, Jacobs BC. Intrathecal anti-GalC antibodies in bickerstaff brain stem encephalitis. *Neuropediatrics* (2015) 46(6):428–30. doi:10.1055/s-0035-1566730
33. Vergori A, Masi G, Donati D, Ginanneschi F, Annunziata P, Cerase A, et al. Listeria meningoencephalitis and anti-GQ1b antibody syndrome. *Infection* (2016) 44(4):543–6. doi:10.1007/s15010-015-0862-y

34. Fisher M. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). *N Engl J Med* (1956) 255(2):57–65. doi:10.1056/NEJM195607122550201
35. Bickerstaff ER. Brain-stem encephalitis; further observations on a grave syndrome with benign prognosis. *Br Med J* (1957) 1(5032):1384–7. doi:10.1136/bmj.1.5032.1384
36. Al-Din AN, Anderson M, Bickerstaff ER, Harvey I. Brainstem encephalitis and the syndrome of Miller Fisher: a clinical study. *Brain* (1982) 105(Pt 3): 481–95. doi:10.1093/brain/105.3.481
37. Chiba A, Kusunoki S, Shimizu T, Kanazawa I. Serum IgG antibody to ganglioside GQ1b is a possible marker of Miller Fisher syndrome. *Ann Neurol* (1992) 31(6):677–9. doi:10.1002/ana.410310619
38. Yuki N, Yamada M, Sato S, Ohama E, Kawase Y, Ikuta F, et al. Association of IgG anti-GD1a antibody with severe Guillain-Barre syndrome. *Muscle Nerve* (1993) 16(6):642–7. doi:10.1002/mus.880160610
39. Sano Y, Kanda T. Isolation and properties of endothelial cells forming the blood-nerve barrier. *Methods Mol Biol* (2011) 686:417–25. doi:10.1007/978-1-60761-938-3_21
40. Kanda T. Biology of the blood-nerve barrier and its alteration in immune mediated neuropathies. *J Neurol Neurosurg Psychiatry* (2013) 84(2):208–12. doi:10.1136/jnnp-2012-302312
41. Peltonen S, Alanne M, Peltonen J. Barriers of the peripheral nerve. *Tissue Barriers* (2013) 1(3):e24956. doi:10.4161/tisb.24956
42. Shimizu F, Omoto M, Sano Y, Mastui N, Miyashiro A, Tasaki A, et al. Sera from patients with multifocal motor neuropathy disrupt the blood-nerve barrier. *J Neurol Neurosurg Psychiatry* (2014) 85(5):526–37. doi:10.1136/jnnp-2013-305405
43. Arias I, Sorlozano A, Villegas E, de Dios Luna J, McKenney K, Cervilla J, et al. Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr Res* (2012) 136(1–3):128–36. doi:10.1016/j.schres.2011.10.026
44. Hornig M. The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. *Curr Opin Rheumatol* (2013) 25(4):488–795. doi:10.1097/BOR.0b013e32836208de
45. Goldmann T, Prinz M. Role of microglia in CNS autoimmunity. *Clin Dev Immunol* (2013) 2013:208093. doi:10.1155/2013/208093
46. Graus F, Dalmau J. Paraneoplastic neurological syndromes: diagnosis and treatment. *Curr Opin Neurol* (2007) 20(6):732–7. doi:10.1097/WCO.0b013e3282f189dc
47. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* (2011) 10(1):63–74. doi:10.1016/S1474-4422(10)70253-2
48. Bataller L, Valero C, Diaz R, Froufe A, Garcia-Zarza A, Ribalta T, et al. Cerebellar ataxia associated with neuroendocrine thymic carcinoma and GAD antibodies. *J Neurol Neurosurg Psychiatry* (2009) 80(6):696–7. doi:10.1136/jnnp.2008.161042
49. Arino H, Hoftberger R, Gresa-Arribas N, Martinez-Hernandez E, Armangue T, Kruer MC, et al. Paraneoplastic neurological syndromes and glutamic acid decarboxylase antibodies. *JAMA Neurol* (2015) 72(8):874–81. doi:10.1001/jamaneurol.2015.0749
50. Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* (2004) 127 (Pt 3):701–12. doi:10.1093/brain/awh077
51. Pinatel D, Hivert B, Boucraut J, Saint-Martin M, Rogemond V, Zoupi L, et al. Inhibitory axons are targeted in hippocampal cell culture by anti-Caspr2 autoantibodies associated with limbic encephalitis. *Front Cell Neurosci* (2015) 9:265. doi:10.3389/fncel.2015.00265
52. Tan KM, Lennon VA, Klein CJ, Boeve BF, Pittock SJ. Clinical spectrum of voltage-gated potassium channel autoimmunity. *Neurology* (2008) 70(20):1883–90. doi:10.1212/01.wnl.0000312275.04260.a0
53. Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, Mazia C, et al. Morvan syndrome: clinical and serological observations in 29 cases. *Ann Neurol* (2012) 72(2):241–55. doi:10.1002/ana.23577
54. Vernino S, Lennon VA. Autoantibody profiles and neurological correlations of thymoma. *Clin Cancer Res* (2004) 10(21):7270–5. doi:10.1158/1078-0432.CCR-04-0735
55. Dileepan T, Smith ED, Knowland D, Hsu M, Platt M, Bittner-Eddy P, et al. Group A *Streptococcus* intranasal infection promotes CNS infiltration by streptococcal-specific Th17 cells. *J Clin Invest* (2016) 126(1):303–17. doi:10.1172/JCI80792
56. Brimberg L, Benhar I, Mascaro-Blanco A, Alvarez K, Lotan D, Winter C, et al. Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model of Sydenham chorea and related neuropsychiatric disorders. *Neuropsychopharmacology* (2012) 37(9):2076–87. doi:10.1038/npp.2012.56
57. Yaddanapudi K, Hornig M, Serge R, De Miranda J, Baghban A, Villar G, et al. Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. *Mol Psychiatry* (2010) 15(7):712–26. doi:10.1038/mp.2009.77
58. Lotan D, Benhar I, Alvarez K, Mascaro-Blanco A, Brimberg L, Frenkel D, et al. Behavioral and neural effects of intra-striatal infusion of anti-streptococcal antibodies in rats. *Brain Behav Immun* (2014) 38:249–62. doi:10.1016/j.bbi.2014.02.009
59. Macri S, Ceci C, Onori MP, Invernizzi RW, Bartolini E, Altabella L, et al. Mice repeatedly exposed to Group-A beta-Haemolytic *Streptococcus* show perseverative behaviors, impaired sensorimotor gating, and immune activation in rostral diencephalon. *Sci Rep* (2015) 5:13257. doi:10.1038/srep13257
60. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* (2010) 30(17):5866–75. doi:10.1523/JNEUROSCI.0167-10.2010
61. Wright S, Hashemi K, Stasiak L, Bartram J, Lang B, Vincent A, et al. Epileptogenic effects of NMDAR antibodies in a passive transfer mouse model. *Brain* (2015) 138(Pt 11):3159–67. doi:10.1093/brain/awv257
62. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry* (2014) 19(10):1143–9. doi:10.1038/mp.2013.110
63. Planaguma J, Leypoldt F, Mannara F, Gutierrez-Cuesta J, Martin-Garcia E, Aguilar E, et al. Human *N*-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* (2015) 138(Pt 1):94–109. doi:10.1093/brain/awu310
64. Manto M-U, Laute M-A, Aguera M, Rogemond V, Pandolfo M, Honnorat J. Effects of anti-glutamic acid decarboxylase antibodies associated with neurological diseases.: GAD-Ab and cerebellar function. *Ann Neurol* (2007) 61(6):544–51. doi:10.1002/ana.21123
65. Geis C, Weishaupt A, Hallermann S, Grunewald B, Wessig C, Wultsch T, et al. Stiff person syndrome-associated autoantibodies to amphiphysin mediate reduced GABAergic inhibition. *Brain* (2010) 133(11):3166–80. doi:10.1093/brain/awq253
66. Hackert JK, Muller L, Rohde M, Bien CG, Kohling R, Kirschstein T. Anti-GAD65 containing cerebrospinal fluid does not alter GABAergic transmission. *Front Cell Neurosci* (2016) 10:130. doi:10.3389/fncel.2016.00130
67. Stemmler N, Rohleder K, Malter MP, Widman G, Elger CE, Beck H, et al. Serum from a patient with GAD65 antibody-associated limbic encephalitis did not alter GABAergic neurotransmission in cultured hippocampal networks. *Front Neurol* (2015) 6:189. doi:10.3389/fneur.2015.00189
68. Wang B, Dileepan T, Briscoe S, Hyland KA, Kang J, Khoruts A, et al. Induction of TGF-beta1 and TGF-beta1-dependent predominant Th17 differentiation by group A streptococcal infection. *Proc Natl Acad Sci U S A* (2010) 107(13):5937–42. doi:10.1073/pnas.0904831107
69. Dileepan T, Linehan JL, Moon JJ, Pepper M, Jenkins MK, Cleary PP. Robust antigen specific th17 T cell response to group A *Streptococcus* is dependent on IL-6 and intranasal route of infection. *PLoS Pathog* (2011) 7(9):e1002252. doi:10.1371/journal.ppat.1002252
70. Hoffman KL, Hornig M, Yaddanapudi K, Jabado O, Lipkin WI. A murine model for neuropsychiatric disorders associated with group A beta-hemolytic streptococcal infection. *J Neurosci* (2004) 24(7):1780–91. doi:10.1523/JNEUROSCI.0887-03.2004
71. Cutforth T, DeMille MM, Agalliu I, Agalliu D. CNS autoimmune disease after *Streptococcus pyogenes* infections: animal models, cellular mechanisms and genetic factors. *Future Neurol* (2016) 11(1):63–76. doi:10.2217/fnl.16.4

72. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* (2016) 15(4):391–404. doi:10.1016/S1474-4422(15)00401-9
73. Wurdemann T, Kersten M, Tokay T, Guli X, Kober M, Rohde M, et al. Stereotactic injection of cerebrospinal fluid from anti-NMDA receptor encephalitis into rat dentate gyrus impairs NMDA receptor function. *Brain Res* (2016) 1633:10–8. doi:10.1016/j.brainres.2015.12.027
74. Guasp M, Sola-Valls N, Martinez-Hernandez E, Gil MP, Gonzalez C, Brieva L, et al. Cerebellar ataxia and autoantibodies restricted to glutamic acid decarboxylase 67 (GAD67). *J Neuroimmunol* (2016) 300:15–7. doi:10.1016/j.jneuroim.2016.09.019
75. Haselmann H, Ropke L, Werner C, Kunze A, Geis C. Interactions of human autoantibodies with hippocampal GABAergic synaptic transmission – analyzing antibody-induced effects ex vivo. *Front Neurol* (2015) 6:136. doi:10.3389/fneur.2015.00136
76. Rodella U, Scorzeto M, Duregotti E, Negro S, Dickinson BC, Chang CJ, et al. An animal model of Miller Fisher syndrome: mitochondrial hydrogen peroxide is produced by the autoimmune attack of nerve terminals and activates Schwann cells. *Neurobiol Dis* (2016) 96:95–104. doi:10.1016/j.nbd.2016.09.005
77. Maier H, Schmidbauer M, Pfausler B, Schmutzhard E, Budka H. Central nervous system pathology in patients with the Guillain-Barre syndrome. *Brain* (1997) 120(Pt 3):451–64. doi:10.1093/brain/120.3.451
78. Okumura A, Ushida H, Maruyama K, Itomi K, Ishiguro Y, Takahashi M, et al. Guillain-Barre syndrome associated with central nervous system lesions. *Arch Dis Child* (2002) 86(4):304–6. doi:10.1136/adc.86.4.304
79. Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* (2010) 9(8):776–85. doi:10.1016/S1474-4422(10)70137-X
80. Kleopa KA, Elman LB, Lang B, Vincent A, Scherer SS. Neuromyotonia and limbic encephalitis sera target mature shaker-type K+ channels: subunit specificity correlates with clinical manifestations. *Brain* (2006) 129 (Pt 6):1570–84. doi:10.1093/brain/awl084
81. Lancaster E, Martinez-Hernandez E, Dalmau J. Encephalitis and antibodies to synaptic and neuronal cell surface proteins. *Neurology* (2011) 77(2):179–89. doi:10.1212/WNL.0b013e318224afde
82. Shillito P, Molenaar PC, Vincent A, Leys K, Zheng W, van den Berg RJ, et al. Acquired neuromyotonia: evidence for autoantibodies directed against K+ channels of peripheral nerves. *Ann Neurol* (1995) 38(5):714–22. doi:10.1002/ana.410380505
83. Kaur C, Rathnasamy G, Ling EA. The choroid plexus in healthy and diseased brain. *J Neuropathol Exp Neurol* (2016) 75(3):198–213. doi:10.1093/jnen/nlv030
84. Liddelow SA, Temple S, Mollgard K, Gehwolf R, Wagner A, Bauer H, et al. Molecular characterisation of transport mechanisms at the developing mouse blood-CSF interface: a transcriptome approach. *PLoS One* (2012) 7(3):e33554. doi:10.1371/journal.pone.0033554
85. Liddelow SA. Development of the choroid plexus and blood-CSF barrier. *Front Neurosci* (2015) 9:32. doi:10.3389/fnins.2015.00032
86. Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, et al. C-C chemokine receptor 6-regulated entry of Th-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol* (2009) 10(5):514–8. doi:10.1038/ni.1716
87. Rudick RA, Eskin TA. Neuropathological features of a lupus-like disorder in autoimmune mice. *Ann Neurol* (1983) 14(3):325–32. doi:10.1002/ana.410140311
88. Zameer A, Hoffman SA. Increased ICAM-1 and VCAM-1 expression in the brains of autoimmune mice. *J Neuroimmunol* (2003) 142(1–2):67–74. doi:10.1016/S0165-5728(03)00262-5
89. Chan OT, Madaio MP, Shlomchik MJ. The central and multiple roles of B cells in lupus pathogenesis. *Immunol Rev* (1999) 169:107–21. doi:10.1111/j.1600-065X.1999.tb01310.x
90. Wen J, Stock AD, Chalmers SA, Putterman C. The role of B cells and autoantibodies in neuropsychiatric lupus. *Autoimmun Rev* (2016) 15(9):890–5. doi:10.1016/j.autrev.2016.07.009
91. Wolak DJ, Thorne RG. Diffusion of macromolecules in the brain: implications for drug delivery. *Mol Pharm* (2013) 10(5):1492–504. doi:10.1021/mp300495e
92. Zhao H, Aoshi T, Kawai S, Mori Y, Konishi A, Ozkan M, et al. Olfactory plays a key role in spatiotemporal pathogenesis of cerebral malaria. *Cell Host Microbe* (2014) 15(5):551–63. doi:10.1016/j.chom.2014.04.008
93. Meredith ME, Salameh TS, Banks WA. Intranasal delivery of proteins and peptides in the treatment of neurodegenerative diseases. *AAPS J* (2015) 17(4):780–7. doi:10.1208/s12248-015-9719-7
94. van Riel D, Verdijk R, Kuiken T. The olfactory nerve: a shortcut for influenza and other viral diseases into the central nervous system. *J Pathol* (2015) 235(2):277–87. doi:10.1002/path.4461
95. Schafer A, Brooke CB, Whitmore AC, Johnston RE. The role of the blood-brain barrier during Venezuelan equine encephalitis virus infection. *J Virol* (2011) 85(20):10682–90. doi:10.1128/JVI.05032-11
96. Munster VJ, Prescott JB, Bushmaker T, Long D, Rosenke R, Thomas T, et al. Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. *Sci Rep* (2012) 2:736. doi:10.1038/srep00736
97. Herbert RP, Harris J, Chong KP, Chapman J, West AK, Chuah MI. Cytokines and olfactory bulb microglia in response to bacterial challenge in the compromised primary olfactory pathway. *J Neuroinflammation* (2012) 9:109. doi:10.1186/1742-2094-9-109
98. Lochhead JJ, Wolak DJ, Pizzo ME, Thorne RG. Rapid transport within cerebral perivascular spaces underlies widespread tracer distribution in the brain after intranasal administration. *J Cereb Blood Flow Metab* (2015) 35(3):371–81. doi:10.1038/jcbfm.2014.215
99. Kumar NN, Gautam M, Lochhead JJ, Wolak DJ, Ithapu V, Singh V, et al. Relative vascular permeability and vascularity across different regions of the rat nasal mucosa: implications for nasal physiology and drug delivery. *Sci Rep* (2016) 6:31732. doi:10.1038/srep31732
100. Kaminski M, Bechmann I, Kiwit J, Glumm J. Migration of monocytes after intracerebral injection. *Cell Adh Migr* (2012) 6(3):164–7. doi:10.4161/cam.20281
101. Mohammad MG, Tsai VW, Ruitenberg MJ, Hassanpour M, Li H, Hart PH, et al. Immune cell trafficking from the brain maintains CNS immune tolerance. *J Clin Invest* (2014) 124(3):1228–41. doi:10.1172/JCI71544
102. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and dysfunction of the blood-brain barrier. *Cell* (2015) 163(5):1064–78. doi:10.1016/j.cell.2015.10.067
103. Knowland D, Arac A, Sekiguchi KJ, Hsu M, Lutz SE, Perrino J, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron* (2014) 82(3):603–17. doi:10.1016/j.neuron.2014.03.003
104. Vajkoczy P, Laschinger M, Engelhardt B. Alpha4-integrin-VCAM-1 binding mediates G protein-independent capture of encephalitogenic T cell blasts to CNS white matter microvessels. *J Clin Invest* (2001) 108(4):557–65. doi:10.1172/jci12440
105. Lengfeld JE, Lutz SE, Smith JR, Diaconu C, Scott C, Kofman SB, et al. Endothelial Wnt/beta-catenin signaling reduces immune cell infiltration in multiple sclerosis. *Proc Natl Acad Sci U S A* (2017) 114(7):E1168–77. doi:10.1073/pnas.1609905114
106. Bartanusz V, Jezova D, Alajajian B, Digicaylioglu M. The blood-spinal cord barrier: morphology and clinical implications. *Ann Neurol* (2011) 70(2):194–206. doi:10.1002/ana.22421
107. Cruz-Orengo L, Daniels BP, Dorsey D, Basak SA, Grajales-Reyes JG, McCandless EE, et al. Enhanced sphingosine-1-phosphate receptor 2 expression underlies female CNS autoimmunity susceptibility. *J Clin Invest* (2014) 124(6):2571–84. doi:10.1172/jci73408
108. Tavazoie M, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, Zaidi B, et al. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* (2008) 3(3):279–88. doi:10.1016/j.stem.2008.07.025
109. Winkler CW, Race B, Phillips K, Peterson KE. Capillaries in the olfactory bulb but not the cortex are highly susceptible to virus-induced vascular leak and promote viral neuroinvasion. *Acta Neuropathol* (2015) 130(2):233–45. doi:10.1007/s00401-015-1433-0
110. Colin-Castelan D, Ramirez-Santos J, Gutierrez-Ospina G. Differential vascular permeability along the forebrain ventricular neurogenic niche in the adult murine brain. *J Neurosci Res* (2016) 94(2):161–9. doi:10.1002/jnr.23682
111. Sajja RK, Rahman S, Cucullo L. Drugs of abuse and blood-brain barrier endothelial dysfunction: a focus on the role of oxidative stress. *J Cereb Blood Flow Metab* (2016) 36(3):539–54. doi:10.1177/0271678X15616978

112. Argaw AT, Zhang Y, Snyder BJ, Zhao M-L, Kopp N, Lee SC, et al. IL-1beta regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *J Immunol* (2006) 177(8):5574–84. doi:10.4049/jimmunol.177.8.5574
113. Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Front Neurol* (2013) 4:32. doi:10.3389/fneur.2013.00032
114. Marchiando AM, Shen L, Graham WV, Weber CR, Schwarz BT, Austin JR II, et al. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *J Cell Biol* (2010) 189(1):111–26. doi:10.1083/jcb.200902153
115. Alvarez JI, Cayrol R, Prat A. Disruption of central nervous system barriers in multiple sclerosis. *Biochim Biophys Acta* (2011) 1812(2):252–64. doi:10.1016/j.bbadis.2010.06.017
116. Stamatovic SM, Keep RF, Wang MM, Jankovic I, Andjelkovic AV. Caveolae-mediated internalization of occludin and claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. *J Biol Chem* (2009) 284(28):19053–66. doi:10.1074/jbc.M109.000521
117. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* (2007) 13(10):1173–5. doi:10.1038/nm1651
118. Alter A, Duddy M, Hebert S, Biernacki K, Prat A, Antel JP, et al. Determinants of human B cell migration across brain endothelial cells. *J Immunol* (2003) 170(9):4497–505. doi:10.4049/jimmunol.170.9.4497
119. Lehmann-Horn K, Sagan SA, Bernard CC, Sobel RA, Zamvil SS. B-cell very late antigen-4 deficiency reduces leukocyte recruitment and susceptibility to central nervous system autoimmunity. *Ann Neurol* (2015) 77(5):902–8. doi:10.1002/ana.24387
120. Michel L, Touil H, Pikor NB, Gommerman JL, Prat A, Bar-Or A. B cells in the multiple sclerosis central nervous system: trafficking and contribution to CNS-compartmentalized inflammation. *Front Immunol* (2015) 6:636. doi:10.3389/fimmu.2015.00636
121. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol* (2004) 14(2):164–74. doi:10.1111/j.1750-3639.2004.tb00049.x
122. Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med* (2009) 6(1):e1. doi:10.1371/journal.pmed.0060001
123. Claes N, Fraussen J, Stinissen P, Hupperts R, Somers V. B cells are multifunctional players in multiple sclerosis pathogenesis: insights from therapeutic interventions. *Front Immunol* (2015) 6:642. doi:10.3389/fimmu.2015.00642
124. D'Agostino PM, Kwak C, Vecchiarelli HA, Toth JG, Miller JM, Masheeb Z, et al. Viral-induced encephalitis initiates distinct and functional CD103+ CD11b+ brain dendritic cell populations within the olfactory bulb. *Proc Natl Acad Sci U S A* (2012) 109(16):6175–80. doi:10.1073/pnas.1203941109
125. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* (2011) 10(8):759–72.
126. Baburamani AA, Ek CJ, Walker DW, Castillo-Melendez M. Vulnerability of the developing brain to hypoxic-ischemic damage: contribution of the cerebral vasculature to injury and repair? *Front Physiol* (2012) 3:424. doi:10.3389/fphys.2012.00424
127. Hara K, Yoshizuka M, Doi Y, Fujimoto S. Effect of bis (tributyl tin) oxide on permeability of the blood-brain barrier: a transient increase. *Occup Environ Med* (1994) 51(11):735–8. doi:10.1136/oem.51.11.735
128. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* (2014) 6(263):263ra158. doi:10.1126/scitranslmed.3009759
129. Yoshio T, Okamoto H, Hirohata S, Minota S. IgG anti-NR2 glutamate receptor autoantibodies from patients with systemic lupus erythematosus activate endothelial cells. *Arthritis Rheum* (2013) 65(2):457–63. doi:10.1002/art.37745
130. Odaka M, Yuki N, Yamada M, Koga M, Takemi T, Hirata K, et al. Bickerstaff's brainstem encephalitis: clinical features of 62 cases and a subgroup associated with Guillain-Barre syndrome. *Brain* (2003) 126(Pt 10):2279–90. doi:10.1093/brain/awg233
131. Snider LA, Lougee L, Slattery M, Grant P, Swedo SE. Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders. *Biol Psychiatry* (2005) 57(7):788–92. doi:10.1016/j.biopsych.2004.12.035
132. Williams KA, Swedo SE, Farmer CA, Grantz H, Grant PJ, D'Souza P, et al. Randomized, controlled trial of intravenous immunoglobulin for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *J Am Acad Child Adolesc Psychiatry* (2016) 55(10):860e–7e. doi:10.1016/j.jaac.2016.06.017
133. Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias lecture. *Neurology* (2016) 87(23):2471–82. doi:10.1212/WNL.0000000000003414
134. Nathoo N, Jalal H, Natah SS, Zhang Q, Wu Y, Dunn JF. Hypoxia and inflammation-induced disruptions of the blood-brain and blood-cerebrospinal fluid barriers assessed using a novel T1-based MRI method. *Acta Neurochir Suppl* (2016) 121:23–8. doi:10.1007/978-3-319-18497-5_5
135. Mulkey SB, Glasier CM, El-Nabbout B, Walters WD, Ionita C, McCarthy MH, et al. Nerve root enhancement on spinal MRI in pediatric Guillain-Barre syndrome. *Pediatr Neurol* (2010) 43(4):263–9. doi:10.1016/j.pediatrneurol.2010.05.011
136. Yikilmaz A, Doganay S, Gumus H, Per H, Kumandas S, Coskun A. Magnetic resonance imaging of childhood Guillain-Barre syndrome. *Childs Nerv Syst* (2010) 26(8):1103–8. doi:10.1007/s00381-010-1197-8
137. Ashikari Y, Kobayashi S, Tago A, Yoneyama M, Ito M, Fukuda K, et al. A case of Guillain-Barre syndrome with meningeal irritation. *Brain Dev* (2016) 38(1):163–6. doi:10.1016/j.braindev.2015.06.001
138. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* (1996) 380(6573):435–9. doi:10.1038/380435a0
139. Liebner S, Corada M, Bangsow T, Babbage J, Taddei A, Czupalla CJ, et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *J Cell Biol* (2008) 183(3):409–17. doi:10.1083/jcb.200806024
140. Stenman JM, Rajagopal J, Carroll TJ, Ishibashi M, McMahon J, McMahon AP. Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature. *Science* (2008) 322(5905):1247–50. doi:10.1126/science.1164594
141. Daneman R, Agalliu D, Zhou L, Kuhnert F, Kuo CJ, Barres BA. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. *Proc Natl Acad Sci U S A* (2009) 106(2):641–6. doi:10.1073/pnas.0805165106
142. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* (2013) 19(12):1584–96. doi:10.1038/nm.3407
143. Alvarez JI, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, Terouz S, et al. The hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science* (2011) 334(6063):1727–31. doi:10.1126/science.1206936
144. Beurel E, Kaidanovich-Beilin O, Yeh WI, Song L, Palomo V, Michalek SM, et al. Regulation of Th1 cells and experimental autoimmune encephalomyelitis by glycogen synthase kinase-3. *J Immunol* (2013) 190(10):5000–11. doi:10.4049/jimmunol.1203057
145. Licht-Murava A, Paz R, Vaks L, Avrahami L, Plotkin B, Eisenstein M, et al. A unique type of GSK-3 inhibitor brings new opportunities to the clinic. *Sci Signal* (2016) 9(454):ra110. doi:10.1126/scisignal.aah7102


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 05 July 2017
doi: 10.3389/fimmu.2017.00752

# Neuronal Surface Autoantibodies in Neuropsychiatric Disorders: Are There Implications for Depression?

*Shenghua Zong, Carolin Hoffmann, Marina Mané-Damas, Peter Molenaar, Mario Losen and Pilar Martinez-Martinez**

*Division Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands*

Autoimmune diseases are affecting around 7.6–9.4% of the general population. A number of central nervous system disorders, including encephalitis and severe psychiatric disorders, have been demonstrated to associate with specific neuronal surface autoantibodies (NSAbs). It has become clear that specific autoantibodies targeting neuronal surface antigens and ion channels could cause severe mental disturbances. A number of studies have focused or are currently investigating the presence of autoantibodies in specific mental conditions such as schizophrenia and bipolar disorders. However, less is known about other conditions such as depression. Depression is a psychiatric disorder with complex etiology and pathogenesis. The diagnosis criteria of depression are largely based on symptoms but not on the origin of the disease. The question which arises is whether in a subgroup of patients with depression, the symptoms might be caused by autoantibodies targeting membrane-associated antigens. Here, we describe how autoantibodies targeting membrane proteins and ion channels cause pathological effects. We discuss the physiology of these antigens and their role in relation to depression. Finally, we summarize a number of studies detecting NSAbs with a special focus on cohorts that include depression diagnosis and/or show depressive symptoms.

**Keywords: neuronal surface autoantibodies, neuropsychiatric disorders, depression, pathogenicity, immunoglobulin, neurotransmitter receptor, ion channel, blood–brain barrier**

OPEN ACCESS

***Edited by:***
*Fabienne Brilot, University of Sydney, Australia*

***Reviewed by:***
*Anna Fogdell-Hahn, Karolinska Institutet, Sweden*
*Richa Hanamsagar, Massachusetts General Hospital, United States*

***Correspondence:**
*Pilar Martinez-Martinez*
*p.martinez@maastrichtuniversity.nl*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *07 March 2017*
***Accepted:*** *13 June 2017*
***Published:*** *05 July 2017*

***Citation:***
*Zong S, Hoffmann C, Mané-Damas M, Molenaar P, Losen M and Martinez-Martinez P (2017) Neuronal Surface Autoantibodies in Neuropsychiatric Disorders: Are There Implications for Depression? Front. Immunol. 8:752. doi: 10.3389/fimmu.2017.00752*

## INTRODUCTION

Neuronal surface autoantibodies (NSAbs) have been described mainly in autoimmune encephalitis, a group of newly defined neuroimmunological disorders (1). Those autoantibodies target essential neurotransmitter receptors, ion channels, or associated proteins on the membrane of neuronal cells, such as *N*-methyl-D-aspartate receptor (NMDAR) (2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) (3, 4), metabotropic glutamate receptor 1 (mGluR1) (5), metabotropic glutamate receptor 5 (mGluR5) (6), $GABA_B$ receptor ($GABA_BR$) (7), $GABA_A$ receptor ($GABA_AR$) (8–10), leucine-rich, glioma inactivated 1 (LGI1) and contactin-associated protein-like 2 (Caspr2) (11), dipeptidyl aminopeptidase-like protein 6 (DPPX) (12–14), and dopamine receptor $D_2$ (D2R) (15). Antibody-positive cases are associated with a spectrum of neurological disorders including limbic encephalitis, neuromyotonia, Morvan's syndrome, epilepsy, and psychiatric disorders (16–19).

Depression is a psychiatric disorder with complex etiology and pathogenesis. The International Classification of Diseases and The Diagnostic and Statistical Manual of Mental Disorders are widely used for the diagnoses of this disorder, based on symptoms but not on the cause of the disease. There are several theories about the causes of depression and immune dysregulation is one of them. The relationship between the immune system and depression has been widely discussed. To date, most research has focused on pro-inflammatory cytokines and a few reviews also propose a direct link between autoantibodies and depression (20, 21). Studies investigating the presence of autoantibodies in depression have focused in those targeting peripheral organs like the thyroid and intracellular antigens such as antinuclear antibodies and ribosomal-P antibodies (21–25). During the past decade, it has become clear that NSAbs could cause severe neuropsychiatric disorders. Since some of the NSAbs interfere with neurotransmission pathways related to depression (26–28), a subtype of depression may be caused by antibody-mediated autoimmunity and, therefore, might potentially respond to immunotherapy. In the current review, we summarize the literature about NSAbs in autoimmune encephalitis and psychiatric disorders, with a special focus on what is known regarding NSAbs in depression, evaluate the techniques used and how results can be interpreted, and identify research gaps. Together, we aim to provide insight into the potential role of NSAbs in depression based on the function of relevant neurotransmitter receptors and ion channels as well as autoantibody effector mechanisms.

## HOW NSAbs REACH THE CENTRAL NERVES SYSTEM (CNS)

Because neuronal surface proteins are the target of the autoantibodies discussed in this review, it is important to first understand how those autoantibodies get access to the CNS. Now it is widely accepted that the CNS is targeted by the immune system, yet the mechanism how autoantibodies go through the blood–brain barrier (BBB) is still unclear. Under normal conditions, immunoglobulins go through the BBB at a very low rate; a good example is immunoglobulin G (IgG). IgG concentration in the cerebrospinal fluid (CSF) is approximately 1% of the levels in the peripheral circulation (29–31). This indicates that once the autoantibodies reach the CNS they can cause disease as it has been observed in autoimmune encephalitis. In certain situations, like inflammation, for example, during the group A Streptococcus infection, specific Th17 cells could migrate into the brain through the cribriform plate along olfactory sensory axons. The Th17 cells expressed IL-17A which induced endothelial tight junction breakdown, increasing BBB permeability and facilitating the penetration of IgG in the brain (32). Additionally, the BBB may become leaky because of stroke, brain trauma, hemorrhages, microangiopathy, or brain tumors, and antibody penetration rate might increase. In this regard, a study has reported that autoantibodies to NMDAR (anti-NMDAR) seropositive schizophrenia patients with a history of neurotrauma or birth complications had more severe neurological symptoms than seronegative patients. And intravenous injections of extracted Ig fractions (IgG, IgA, or IgM) from anti-NMDAR seropositive patients to BBB leaky (ApoE−/−) mice could induce a psychosis-related response (33). A further study confirmed that APOE4 carrier status and anti-NMDAR seropositivity together were significantly associated with schizoaffective disorder (34). Those results indicate the importance of the BBB for anti-NMDAR-mediated pathology.

Besides, intrathecal synthesis is another possible source for autoantibodies in the CNS. B-cells can migrate to the brain and produce autoantibodies locally (35–37). This is also important to keep in mind when thinking about therapy because any potential drug against B cells has to pass the BBB to be effective. The evidence is mainly from studies analyzing autoantibodies in serum and CSF from encephalitis patients. It has been reported that in some encephalitis patients, autoantibodies targeting the NMDAR, AMPAR, $GABA_BR$, DPPX, mGluR1, or mGluR5 were found only in the CSF (38). A postmortem study showed the presence of CD138+ plasma cells in the brain of NMDAR encephalitis patients, suggesting intrathecal synthesis of autoantibodies (36). Intrathecal antibody synthesis was also described in a case with autoantibodies against the mGluR1 where the patient did not respond to immunotherapy, while serum antibody levels dropped but CSF levels were still high (39). Other NSAbs, such as autoantibodies to LGI1, Caspr2, glycine receptor, and $GABA_AR$ may, in rare instances, be identified only in serum but be absent in CSF (38). However, if the autoantibodies are immunoabsorbed by the antigen in the brain, they might still have effects and play a pathogenic role even they are not detectable in the CSF (40).

## IgG EFFECTOR FUNCTIONS

Antibodies (or Igs) are produced by plasma B cells. They are defined as IgM, IgG, IgA, IgD, and IgE isotypes according to heavy chain C domains. Different types of NSAbs (IgM, IgA, and IgG) have been found so far; IgG autoantibodies are considered the most pathogenic (1, 10, 33). IgG, composed of two paired heavy chain and light chain, is the major antibody in body fluid and a crucial player in the humoral immune response. In humans, four IgG isotypes (IgG1–4) exist, which have different ability to activate the complement system (41). IgG1–3 mediate pro-inflammatory activities, while IgG4 also has anti-inflammatory activities (42). The functions of IgG effector in myasthenia gravis (MG) and other well-studied autoimmune disorders are schematically illustrated in **Figure 1**.

### Antigenic Modulation

Antibodies of the IgG1–3 subtypes are able to cross-link the antigens because of their bivalent nature, whereas the IgG4 subtype loses this ability after the fab-arm exchange with other unrelated IgG4 molecules (43). Cross-linking autoantibodies are believed to bring the antigens close together on the cell membrane and promote the degradation of the ligand–receptor complex (44). In the case of MG, antiacetylcholine receptor autoantibodies (anti-AChR), mainly IgG1 and IgG3, are able to cross-link adjacent AChR molecules, leading to rapid internalization by endocytosis and AChR degradation (45, 46). Previous studies indicated that anti-NMDAR, IgG1–3, led to a reduction in the synaptic and extrasynaptic receptors and further decreased the

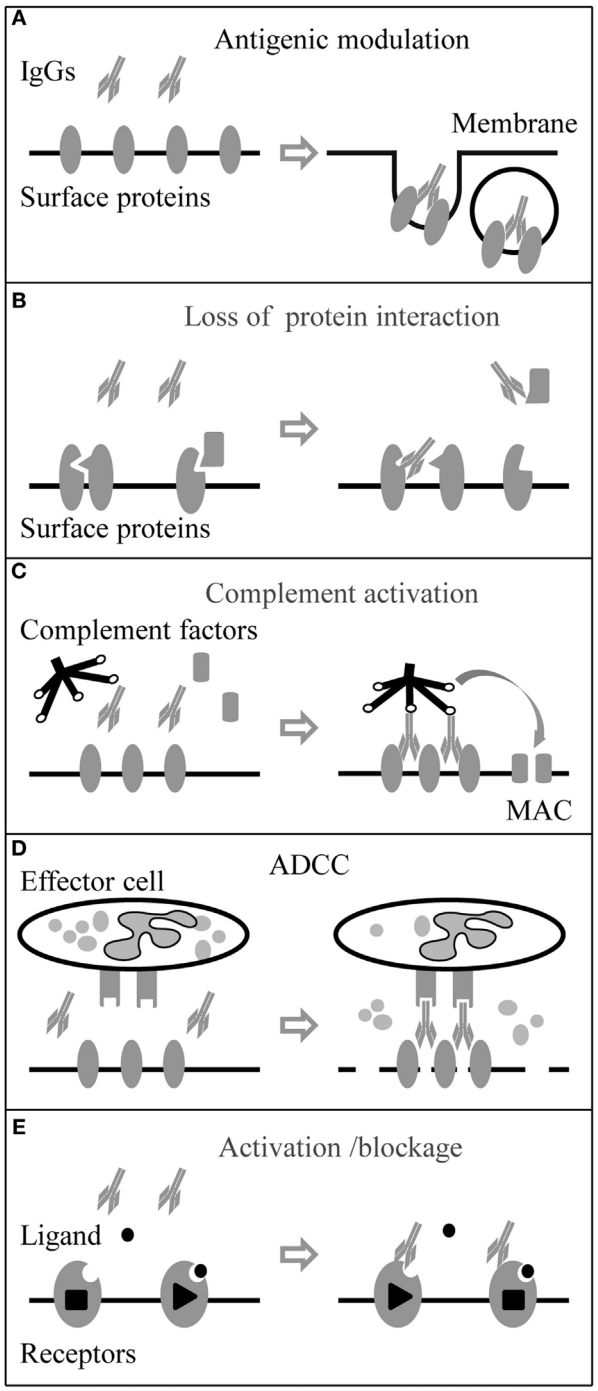



**FIGURE 1** | Immunoglobulin G (IgG) autoantibody effector mechanisms. Neuronal surface proteins like G-protein coupled receptors, ion channels, and associated proteins can be the targets of autoantibodies. **(A)** Autoantibodies can directly target surface proteins and induce their internalization by cross-linking of the antigens. **(B)** Autoantibodies can also target associate proteins and block protein–protein interaction. **(C)** Autoantibodies (IgG3 > IgG1 > IgG2) can activate the complement system and form the membrane attack complex (MAC) leading to damage of the membrane. **(D)** Autoantibodies binding to effector cell with Fc receptors (FcRs) can trigger antibody-dependent cell-mediated cytotoxicity (ADCC). **(E)** In addition, autoantibodies can be agonists or antagonists and activate or block the function of membrane receptors.

synaptic plasticity and transmission (47–50). Anti-$GABA_AR$, IgG1 and IgG3, had a similar effect with a reduction of $GABA_AR$ clusters in both synaptic and extrasynaptic areas (8–10). Also, application of anti-AMPAR (GluR1/2) to neuronal cultures significantly decreased the number of AMPAR clusters at synaptic and extrasynaptic areas by increasing the internalization of AMPAR clusters; the IgG subclasses were not analyzed in these studies (4, 51).

## Complement Activation

IgG1–3 can activate the complement system by forming the membrane attack complex (MAC) and leading to membrane damage of targeted cells. Still in MG, anti-AChR binding to AChRs, which are densely packed in the folds of the postsynaptic membrane of the neuromuscular junction, results in a very high density of AChR-bound autoantibodies and hence a very tightly packed Fc region. The complement system is activated with high efficiency and as a result, MAC is formed in the postsynaptic membrane. Together with antigenic modulation, complement activation causes severe endplate membrane damage (45, 52). Brain biopsy findings support that complement activation and MAC deposition happen associated with acute neuronal cell death in anti-voltage-gated potassium channel (VGKC) complex encephalitis and Rasmussen's encephalitis (53, 54).

## Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Antibody-dependent cell-mediated cytotoxicity is the process when cytotoxic effector cells of the immune system kill the antibody targeted cell by the releasing cytotoxic granules or by expressing cell death-inducing molecules. The process is activated when the Fc receptors (FcRs) on the effector cell surface bind to Fc region of target-bound antibodies (IgG, IgA, or IgE subtypes). Those effector cells include natural killer cells, monocytes, macrophages, neutrophils, eosinophils, and dendritic cells. In humans, the IgG1 subtype has the ability to strongly trigger ADCC and is used widely in therapy for certain types of cancer (55, 56). Neuromyelitis optica (NMO) is a severe inflammatory demyelinating disease in CNS, and autoantibodies against aquaporin-4 (anti-AQP4), a water channel on astrocyte play a role in the pathology of NMO by triggering complement activation and ADCC (57). *In vitro*, NMO patient serum and CSF IgG induced ADCC of glial cells transfected with AQP4 (58). *In vivo*, injection of anti-AQP4 produced large NMO lesions in mice, with the loss of AQP4 and GFAP immunoreactivity, inflammation, and demyelination. Those pathologies were largely reduced when FcγIII receptor deficient mice were used or when normal mice were injected with Fcγ receptor blocking antibody (59).

## Loss of Receptor or Ion Channel-Associated Proteins

Autoantibodies can target receptor or ion channel-associated proteins. As a result, the protein–protein interaction between the receptor and the associated protein is interrupted with the consequence that those receptors or ion channels become dysfunctional. Autoantibodies to muscle-specific kinase (anti-MuSK) are

another type of autoantibodies involved in the pathogenicity of MG. Anti-MuSK (predominant IgG4) binds to an extracellular epitope on MuSK at the neuromuscular junction, inhibits the pathway involved in the clustering of the AChRs in the membrane, and leads to failure of neuromuscular transmission (43). Autoantibodies to LGI1, a VGKC complex-associated protein, play a similar role, resulting in reduced VGKC function at CNS synapses and increased cell excitability (60). Besides, anti-LGI1 also interferes with other surface receptors. LGI1 interacts with the ADAM22/23, epilepsy-related transmembrane proteins, and regulates AMPAR-mediated synaptic transmission in the hippocampus (61, 62). Additionally, an *in vitro* study showed that anti-LGI1 from encephalitis patients blocked the binding of LGI1 to ADAM22 by neutralizing the ADAM22-binding domain of LGI1. The loss of LGI1-ADAM22 interaction could further reduce synaptic AMPAR, which indirectly associates with ADAM22 (63). Importantly, this indicates that besides their direct effect on ion channel/receptors, autoantibodies may interfere with protein–protein interaction and have consequences for synapse formation, function, and maintenance.

### Activation, Inactivation, and Functional Receptor Blockage of the Receptors

Autoantibodies may activate, inactivate, or block ion channels and neurotransmitter G protein-coupled receptors (64). Serum IgG from MG patients has been shown to block the ACh binding sites in cultured mammalian muscle cells (65) and caused acute and severe muscle weakness in rodents, independent of inflammation or necrosis (66). Autoantibodies against the γ subunit of the AChR which is only present in embryonic forms of the receptor have been reported in some cases to block the AChR function and cause arthrogryposis multiplex congenita (67). Conversely, AChR antibodies can also induce prolonged open time of the AChR leading to muscle weakness by excitotoxicity at the neuromuscular junction (68). Anti-AMPAR (GluR3B subunit) autoantibodies (anti-AMPA-GluR3B) can activate AMPAR that contains the GluR3B subunit, leading to the spontaneous occurrence of ion currents (69, 70). In an animal study, anti-AMPA-GluR3B produced following immunization with the GluR3B peptide bonded cultured neurons, evoked GluR ion channel activity, and killed neurons by "excitotoxicity" (71). When autoantibodies target G-protein-coupled receptors, they can interfere with signaling pathways, which might lead to slow effector responses. An example is Graves' disease, where autoantibodies against the thyroid-stimulating hormone (TSH) receptor stimulate the synthesis of thyroid hormone, which is produced in excess and results in hyperthyroidism. Additionally, there are anti-TSH receptor antibodies that block the signal transduction and consequently reduce thyroid hormone production by targeting different epitopes of the receptor (72).

## THE TARGETS OF NSAbs ARE RELEVANT IN THE PATHOLOGY OF DEPRESSION

Monoamine imbalance is the main biochemical postulate of depression. Both serotonergic neurotransmission and dopaminergic neurotransmission play important roles in causing depressive symptoms (73). Genetic studies suggest that polymorphisms within genes that encode for 1A serotonin receptor (5-HT1A) and D4 dopamine receptor, increase the risk of major depressive disorder (MDD) (74). 5-HT1A (75, 76) and D2DR (77, 78) levels are decreased in this disorder and both are the targets of several antidepressants (79).

Increasing evidence supports that glutamatergic and GABAergic systems are also involved in depression (27, 28). Glutamate is the predominant excitatory neurotransmitter in the CNS (80, 81). Blockade of glutamate uptake from the synapse has been reported to reduce sensitivity to reward, a symptom of depression (82). Ketamine and other NMDAR antagonists have antidepressant effects (83). Antidepressants such as imipramine can enhance the synaptic expression of GluR1, a subunit of AMPAR (84).

Interestingly, GABA concentration is reduced in cortical brain and CSF in MDD and this deficit could be reversed by chronic treatment with selective serotonin reuptake inhibitors and electroconvulsive therapy (85–87). Studies reported that cortical GABA(A)-benzodiazepine receptor complex affinity and/or number were reduced in MDD. Additionally, mice heterozygous for the γ2 subunit of $GABA_AR$ (γ2+/−) exhibited anxious–depressive behavior (88, 89). In this model, $GABA_AR$ numbers were unaltered, but had reduced benzodiazepine binding sites.

Thus, if the abovementioned neurotransmitter receptors or relevant proteins are targeted by autoantibodies, including ion channels and associated proteins, they could potentially cause depression-like symptoms. Below, we summarize NSAbs that target antigens which are relevant in the pathology of depression (for an illustration see **Figure 2**).

## EVIDENCE OF NSAbs IN DEPRESSION

### Anti-Glutamate Receptor Autoantibodies

#### Anti-NMDAR

The NMDAR, as an ionotropic glutamate receptor, contains two GluN1 and two GluN2 (A–D) subunits (alternatively called NR1 and NR2) forming heterotetramers. The subunit GluN2 can be replaced by the GluN3 (A/B) subunit, which has an inhibitory effect on receptor activity (90, 91). NMDAR has a variety of physiological roles and any dysfunctions, either enhanced or decreased activity, may result in neuropsychiatric disorders such as schizophrenia, bipolar disorder, MDD, substance-induced psychosis, Huntington's disease, Alzheimer's disease, and neuropsychiatric systemic lupus erythematosus (NPSLE) (92). In addition, higher gene expression levels of NR1 and NR2 (A–D) are detected in female patients with MDD (93). Prolonged inhibition of the NMDAR by phencyclidine leads to memory loss, thought disorder, depression, and personality changes (94). Antagonists of the NMDAR like ketamine also have rapid antidepressant effects (95, 96). All in all, these studies suggest that NMDAR plays a critical role in psychiatric disorders including depression.

Anti-NMDARs in autoimmune encephalitis were first described in three patients with ovarian teratoma and commonly presenting with psychiatric symptoms followed by neurological

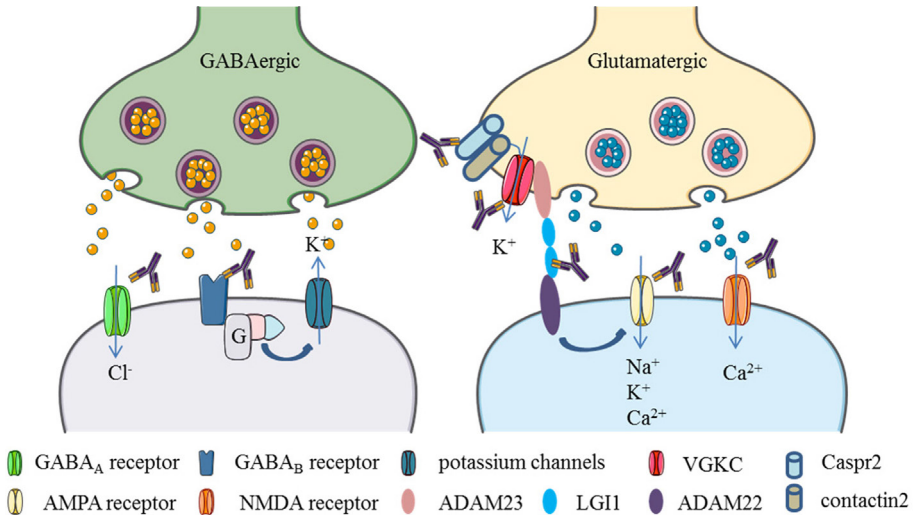



**FIGURE 2** | Neuronal surface autoantibodies target neuronal receptors, ion channels, and/or associate proteins that commonly affect GABA and glutamate transmission in the brain. (1) GABA receptor activation causes chloride anions influx and potassium flow-out, resulting in the hyperpolarization of the postsynaptic neurons. Autoantibodies to $GABA_A$ or $GABA_B$ receptors cause internalization of those membrane proteins and block the GABA transmission, leading to excitation of the postsynaptic neurons. (2) Glutamate receptors activation causes polarization of the postsynaptic neurons by positive ions (Ca2+, Na+, K+) influx. Autoantibodies to NMDA and AMPA receptors drive internalization of those receptors and block the glutamate transmission. (3) Potassium channels can be activated by $GABA_B$ receptors through G proteins. Some proteins like leucine-rich, glioma inactivated 1 (LGI1) and contactin-associated protein-like 2 (Caspr2), contactin 2, ADAM22, and ADAM23 are associated with voltage-gated potassium channels (VGKCs). LGI1 can enhance AMPA receptor-mediated synaptic transmission by binding to ADAM22. Autoantibodies target to those associate proteins would cause VGKCs or AMPA receptor dysfunction (Elements are partly adapted from Servier Medical Art. http://smart.servier.com/).

manifestations including seizures, movement disorder, and dysfunction of the autonomous nervous system (2). The methods used for detection were immunohistochemistry (IHC) on rat brain tissues, immunocytochemistry on live hippocampal neurons, and fixed cell-based assay (CBA). The autoantibodies identified were present both in CSF and serum. Later studies revealed that the extracellular N-terminal domain of the NR1 subunit is the main epitope of those autoantibodies (97). A case series showed that in more than two-thirds of cases with NMDAR encephalitis patients were initially seen by psychiatrists or admitted to psychiatric centers because they showed prominent psychiatric symptoms including anxiety, agitation, bizarre behavior, delusional or paranoid thoughts, and visual or auditory hallucinations (98). Consequently, researchers broaden the search for anti-NMDAR to psychiatric disorders, mainly first episode psychosis. Bipolar and MDDs were usually included as psychiatric disorder controls. One meta-analysis indicated higher odds of anti-NMDAR in psychotic and affective disorders (99). An affective disorder cohort consisting of 148 patients was screened for anti-NMDAR, in which 24 (16.2%) were seropositive (5 were IgG, 15 IgA, and 7 IgM). The prevalence in this cohort was higher than in healthy controls (10.8%) (34). In this study, the method used was fixed CBA and the dilution of serum used was from 1 in 10 and titers for positive cases were double-determined in two laboratories. The results have been criticized because of the much higher prevalence of anti-NMDAR in healthy control than in other groups' study results (34, 100, 101). Further complementary investigations, using a dilution of 1:320, identified a lower percentage of positive individuals in a cohort of depression patients. Anti-NMDAR (IgG, IgA, and IgM) were found to be 4.1% in depression, still higher than healthy control (1.7%) at the significant level (33, 99). The author explained the increased number of seropositive anti-NMDAR cases in affective disorder cohort by the fact that the mean age of the affective disorder group was higher than in the control group (autoantibody prevalence is generally increasing with age) (33). Another study using same methods found 10.6% (1.9% IgG) positive for anti-NMDAR affective disorder cohort ($n = 310$) but no significant difference for healthy control (102). Additionally, another study analyzed a depression cohort ($n = 70$) and found two (2.9%) seropositive patients for NMDAR (both IgA) and one seropositive (0.4%) (IgM) result in a healthy control ($n = 230$), so none of them were IgG (101). The experiment was replicated and higher numbers of seropositive cases were found both in healthy controls and the disease groups (103). Early studies by Dickerson et al. (104) (ELISA, peptide of NR2, $n = 28$) and Zandi et al. (105) using variations of the methodology (live CBA) did not report any positive results in depression cohorts. Passive transfer of anti-NMDAR (NR1) to mice could cause depressive-like symptoms (106). However, the correlation of symptoms in animal models with those observed in humans needs to be further demonstrated (107).

In contrast to anti-NMDAR in autoimmune encephalitis which mainly targets the NR1 subunit, Lapteva and colleagues found that autoantibodies targeting the NR2 subunit of NMDAR were associated with depression in systemic lupus erythematosus (SLE) patients (108). In fact, anti-NR2A/B autoantibodies were thought to be a subset of the anti-double-stranded DNA (dsDNA) antibodies (109). The epitope identified to be targeted by the

antibodies in this study was a pentapeptide Asp/Glu-Trp-Asp/Glu-Tyr-Ser/Gly. This sequence present on the NR2A/B subunit is a mimotope of anti-dsDNA. This was confirmed by showing that affinity-purified antibodies from SLE patients targeting this peptide also bind to dsDNA (109, 110). Moreover, those autoantibodies mediated apoptotic death of neurons *in vivo* and *in vitro* (109). Several studies have investigated the role of anti-NR2 in NPSLE and found that the antibody may lead to dysfunction of NMDAR *in vitro* and that passive transfer of anti-NR2 in animals induced neuronal apoptosis and affects animal memory and cognitive ability (111, 112).

Anti-NMDAR autoantibodies in depression are still questionable since most of these studies considered the depression cohorts as control groups and numbers were relatively small. Variations in the methodology make it difficult to compare results from different groups, which is a common fact that should be kept in mind through this review. In particular, the methodology varies among studies (CBA or ELISA), or the same methodology is used with different experimental conditions (fixed or live CBA) by different groups, different subunits of the antigens are employed (NR1, NR1, and NR2a/b together in CBA, NR2 peptide in ELISA), different body fluids (serum, plasma, or CSF), different immunoglobulins detected (IgG, IgA, and/or IgM) and different dilutions of the sample used (from 1:10 to 1:320) (17).

### Anti-AMPAR

AMPAR is another ionotropic glutamate receptor which mediates the fast excitatory neurotransmission in the CNS (113). The majority of AMPAR are tetramers composed of two GluR2 and either two GluR1, three, or four subunits that combine in a brain region-dependent manner (114, 115). GluR1/2 and GluR2/3 receptors are highly expressed in the synaptic CA3-CA1 areas of the hippocampus. Besides, they are also expressed in cerebellum and caudate putamen (116).

Lai and colleagues first reported autoantibodies to AMPAR (GluR1 and GluR2 subunits) in limbic encephalitis (4). The clinical features of this type of autoimmune encephalitis are short-term memory deficits, emotional/behavioral changes, and seizures, frequent association with paraneoplastic disease, treatment responsiveness and has a tendency to relapse (4). GluR3 has been identified as an autoantigen in Rasmussen's encephalitis in which the clinical characteristics of these patients were mainly epilepsy and language problems (117, 118). An anti-AMPAR (GluR1)-positive case was reported with breast ductal infiltrating adenocarcinoma that showed behavioral changes, depressed mood, and memory loss during the process of the disease without seizures (3). In contrast, screening for anti-AMPAR (GluR1 and GluR2) in a depression cohort ($n < 380$) by fixed CBA using 1:10 diluted serum did not report any positive cases (101, 102).

## Anti-GABA Receptor Autoantibodies

### Anti-$GABA_A$ Receptor

$GABA_AR$ are ionotropic receptors and GABA is the ligand. There are several subunit isoforms (α, β, and γ) for the $GABA_AR$, which determine the receptor's agonist affinity, chance of opening, conductance, and other properties. Subunits of $GABA_AR$ have a different distribution in the brain and may respond with a different sensitivity to GABA, leading to a different function. A decline in $GABA_AR$ signaling triggers hyperactivity in neurological disorders such as insomnia, anxiety, and epilepsy.

Autoantibodies to $GABA_AR$ were recently identified in autoimmune encephalitis. The clinical features varied in different studies. Petit-Pedrol et al. reported a series of 18 patients with anti-$GABA_AR$, of whom 6 had high titer antibodies detected both in blood and CSF and showed severe encephalitis and refractory seizures (8). The other 12 patients with lower titers in serum had different diagnoses. Six showed encephalitis with seizures, four had stiff-person syndrome, and two had opsoclonus-myoclonus. Anti-$GABA_AR$ in lower titers was also found in 5 of these 12. The autoantibodies targeted α1 and β3 subunits and caused selective reduction of the synaptic $GABA_AR$ (8). Two anti-$GABA_AR$ encephalitis patients were reported and their autoantibodies targeted the β3 subunits (9). Later, a study identified the main antigens as α1/γ2 in a group of patients with seizures and cognitive or neuropsychiatric problems. Some of these patients had mood changes (2 in 11 showed depression symptoms and the autoantibodies targeted to α1 or undefined; 3 showed anxiety and the autoantibodies targeted to α1, γ2, or undefined subunits) (10). A cohort of purely depression disorders has not been tested so far.

### Anti-$GABA_B$ Receptor

$GABA_B$ receptors are metabotropic transmembrane receptors that are linked to G-protein-gated potassium channels (119). There are two $GABA_BR$ subtypes, $GABA_{B1}R$ and $GABA_{B2}R$, assembling into functional heterogenic complexes (120, 121). $GABA_{B1}R(-/-)$ mice, which lack functional GABA(B) receptors, showed more anxiety and decreased immobility (antidepressant-like behavior), and $GABA_BR$ selective antagonist CGP56433A showed antidepressant effects as well (122).

Autoantibodies to the $GABA_BR$ (anti-$GABA_BR$) were reported in limbic encephalitis (15 in 410 cases) (7). In all patients, autoantibodies to $GABA_BR$ targeted the $GABA_{B1}R$ and only one targeted $GABA_{B2}R$ additionally (123, 124). If anti-$GABA_BR$ inactivates synaptic and extrasynaptic $GABA_BR$, it could potentially cause anxiety but not depression. Additionally, one anti-$GABA_BR$ (B1/B2) positive patient was found in a depression cohort ($n < 310$) by fixed CBA using 1:10 diluted serum with all the controls being seronegative ($n > 1{,}693$) (102). To date, there are only limited studies that focus on this antigen and further investigations should be performed to extend the knowledge about $GABA_BR$ autoantibody effector mechanisms.

## Anti-Monoamine Receptor Autoantibodies

### Anti-5-HT1A Receptor and anti-D2 Antibodies

The 5-HT1A receptor is a subtype of serotonin receptor expressed widely in the limbic system and has implications in the control of mood, cognition, and memory (125). D2R is a dopamine receptor and has long isoforms (located mainly on the postsynaptic membrane) and short isoforms (mainly on the presynaptic membrane), coded by alternative splicing of the same DRD2 gene (126). It is highly expressed in basal ganglia and also cortex, hippocampus, and in substantia nigra and is involved in synaptic plasticity and memory formation (127). Both receptors are coupled with

G-proteins that inhibit adenylyl cyclase, as well as other second messenger cascades (125, 128).

The presence in serum of IgG autoantibodies against 5-HT1A (anti-5-HT1A) and dopamine receptor $D_2$ (anti-D2R) in psychiatric disorders was studied by radioimmunoassay (RIA) (129). 7.9% of the mood disorder patients including 33 MDD had anti-5-HT1A and 9.5% had anti-D2R compared to healthy controls which were seronegative for these autoantibodies. Anti-D2R was significantly associated with the severity of guilt feeling and depressive mood. To our knowledge, no further experiments have been reported detecting or investigating the role of anti-5-HT1A in psychiatric disorders.

Immunoglobulin G autoantibodies against D2R were identified by flow cytometry CBA with a cutoff at three SDs above the control mean using transfected HEK cells in a subgroup of children with basal ganglia encephalitis (15). 12 of 17 children (aged 0.4–15 years, nine males) with basal ganglia encephalitis had anti-D2R, compared with 0 in 67 controls. The 12 anti-D2R-positive patients had movement disorders and psychiatric disturbance characterized by Parkinsonism, dystonia, chorea, emotional lability, attention deficit, and psychosis. A later study showed a specific and significant reduction of D2R when transfected cells were incubated with anti-D2R, and the extracellular N-terminus of D2R was revealed as the main immunogenic region (130). 3 anti-D2R-positive cases out of 43 were reported in first episode of acute psychosis in children and the 17 controls studied were seronegative (131). This is the first report of serum IgG autoantibodies to surface D2R in pediatric patients with isolated psychosis. And three of the patients were previously diagnosed with other types of mental disorders: one patient had attention-deficit/hyperactivity disorder, behavior disorder, one had depression and anxiety, prematurity, and one had anorexia nervosa (131).

## Anti-VGKC Complex and Associated Protein Autoantibodies

### Anti-LGI1, Anti-Caspr2, and Anti-DPPX

Voltage-gated potassium channels, typically formed by four different α subunits (there are 40 α subunits known), each associated with a β subunit (more than 12 β auxiliary proteins to α subunits), play a crucial role in returning the depolarized cell such as neurons to a resting state (26, 132). Typically, they are tetramers of four α subunits arranged as a ring, each contributing to the wall of the transmembrane K+ pore. Additionally, there are other associated proteins like LGI1, Caspr2, contactin 2, ADAM22, and ADAM23, which can affect the function of VGKC and AMPAR (mentioned in the antibody effector function section) (133).

Autoantibodies to the VGKC complex (anti-VGKC complex) have been known for a long time and are involved in the pathogenesis of neuromyotonia, Morvan's syndrome, epilepsy, and limbic encephalitis (26, 134, 135). In recent years, researchers identified by CBA and IHC that the VGKC-associated proteins LGI1 and Caspr2 are actually the main targets in autoimmune encephalitis. Kv4.2, a subtype of VGKC, is widely expressed in the CNS and autoantibodies directed against DPPX (an auxiliary subunit of Kv4.2 channels) (anti-DPPX) was also identified, yet in approximately 19% of the seropositive cases for the VGKC complex by RIA the antigen/s remain unknown (11, 14). Epilepsy and limbic encephalitis are more frequently related to anti-LGI1, while peripheral nerve hyperexcitability disorders, like Morvan's syndrome, are more common in anti-Caspr2-positive cases (136). Anti-LGI1 patients present a clinical spectrum of confusion, depression, paranoia, behavior disturbances, visual hallucinations, and dementia at onset of the disease (137–139). Two seropositive (one IgG type) anti-Caspr2 were found in a cohort of 310 patients with affective disorders, while in the same study, no anti-LGI1 and anti-DPPX seropositive cases were reported (102). The largest described cohort of anti-DPPX (IgG)-positive patients consisted of 20 cases. Those sera or CSF-positive cases were found in patients referred for evaluation of paraneoplastic neurologic autoimmunity (totally tested 83) and 41,812 samples submitted for evaluation of neural autoantibodies (0.02% positive anti-DPPX). Out of the 20 anti-DPPX-positive patients, 20% showed depressive symptoms (14).

## TAKE-HOME MESSAGE

Although an increasing number of studies have substantially improved our knowledge on autoimmunity in the CNS, still large controversy exists, especially due to the variation in the methodology used. Also, our knowledge is largely based on findings from autoimmune encephalitis cohorts. There are several methodological aspects which have to be considered when detecting NSAbs in psychiatric disorders, especially in depression or other mood disorders. First, the antigens targeted by the autoantibodies can be composed of several subunits. Autoantibodies against each of the subunits can have different clinical significance and implications (1). A good example is the detection of NMDA NR1 antibodies and N2A/B antibodies. Anti-NR1 is believed to be pathogenic in NMDAR encephalitis (97). However, anti-N2A/B plays a role in NPSLE (108). When autoantibodies target different subunits of other glutamate receptors or GABA receptors, they may cause different clinical symptoms. At the same time, most NSAbs target epitopes only if the antigens are expressed in their native conformation. Techniques like CBA, IHC of brain sections optimized to detect membrane proteins (rodent), and immunocytochemistry of cultures of rodent live hippocampal neurons fit this requirement. Third, different concentrations of the same autoantibody might have different effects and biological relevance. For example, high titers of anti-$GABA_AR$ are specific for severe encephalitis and refractory seizures patients and low titers present in a broad range of neurology disorders and may lack specificity (8). Another aspect which needs to be taken into account is the value of serum and CSF for detecting autoantibodies. The use of CSF for detecting NSAbs in depression has not been evaluated to date. Finally, NSAbs should be tested in a "panel", rather than a single one because of the overlap between symptoms and signs of different autoimmune encephalitis and psychotic disorders (140). Also, the coexistence of several NSAbs may occur in individual patients and cause combined manifestations (9, 141, 142).

To summarize, NSAbs, targeting important neuronal receptors or interfering with ion channels and associated protein function, are responsible for psychiatric symptoms in autoimmune encephalitis cases. At the moment, several studies reported the

presence of anti-NMDAR (NR1 and NR2B), anti-5-HT1A, and D2R in depression cohorts. However, due to the heterogeneity of the methodology, variation in the samples used, and the limited cohort size, there is insufficient evidence to support those NSAbs can cause depression without other obvious neurological symptoms. In the future, large cohorts, longitudinal studies need to be performed using sensitive, quantitative, and reproducible methods without loss of antigen conformation. Finally, analysis of autoantibodies targeting neuronal surface antigens relevant to the pathology of depression should be performed.

## AUTHOR CONTRIBUTIONS

SZ contributes in the design, writing, and correcting of the paper. CH and MD contributed the writing and corrections; PM helped with the corrections of the review; ML helped with design and the corrections of the review; and PM-M supervised the design and helped to write and correct the review.

## FUNDING

The authors thank the Netherlands Organization for Scientific Research "Graduate School of Translational Neuroscience Program" (022005019), the Brain Foundation of the Netherlands (KS2012(1)-157), and the ZonMW NWO Program Translationeel onderzoek (40-41200-98-9257) as well as the Veni Fellowship of the Netherlands Organization for Scientific Research (916.10.148), a fellowship of the Brain Foundation of the Netherlands (FS2008(1)-28), the Prinses Beatrix Fonds (Project WAR08-12), and the China Scholarship Council (201507720015).

## REFERENCES

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* (2016) 15(4):391–404. doi:10.1016/S1474-4422(15)00401-9
2. Dalmau J, Tüzün E, Wu H, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* (2007) 61(1):25–36. doi:10.1002/ana.21050
3. Bataller L, Galiano R, García-Escrig M, Martínez B, Sevilla T, Blasco R, et al. Reversible paraneoplastic limbic encephalitis associated with antibodies to the AMPA receptor. *Neurology* (2010) 74(3):265–7. doi:10.1212/WNL.0b013e3181cb3e52
4. Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol* (2009) 65(4):424–34. doi:10.1002/ana.21589
5. Savitz JB, Drevets WC. Neuroreceptor imaging in depression. *Neurobiol Dis* (2013) 52:49–65. doi:10.1016/j.nbd.2012.06.001
6. Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, et al. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nucl Med Biol* (2007) 34(7):865–77. doi:10.1016/j.nucmedbio.2007.06.008
7. Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, et al. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* (2010) 9(1):67–76. doi:10.1016/S1474-4422(09)70324-2
8. Petit-Pedrol M, Armangue T, Peng X, Bataller L, Cellucci T, Davis R, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABA(A) receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *Lancet Neurol* (2014) 13(3):276–86. doi:10.1016/S1474-4422(13)70299-0
9. Ohkawa T, Satake S, Yokoi N, Miyazaki Y, Ohshita T, Sobue G, et al. Identification and characterization of GABA(A) receptor autoantibodies in autoimmune encephalitis. *J Neurosci* (2014) 34(24):8151. doi:10.1523/JNEUROSCI.4415-13.2014
10. Pettingill P, Kramer HB, Coebergh JA, Pettingill R, Maxwell S, Nibber A, et al. Antibodies to GABAA receptor α1 and γ2 subunits clinical and serologic characterization. *Neurology* (2015) 84(12):1233–41. doi:10.1212/WNL.0000000000001326
11. Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* (2010) 133(9):2734–48. doi:10.1093/brain/awq213
12. Boronat A, Gelfand JM, Gresa-Arribas N, Jeong H-Y, Walsh M, Roberts K, et al. Encephalitis and antibodies to DPPX, a subunit of Kv4.2 potassium channels. *Ann Neurol* (2013) 73(1):120–8. doi:10.1002/ana.23756
13. Balint B, Jarius S, Nagel S, Haberkorn U, Probst C, Blöcker IM, et al. Progressive encephalomyelitis with rigidity and myoclonus: a new variant with DPPX antibodies. *Neurology* (2014) 82(17):1521–8. doi:10.1212/WNL.0000000000000372
14. Tobin WO, Lennon VA, Komorowski L, Probst C, Clardy SL, Aksamit AJ, et al. DPPX potassium channel antibody: frequency, clinical accompaniments, and outcomes in 20 patients. *Neurology* (2014) 83(20):1797–803. doi:10.1212/WNL.0000000000000991
15. Dale RC, Merheb V, Pillai S, Wang D, Cantrill L, Murphy TK, et al. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain* (2012) 135(11):3453–68. doi:10.1093/brain/aws256
16. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* (2011) 10(8):759–72. doi:10.1016/S1474-4422(11)70096-5
17. Lennox BR, Palmer-Cooper EC, Pollak T, Hainsworth J, Marks J, Jacobson L, et al. Prevalence and clinical characteristics of serum neuronal cell surface antibodies in first-episode psychosis: a case-control study. *Lancet Psychiatry* (2017) 4:42–8. doi:10.1016/s2215-0366(16)30375-3
18. Pollak TA, McCormack R, Peakman M, Nicholson TR, David AS. Prevalence of anti-N-methyl-D-aspartate (NMDA) receptor antibodies in patients with schizophrenia and related psychoses: a systematic review and meta-analysis. *Psychol Med* (2014) 44(12):2475–87. doi:10.1017/S003329171300295X
19. León-Caballero J, Pacchiarotti I, Murru A, Valentí M, Colom F, Benach B, et al. Bipolar disorder and antibodies against the N-methyl-D-aspartate receptor: a gate to the involvement of autoimmunity in the pathophysiology of bipolar illness. *Neurosci Biobehav Rev* (2015) 55:403–12. doi:10.1016/j.neubiorev.2015.05.012
20. Chen Y, Jiang T, Chen P, Ouyang J, Xu G, Zeng Z, et al. Emerging tendency towards autoimmune process in major depressive patients: a novel insight from Th17 cells. *Psychiatry Res* (2011) 188(2):224–30. doi:10.1016/j.psychres.2010.10.029
21. Iseme RA, McEvoy M, Kelly B, Agnew L, Attia J, Walker FR. Autoantibodies and depression: evidence for a causal link? *Neurosci Biobehav Rev* (2014) 40:62–79. doi:10.1016/j.neubiorev.2014.01.008
22. Dama M, Steiner M, Van Lieshout R. Thyroid peroxidase autoantibodies and perinatal depression risk: a systematic review. *J Affect Disord* (2016) 198:108–21. doi:10.1016/j.jad.2016.03.021
23. Bai R, Liu S, Zhao Y, Cheng Y, Li S, Lai A, et al. Depressive and anxiety disorders in systemic lupus erythematosus patients without major neuropsychiatric manifestations. *J Immunol Res* (2016) 2016:2829018. doi:10.1155/2016/2829018
24. Laske C, Zank M, Klein R, Stransky E, Batra A, Buchkremer G, et al. Autoantibody reactivity in serum of patients with major depression, schizophrenia and healthy controls. *Psychiatry Res* (2008) 158(1):83–6. doi:10.1016/j.psychres.2006.04.023
25. Postal M, Appenzeller S. The importance of cytokines and autoantibodies in depression. *Autoimmun Rev* (2015) 14(1):30–5. doi:10.1016/j.autrev.2014.09.001
26. Gutman GA, Chandy KG, Grissmer S, Lazdunski M, Mckinnon D, Pardo LA, et al. International Union of Pharmacology. LIII. Nomenclature and molecular

relationships of voltage-gated potassium channels. *Pharmacol Rev* (2005) 57(4):473–508. doi:10.1124/pr.57.4.10
27. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology* (2012) 62(1):63–77. doi:10.1016/j.neuropharm.2011.07.036
28. Möhler H. The GABA system in anxiety and depression and its therapeutic potential. *Neuropharmacology* (2012) 62(1):42–53. doi:10.1016/j.neuropharm.2011.08.040
29. Cutler RWP, Watters GV, Hammerstad JP. The origin and turnover rates of cerebrospinal fluid albumin and gamma-globulin in man. *J Neurol Sci* (1970) 10(3):259–68. doi:10.1016/0022-510X(70)90154-1
30. Reiber H. Flow rate of cerebrospinal fluid (CSF) – a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. *J Neurol Sci* (1994) 122(2):189–203. doi:10.1016/0022-510X(94)90298-4
31. Hoffmann C, Zong S, Mané-Damas M, Molenaar P, Losen M, Martinez-Martinez P. Autoantibodies in neuropsychiatric disorders. *Antibodies* (2016) 5(2):9. doi:10.3390/antib5020009
32. Dileepan T, Smith ED, Knowland D, Hsu M, Platt M, Bittner-Eddy P, et al. Group A streptococcus intranasal infection promotes CNS infiltration by streptococcal-specific Th17 cells. *J Clin Invest* (2016) 126(1):303–17. doi:10.1172/JCI80792
33. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry* (2014) 19(10):1143–9. doi:10.1038/mp.2013.110
34. Hammer C, Zerche M, Schneider A, Begemann M, Nave KA, Ehrenreich H. Apolipoprotein E4 carrier status plus circulating anti-NMDAR1 autoantibodies: association with schizoaffective disorder. *Mol Psychiatry* (2014) 19(10):1054–6. doi:10.1038/mp.2014.52
35. Ransohoff RM, Kivisakk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* (2003) 3(7):569–81. doi:10.1038/nri1130
36. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. *Neurology* (2011) 77(6):589–93. doi:10.1212/WNL.0b013e318228c136
37. Hansen H, Klingbeil C, Dalmau J, Li W, Weißbrich B, Wandinger K. Persistent intrathecal antibody synthesis 15 years after recovering from anti-N-methyl-D-aspartate receptor encephalitis. *JAMA Neurol* (2013) 70(1):117–9. doi:10.1001/jamaneurol.2013.585
38. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann N Y Acad Sci* (2015) 1338(1):94–114. doi:10.1111/nyas.12553
39. Smitt PS, Kinoshita A, De Leeuw B, Moll W, Coesmans M, Jaarsma D, et al. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *N Engl J Med* (2000) 342(1):21–7. doi:10.1056/NEJM200001063420104
40. Castillo-Gomez E, Kästner A, Steiner J, Schneider A, Hettling B, Poggi G, et al. The brain as immunoprecipitator of serum autoantibodies against N-methyl-D-aspartate receptor subunit NR1. *Ann Neurol* (2016) 79(1):144–51. doi:10.1002/ana.24545
41. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* (2014) 5:520. doi:10.3389/fimmu.2014.00520
42. Gomez AM, Van Den Broeck J, Vrolix K, Janssen SP, Lemmens MAM, Van Der Esch E, et al. Antibody effector mechanisms in myasthenia gravis-pathogenesis at the neuromuscular junction. *Autoimmunity* (2010) 43(5–6):353–70. doi:10.3109/08916930903555943
43. Koneczny I, Stevens JAA, De Rosa A, Huda S, Huijbers MG, Saxena A, et al. IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. *J Autoimmun* (2017) 77:104–15. doi:10.1016/j.jaut.2016.11.005
44. Chatenoud L, Bach J-F. Antigenic modulation – a major mechanism of antibody action. *Immunol Today* (1984) 5(1):20–5. doi:10.1016/0167-5699(84)90061-6
45. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N Engl J Med* (1978) 298(20):1116–22. doi:10.1056/NEJM197805182982004
46. Pumplin D, Drachman D. Myasthenic patients' IgG causes redistribution of acetylcholine receptors: freeze-fracture studies. *J Neurosci* (1983) 3(3):576–84.
47. Mikasova L, De Rossi P, Bouchet D, Georges F, Rogemond V, Didelot A, et al. Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain* (2012) 135(5):1606–21. doi:10.1093/brain/aws092
48. Jantzen SU, Ferrea S, Wach C, Quasthoff K, Illes S, Scherfeld D, et al. In vitro neuronal network activity in NMDA receptor encephalitis. *BMC Neurosci* (2013) 14:17. doi:10.1186/1471-2202-14-17
49. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* (2010) 30(17):5866–75. doi:10.1523/jneurosci.0167-10.2010
50. Tüzün E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J. Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathol* (2009) 118(6):737–43. doi:10.1007/s00401-009-0582-4
51. Peng X, Hughes EG, Moscato EH, Parsons TD, Dalmau J, Balice-Gordon RJ. Cellular plasticity induced by anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor encephalitis antibodies. *Ann Neurol* (2015) 77(3):381–98. doi:10.1002/ana.24293
52. Engel AG, Arahata K. The membrane attack complex of complement at the endplate in myasthenia gravisa. *Ann N Y Acad Sci* (1987) 505(1):326–32. doi:10.1111/j.1749-6632.1987.tb51301.x
53. Bien CG, Vincent A, Barnett MH, Becker AJ, Blümcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* (2012) 135(5):1622–38. doi:10.1093/brain/aws082
54. Whitney KD, Andrews PI, McNamara JO. Immunoglobulin G and complement immunoreactivity in the cerebral cortex of patients with Rasmussen's encephalitis. *Neurology* (1999) 53(4):699. doi:10.1212/wnl.53.4.699
55. Ochoa MC, Minute L, Rodriguez I, Garasa S, Perez-Ruiz E, Inoges S, et al. Antibody-dependent cell cytotoxicity: immunotherapy strategies enhancing effector NK cells. *Immunol Cell Biol* (2017) 95:347–55. doi:10.1038/icb.2017.6
56. Teillaud J-L. *Antibody-Dependent Cellular Cytotoxicity (ADCC)*. eLS, John Wiley & Sons, Ltd (2012). doi:10.1002/9780470015902.a0000498.pub2
57. Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* (2002) 125(7):1450–61. doi:10.1093/brain/awf151
58. Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C, et al. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol* (2009) 66(5):617–29. doi:10.1002/ana.21802
59. Ratelade J, Asavapanumas N, Ritchie AM, Wemlinger S, Bennett JL, Verkman AS. Involvement of antibody-dependent cell-mediated cytotoxicity in inflammatory demyelination in a mouse model of neuromyelitis optica. *Acta Neuropathol* (2013) 126(5):699–709. doi:10.1007/s00401-013-1172-z
60. Lalic T, Pettingill P, Vincent A, Capogna M. Human limbic encephalitis serum enhances hippocampal mossy fiber-CA3 pyramidal cell synaptic transmission. *Epilepsia* (2011) 52(1):121–31. doi:10.1111/j.1528-1167.2010.02756.x
61. Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science* (2006) 313(5794):1792–5. doi:10.1126/science.1129947
62. Fukata Y, Lovero KL, Iwanaga T, Watanabe A, Yokoi N, Tabuchi K, et al. Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proc Natl Acad Sci U S A* (2010) 107(8):3799–804. doi:10.1073/pnas.0914537107
63. Ohkawa T, Fukata Y, Yamasaki M, Miyazaki T, Yokoi N, Takashima H, et al. Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J Neurosci* (2013) 33(46):18161–74. doi:10.1523/JNEUROSCI.3506-13.2013
64. Vincent A. Unravelling the pathogenesis of myasthenia gravis. *Nat Rev Immunol* (2002) 2(10):797–804. doi:10.1038/nri916
65. Howard FM, Lennon VA, Finley JON, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis. *Ann N Y Acad Sci* (1987) 505(1):526–38. doi:10.1111/j.1749-6632.1987.tb51321.x
66. Gomez CM, Richman DP. Anti-acetylcholine receptor antibodies directed against the alpha-bungarotoxin binding site induce a unique form of experimental myasthenia. *Proc Natl Acad Sci U S A* (1983) 80(13):4089–93. doi:10.1073/pnas.80.13.4089

67. Riemersma S, Vincent A, Beeson D, Newland C, Hawke S, Vernet-der Garabedian B, et al. Association of arthrogryposis multiplex congenita with maternal antibodies inhibiting fetal acetylcholine receptor function. *J Clin Invest* (1996) 98(10):2358–63. doi:10.1172/JCI119048
68. Wintzen AR, Plomp JJ, Molenaar PC, van Dijk JG, van Kempen GTH, Vos RM, et al. Acquired slow–channel syndrome: a form of myasthenia gravis with prolonged open time of the acetylcholine receptor channel. *Ann Neurol* (1998) 44(4):657–64. doi:10.1002/ana.410440412
69. Levite M, Ganor Y. Autoantibodies to glutamate receptors can damage the brain in epilepsy, systemic lupus erythematosus and encephalitis. *Expert Rev Neurother* (2008) 8(7):1141–60. doi:10.1586/14737175.8.7.1141
70. Levite M. Glutamate receptor antibodies in neurological diseases: anti-AMPA-GluR3 antibodies, anti-NMDA-NR1 antibodies, anti-NMDA-NR2A/B antibodies, anti-mGluR1 antibodies or anti-mGluR5 antibodies are present in subpopulations of patients with either: epilepsy, encephalitis, cerebellar ataxia, systemic lupus erythematosus (SLE) and neuropsychiatric SLE, Sjogren's syndrome, schizophrenia, mania or stroke. These autoimmune anti-glutamate receptor antibodies can bind neurons in few brain regions, activate glutamate receptors, decrease glutamate receptor's expression, impair glutamate-induced signaling and function, activate blood brain barrier endothelial cells, kill neurons, damage the brain, induce behavioral/psychiatric/cognitive abnormalities and ataxia in animal models, and can be removed or silenced in some patients by immunotherapy. *J Neural Transm* (2014) 121(8):1029–75. doi:10.1007/s00702-014-1193-3
71. Levite M, Fleidervish IA, Schwarz A, Pelled D, Futerman AH. Autoantibodies to the glutamate receptor kill neurons via activation of the receptor ion channel. *J Autoimmun* (1999) 13(1):61–72. doi:10.1006/jaut.1999.0301
72. Saravanan P, Dayan CM. Thyroid autoantibodies. *Endocrinol Metab Clin North Am* (2001) 30(2):315–37. doi:10.1016/S0889-8529(05)70189-4
73. Elhwuegi AS. Central monoamines and their role in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2004) 28(3):435–51. doi:10.1016/j.pnpbp.2003.11.018
74. Gatt JM, Burton KLO, Williams LM, Schofield PR. Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. *J Psychiatr Res* (2015) 60:1–13. doi:10.1016/j.jpsychires.2014.09.014
75. Savitz J, Lucki I, Drevets WC. 5-HT1A receptor function in major depressive disorder. *Prog Neurobiol* (2009) 88(1):17–31. doi:10.1016/j.pneurobio.2009.01.009
76. Kim D, Chung S, Lee SH, Koo JH, Lee J-H, Jahng JW. Decreased expression of 5-HT1A in the circumvallate taste cells in an animal model of depression. *Arch Oral Biol* (2017) 76:42–7. doi:10.1016/j.archoralbio.2017.01.005
77. Park SK, Nguyen MD, Fischer A, Luke MP-S, Affar EB, Dieffenbach PB, et al. Par-4 links dopamine signaling and depression. *Cell* (2005) 122(2):275–87. doi:10.1016/j.cell.2005.05.031
78. Glantz LA, Gilmore JH, Overstreet DH, Salimi K, Lieberman JA, Jarskog LF. Pro-apoptotic Par-4 and dopamine D2 receptor in temporal cortex in schizophrenia, bipolar disorder and major depression. *Schizophr Res* (2010) 118(1–3):292–9. doi:10.1016/j.schres.2009.12.027
79. Citrome L. Brexpiprazole: a new dopamine $D_2$ receptor partial agonist for the treatment of schizophrenia and major depressive disorder. *Drugs Today (Barc)* (2015) 51(7):397–414. doi:10.1358/dot.2015.51.7.2358605
80. Cotman CW, Monaghan DT, Ottersen OP, Storm-Mathisen J. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends Neurosci* (1987) 10(7):273–80. doi:10.1016/0166-2236(87)90172-X
81. Stevens CF. A finger on brain receptors. *Nature* (1989) 342(6250):620–1. doi:10.1038/342620a0
82. Bechtholt-Gompf AJ, Walther HV, Adams MA, Carlezon WA, Öngür D, Cohen BM. Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. *Neuropsychopharmacology* (2010) 35(10):2049–59. doi:10.1038/npp.2010.74
83. Iadarola ND, Niciu MJ, Richards EM, Vande Voort JL, Ballard ED, Lundin NB, et al. Ketamine and other N-methyl-D-aspartate receptor antagonists in the treatment of depression: a perspective review. *Ther Adv Chronic Dis* (2015) 6(3):97–114. doi:10.1177/2040622315579059
84. Du J, Suzuki K, Wei Y, Wang Y, Blumenthal R, Chen Z, et al. The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders. *Neuropsychopharmacology* (2006) 32(4):793–802. doi:10.1038/sj.npp.1301178
85. Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA, et al. Reduced cortical γ-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* (1999) 56(11):1043–7. doi:10.1001/archpsyc.56.11.1043
86. Sanacora G, Mason GF, Rothman DL, Krystal JH. Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. *Am J Psychiatry* (2002) 159(4):663–5. doi:10.1176/appi.ajp.159.4.663
87. Sanacora G, Mason GF, Rothman DL, Hyder F, Ciarcia JJ, Ostroff RB, et al. Increased cortical GABA concentrations in depressed patients receiving ECT. *Am J Psychiatry* (2003) 160(3):577–9. doi:10.1176/appi.ajp.160.3.577
88. Klumpers UM, Veltman DJ, Drent ML, Boellaard R, Comans EF, Meynen G, et al. Reduced parahippocampal and lateral temporal GABAA-[11C] flumazenil binding in major depression: preliminary results. *Eur J Nucl Med Mol Imaging* (2010) 37(3):565–74. doi:10.1007/s00259-009-1292-9
89. Shen Q, Lal R, Luellen BA, Earnheart JC, Andrews AM, Luscher B. γ-Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. *Biol Psychiatry* (2010) 68(6):512–20. doi:10.1016/j.biopsych.2010.04.024
90. Vyklicky V, Korinek M, Smejkalova T, Balik A, Krausova B, Kaniakova M, et al. Structure, function, and pharmacology of NMDA receptor channels. *Physiol Res* (2014) 63:S191.
91. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* (2010) 62(3):405–96. doi:10.1124/pr.109.002451
92. Lakhan SE, Caro M, Hadzimichalis N. NMDA receptor activity in neuropsychiatric disorders. *Front Psychiatry* (2013) 4:52. doi:10.3389/fpsyt.2013.00052
93. Martucci L, Wong AHC, De Luca V, Likhodi O, Wong GWH, King N, et al. N-methyl-D-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: polymorphisms and mRNA levels. *Schizophr Res* (2006) 84(2–3):214–21. doi:10.1016/j.schres.2006.02.001
94. Ellison G. The N-methyl-D-aspartate antagonists phencyclidine, ketamine and dizocilpine as both behavioral and anatomical models of the dementias. *Brain Res Rev* (1995) 20(2):250–67. doi:10.1016/0165-0173(94)00014-G
95. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* (2000) 47(4):351–4. doi:10.1016/S0006-3223(99)00230-9
96. Han Y, Chen J, Zou D, Zheng P, Li Q, Wang H, et al. Efficacy of ketamine in the rapid treatment of major depressive disorder: a meta-analysis of randomized, double-blind, placebo-controlled studies. *Neuropsychiatr Dis Treat* (2016) 12:2859. doi:10.2147/NDT.S117146
97. Gleichman AJ, Spruce LA, Dalmau J, Seeholzer SH, Lynch DR. Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. *J Neurosci* (2012) 32(32):11082–94. doi:10.1523/JNEUROSCI.0064-12.2012
98. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* (2008) 7(12):1091–8. doi:10.1016/S1474-4422(08)70224-2
99. Pearlman DM, Najjar S. Meta-analysis of the association between N-methyl-D-aspartate receptor antibodies and schizophrenia, schizoaffective disorder, bipolar disorder, and major depressive disorder. *Schizophr Res* (2014) 157(1–3):249–58. doi:10.1016/j.schres.2014.05.001
100. Titulaer M, Dalmau J. Antibodies to NMDA receptor, blood-brain barrier disruption and schizophrenia: a theory with unproven links. *Mol Psychiatry* (2014) 19(10):1054. doi:10.1038/mp.2014.25
101. Steiner J, Walter M, Glanz W, Sarnyai Z, Bernstein H, Vielhaber S, et al. Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of igg nr1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis. *JAMA Psychiatry* (2013) 70(3):271–8. doi:10.1001/2013.jamapsychiatry.86

102. Dahm L, Ott C, Steiner J, Stepniak B, Teegen B, Saschenbrecker S, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann Neurol* (2014) 76(1):82–94. doi:10.1002/ana.24189
103. Steiner J, Teegen B, Schiltz K, Bernstein H, Stoecker W, Bogerts B. Prevalence of N-methyl-D-aspartate receptor autoantibodies in the peripheral blood: healthy control samples revisited. *JAMA Psychiatry* (2014) 71(7):838–9. doi:10.1001/jamapsychiatry.2014.469
104. Dickerson F, Stallings C, Origoni A, Vaughan C, Katsafanas E, Khushalani S, et al. A combined marker of inflammation in individuals with mania. *PLoS One* (2013) 8(9):e73520. doi:10.1371/journal.pone.0073520
105. Zandi MS, Irani SR, Lang B, Waters P, Jones PB, McKenna P, et al. Disease-relevant autoantibodies in first episode schizophrenia. *J Neurol* (2011) 258(4):686–8. doi:10.1007/s00415-010-5788-9
106. Planagumà J, Leypoldt F, Mannara F, Gutiérrez-Cuesta J, Martín-García E, Aguilar E, et al. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* (2015) 138(1):94–109. doi:10.1093/brain/awu310
107. Desestret V, Pascual O, Honnorat J. A mouse model of autoimmune encephalitis. *Brain* (2015) 138(1):5–8. doi:10.1093/brain/awu342
108. Lapteva L, Nowak M, Yarboro CH, Takada K, Roebuck-Spencer T, Weickert T, et al. Anti-N-methyl-D-aspartate receptor antibodies, cognitive dysfunction, and depression in systemic lupus erythematosus. *Arthritis Rheum* (2006) 54(8):2505–14. doi:10.1002/art.22031
109. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med* (2001) 7(11):1189–93. doi:10.1038/nm1101-1189
110. Putterman C, Diamond B. Immunization with a peptide surrogate for double-stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. *J Exp Med* (1998) 188(1):29–38. doi:10.1084/jem.188.1.29
111. Kowal C, DeGiorgio LA, Lee JY, Edgar MA, Huerta PT, Volpe BT, et al. Human lupus autoantibodies against NMDA receptors mediate cognitive impairment. *Proc Natl Acad Sci U S A* (2006) 103(52):19854–9. doi:10.1073/pnas.0608397104
112. Chang EH, Volpe BT, Mackay M, Aranow C, Watson P, Kowal C, et al. Selective impairment of spatial cognition caused by autoantibodies to the N-methyl-D-aspartate receptor. *EBioMedicine* (2015) 2(7):755–64. doi:10.1016/j.ebiom.2015.05.027
113. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu Rev Cell Dev Biol* (2007) 23(1):613–43. doi:10.1146/annurev.cellbio.23.090506.123516
114. Palmer CL, Cotton L, Henley JM. The molecular pharmacology and cell biology of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Pharmacol Rev* (2005) 57(2):253–77. doi:10.1124/pr.57.2.7
115. Greger IH, Ziff EB, Penn AC. Molecular determinants of AMPA receptor subunit assembly. *Trends Neurosci* (2007) 30(8):407–16. doi:10.1016/j.tins.2007.06.005
116. Sprengel R. Role of AMPA receptors in synaptic plasticity. *Cell Tissue Res* (2006) 326(2):447–55. doi:10.1007/s00441-006-0275-4
117. Rogers S, Andrews P, Gahring L, Whisenand T, Cauley K, Crain B, et al. Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science* (1994) 265(5172):648–51. doi:10.1126/science.8036512
118. Mantegazza R, Bernasconi P, Baggi F, Spreafico R, Ragona F, Antozzi C, et al. Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J Neuroimmunol* (2002) 131(1–2):179–85. doi:10.1016/S0165-5728(02)00261-8
119. Emson PC. GABAB receptors: structure and function. *Prog Brain Res* (2007) 160: 43–57. In: James M, Tepper EDA, Bolam JP, editors. Elsevier. doi:10.1016/S0079-6123(06)60004-6
120. Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, et al. GABAB-receptor subtypes assemble into functional heteromeric complexes. *Nature* (1998) 396(6712):683–7. doi:10.1038/25360
121. Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, et al. GABAB receptors function as a heteromeric assembly of the subunits GABABR1 and GABABR2. *Nature* (1998) 396(6712):674–9. doi:10.1038/24050
122. Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for GABAB receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology* (2004) 29(6):1050–62. doi:10.1038/sj.npp.1300413
123. Batocchi AP, Marca GD, Frisullo G. Complement-mediated cytotoxicity of antibodies to the GABAB receptor. *Lancet Neurol* (2010) 9(4):343. doi:10.1016/S1474-4422(10)70070-3
124. Lancaster E, Lai M, Dalmau J. Complement-mediated cytotoxicity of antibodies to the GABAB receptor. *Lancet Neurol* (2010) 9(4):343; author reply 343–4. doi:10.1016/S1474-4422(10)70071-5
125. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* (1999) 38(8):1083–152. doi:10.1016/S0028-3908(99)00010-6
126. Usiello A, Baik J-H, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, et al. Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* (2000) 408(6809):199–203. doi:10.1038/35041572
127. Beaulieu J-M, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* (2011) 63(1):182–217. doi:10.1124/pr.110.002642
128. Vallar L, Meldolesi J. Mechanisms of signal transduction at the dopamine D2 receptor. *Trends Pharmacol Sci* (1989) 10(2):74–7. doi:10.1016/0165-6147(89)90082-5
129. Tanaka S, Matsunaga H, Kimura M, Tatsumi K-I, Hidaka Y, Takano T, et al. Autoantibodies against four kinds of neurotransmitter receptors in psychiatric disorders. *J Neuroimmunol* (2003) 141(1–2):155–64. doi:10.1016/S0165-5728(03)00252-2
130. Sinmaz N, Tea F, Pilli D, Zou A, Amatoury M, Nguyen T, et al. Dopamine-2 receptor extracellular N-terminus regulates receptor surface availability and is the target of human pathogenic antibodies from children with movement and psychiatric disorders. *Acta Neuropathol Commun* (2016) 4:126. doi:10.1186/s40478-016-0397-1
131. Pathmanandavel K, Starling J, Merheb V, Ramanathan S, Sinmaz N, Dale RC, et al. Antibodies to surface dopamine-2 receptor and N-methyl-D-aspartate receptor in the first episode of acute psychosis in children. *Biol Psychiatry* (2015) 77(6):537–47. doi:10.1016/j.biopsych.2014.07.014
132. Pongs O, Leicher T, Berger M, Roeper J, BÄHring R, Wray D, et al. Functional and molecular aspects of voltage-gated K+ channel β subunits. *Ann N Y Acad Sci* (1999) 868(1):344–55. doi:10.1111/j.1749-6632.1999.tb11296.x
133. Long SB, Campbell EB, MacKinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science* (2005) 309(5736):897–903. doi:10.1126/science.1116269
134. Hart IK, Waters C, Vincent A, Newland C, Beeson D, Pongs O, et al. Autoantibodies detected to expressed K+ channels are implicated in neuromyotonia. *Ann Neurol* (1997) 41(2):238–46. doi:10.1002/ana.410410215
135. Vincent A. Autoimmune channelopathies: John Newsom-Davis's work and legacy: a summary of the Newsom-Davis memorial lecture 2008. *J Neuroimmunol* (2008) 201–202:245–9. doi:10.1016/j.jneuroim.2008.07.007
136. Irani SR, Gelfand JM, Al-Diwani A, Vincent A. Cell-surface central nervous system autoantibodies: clinical relevance and emerging paradigms. *Ann Neurol* (2014) 76(2):168–84. doi:10.1002/ana.24200
137. Mayasi Y, Takhtani D, Garg N. Leucine-rich glioma-inactivated protein 1 antibody encephalitis: a case report. *Neurol Neuroimmunol Neuroinflamm* (2014) 1(4):e51. doi:10.1212/NXI.0000000000000051
138. Casault C, Alikhani K, Pillay N, Koch M. Jerking & confused: leucine-rich glioma inactivated 1 receptor encephalitis. *J Neuroimmunol* (2015) 289:84–6. doi:10.1016/j.jneuroim.2015.10.010
139. Messelmani M, Fekih-Mrissa N, Zaouali J, Mrissaa R. Limbic encephalitis associated with leucine-rich glioma-inactivated 1 antibodies. *Ann Saudi Med* (2015) 35(1):76. doi:10.5144/0256-4947.2015.76
140. Al-Diwani A, Pollak TA, Langford AE, Lennox BR. Synaptic and neuronal autoantibody-associated psychiatric syndromes: controversies and hypotheses. *Front Psychiatry* (2017) 8:13. doi:10.3389/fpsyt.2017.00013
141. Gresa-Arribas N, Ariño H, Martínez-Hernández E, Petit-Pedrol M, Sabater L, Saiz A, et al. Antibodies to inhibitory synaptic proteins in neurological syndromes associated with glutamic acid decarboxylase autoimmunity. *PLoS One* (2015) 10(3):e0121364. doi:10.1371/journal.pone.0121364

142. Sinmaz N, Amatoury M, Merheb V, Ramanathan S, Dale RC, Brilot F. Autoantibodies in movement and psychiatric disorders: updated concepts in detection methods, pathogenicity, and CNS entry. *Ann N Y Acad Sci* (2015) 1351(1):22–38. doi:10.1111/nyas.12764

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 07 June 2017
doi: 10.3389/fimmu.2017.00652

# Expanding Role of T Cells in Human Autoimmune Diseases of the Central Nervous System

*Deepti Pilli[1†], Alicia Zou[1†], Fiona Tea[1], Russell C. Dale[1,2] and Fabienne Brilot[1,2*]*

[1] Brain Autoimmunity Group, Institute for Neuroscience and Muscle Research, The Kids Research Institute at The Children's Hospital at Westmead, University of Sydney, Sydney, NSW, Australia, [2] Brain and Mind Centre, University of Sydney, Sydney, NSW, Australia

It is being increasingly recognized that a dysregulation of the immune system plays a vital role in neurological disorders and shapes the treatment of the disease. Aberrant T cell responses, in particular, are key in driving autoimmunity and have been traditionally associated with multiple sclerosis. Yet, it is evident that there are other neurological diseases in which autoreactive T cells have an active role in pathogenesis. In this review, we report on the recent progress in profiling and assessing the functionality of autoreactive T cells in central nervous system (CNS) autoimmune disorders that are currently postulated to be primarily T cell driven. We also explore the autoreactive T cell response in a recently emerging group of syndromes characterized by autoantibodies against neuronal cell-surface proteins. Common methodology implemented in T cell biology is further considered as it is an important determinant in their detection and characterization. An improved understanding of the contribution of autoreactive T cells expands our knowledge of the autoimmune response in CNS disorders and can offer novel methods of therapeutic intervention.

**Keywords: autoreactive T cells, central nervous system autoimmune diseases, neuroimmunology, autoantibodies, multiple sclerosis, T cell detection**

OPEN ACCESS

***Edited by:***
*Jens Geginat, Istituto Nazionale Genetica Molecolare (INGM), Italy*

***Reviewed by:***
*Richa Hanamsagar, Massachusetts General Hospital, United States*
*Anne Kathrin Mausberg, Essen University Hospital, Germany*

***Correspondence:**
*Fabienne Brilot*
*fabienne.brilot@sydney.edu.au*

*[†]These authors have contributed equally to this work.*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *31 January 2017*
***Accepted:*** *17 May 2017*
***Published:*** *07 June 2017*

***Citation:***
*Pilli D, Zou A, Tea F, Dale RC and Brilot F (2017) Expanding Role of T Cells in Human Autoimmune Diseases of the Central Nervous System. Front. Immunol. 8:652. doi: 10.3389/fimmu.2017.00652*

## INTRODUCTION

Autoimmunity is believed to be the underlying cause in a growing number of neurological disorders. Although the precise mechanisms that trigger autoimmunity have not been fully elucidated, it is known that a dysregulation in T cells is a key component, given their constitutive role in immunosurveillance (1). The archetypal neurological disease mediated primarily by T cells is multiple sclerosis (MS) (2, 3). It has been studied extensively for many years in both humans and animal models, and an informed understanding of MS has laid the groundwork for further studies in other suspected autoimmune neurological disorders. In particular, Rasmussen's encephalitis (RE) (4) and a spectrum of paraneoplastic syndromes (5, 6) are hypothesized to be T cell driven. In other disorders, like amytrophic lateral sclerosis (ALS), T cells may conversely play a neuroprotective role (7). In addition to a dysfunctional cellular immunity, effector molecules of humoral immunity, such as autoantibodies, may concomitantly participate in autoimmunity. Although paraneoplastic syndromes have been associated with specific autoantibodies, the search for autoantibodies in autoimmune central nervous system (CNS) diseases such as MS, RE, and ALS is still ongoing.

Indeed, in recent years, a growing number of autoantibodies targeting neuronal receptors or synaptic proteins of the CNS are proving to be useful biomarkers of various neurological

diseases treatable with immunotherapy (8–15). This has spurred intensive investigations to understand the mechanisms behind autoantibody responses, with emerging evidence suggesting a pathogenic role. However, it is well established that the production and sustenance of immunoglobulin-G (IgG) autoantibodies and autoantibody-producing B cells necessitates the involvement of T cells reactive against a shared protein antigen (16–19). Although this aspect of adaptive immunity has been explored less thoroughly in autoantibody-associated neuroimmune disorders, this premise has broadened studies to focus on cellular responses in the following autoantibody-associated diseases: neuromyelitis optica (NMO), acute disseminated encephalomyelitis (ADEM), stiff person syndrome (SPS), and anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis.

In this review, we explore the accumulating evidence of cellular immune responses in various disorders of the CNS that are predominantly T cell-driven, as well as the more newly classified group of autoantibody-associated syndromes. In particular, we focus on findings in humans, as many studies conducted in animal models are reviewed elsewhere or have been recently reviewed (1, 20–23). Common methods implemented in the study of T cell biology are also evaluated.

## IMMUNOSURVEILLANCE OF T CELLS IN THE CNS

The CNS has been traditionally viewed as an immune privilege site that is inaccessible to T cells and other immune cells. However, it is now well recognized that T cells actively survey the CNS in the healthy state to ensure host defense against infections. Central and effector memory T cells constantly patrol the brain and spinal cord for pathogens *via* the cerebrospinal fluid (CSF) that bathes these structures (24–26). In fact, around 80% of immune cells in the CSF are T cells (27). As they travel through the subarachnoid space between the meninges, T cells interact with resident antigen-presenting cells (APCs) to sample antigens, including parenchyma-derived antigens in the interstitial fluid that drains into the CSF (28). Memory T cells can then be restimulated upon recognition of a pathogen as part of the host response.

Moreover, recent evidence confirms the presence of lymphatic vessels within the meninges of healthy mice that resemble traditional lymphatic vessels found in the periphery, both structurally and functionally (29, 30). These meningeal vessels line the dural sinuses and drain cells and fluid of the subarachnoid space directly into the deep cervical lymph node. Notably, T cells were identified in these meningeal lymphatics (30), indicating a travel route between the CNS and lymph nodes in the steady state. Together, this refutes previous notions that immune cell entry into the CNS was restricted by the apparent absence of lymphatic drainage, and further supports the concept of immunosurveillance by T cells in the CNS.

Although the blood–brain barrier (BBB) and blood–CSF barrier shielding the CNS were seen to be another mechanism exempting the CNS from immune monitoring, various adhesion molecules on their surface enable T cell migration. Egress from the blood to the CSF is dependent on the expression of P-selectin in choroid plexus stroma vessels and meningeal vessels (24, 31). In addition, the interaction of α4β1 integrin with vascular cell adhesion molecule 1 (VCAM1) on endothelial cells of the BBB is important in facilitating T cell movement into the perivascular space, as evidenced by the efficacy of natalizumab in reducing inflammation in MS (32–34).

As will be discussed later, the importance of immunosurveillance in maintaining homeostasis in the CNS is particularly evident when it is disrupted by immunosuppression. Under immunosuppression, the mobilization of immune cells into the CNS is hindered, making the body more susceptible to opportunistic infections by agents such as, JC polyoma virus (JCV), herpes simplex virus, toxoplasmosis, and *Cryptococcus* (35–37). With the host immune response dampened and the CNS unguarded, the pathogenic response goes unchecked, leading to potentially fatal diseases.

## T CELL-MEDIATED CNS DISEASES

### Multiple Sclerosis

Multiple sclerosis is a common chronic inflammatory disease of the CNS resulting in the demyelination of neurons. Damage to the myelin sheath surrounding neuronal axons leads to the progressive loss of neurological function and affects over two million people globally (38, 39). The majority of MS patients (85%) experience a relapsing-remitting disease course (40), who can transition into a secondary progressive disease form after approximately 10 years of primary disease (41, 42). The remaining 15% of patients follow a primary progressive disease course characterized by a steady decline in neurological function from the initial attack (38). Lesions, or plaques, are traditionally thought to present in the white matter of the brain and spinal cord. However, recent studies have shown gray matter lesions to accrue through the MS disease course and dominate in progressive disease (43–49).

The current consensus argues in favor of MS as an autoimmune disease mediated by self-reactive, myelin-specific T cells (1, 38, 46), with additional components of genetic susceptibility and environmental factors (41, 50). In terms of genetic susceptibility, MS has been strongly associated with different HLA class II haplotypes, including HLA-DR15 and HLA-DQ6, although their contributions to clinical disease have yet to be uncovered (51–53). It is hypothesized that these MHC molecules are able to present target autoantigens to autoreactive components of the adaptive immune system (54, 55). Several immune system genes have also been implicated in MS disease susceptibility, including those that code for IL-17 and IL-2 receptor (51, 52).

However, genetics only partially contribute to the risk of MS disease development. A significant proportion of disease risk can be directly correlated with various lifestyle and environmental factors including vitamin D deficiency, Epstein-Barr virus (EBV) infection, and smoking (56, 57). Epidemiological studies show that there is a strong association between MS prevalence and the angle of latitude. This trend may be attributed to exposure to solar radiation and vitamin D, with vitamin D-deficient individuals more prone to developing disease (58, 59). Several studies have

also shown that MS patients with lower serum levels of vitamin D to be more susceptible to relapses (60–63). Additionally, EBV infection has been strongly linked to MS disease initiation (64). While up to 95% of the general population is seropositive for the virus by early adulthood, the risk of MS will be 15 fold greater in the seropositive population than the seronegative cohort (65). Certain sequences of EBV have been hypothesized to share homology with components of the CNS, suggesting that MS autoimmunity may be initiated by molecular mimicry (66, 67).

The MS autoimmune hypothesis is supported by the predominant presence of activated T cells in active plaques (41, 50, 65, 68–70). Myelin-specific T cells are first activated in the peripheral compartment, after which they cross the BBB into the CNS as they gain the expression of the appropriate adhesion molecules and homing receptors (55, 71). Once inside the CNS, T cells are then reactivated by CNS autoantigens presented by CNS-resident APCs, contributing to the clinical disease and demyelination (72, 73).

$CD4^+$ T cells have been the focus of MS autoimmunity for decades, as MHC class II-restricted T cells are preferentially activated by EAE disease induction (38). Self-reactive $CD4^+$ T cells have been shown to recognize proteins of the myelin sheath, including myelin basic protein (MBP) (74, 75), myelin-associated glycoprotein (MAG) (76, 77), and myelin oligodendrocyte glycoprotein (MOG) (78) in both MS patients and healthy donors (69). However, $CD4^+$ T cells in MS patients display an activated or memory phenotype with increased avidity to myelin proteins, compared to naive myelin-specific $CD4^+$ T cells isolated from controls (79–82). Previously, myelin-specific $CD4^+$ T cells in MS were thought to contribute to Th1-mediated inflammation, in contrast to the Th2-mediated response of myelin-reactive T cells isolated from healthy donors (83, 84). However, recent studies have demonstrated the importance of IL-23 in MS. IL-23 is necessary for the regulation of the proinflammatory IL-17-secreting Th17 cell lineage, which have been described as the pathogenic mediators of several autoimmune diseases (85–87). This is supported by the upregulation of IL-17 gene expression in the brain lesions of MS patients as measured by microarray analysis (88, 89). The levels of Th17 cells in the CSF of relapsing MS patients were elevated in comparison to non-inflammatory neurological disease controls, whereas there were no differences in the percentages of IFN-γ-secreting Th1 cells (90). Th17 cells were also raised in the peripheral compartments of MS patients during relapses, implicating their possible relevance to disease activity (91, 92). Th17 cells were additionally demonstrated to home to active regions of lesions and areas of inflammatory demyelination, and are a major constituent of perivascular cuffs (93). The high expression of granzyme B by myelin-specific Th17 cells also promotes the death of human neurons (94). These works combined strongly insinuates Th17 cells as a potential mediator of MS pathogenesis.

The focus of research in MS has recently shifted from a predominantly $CD4^+$ T cell field to include $CD8^+$ cytotoxic T cells as a novel effector cell type in MS pathology (95, 96). $CD8^+$ T cells have been shown to outnumber $CD4^+$ T cells in MS plaques up to 10-fold at all stages of disease progression (47, 97–100). Oligoclonal expansion within the $CD8^+$ T cell compartment is elevated compared to $CD4^+$ T cells in lesions, CSF, and peripheral blood of MS patients (95, 96, 101). In contrast to its constitutive low expression in the CNS, MHC class I is highly upregulated on neurons and glial cells within MS lesions, which proposes that $CD8^+$ T cells may be interacting with these cells (100, 102, 103). A significant number of activated or memory $CD8^+$ T cells are capable of secreting the proinflammatory cytokine, IL-17, similar to the Th17 cells mentioned earlier (93). Histological analysis of MS lesions has also revealed that granzyme B-positive $CD8^+$ T cells are often located adjacent to regions of demyelination (47, 104, 105). Expectedly, the levels of $CD8^+$ T cells within lesions have been positively correlated with the magnitude of axonal injury (106). These findings encourage the hypothesis that $CD8^+$ T cells play a role in the demyelination of axons in MS lesions.

The monoclonal antibody natalizumab is successfully used as an immunosuppressant in diseases such as MS. Natalizumab therapy is administered to MS patients who are unresponsive to first-line immunotherapies, as well as those with severe clinical disease (107). It has shown a 68% reduction in the annualized relapse rate of MS patients and has decreased the probability of sustained disability progression by 42% over the course of 2 years (108). Natalizumab targets the α4β1 integrin on T cells, thereby preventing T cells binding to VCAM1 on endothelial cells of the BBB and subsequent egress into the CNS. This is evidenced by the significant decline of several populations of T cells in the CSF of natalizumab-treated MS patients compared to controls (34). As such, immune surveillance within the CNS is compromised, which can lead to various inherited or acquired immune deficiencies (37). In particular, the use of natalizumab has been associated with opportunistic infections, most significantly JCV infection or reactivation leading to potentially lethal progressive multifocal leukoencephalopathy (inflammation of white matter in the brain) in 4 out of 1,000 treated patients (37).

Based on a review of the literature, it seems apparent that MS is a multifaceted autoimmune disease with potential contributions from Th17 cells and $CD8^+$ T cells in demyelination (**Table 1**). Although T cell dependency is well established, the quest for potential autoantibodies in MS is still going strong (109). Popularly studied autoantigens in this field include MOG and aquaporin 4 (AQP4), although extensive research into these targets reveal that they are not, in fact, associated with MS (11, 110).

## Rasmussen's Encephalitis

Rasmussen's encephalitis is a chronic pediatric inflammatory neurological disorder characterized by drug-resistant focal seizures, unihemispheric inflammation and atrophy, and unilateral movement disorders accompanied by progressive neurological decline (112, 123, 124). Lymphocytic and microglial nodules are commonly observed upon histopathological analysis of RE brain specimens, along with perivascular cuffing of infiltrating T cells, neuron and astrocyte death, and gliosis of the diseased hemisphere (4, 124, 125). RE has not yet been associated with any disease-specific autoantibodies, and the presence of autoantibodies to glutamate receptor 3 is secondary to and not causative of disease (124, 126–129). In fact, RE has been hypothesized to be a T cell-mediated disease based on the dominant influx of $CD8^+$ T cells into active brain lesions at the initiation of disease (4) (**Table 1**). 7% of these infiltrating $CD8^+$ cells are granzyme

**TABLE 1** | Summary of findings of T cell activity in T cell-associated central nervous system diseases.

| Disease | T cell antigen | Implicated T cell subset(s) and dysregulation of associated cytokines, chemokines, and other inflammatory mediators | HLA associations | Associated antibody | Reference |
|---|---|---|---|---|---|
| Multiple sclerosis | MBP, MAG, MOG | Th17: IL-23, IL-17, granzyme B<br>$CD8^+$ T cells: IL-17, granzyme B | HLA-DR15, HLA-DQ-6 | Unknown | Andersson et al. (76), Cua et al. (85), Hafler et al. (51), International Multiple Sclerosis Genetics et al. (52), Montes et al. (88), Olsson and Hillert (53), Pette et al. (74), Raine et al. (89), Tsuchida et al. (77), Valli et al. (75), Zhang et al. (78) |
| Rasmussen's encephalitis | Unknown | $CD8^+$ T cells: granzyme B<br>Unknown source: IL-6, TNF-α, IFN-γ | HLA-DR6 (possible) | Unknown | Andermann et al. (111), Bien et al. (112), Takahashi et al. (113), Tekgul et al. (114) |
| Paraneoplastic syndromes | Hu, Ma2, Yo | $CD8^+$ T cells: granzyme B<br>Unknown source: IFN-α, IL-12 | Hu: HLA-DR3, HLA-DQ2<br>Yo:HLA-A24 | Hu, Ma2, Yo, CRMP5/CV2, amphiphysin | Benyahia et al. (115), Darnell et al. (116), De Graaf et al. (117), Domschke et al. (118), Leypoldt and Wandinger (119), Rousseau et al. (120), Tanaka et al. (121), Tanaka and Tanaka (122) |

B-positive, and vesicles were often found positioned adjacent to MHC class I-expressing neurons and astrocytes with their granules polarized toward their target(s), suggesting a cytotoxic T cell-mediated disease course of RE (112). In addition to increased levels of granzyme B at initial stages of disease (113), Tekgul et al. revealed raised concentrations of the cytokine IL-6 in the CSF of RE patients compared to controls (114). A correlation was then established between the magnitude of neuronal death and inflammation with the level of IL-6 in the CNS of these patients, based on magnetic resonance spectroscopy (114). The overproduction of IL-6 has been attributed to overstimulation of TNF-α early in disease (113). Takahashi et al. have also shown that excessive IFN-γ production during the early stages of disease induces the secretion of IL-12 from macrophages (113).

Analysis of the T cell receptor (TCR) repertoire in the CNS and periphery of RE patients revealed clonal expansions of $CD8^+$ T cells in both compartments, suggesting the presence of an antigen-specific T cell response (130). This is in contrast to normal TCR distribution in stroke patient controls (130). The number of peripheral $CD8^+$ T cell clones has also been shown to correlate with the magnitude of unihemispheric atrophy (131). Although the disease epitope for RE has not yet been elucidated, the identification of a $CD8^+$ T cell-mediated response in this disease expands potential treatment options, as seizures may be refractory and poorly responsive to anti-epileptic drugs (124). As a result, some patients may require invasive procedures such as hemispherectomy to regulate seizure frequency (124). Novel T cell-specific immunotherapies, like T cell blockade from the CNS with natalizumab, are therefore a promising alternative (124, 132, 133).

## Paraneoplastic Syndromes

Diseases in which the body's immune system is altered in response to cancer are termed paraneoplastic syndromes. When paraneoplastic syndromes disturb the CNS, the effects can be far more severe than the initiating tumor, with significant disability taking hold over short periods of time (134). In CNS paraneoplastic syndromes, paraneoplastic antibodies are present at higher titers in CSF versus serum, insinuating that they are synthesized intrathecally (135). These onconeuronal IgG antibodies target intracellular neuronal antigens expressed ectopically by the tumor (136). Paraneoplastic antibodies are important biomarkers of disease, but appear unrelated to pathogenesis (137). Instead, pathogenesis may be mediated by T cells targeting the same autoantigens as the onconeural antibodies present (5) (**Table 1**). This hypothesis is supported by the presence of disease-specific T cells in the peripheral blood and CSF of patients with anti-Yo (cdr2) (116) and anti-Hu antibodies (115, 120). Extensive T cell infiltration into the CNS in patients with anti-Ma2 (138) and anti-Hu antibody-associated paraneoplastic encephalitis (139) has also been observed, and along with poor responses to humoral immunotherapies (140–142), supports a T cell-mediated pathogenesis of CNS paraneoplastic syndromes.

$CD8^+$ cytotoxic T cells have been implicated in paraneoplastic limbic encephalitis and are associated with autoantibodies against intracellular antigens, mainly Hu (139) and Ma2, as well as CRMP5/CV2 and amphiphysin (5). In comparison to encephalitides with neuronal cell-surface antigen-directed autoantibodies, T cells in anti-Ma2 and anti-Hu paraneoplastic encephalitis are preferentially skewed toward a $CD8^+$ phenotype, with a significantly higher number of activated cytotoxic granzyme B-positive cells found in close proximity to injured neurons (143, 144).

There is a limited number of studies that detail the cytokine profile in paraneoplastic patients. Autoreactive T cells in paraneoplastic breast cancer patients were found in association with elevated intratumoural levels of IFN-α and IL-12, a correlation unseen in antibody-negative breast cancer patients (118). As IL-12 is concomitant with T cell activation and function, the increase of this cytokine likely promotes the expansion of autoreactive T cells in paraneoplastic syndromes (118, 145). These results collectively suggest that paraneoplastic encephalitides are mediated by cytotoxic, antigen-specific $CD8^+$ T cells, in which onconeuronal antibodies may exist as an epiphenomenon.

## Amyotrophic Lateral Sclerosis

Amytrophic lateral sclerosis is a neurodegenerative disease of the motor neurons resulting in progressive muscle paralysis. The

underlying mechanisms of ALS have not yet been elucidated, and therapies to modify or delay the advancement of the disease are still being trialed. Intriguingly, recent studies have shown that $CD4^+$ T cells infiltrating the spinal cord in ALS patients and mice lie adjacent to degenerating motor neurons and activated microglia (7, 146–148). However, global immunosuppression does not appear to be effective in ALS treatment, suggesting that these T cells may, in fact, rescue motor neuron death (7). There have been several studies investigating the neuroprotective mechanisms of $CD4^+$ T cells following injury (7, 149–152). The data collected thus far suggests that $CD4^+$ T cells in ALS mediate motor neuron survival in a highly regulated process (7).

## T CELLS IN ANTIBODY-ASSOCIATED CNS DISEASES

### Neuromyelitis Optica

The most compelling evidence for autoreactive T cell involvement in an autoantibody-associated disease comes from studies in neuromyelitis optica (**Table 2**). NMO is an aggressive demyelinating disease that is distinguished from MS by the presence of specific IgG1 antibodies against AQP4 (153, 154), a water channel abundantly expressed by astrocytes in the CNS. Anti-AQP4 antibodies are detected in a significant proportion (up to 75%) of NMO patients (110, 155) and have become an important diagnostic tool. However, involvement of other immune mechanisms has been theorized as several lines of evidence, while inconclusive, indicate that anti-AQP4 antibodies alone do not induce complete pathogenesis. For example, there are incongruences in NMO induction in animal models by passive transfer of anti-AQP4 IgG alone (156–158), and high titers of anti-AQP4 antibodies were detected in humans during remission (159, 160). Furthermore, B cell-targeted immunotherapies do not always ameliorate the disease (161, 162).

Since the early description of $CD3^+$ T cells in active NMO lesions (189), there is mounting evidence of cellular involvement in NMO. In fact, activated T cells infiltrate NMO patient-derived lesions (190) and clonal expansion of T cells was reported (191). Efforts have been made to define the immunodominant epitope by identifying which peptide from a human AQP4 (hAQP4) peptide library induced the greatest T cell proliferation when cultured with peripheral blood mononuclear cells (PBMCs) from anti-AQP4 antibody-positive NMO patients compared to MS subjects and healthy controls (165, 168, 169). However, further studies are required to precisely define the dominant target region as these epitopes differed greatly between studies. This discrepancy could be due to different populations with varied HLA associations, or different stages of the disease in which subjects were sampled. Indeed, a longitudinal analysis of NMO patients has demonstrated a change in reactivity and specificity of T cells toward the hAQP4 peptides over time (168). Relapses in the disease were associated with elevated $CD69^+$ activated T cells compared to remission (165), highlighting the possible intermittent role of T cells during an NMO attack, and thus emphasizing the importance of understanding the T cell response to monitor the disease course.

Cytokine profiling helps elucidate the functional properties of AQP4-specific T cells and has revealed that these cells exhibit predominantly a Th17 bias but also a Th1 response. Compared to MS patients or healthy controls, increased secretion of IL-17, IL-10, IL-6, and IFN-γ have been reported in the CSF (166, 167), peripheral blood (168, 169, 192), and epitope-specific T cell lines derived from NMO patients (168). Secretion of IL-17 from Th17-biased AQP4-specific T cells promoted neutrophil infiltration, which was consistent with pathological findings (169, 189). In particular, elevated IL-6, a cytokine important for Th17 differentiation, may promote survival of AQP4-specific Th17 cells while suppressing $FOXP3^+$ Treg function (193–195). Furthermore, tocilizumab, a monoclonal antibody against IL-6 receptor, ameliorated the disease in NMO patients unresponsive to standard immunotherapy (196, 197).

Genetics may be a determinant of autoimmunity and indeed, there appears to be a HLA haplotype association in NMO. Depending on the ethnicity of the cohort, there is an over-representation of HLA-DRB1*03, HLA-DRB3, or

**TABLE 2** | Summary of findings of T cell activity in antibody-associated central nervous system diseases.

| Disease | T cell antigen | Implicated $CD4^+$ T cell subset(s) and dysregulation of associated cytokines and chemokines | HLA associations | Associated antibody | Reference |
|---|---|---|---|---|---|
| Neuromyelitis optica | AQP4 | Th1: IFN-γ; Th17: IL-17, IL-6, IL-10 | HLA-DRB1*03, HLA-DRB3, HLA-DP1*0501 | Anti-AQP4 IgG | Brum et al. (163), Deschamps et al. (164), Matsuya et al. (165), Tanaka et al. (166), Uzawa et al. (167), Vaknin-Dembinsky et al. (168), Varrin-Doyer et al. (169), Wang et al. (170), Zephir et al. (171) |
| Acute disseminated encephalomyelitis | Unknown | Th1: IFN-γ, TNF-α, IL-2; Th2; IL-4, IL-6, G-CSF, IL-10; Th17: IL-17, IL-6, G-CSF, IL-10; Chemokines: CXCL10, CCL1, CCL7, CCL22 | Unknown | Anti-MOG IgG | Dale and Morovat (172), Ichiyama et al. (173), Ishizu et al. (174), Jorens et al. (175), Pohl-Koppe et al. (176), Yoshitomi et al. (177) |
| Stiff person syndrome | GAD65 | Th1: IFN-γ; Th2: IL-13, IL-4, IL-5 | HLA-DQB*0201, HLA-DRB1*0301 | Anti-GAD IgG | Costa et al. (178), Hanninen et al. (179), Hummel et al. (180), Pugliese et al. (181), Schloot et al. (182), Skorstad et al. (183) |
| Anti-NMDAR encephalitis | Unknown | Th1: IFN-γ, TNF-α; Th17: IL-17, IL-6, IL-23; Chemokines: CXCL10 | Unknown | Anti-NMDAR IgG | Byun et al. (184), Kothur et al. (185), Lee et al. (186), Liba et al. (187), Ulusoy et al. (188) |

HLA-DPB1*0501 in anti-AQP4 antibody-positive NMO patients (163, 164, 169–171, 198). Interestingly, Varrin-Doyer et al. demonstrated that the hAQP4 epitope they identified induced the highest T cell reactivity in NMO patients that were HLA-DR carriers (169). However, there needs to be more definitive analysis as a distinct HLA allele could not be determined based on the T cell response to a different set of AQP4 epitopes (165).

While the triggers of autoimmunity remain elusive, like in many other autoimmune diseases, molecular mimicry has been implicated in the generation of AQP4-specific T cells. In addition to proposing AQP4-specific T cell epitopes, Varrin-Doyer et al. revealed a 90% homology between the immunodominant AQP4 epitope and *Clostridium perfringens* adenosine triphosphate-binding cassette transporter permease, and a 60–70% homology to other commensal and pathogenic *Clostridium* species (169). Not only could these microbes serve to display cross-reactive determinants, the *Clostridium* species may also augment a Th17-biased response as demonstrated in mice (169, 199). Nevertheless, further investigations into molecular mimicry are required to ascertain the extent of its contribution to the development of AQP4-specific T cells.

## Acute Disseminated Encephalomyelitis

Acute disseminated encephalomyelitis is a monophasic inflammatory demyelinating disease predominantly affecting children. It can have postinfectious origins but in a subset of patients (27–47%) (200), extensive evidence implicates pathogenic autoantibodies against MOG, a protein on the outer surface of the myelin sheath (201–206). Interestingly, findings predating the discovery of anti-MOG antibodies in ADEM (205) provide support for an autoimmune T cell response.

The majority of literature supporting T cell involvement in ADEM stems indirectly from analyses of chemokines and cytokines (**Table 2**). Concurrent recruitment of Th1 and Th2 cells has been proposed as there was an increase in their signature chemokines, CXCL10, CCL1, CCL7, and CCL22 in the CSF of adults with ADEM compared to MS and healthy controls and was correlated with an increase in pleocytosis (207). Dysregulation in cytokine production was not distinguished in adults, but IFN-γ, TNF-α, IL-2, IL-10, IL-6, and G-CSF were upregulated in separate pediatric ADEM cohorts (172–176), further supporting the contribution of Th1 and Th2 cells. Pohl-Koppe et al. hypothesized that Th1 cells contribute to the deleterious effects of the disease, while Th2 cells predominate in the recovery of ADEM as they reported an absence of IFN-γ but an increase in IL-4 in patients during the recovery phase (176). Consistent with this, there was increased IFN-$\gamma^+$CD3$^+$ T cells in the peripheral blood during the acute stage of ADEM (177).

Conversely, as IL-6, G-CSF, and IL-10 are pleiotropic, their elevation along with IL-17A, but little Th1 and no Th2 cytokines, in the CSF of anti-MOG antibody-positive children favors a Th17 phenotype (208). Interestingly, this increase in Th17 cytokines correlated with an increase in B cell-associated cytokines and chemokines, suggesting possible interactions between multiple cell types in mediating demyelination (208). Likewise, CSF IL-6 levels correlated with the presence of plasma anti-MOG antibodies in acquired demyelinating syndromes like ADEM (209). It can then be proposed that, like in NMO, IL-6 signaling is a suitable target for treatment in anti-MOG antibody-positive patients resistant to conventional immunotherapy (196, 197). These preliminary, albeit conflicting, reports of functional helper T cells warrant investigations into autoreactive T cells themselves, but also in combination with the recent developments in anti-MOG antibodies to assess the interplay between the humoral and cellular components of the autoimmune response in ADEM (201, 204, 210).

## Stiff Person Syndrome and Other Anti-Glutamic Acid Decarboxylase Glutamic Acid Decarboxylase (GAD) Antibody-Associated Neurological Disorders

Markedly high titers of autoantibodies against glutamic acid decarboxylase (GAD) are a hallmark of non-paraneoplastic SPS and variants of cerebellar ataxia, limbic encephalitis, and epilepsy (211–214). As GAD is an enzyme involved in the synthesis of the inhibitory neurotransmitter γ–aminobutyric (GABA), the current hypothesis is that anti-GAD antibodies disrupt GABAergic signaling. Indeed, *in vitro* and *in vivo* studies demonstrate the potential pathogenicity of anti-GAD antibodies (215–217). The salient question remains, however, of the mechanism underlying autoantibody recognition of a cytoplasmic antigen like GAD, which is unlike other known extracellular antigens targeted by pathogenic autoantibodies (218).

Given the variety of anti-GAD antibody-associated neurological disorders, it is plausible that antigen-specific T cells play an additional role in pathogenesis that differentiates the diseases (**Table 2**). This is an important aspect for investigation but there is a paucity of studies examining cellular mechanisms despite reports of CNS infiltration of lymphocytes in these patients (219). In a study comparing SPS with cerebellar ataxia associated with polyendocrine autoimmunity (CAPA), both cohorts presented with high titers of anti-GAD antibodies (178). Yet, cell proliferation and the percentage of HLADR$^+$CD3$^+$ activated T cells in response to GAD65 protein was significantly greater in SPS but not CAPA. Monitoring the course of SPS revealed a constant reactivity of CD4$^+$ T cells against GAD65, which notably correlated with high anti-GAD antibody titers (179). A few other groups have identified GAD65-specific T cells in the blood but these were weakly responsive to GAD65 (180, 182, 220, 221). To this end, Skorstad et al. argue that GAD65-specific T cells largely reside in the CNS along with B cells to collaborate in the intrathecal production of anti-GAD antibodies as they were more successful in identifying and cloning GAD65-specific T cells from CSF than from blood (183). Furthermore, using overlapping GAD65 peptides, putative T cell epitopes have been identified but differ between studies and depending on whether T cell lines were generated from the blood or CSF (179, 182, 183, 220). As has been demonstrated with anti-GAD65 antibody epitopes (222) [recently reviewed in Ref. (223)], differences in T cell epitopes have been shown to be a distinguishing factor between SPS and type 1 diabetes, another anti-GAD antibody-associated disease (220, 221).

Exploration of the cytokine environment to determine the phenotype of T cells has indicated largely a Th2 bias. Secretion

of IL-13, IL-4, and IL-5 reported in SPS (179, 182, 183) supports a non-inflammatory environment wherein disease is driven by autoantibodies. IFN-γ production, indicative of a Th1 response, was also recorded (182, 183) and subsequently reduced upon treatment with immunotherapy, and coincided with clinical improvement (180). It was proposed that high levels of IFN-γ production occurs in the early phase of SPS but is later exceeded by significant production of IL-13, alluding to a shift from Th1 to Th2 (179). While low or undetectable in SPS, there was a notable production of IFN-γ, and hence a dominant Th1 response is observed in CAPA (178) and type 1 diabetes (220).

T cell involvement is further supported by preliminary findings on HLA allele correlations in SPS. Pugliese et al. described a strong association between SPS and carriers of HLA-DQB1*0201 haplotype (181). HLA-DRB1*0301 has additionally been proposed as a correlate of SPS but the validity of this finding is hampered by small sample size (178, 179).

## Anti-NMDAR Encephalitis

Anti-NMDAR encephalitis is the prototypic autoimmune encephalitis associated with autoantibodies against cell-surface antigens. Discovery of the specific anti-NMDAR antibody (224) has sparked considerable interest in humoral mechanisms of this disease [recently reviewed in Ref. (225)], leading to the current hypothesis that anti-NMDAR antibodies exhibit pathogenic effects *via* internalization of the surface receptor, thereby resulting in reversible NMDAR hypofunction (226–228).

To date, there are limited and small studies investigating cellular responses in anti-NMDAR encephalitis but nevertheless, prompt further exploration (**Table 2**). Evidence of T cell involvement derives from cytokine and chemokine profiling and mainly favors a Th17 response. Based on significantly elevated serum levels of IL-17 and IL-6 in anti-NMDAR antibody-positive patients compared to controls (184), Byun et al. hypothesized that undetected Th17 cells secrete IL-17, which promotes a positive feedback loop of IL-6 signaling that facilitates intrathecal antibody production observed in most patients (224, 229). In line with this finding, targeting the IL-6 receptor with tocilizumab in rituximab-resistant patients with suspected autoimmune encephalitis demonstrated marked improvements (186), as was also seen in NMO. Upregulation of serum IL-23 strengthens the case for Th17 activity (188). There appears to be some heterogeneity in T cell lineage as higher levels IFN-γ and TNF-α were observed in the CSF, indicative of a Th1 cytokine dysregulation (185, 187). Consistent with T cell involvement in anti-NMDAR encephalitis was the increased level of T cell-related chemokine CXCL10 in patient CSF, which correlated with CSF pleocytosis (187).

On the other hand, there is contentious evidence of T cell involvement. Immunopathological analysis of brain sections from anti-NMDAR encephalitis patients show some to no evidence of T cell infiltration in the parenchyma and perivascular space, which disqualifies $CD8^+$ cytotoxic T cells as drivers of the disease (143, 230–232). However, this does not preclude the possibility of NMDAR-specific T cell involvement in B cell activation in the periphery, prior to anti-NMDAR antibodies trafficking to the CNS.

# EVALUATING T CELL DETECTION METHODS

Choice of methods is a key determinant in discovering and studying T cell biology. An important consideration is the rarity of antigen-specific T cells, especially the proportion reactive against auto-antigens which is typically less than 0.01% of the total T cell repertoire (233). It is therefore imperative that sensitive yet specific techniques are implemented when analyzing antigen-specific T cells. Widely used T cell detection methods can be broadly distinguished into two categories: techniques that identify and assess specificity and assays that examine the functionality (**Table 3**).

An important tool for identification of antigen-specific T cells is peptide-MHC (pMHC) multimers. This rapidly evolving technology involves the formation of a complex of peptide-loaded MHC monomers *via* biotinylation with a fluorescently labeled streptavidin (**Figure 1**), which increases binding avidity (234) and overcomes the issue of low affinity binding and fast dissociation rate between TCRs and pMHC monomers (235). The value of this method lies in the direct and specific recognition and isolation of T cells *via* flow cytometry that is independent of their biological activity, such as anergic cells that are incapable of proliferation and cytokine production (236–239). However, this is a double-edged sword, as knowledge of the functional characteristics of the identified T cells allows for a deeper understanding of their response. In addition, a major drawback of this approach is that it necessitates knowledge of the T cell epitope and its MHC haplotype association (236). While pMHC multimers are extensively used for study of $CD8^+$ T cells, their use in the study of MHC class II-restricted $CD4^+$ T cells is challenged by the lower frequency of antigen-specific $CD4^+$ T cells in the peripheral blood (240) but also largely by the difficulty in creating them because of variations in MHC structure and TCR affinity (236, 238).

Alternatively, antigen-specific T cells can be directly identified and isolated for downstream functional characterization by probing for activation markers expressed on the surface of T cells upon antigenic stimulation. A major advantage of this technique is that unlike pMHC multimers, it does not require knowledge of the antigenic epitope and associated MHC haplotypes (236) and is effective for studying $CD4^+$ T cells. A multitude of activation markers have been proposed, including CD25, CD69, CD40L, CD134, CD137, and HLA-DR (241–245). Such markers are favorable indicators of an antigenic-specific response as their surface expression is contingent on activation, but absent or minimally expressed in the resting phase, and occurs for a transient period of time. Moreover, like pMHC multimer staining, expression of some activation markers is irrespective of T cell function and its differentiation state (236, 244), allowing for an unbiased characterization of antigen-specific T cells.

Detection of antigen-specific T cells is a vital step, but determining the functional capacity of the identified cells in producing a robust immune response is equally important and relies on functional assays (**Figure 2**). Methods measuring $[^3H]$-thymidine incorporation into lymphocyte DNA (246) and dilution of carboxyfluorescein succinimidyl ester (CFSE) dye bound to amine groups of intracellular molecules (247) during

**TABLE 3** | Evaluation of major techniques used in the analysis of human antigen-specific T cells.

| Technique | Advantages | Disadvantages |
|---|---|---|
| **Identification of antigen-specific T cells** | | |
| Peptide-MHC (pMHC) multimers | • Highly specific interaction between T cell receptor and its cognate antigenic peptide presented by the multimer<br>• Independent of functional status of cells<br>• Labeled T cells can be isolated and purified for further characterization | • Requires prior knowledge of epitope and its HLA haplotype restriction<br>• Does not provide functional details of identified antigen-specific T cell<br>• More difficult to develop multimers for $CD4^+$ T cells |
| Detection *via* activation markers | • Independent of epitope and HLA haplotype restriction<br>• Allows characterization of all antigen-specific T cells, irrespective of subtype<br>• Identified cells are viable, allowing for isolation and purification for further characterization | • Unless appropriate activation markers are selected, results may be confounded by marker expression on non-stimulated T cells and bystander activation |
| **Functional assays** | | |
| $[^3H]$-thymidine incorporation | • Demonstrates the proliferative capacity of antigen-specific T cells<br>• Allows for detection of numerous antigen-specific T cells | • Source of cytokine is not available, making it an indirect method of T cell detection<br>• Phenotype of proliferative cells cannot be determined<br>• Results may be confounded by bystander activation<br>• Frequency of T cells in original sample cannot be elucidated |
| Carboxyfluorescein succinimidyl ester (CFSE) dilution assay | • Demonstrates the proliferative capacity of antigen-specific T cells<br>• Allows for detection of numerous antigen-specific T cells<br>• If used in conjunction with antibodies against activation markers, the phenotype of the proliferative cells may be determined | • An indirect method of T cell detection if CFSE used alone<br>• Frequency of T cells in original sample may be confounded by bystander activation<br>• CFSE may interfere with normal cellular processes |
| Enzyme-linked immunospot (ELISPOT) | • Can enumerate cells capable of secreting cytokine of interest and categorize them into likely T cell subsets<br>• Can characterize cytokine kinetics based on spot morphology<br>• Highly sensitive, even in small samples | • Selection of cytokine for analysis is based on hypothesis of its relevance<br>• Restricted to analysis of maximum two cytokines per experiment<br>• Frequency of antigen-specific T cells may be underestimated due to non-functional cells and also possible secretion of cytokines other than that being tested by assay<br>• Source of cytokine is not available, making it an indirect method of T cell detection |
| Intracellular cytokine staining (ICS) | • Allows simultaneous determination of cytokines produced and phenotype of cells producing the cytokines, if antibodies against activation markers used in conjunction<br>• Quantify cytokine produced per cell | • Selection of cytokine for analysis is based on hypothesis of its relevance<br>• Requires larger sample than ELISPOT<br>• Cells not viable for further analysis due to fixation and permeabilization |

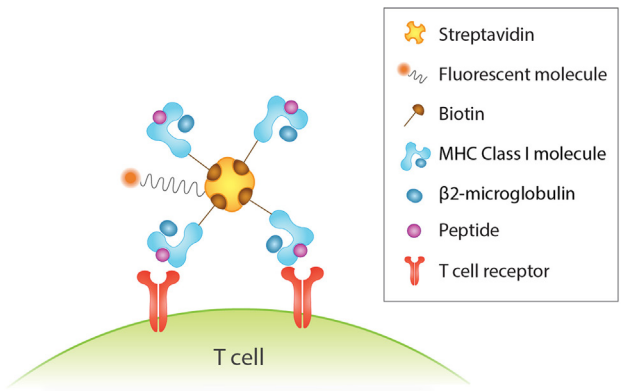



**FIGURE 1** | Detection of human antigen-specific T cells with peptide-MHC (pMHC) multimer. Binding four pMHC monomers, for instance, *via* biotin–streptavidin interactions increases binding avidity between antigen and T cell receptor. This in turn enhances the sensitivity and specificity of antigen-specific T cells detected by flow cytometry analysis *via* the fluorescent streptavidin.

cell division have been a mainstay for evaluating the proliferation of lymphocytes in response to antigens. These procedures circumvent the issue of low frequency of target cells. However, as it is often PBMCs that are cultured, and not sorted T cells, there is the possibility of bystander activation, which decreases the specificity of the response observed and the frequency of the antigen-specific T cells cannot be accurately extrapolated. In the case of $[^3H]$-thymidine incorporation assay, the proliferative cell subpopulation cannot be phenotyped, whereas with CFSE dilution assay, cell subpopulations may be delineated with surface markers and flow cytometry analysis. However, the dye can interfere with expression of activation markers (248).

Functional assays that examine cytokine production allows for classification of the cells into different subsets that are distinguished by different effector functions. This is particularly valuable for $CD4^+$ T cells that can be categorized as Th1, Th2, and Th17 cells, for example. Two classic procedures that assess cytokine production are enzyme-linked immunospot (ELISPOT) and intracellular cytokine staining (ICS) (**Figure 2**). While an ELISPOT detects secreted cytokines induced by antigens, ICS

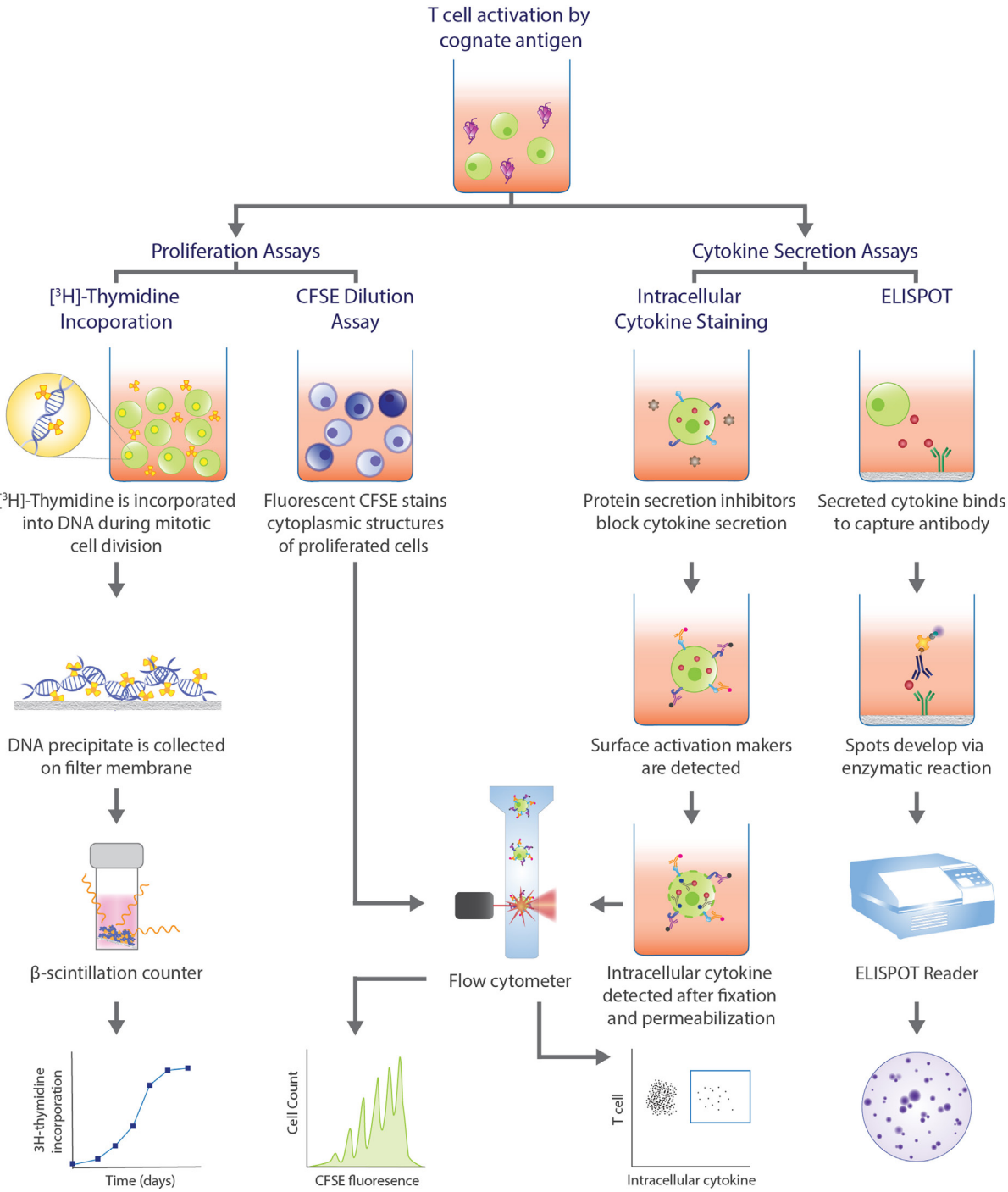



**FIGURE 2** | Functional assays commonly used in human T cell studies. Functional assays can be categorized into those that assess proliferative capacity of antigen-specific T cells and assays that analyze cytokine profiles upon T cell recognition of cognate antigen and subsequent activation. Proliferation assays can measure the amount of radioactive [3H]-thymidine incorporated into the DNA during cell division, with greater radioactivity indicating greater cell division. Alternatively, the level of fluorescence emitted by cells stained with carboxyfluorescein succinimidyl ester (CFSE) can be detected by flow cytometry, with greater number divisions correlating with lower fluorescence. In intracellular cytokine staining (ICS), protein secretion inhibitors, such as brefeldin A or monensin, allows for examination of cytokine production within a cell. Staining surface activation markers allows for phenotyping. Following fixation and permeabilization, the trapped intracellular cytokines are stained with fluorescent antibodies which can be detected *via* flow cytometry. Enzyme-linked immunospot (ELISPOT) is a popular method to assess cytokine secretion. The cytokine of interest secreted from an activated T cell is bound to a capture antibody on a PVDF bottom well. A biotinylated detection antibody also binds to the cytokine and facilitates the interaction between streptavidin-conjugated enzyme and its substrate to produce a color spot. Spots are quantified with a ELISPOT plate reader. Each spot represents one reactive cell.

reveals cytokine production within the golgi/ER bodies upon permeabilization of the cell and treatment with protein secretion inhibitors, such as brefeldin A or monensin. Both techniques are sensitive for detection of antigen-specific T cells and allow for enumeration and characterization at a single-cell level. However, as both techniques also depend on postulating cytokines relevant for the disease, it is possible that the frequency of cytokine-producing cells is underestimated if cells secrete cytokines other than the one of interest. Additionally, the breadth of cytokine analysis is limited in an ELISPOT to only two cytokines for a given experiment (249). The source of the cytokine cannot be determined with an ELISPOT, making it an indirect method of T cell identification. Conversely, ICS allows for the simultaneous detection of cytokines and the phenotype of the cytokine-producing cells by the addition of activation marker fluorescent antibodies. Hence, pairing with pMHC multimer or activation marker staining enhances the specificity of the reaction observed and provides a complete assessment of the antigen-specific response.

Pre-enrichment is a modification often made to the above methods to overcome the issue of low target cells and further improve the sensitivity offered by flow cytometry. A common approach is *in vitro* expansion, wherein PBMCs are cultured in the presence of the antigen over one to two weeks to preferentially grow antigen-specific T cells. However, similar to proliferation assays, a drawback of this procedure is the risk of activating non-T cells present in the sample, thereby increasing background and reducing the accuracy of the quoted frequency of antigen-specific T cells (236). Alternatively, enriching the target cell population *via* magnetic separation greatly increases sensitivity and provides insight into previously unidentified T cell subpopulations, provided that highly specific markers were utilized, whether that be pMHC multimers or activation markers (236, 238).

Employing the right technique may lead to discovery of T cells in disease. Each method explores different aspects of T cell biology. Taking this into consideration and the rarity of antigen-specific T cells, it is advantageous to integrate the various approaches for a more reliable and holistic understanding of the cellular mechanisms at play.

## FUTURE DIRECTIONS

Cellular immunity is a key player in the autoimmune response, as evidenced by the growing number of studies in both T cell-mediated and antibody-associated CNS disorders. Yet, there is much to be learnt of T cell contribution to the complexities of CNS autoimmunity. Investigating the underlying cellular mechanisms can deepen our understanding of disease pathogenesis, especially in the expanding range of neurological diseases recently associated with antibodies, in patients seronegative for antibodies but suspected to have immune dysregulation, in differentiating clinically similar diseases with heterogeneous pathology, and in conditions currently classified as idiopathic. Importantly, this new found knowledge can lead to the development of improved diagnostic tools and also translate into novel immunotherapeutics that are more targeted against T cells or their cytokines, like tocilizumab and IL-17-directed secukinumab (250), which can be more effective than the current treatment regime given the unique environment of the CNS.

## AUTHOR CONTRIBUTIONS

DP and AZ performed the literature review, wrote the manuscript, and contributed equally to the work. DP and FT designed the figures. FB conceived the concept, critically revised, and oversaw the process of manuscript preparation. All authors read, edited, and approved the final manuscript.

## ACKNOWLEDGMENTS

The authors would like to thank remaining members of the Brain Autoimmunity Group for their valuable comments on the manuscript.

## FUNDING

The authors received funding from the Australian National Health and Medical Research Council (NHMRC), Multiple Sclerosis Research Australia, Star Scientific Foundation, Tourette Syndrome Association (USA), and the Petre Foundation (AU).

## REFERENCES

1. Goverman J. Autoimmune T cell responses in the central nervous system. *Nat Rev Immunol* (2009) 9(6):393–407. doi:10.1038/nri2550
2. Compston A, Coles A. Multiple sclerosis. *Lancet* (2008) 372(9648):1502–17. doi:10.1016/S0140-6736(08)61620-7
3. Hemmer B, Kerschensteiner M, Korn T. Role of the innate and adaptive immune responses in the course of multiple sclerosis. *Lancet Neurol* (2015) 14(4):406–19. doi:10.1016/S1474-4422(14)70305-9
4. Farrell MA, Droogan O, Secor DL, Poukens V, Quinn B, Vinters HV. Chronic encephalitis associated with epilepsy: immunohistochemical and ultrastructural studies. *Acta Neuropathol* (1995) 89(4):313–21. doi:10.1007/BF00309624
5. Dalmau J, Rosenfeld MR. Paraneoplastic syndromes of the CNS. *Lancet Neurol* (2008) 7(4):327–40. doi:10.1016/S1474-4422(08)70060-7
6. Melzer N, Meuth SG, Wiendl H. Paraneoplastic and non-paraneoplastic autoimmunity to neurons in the central nervous system. *J Neurol* (2013) 260(5):1215–33. doi:10.1007/s00415-012-6657-5
7. Byram SC, Carson MJ, DeBoy CA, Serpe CJ, Sanders VM, Jones KJ. CD4-positive T cell-mediated neuroprotection requires dual compartment antigen presentation. *J Neurosci* (2004) 24(18):4333–9. doi:10.1523/JNEUROSCI.5276-03.2004
8. Dalmau J, Rosenfeld MR. Autoimmune encephalitis update. *Neuro Oncol* (2014) 16(6):771–8. doi:10.1093/neuonc/nou030
9. Hoftberger R. Neuroimmunology: an expanding frontier in autoimmunity. *Front Immunol* (2015) 6:206. doi:10.3389/fimmu.2015.00206
10. Irani SR, Gelfand JM, Al-Diwani A, Vincent A. Cell-surface central nervous system autoantibodies: clinical relevance and emerging paradigms. *Ann Neurol* (2014) 76(2):168–84. doi:10.1002/ana.24200
11. Ramanathan S, Dale RC, Brilot F. Anti-MOG antibody: the history, clinical phenotype, and pathogenicity of a serum biomarker for demyelination. *Autoimmun Rev* (2016) 15(4):307–24. doi:10.1016/j.autrev.2015.12.004
12. Ramanathan S, Mohammad SS, Brilot F, Dale RC. Autoimmune encephalitis: recent updates and emerging challenges. *J Clin Neurosci* (2014) 21(5):722–30. doi:10.1016/j.jocn.2013.07.017

13. Sinmaz N, Amatoury M, Merheb V, Ramanathan S, Dale RC, Brilot F. Autoantibodies in movement and psychiatric disorders: updated concepts in detection methods, pathogenicity, and CNS entry. *Ann N Y Acad Sci* (2015) 1351:22–38. doi:10.1111/nyas.12764
14. van Coevorden-Hameete MH, de Graaff E, Titulaer MJ, Hoogenraad CC, Sillevis Smitt PA. Molecular and cellular mechanisms underlying anti-neuronal antibody mediated disorders of the central nervous system. *Autoimmun Rev* (2014) 13(3):299–312. doi:10.1016/j.autrev.2013.10.016
15. Zuliani L, Graus F, Giometto B, Bien C, Vincent A. Central nervous system neuronal surface antibody associated syndromes: review and guidelines for recognition. *J Neurol Neurosurg Psychiatry* (2012) 83(6):638–45. doi:10.1136/jnnp-2011-301237
16. Crotty S. A brief history of T cell help to B cells. *Nat Rev Immunol* (2015) 15(3):185–9. doi:10.1038/nri3803
17. Mitchison NA. T-cell–B-cell cooperation. *Nat Rev Immunol* (2004) 4(4): 308–12. doi:10.1038/nri1334
18. Toellner KM, Luther SA, Sze DM, Choy RK, Taylor DR, MacLennan IC, et al. T helper 1 (Th1) and Th2 characteristics start to develop during T cell priming and are associated with an immediate ability to induce immunoglobulin class switching. *J Exp Med* (1998) 187(8):1193–204. doi:10.1084/jem.187.8.1193
19. Vinuesa CG, Linterman MA, Yu D, MacLennan IC. Follicular helper T cells. *Annu Rev Immunol* (2016) 34:335–68. doi:10.1146/annurev-immunol-041015-055605
20. McPherson RC, Anderton SM. Adaptive immune responses in CNS autoimmune disease: mechanisms and therapeutic opportunities. *J Neuroimmune Pharmacol* (2013) 8(4):774–90. doi:10.1007/s11481-013-9453-9
21. Petermann F, Korn T. Cytokines and effector T cell subsets causing autoimmune CNS disease. *FEBS Lett* (2011) 585(23):3747–57. doi:10.1016/j.febslet.2011.03.064
22. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine* (2015) 74(1):5–17. doi:10.1016/j.cyto.2014.09.011
23. Sie C, Korn T, Mitsdoerffer M. Th17 cells in central nervous system autoimmunity. *Exp Neurol* (2014) 262(Pt A):18–27. doi:10.1016/j.expneurol.2014.03.009
24. Kivisakk P, Mahad DJ, Callahan MK, Trebst C, Tucky B, Wei T, et al. Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin. *Proc Natl Acad Sci U S A* (2003) 100(14):8389–94. doi:10.1073/pnas.1433000100
25. Ransohoff RM, Kivisakk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* (2003) 3(7):569–81. doi:10.1038/nri1130
26. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* (1999) 401(6754):708–12. doi:10.1038/44385
27. Svenningsson A, Andersen O, Edsbagge M, Stemme S. Lymphocyte phenotype and subset distribution in normal cerebrospinal fluid. *J Neuroimmunol* (1995) 63(1):39–46. doi:10.1016/0165-5728(95)00126-3
28. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat Rev Immunol* (2012) 12(9): 623–35. doi:10.1038/nri3265
29. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med* (2015) 212(7):991–9. doi:10.1084/jem.20142290
30. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature* (2015) 523(7560):337–41. doi:10.1038/nature14432
31. Carrithers MD, Visintin I, Kang SJ, Janeway CA Jr. Differential adhesion molecule requirements for immune surveillance and inflammatory recruitment. *Brain* (2000) 123(Pt 6):1092–101. doi:10.1093/brain/123.6.1092
32. Khatri BO, Man S, Giovannoni G, Koo AP, Lee JC, Tucky B, et al. Effect of plasma exchange in accelerating natalizumab clearance and restoring leukocyte function. *Neurology* (2009) 72(5):402–9. doi:10.1212/01.wnl.0000341766.59028.9d
33. Man S, Tucky B, Bagheri N, Li X, Kochar R, Ransohoff RM. Alpha4 integrin/FN-CS1 mediated leukocyte adhesion to brain microvascular endothelial cells under flow conditions. *J Neuroimmunol* (2009) 210(1–2):92–9. doi:10.1016/j.jneuroim.2009.03.008
34. Stuve O, Marra CM, Jerome KR, Cook L, Cravens PD, Cepok S, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann Neurol* (2006) 59(5):743–7. doi:10.1002/ana.20858
35. Ellwardt E, Walsh JT, Kipnis J, Zipp F. Understanding the role of T cells in CNS homeostasis. *Trends Immunol* (2016) 37(2):154–65. doi:10.1016/j.it.2015.12.008
36. Kappos L, Bates D, Edan G, Eraksoy M, Garcia-Merino A, Grigoriadis N, et al. Natalizumab treatment for multiple sclerosis: updated recommendations for patient selection and monitoring. *Lancet Neurol* (2011) 10(8):745–58. doi:10.1016/S1474-4422(11)70149-1
37. Warnke C, Olsson T, Hartung HP. PML: the dark side of immunotherapy in multiple sclerosis. *Trends Pharmacol Sci* (2015) 36(12):799–801. doi:10.1016/j.tips.2015.09.006
38. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* (2015) 15(9):545–58. doi:10.1038/nri3871
39. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* (2005) 58(6):840–6. doi:10.1002/ana.20703
40. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* (1996) 46(4):907. doi:10.1212/WNL.46.4.907
41. Steinman L. A molecular trio in relapse and remission in multiple sclerosis. *Nat Rev Immunol* (2009) 9(6):440–7. doi:10.1038/nri2548
42. Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J, et al. The natural history of multiple sclerosis: a geographically based study. 2. Predictive value of the early clinical course. *Brain* (1989) 112(Pt 6):1419–28. doi:10.1093/brain/112.6.1419
43. BØ L, Vedeler CA, Nyland HI, Trapp BD, MØRk SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J Neuropathol Exp Neurol* (2003) 62(7):723–32. doi:10.1093/jnen/62.7.723
44. Calabrese M, De Stefano N, Atzori M, Bernardi V, Mattisi I, Barachino L, et al. Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. *Arch Neurol* (2007) 64(10):1416–22. doi:10.1001/archneur.64.10.1416
45. Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. *Ann Neurol* (2008) 64(3):255–65. doi:10.1002/ana.21436
46. Huseby ES, Kamimura D, Arima Y, Parello CS, Sasaki K, Murakami M. Role of T cell-glial cell interactions in creating and amplifying central nervous system inflammation and multiple sclerosis disease symptoms. *Front Cell Neurosci* (2015) 9:295. doi:10.3389/fncel.2015.00295
47. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. *Brain Pathol* (2007) 17(2):210–8. doi:10.1111/j.1750-3639.2007.00064.x
48. Ontaneda D, Hyland M, Cohen JA. Multiple sclerosis: new insights in pathogenesis and novel therapeutics. *Annu Rev Med* (2012) 63(1):389–404. doi:10.1146/annurev-med-042910-135833
49. Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol* (2001) 50(3):389. doi:10.1002/ana.1123
50. Martin R, McFarland HF. Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol* (2007) 8(9):913–9. doi:10.1038/ni1507
51. Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* (2007) 357(9):851–62. doi:10.1056/NEJMoa073493
52. International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* (2011) 476(7359):214–9. doi:10.1038/nature10251
53. Olsson T, Hillert J. The genetics of multiple sclerosis and its experimental models. *Curr Opin Neurol* (2008) 21(3):255–60. doi:10.1097/WCO.0b013e3282fd10cc
54. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glanville J, Dobbins J, et al. Deconstructing the peptide-MHC specificity of T cell recognition. *Cell* (2014) 157(5):1073–87. doi:10.1016/j.cell.2014.03.047
55. Huseby ES, Huseby PG, Shah S, Smith R, Stadinski BD. Pathogenic CD8 T cells in multiple sclerosis and its experimental models. *Front Immunol* (2012) 3:64. doi:10.3389/fimmu.2012.00064

56. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* (2017) 13(1):25–36. doi:10.1038/nrneurol.2016.187
57. van der Mei I, Lucas RM, Taylor BV, Valery PC, Dwyer T, Kilpatrick TJ, et al. Population attributable fractions and joint effects of key risk factors for multiple sclerosis. *Mult Scler* (2016) 22(4):461–9. doi:10.1177/1352458515594040
58. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* (2006) 296(23):2832–8. doi:10.1001/jama.296.23.2832
59. Munger KL, Zhang SM, O'Reilly E, Hernan MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology* (2004) 62(1):60–5. doi:10.1212/01.WNL.0000101723.79681.38
60. Mowry EM, Krupp LB, Milazzo M, Chabas D, Strober JB, Belman AL, et al. Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. *Ann Neurol* (2010) 67(5):618–24. doi:10.1002/ana.21972
61. Mowry EM, Waubant E, McCulloch CE, Okuda DT, Evangelista AA, Lincoln RR, et al. Vitamin D status predicts new brain magnetic resonance imaging activity in multiple sclerosis. *Ann Neurol* (2012) 72(2):234–40. doi:10.1002/ana.23591
62. Runia TF, Hop WC, de Rijke YB, Buljevac D, Hintzen RQ. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. *Neurology* (2012) 79(3):261–6. doi:10.1212/WNL.0b013e31825fdec7
63. Simpson S Jr, Taylor B, Blizzard L, Ponsonby AL, Pittas F, Tremlett H, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. *Ann Neurol* (2010) 68(2):193–203. doi:10.1002/ana.22043
64. Ascherio A. Environmental factors in multiple sclerosis. *Expert Rev Neurother* (2013) 13(12 Suppl):3–9. doi:10.1586/14737175.2013.865866
65. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann Neurol* (2007) 61(4):288–99. doi:10.1002/ana.21117
66. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. *Clin Rev Allergy Immunol* (2012) 42(1):102–11. doi:10.1007/s12016-011-8294-7
67. Wandinger K, Jabs W, Siekhaus A, Bubel S, Trillenberg P, Wagner H, et al. Association between clinical disease activity and *Epstein-Barr virus* reactivation in MS. *Neurology* (2000) 55(2):178–84. doi:10.1212/WNL.55.2.178
68. Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, et al. Dysregulated *Epstein-Barr virus* infection in the multiple sclerosis brain. *J Exp Med* (2007) 204(12):2899–912. doi:10.1084/jem.20071030
69. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* (2005) 23(1):683–747. doi:10.1146/annurev.immunol.23.021704.115707
70. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, et al. *Epstein-Barr virus* infection is not a characteristic feature of multiple sclerosis brain. *Brain* (2009) 132(12):3318–28. doi:10.1093/brain/awp200
71. Kidd G, Kivisäkk P, Ransohoff RM. Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* (2003) 3(7):569–81. doi:10.1038/nri1130
72. Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev* (2006) 213(1):48–65. doi:10.1111/j.1600-065X.2006.00441.x
73. González H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflammation* (2014) 11(1):201. doi:10.1186/s12974-014-0201-8
74. Pette M, Fujita K, Wilkinson D, Altmann DM, Trowsdale J, Giegerich G, et al. Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. *Proc Natl Acad Sci U S A* (1990) 87(20):7968–72. doi:10.1073/pnas.87.20.7968
75. Valli A, Sette A, Kappos L, Oseroff C, Sidney J, Miescher G, et al. Binding of myelin basic protein peptides to human histocompatibility leukocyte antigen class II molecules and their recognition by T cells from multiple sclerosis patients. *J Clin Invest* (1993) 91(2):616–28. doi:10.1172/JCI116242
76. Andersson M, Yu M, Söderström M, Weerth S, Baig S, Linington C, et al. Multiple MAG peptides are recognized by circulating T and B lymphocytes in polyneuropathy and multiple sclerosis. *Eur J Neurol* (2002) 9(3):243–51. doi:10.1046/j.1468-1331.2002.00391.x
77. Tsuchida T, Parker KC, Turner RV, McFarland HF, Coligan JE, Biddison WE. Autoreactive CD8+ T-cell responses to human myelin protein-derived peptides. *Proc Natl Acad Sci U S A* (1994) 91(23):10859–63. doi:10.1073/pnas.91.23.10859
78. Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner HL, Hafler DA. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J Exp Med* (1994) 179(3):973–84. doi:10.1084/jem.179.3.973
79. Bielekova B, Sung M-H, Kadom N, Simon R, McFarland H, Martin R. Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. *J Immunol* (2004) 172(6):3893. doi:10.4049/jimmunol.172.6.3893
80. Lovett-Racke AE, Trotter JL, Lauber J, Perrin PJ, June CH, Racke MK. Decreased dependence of myelin basic protein-reactive T cells on CD28-mediated costimulation in multiple sclerosis patients. A marker of activated/memory T cells. *J Clin Invest* (1998) 101(4):725–30. doi:10.1172/JCI1528
81. Scholz C, Patton KT, Anderson DE, Freeman GJ, Hafler DA. Expansion of autoreactive T cells in multiple sclerosis is independent of exogenous B7 costimulation. *J Immunol* (1998) 160(3):1532.
82. Zang YCQ, Li S, Rivera VM, Hong J, Robinson RR, Breitbach WT, et al. Increased CD8+ cytotoxic T cell responses to myelin basic protein in multiple sclerosis. *J Immunol* (2004) 172(8):5120. doi:10.4049/jimmunol.172.8.5120
83. Crawford MP, Yan SX, Ortega SB, Mehta RS, Hewitt RE, Price DA, et al. High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood* (2004) 103(11):4222–31. doi:10.1182/blood-2003-11-4025
84. Frohman EM, Racke MK, Raine CS. Multiple sclerosis – the plaque and its pathogenesis. *N Engl J Med* (2006) 354(9):942–55. doi:10.1056/NEJMra052130
85. Cua DJ, Wiekowski M, Sedgwick JD, Chen Y, Murphy CA, Kastelein RA, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* (2003) 421(6924):744–8. doi:10.1038/nature01355
86. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* (2005) 201(2):233–40. doi:10.1084/jem.20041257
87. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* (2006) 441(7090):231–4. doi:10.1038/nature04754
88. Montes M, Zhang X, Berthelot L, Laplaud D-A, Brouard S, Jin J, et al. Oligoclonal myelin-reactive T-cell infiltrates derived from multiple sclerosis lesions are enriched in Th17 cells. *Clin Immunol* (2009) 130(2):133–44. doi:10.1016/j.clim.2008.08.030
89. Raine CS, Strober S, Oksenberg JR, Klonowski P, Langer-Gould A, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* (2002) 8(5):500–8. doi:10.1038/nm0502-500
90. Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. *Brain* (2009) 132(12):3329–41. doi:10.1093/brain/awp289
91. Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. *Ann Neurol* (2009) 65(5):499–509. doi:10.1002/ana.21652
92. Matusevicius D, Kivisäkk P, He B, Kostulas N, Özenci V, Fredrikson S, et al. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. *Mult Scler* (1999) 5(2):101–4. doi:10.1177/135245859900500206
93. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *Am J Pathol* (2008) 172(1):146–55. doi:10.2353/ajpath.2008.070690
94. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* (2007) 13(10):1173–5. doi:10.1038/nm1651
95. Friese MA, Fugger L. Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? *Brain* (2005) 128(Pt 8):1747–63. doi:10.1093/brain/awh578
96. Goverman J, Perchellet A, Huseby ES. The role of CD8(+) T cells in multiple sclerosis and its animal models. *Curr Drug Targets Inflamm Allergy* (2005) 4(2):239. doi:10.2174/1568010053586264

97. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* (2000) 192(3):393–404. doi:10.1084/jem.192.3.393
98. Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J Neurol Sci* (1983) 62(1):219–32. doi:10.1016/0022-510X(83)90201-0
99. Hauser SL, Bhan AK, Gilles F, Kemp M, Kerr C, Weiner HL. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* (1986) 19(6):578–87. doi:10.1002/ana.410190610
100. Traugott U, Reinherz EL, Raine CS. Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science* (1983) 219(4582):308–10. doi:10.1126/science.6217550
101. Junker A, Ivanidze J, Malotka J, Eiglmeier I, Lassmann H, Wekerle H, et al. Multiple sclerosis: T-cell receptor expression in distinct brain regions. *Brain* (2007) 130(11):2789–99. doi:10.1093/brain/awm214
102. Bartlett PF, Wong GHW, Schrader JW, Battye F, Clark-Lewis I. Inducible expression of H-2 and Ia antigens on brain cells. *Nature* (1984) 310(5979):688–91. doi:10.1038/310688a0
103. Neumann H, Cavalie A, Jenne DE, Wekerle H. Induction of MHC class I genes in neurons. *Science* (1995) 269(5223):549–52. doi:10.1126/science.7624779
104. Neumann H, Medana IM, Bauer J, Lassmann H. *Cytotoxic T Lymphocytes in Autoimmune and Degenerative CNS Diseases*. England: Elsevier Ltd (2002). p. 313–9.
105. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Capello E, Mancardi GL, et al. Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. *J Neuropathol Exp Neurol* (2006) 65(2):124–41. doi:10.1097/01.jnen.0000199572.96472.1c
106. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* (2000) 123(Pt 6):1174–83. doi:10.1093/brain/123.6.1174
107. Warnke C, Menge T, Hartung HP, Racke MK, Cravens PD, Bennett JL, et al. Natalizumab and progressive multifocal leukoencephalopathy: what are the causal factors and can it be avoided? *Arch Neurol* (2010) 67(8):923–30. doi:10.1001/archneurol.2010.161
108. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* (2006) 354(9):899–910. doi:10.1056/NEJMoa044397
109. Probstel AK, Sanderson NS, Derfuss T. B cells and autoantibodies in multiple sclerosis. *Int J Mol Sci* (2015) 16(7):16576–92. doi:10.3390/ijms160716576
110. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* (2005) 202(4):473–7. doi:10.1084/jem.20050304
111. Andermann E, Oguni H, Guttmann RD, Osterland CK, Antel JP, Eeg-Olofisson O, et al. Genetic aspects of chronic encephalitis. In: Andermann F, editor. *Chronic Encephalitis and Epilepsy*. Boston: Butterworth-Heinemann (1991). p. 167–75.
112. Bien CG, Urbach H, Deckert M, Schramm J, Wiestler OD, Lassmann H, et al. Diagnosis and staging of Rasmussen's encephalitis by serial MRI and histopathology. *Neurology* (2002) 58(2):250. doi:10.1212/WNL.58.2.250
113. Takahashi Y, Mine J, Kubota Y, Yamazaki E, Fujiwara T. A substantial number of Rasmussen syndrome patients have increased IgG, CD4+ T cells, TNFalpha, and granzyme B in CSF. *Epilepsia* (2009) 50(6):1419–31. doi:10.1111/j.1528-1167.2008.01977.x
114. Tekgul H, Polat M, Kitis O, Serdaroglu G, Tosun A, Terlemez S, et al. T-cell subsets and interleukin-6 response in Rasmussen's encephalitis. *Pediatr Neurol* (2005) 33(1):39–45. doi:10.1016/j.pediatrneurol.2005.01.007
115. Benyahia B, Liblau R, Merle-Béral H, Tourani JM, Dalmau J, Delattre JY. Cell-mediated autoimmunity in paraneoplastic neurological syndromes with anti-Hu antibodies. *Ann Neurol* (1999) 45(2):162–7. doi:10.1002/1531-8249(199902)45:23.0.CO;2-R
116. Darnell JC, Albert ML, Darnell RB. Cdr2, a target antigen of naturally occuring human tumor immunity, is widely expressed in gynecological tumors. *Cancer Res* (2000) 60(8):2136.
117. de Graaf MT, de Beukelaar JW, Haasnoot GW, Levering WH, Rogemond V, Didelot A, et al. HLA-DQ2+ individuals are susceptible to Hu-Ab associated paraneoplastic neurological syndromes. *J Neuroimmunol* (2010) 226(1–2):147–9. doi:10.1016/j.jneuroim.2010.05.035
118. Domschke C, Schuetz F, Ge Y, Rom J, Zorn M, Sinn HP, et al. Determination of paraneoplastic autoimmune responses by tumor cell biology and intratumoral IFN-alpha/IL-12 in breast cancer patients. *Cancer Immunol Immunother* (2011) 60(3):401–11. doi:10.1007/s00262-010-0956-4
119. Leypoldt F, Wandinger KP. Paraneoplastic neurological syndromes. *Clin Exp Immunol* (2014) 175(3):336–48. doi:10.1111/cei.12185
120. Rousseau A, Benyahia B, Dalmau J, Connan F, Guillet JG, Delattre JY, et al. T cell response to Hu-D peptides in patients with anti-Hu syndrome. *J Neurooncol* (2005) 71(3):231–6. doi:10.1007/s11060-004-1723-1
121. Tanaka Y, Suzuki N, Takao M, Ichikawa A, Susumu N, Aoki D. Paraneoplastic cerebellar degeneration with fallopian tube adenocarcinoma. *Gynecol Oncol* (2005) 99(2):500–3. doi:10.1016/j.ygyno.2005.06.064
122. Tanaka M, Tanaka K. HLA A24 in paraneoplastic cerebellar degeneration with anti-Yo antibody. *Neurology* (1996) 47(2):606–7. doi:10.1212/WNL.47.2.606
123. Rasmussen T, Olszewski J, Lloydsmith D. Focal seizures due to chronic localized encephalitis. *Neurology* (1958) 8(6):435–45. doi:10.1212/WNL.8.6.435
124. Varadkar S, Bien CG, Kruse CA, Jensen FE, Bauer J, Pardo CA, et al. Rasmussen's encephalitis: clinical features, pathobiology, and treatment advances. *Lancet Neurol* (2014) 13(2):195. doi:10.1016/S1474-4422(13)70260-6
125. Bien CG, Granata T, Antozzi C, Cross JH, Dulac O, Kurthen M, et al. Pathogenesis, diagnosis and treatment of Rasmussen encephalitis: a European consensus statement. *Brain* (2005) 128(Pt 3):454–71. doi:10.1093/brain/awh415
126. Mantegazza R, Bernasconi P, Baggi F, Spreafico R, Ragona F, Antozzi C, et al. Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J Neuroimmunol* (2002) 131(1):179–85. doi:10.1016/S0165-5728(02)00261-8
127. Rogers SW, Andrews PI, Gahring LC, Whisenand T, Cauley K, Crain B, et al. Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science* (1994) 265(5172):648–51. doi:10.1126/science.8036512
128. Watson R, Jiang Y, Bermudez I, Houlihan L, Clover L, McKnight K, et al. Absence of antibodies to glutamate receptor type 3 (GluR3) in Rasmussen encephalitis. *Neurology* (2004) 63(1):43–50. doi:10.1212/01.WNL.0000132651.66689.0F
129. Wiendl H, Bien CG, Bernasconi P, Fleckenstein B, Elger CE, Dichgans J, et al. GluR3 antibodies: prevalence in focal epilepsy but no specificity for Rasmussen's encephalitis. *Neurology* (2001) 57(8):1511. doi:10.1212/WNL.57.8.1511
130. Schwab N, Bien CG, Waschbisch A, Becker A, Vince GH, Dornmair K, et al. CD8+ T-cell clones dominate brain infiltrates in Rasmussen encephalitis and persist in the periphery. *Brain* (2009) 132(5):1236–46. doi:10.1093/brain/awp003
131. Schneider-Hohendorf T, Mohan H, Bien CG, Breuer J, Becker A, Gorlich D, et al. CD8(+) T-cell pathogenicity in Rasmussen encephalitis elucidated by large-scale T-cell receptor sequencing. *Nat Commun* (2016) 7:11153. doi:10.1038/ncomms11153
132. Bien CG, Schramm J. Treatment of Rasmussen encephalitis half a century after its initial description: promising prospects and a dilemma. *Epilepsy Res* (2009) 86(2):101–12. doi:10.1016/j.eplepsyres.2009.06.001
133. Bittner S, Simon OJ, Göbel K, Bien CG, Meuth SG, Wiendl H. Rasmussen encephalitis treated with natalizumab. *Neurology* (2013) 81(4):395–7. doi:10.1212/WNL.0b013e31829c5ceb
134. Gultekin SH, Rosenfeld MR, Voltz R, Eichen J, Posner JB, Dalmau J. Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumour association in 50 patients. *Brain* (2000) 123(Pt 7):1481–94. doi:10.1093/brain/123.7.1481
135. Furneaux HM, Rosenblum MK, Dalmau J, Wong E, Woodruff P, Graus F, et al. Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *N Engl J Med* (1990) 322(26):1844–51. doi:10.1056/NEJM199006283222604
136. McKeon A, Pittock SJ. Paraneoplastic encephalomyelopathies: pathology and mechanisms. *Acta Neuropathol* (2011) 122(4):381–400. doi:10.1007/s00401-011-0876-1
137. Albert ML, Austin LM, Darnell RB. Detection and treatment of activated T cells in the cerebrospinal fluid of patients with paraneoplastic

cerebellar degeneration. *Ann Neurol* (2000) 47(1):9. doi:10.1002/1531-8249(200001)47:13.3.CO;2-9
138. Blumenthal DT, Salzman KL, Digre KB, Jensen RL, Dunson WA, Dalmau J. Early pathologic findings and long-term improvement in anti-Ma2-associated encephalitis. *Neurology* (2006) 67(1):146–9. doi:10.1212/01.wnl.0000223647.83708.20
139. Bernal F, Graus F, Pifarré À, Saiz A, Benyahia B, Ribalta T. Immunohistochemical analysis of anti-Hu-associated paraneoplastic encephalomyelitis. *Acta Neuropathol* (2002) 103(5):509–15. doi:10.1007/s00401-001-0498-0
140. Graus F, Vega F, Delattre JY, Bonaventura I, Reñé R, Arbaiza D, et al. Plasmapheresis and antineoplastic treatment in CNS paraneoplastic syndromes with antineuronal autoantibodies. *Neurology* (1992) 42(3 Pt 1):536–40. doi:10.1212/WNL.42.3.536
141. Shams'ili S, Grefkens J, Leeuw B, Bent M, Hooijkaas H, Holt B, et al. Paraneoplastic cerebellar degeneration associated with antineuronal antibodies: analysis of 50 patients. *Brain* (2003) 126(Pt 6):1409–18. doi:10.1093/brain/awg133
142. Uchuya M, Graus F, Vega F, Reñé R, Delattre JY. Intravenous immunoglobulin treatment in paraneoplastic neurological syndromes with antineuronal autoantibodies. *J Neurol Neurosurg Psychiatry* (1996) 60(4):388–92. doi:10.1136/jnnp.60.4.388
143. Bien CG, Vincent A, Barnett MH, Becker AJ, Blümcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* (2012) 135(Pt 5):1622–38. doi:10.1093/brain/aws082
144. Melzer N, Meuth SG, Wiendl H. CD8+ T cells and neuronal damage: direct and collateral mechanisms of cytotoxicity and impaired electrical excitability. *FASEB J* (2009) 23(11):3659–73. doi:10.1096/fj.09-136200
145. Sommerfeldt N, Schutz F, Sohn C, Forster J, Schirrmacher V, Beckhove P. The shaping of a polyvalent and highly individual T-cell repertoire in the bone marrow of breast cancer patients. *Cancer Res* (2006) 66(16):8258–65. doi:10.1158/0008-5472.CAN-05-4201
146. Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc Natl Acad Sci U S A* (2008) 105(40):15558–63. doi:10.1073/pnas.0807419105
147. Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, et al. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. *Proc Natl Acad Sci U S A* (2008) 105(46):17913–8. doi:10.1073/pnas.0804610105
148. Mantovani S, Garbelli S, Pasini A, Alimonti D, Perotti C, Melazzini M, et al. Immune system alterations in sporadic amyotrophic lateral sclerosis patients suggest an ongoing neuroinflammatory process. *J Neuroimmunol* (2009) 210(1–2):73–9. doi:10.1016/j.jneuroim.2009.02.012
149. Carson MJ. Microglia as liaisons between the immune and central nervous systems: functional implications for multiple sclerosis. *Glia* (2002) 40(2):218–31. doi:10.1002/glia.10145
150. Hammarberg H, Lidman O, Lundberg C, Eltayeb SY, Gielen AW, Muhallab S, et al. Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS-infiltrating T and natural killer cells. *J Neurosci* (2000) 20(14):5283–91.
151. Moalem G, Monsonego A, Shani Y, Cohen IR, Schwartz M. Differential T cell response in central and peripheral nerve injury: connection with immune privilege. *FASEB J* (1999) 13(10):1207–17.
152. Serpe CJ, Coers S, Sanders VM, Jones KJ. CD4+ T, but not CD8+ or B, lymphocytes mediate facial motoneuron survival after facial nerve transection. *Brain Behav Immun* (2003) 17(5):393–402. doi:10.1016/S0889-1591(03)00028-X
153. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
154. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* (2006) 66(10):1485–9. doi:10.1212/01.wnl.0000216139.44259.74
155. Waters P, Jarius S, Littleton E, Leite MI, Jacob S, Gray B, et al. Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transverse myelitis. *Arch Neurol* (2008) 65(7):913–9. doi:10.1001/archneur.65.7.913
156. Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C, et al. Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. *Ann Neurol* (2009) 66(5):617–29. doi:10.1002/ana.21802
157. Bradl M, Misu T, Takahashi T, Watanabe M, Mader S, Reindl M, et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. *Ann Neurol* (2009) 66(5):630–43. doi:10.1002/ana.21837
158. Kinoshita M, Nakatsuji Y, Kimura T, Moriya M, Takata K, Okuno T, et al. Anti-aquaporin-4 antibody induces astrocytic cytotoxicity in the absence of CNS antigen-specific T cells. *Biochem Biophys Res Commun* (2010) 394(1):205–10. doi:10.1016/j.bbrc.2010.02.157
159. Jarius S, Aboul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. *Brain* (2008) 131(Pt 11):3072–80. doi:10.1093/brain/awn240
160. Nishiyama S, Ito T, Misu T, Takahashi T, Kikuchi A, Suzuki N, et al. A case of NMO seropositive for aquaporin-4 antibody more than 10 years before onset. *Neurology* (2009) 72(22):1960–1. doi:10.1212/WNL.0b013e3181a82621
161. Jacob A, Weinshenker BG, Violich I, McLinskey N, Krupp L, Fox RJ, et al. Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients. *Arch Neurol* (2008) 65(11):1443–8. doi:10.1001/archneur.65.11.noc80069
162. Kim SH, Kim W, Li XF, Jung IJ, Kim HJ. Repeated treatment with rituximab based on the assessment of peripheral circulating memory B cells in patients with relapsing neuromyelitis optica over 2 years. *Arch Neurol* (2011) 68(11):1412–20. doi:10.1001/archneurol.2011.154
163. Brum DG, Barreira AA, dos Santos AC, Kaimen-Maciel DR, Matiello M, Costa RM, et al. HLA-DRB association in neuromyelitis optica is different from that observed in multiple sclerosis. *Mult Scler* (2010) 16(1):21–9. doi:10.1177/1352458509350741
164. Deschamps R, Paturel L, Jeannin S, Chausson N, Olindo S, Bera O, et al. Different HLA class II (DRB1 and DQB1) alleles determine either susceptibility or resistance to NMO and multiple sclerosis among the French Afro-Caribbean population. *Mult Scler* (2011) 17(1):24–31. doi:10.1177/1352458510382810
165. Matsuya N, Komori M, Nomura K, Nakane S, Fukudome T, Goto H, et al. Increased T-cell immunity against aquaporin-4 and proteolipid protein in neuromyelitis optica. *Int Immunol* (2011) 23(9):565–73. doi:10.1093/intimm/dxr056
166. Tanaka M, Matsushita T, Tateishi T, Ochi H, Kawano Y, Mei FJ, et al. Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. *Neurology* (2008) 71(13):974–81. doi:10.1212/01.wnl.0000326589.57128.c3
167. Uzawa A, Mori M, Ito M, Uchida T, Hayakawa S, Masuda S, et al. Markedly increased CSF interleukin-6 levels in neuromyelitis optica, but not in multiple sclerosis. *J Neurol* (2009) 256(12):2082–4. doi:10.1007/s00415-009-5274-4
168. Vaknin-Dembinsky A, Brill L, Kassis I, Petrou P, Ovadia H, Ben-Hur T, et al. T-cell responses to distinct AQP4 peptides in patients with neuromyelitis optica (NMO). *Mult Scler Relat Disord* (2016) 6:28–36. doi:10.1016/j.msard.2015.12.004
169. Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, Nelson PA, Stroud RM, Cree BA, et al. Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize *Clostridium* ABC transporter. *Ann Neurol* (2012) 72(1):53–64. doi:10.1002/ana.23651
170. Wang H, Dai Y, Qiu W, Zhong X, Wu A, Wang Y, et al. HLA-DPB1 0501 is associated with susceptibility to anti-aquaporin-4 antibodies positive neuromyelitis optica in southern Han Chinese. *J Neuroimmunol* (2011) 233(1–2):181–4. doi:10.1016/j.jneuroim.2010.11.004
171. Zephir H, Fajardy I, Outteryck O, Blanc F, Roger N, Fleury M, et al. Is neuromyelitis optica associated with human leukocyte antigen? *Mult Scler* (2009) 15(5):571–9. doi:10.1177/1352458508102085
172. Dale RC, Morovat A. Interleukin-6 and oligoclonal IgG synthesis in children with acute disseminated encephalomyelitis. *Neuropediatrics* (2003) 34(3):141–5. doi:10.1055/s-2003-41281
173. Ichiyama T, Shoji H, Kato M, Sawaishi Y, Ozawa H, Matsubara T, et al. Cerebrospinal fluid levels of cytokines and soluble tumour necrosis factor receptor in acute disseminated encephalomyelitis. *Eur J Pediatr* (2002) 161(3):133–7. doi:10.1007/s00431-001-0888-2
174. Ishizu T, Minohara M, Ichiyama T, Kira R, Tanaka M, Osoegawa M, et al. CSF cytokine and chemokine profiles in acute disseminated encephalomyelitis. *J Neuroimmunol* (2006) 175(1–2):52–8. doi:10.1016/j.jneuroim.2006.03.020
175. Jorens PG, VanderBorght A, Ceulemans B, Van Bever HP, Bossaert LL, Ieven M, et al. Encephalomyelitis-associated antimyelin autoreactivity

induced by streptococcal exotoxins. *Neurology* (2000) 54(7):1433–41. doi:10.1212/WNL.54.7.1433
176. Pohl-Koppe A, Burchett SK, Thiele EA, Hafler DA. Myelin basic protein reactive Th2 T cells are found in acute disseminated encephalomyelitis. *J Neuroimmunol* (1998) 91(1–2):19–27. doi:10.1016/S0165-5728(98)00125-8
177. Yoshitomi T, Matsubara T, Nishikawa M, Katayama K, Ichiyama T, Hayashi T, et al. Increased peripheral blood interferon gamma-producing T cells in acute disseminated encephalomyelitis. *J Neuroimmunol* (2000) 111(1–2):224–8. doi:10.1016/S0165-5728(00)00358-1
178. Costa M, Saiz A, Casamitjana R, Castaner MF, Sanmarti A, Graus F, et al. T-cell reactivity to glutamic acid decarboxylase in stiff-man syndrome and cerebellar ataxia associated with polyendocrine autoimmunity. *Clin Exp Immunol* (2002) 129(3):471–8. doi:10.1046/j.1365-2249.2002.01931.x
179. Hanninen A, Soilu-Hanninen M, Hampe CS, Deptula A, Geubtner K, Ilonen J, et al. Characterization of CD4+ T cells specific for glutamic acid decarboxylase (GAD65) and proinsulin in a patient with stiff-person syndrome but without type 1 diabetes. *Diabetes Metab Res Rev* (2010) 26(4):271–9. doi:10.1002/dmrr.1083
180. Hummel M, Durinovic-Bello I, Bonifacio E, Lampasona V, Endl J, Fessele S, et al. Humoral and cellular immune parameters before and during immunosuppressive therapy of a patient with stiff-man syndrome and insulin dependent diabetes mellitus. *J Neurol Neurosurg Psychiatry* (1998) 65(2):204–8. doi:10.1136/jnnp.65.2.204
181. Pugliese A, Solimena M, Awdeh ZL, Alper CA, Bugawan T, Erlich HA, et al. Association of HLA-DQB1*0201 with stiff-man syndrome. *J Clin Endocrinol Metab* (1993) 77(6):1550–3. doi:10.1210/jcem.77.6.8263140
182. Schloot NC, Batstra MC, Duinkerken G, De Vries RR, Dyrberg T, Chaudhuri A, et al. GAD65-reactive T cells in a non-diabetic stiff-man syndrome patient. *J Autoimmun* (1999) 12(4):289–96. doi:10.1006/jaut.1999.0280
183. Skorstad G, Hestvik AL, Vartdal F, Holmoy T. Cerebrospinal fluid T cell responses against glutamic acid decarboxylase 65 in patients with stiff person syndrome. *J Autoimmun* (2009) 32(1):24–32. doi:10.1016/j.jaut.2008.10.002
184. Byun JI, Lee ST, Moon J, Jung KH, Sunwoo JS, Lim JA, et al. Distinct intrathecal interleukin-17/interleukin-6 activation in anti-*N*-methyl-D-aspartate receptor encephalitis. *J Neuroimmunol* (2016) 297:141–7. doi:10.1016/j.jneuroim.2016.05.023
185. Kothur K, Wienholt L, Mohammad SS, Tantsis EM, Pillai S, Britton PN, et al. Utility of CSF cytokine/chemokines as markers of active intrathecal inflammation: comparison of demyelinating, anti-NMDAR and enteroviral encephalitis. *PLoS One* (2016) 11(8):e0161656. doi:10.1371/journal.pone.0161656
186. Lee WJ, Lee ST, Moon J, Sunwoo JS, Byun JI, Lim JA, et al. Tocilizumab in autoimmune encephalitis refractory to rituximab: an institutional cohort study. *Neurotherapeutics* (2016) 13(4):824–32. doi:10.1007/s13311-016-0442-6
187. Liba Z, Kayserova J, Elisak M, Marusic P, Nohejlova H, Hanzalova J, et al. Anti-*N*-methyl-D-aspartate receptor encephalitis: the clinical course in light of the chemokine and cytokine levels in cerebrospinal fluid. *J Neuroinflammation* (2016) 13(1):55. doi:10.1186/s12974-016-0507-9
188. Ulusoy C, Tuzun E, Kurtuncu M, Turkoglu R, Akman-Demir G, Eraksoy M. Comparison of the cytokine profiles of patients with neuronal-antibody-associated central nervous system disorders. *Int J Neurosci* (2012) 122(6):284–9. doi:10.3109/00207454.2011.648762
189. Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* (2002) 125(Pt 7):1450–61. doi:10.1093/brain/awf151
190. Pohl M, Kawakami N, Kitic M, Bauer J, Martins R, Fischer MT, et al. T cell-activation in neuromyelitis optica lesions plays a role in their formation. *Acta Neuropathol Commun* (2013) 1:85. doi:10.1186/2051-5960-1-85
191. Warabi Y, Yagi K, Hayashi H, Matsumoto Y. Characterization of the T cell receptor repertoire in the Japanese neuromyelitis optica: T cell activity is up-regulated compared to multiple sclerosis. *J Neurol Sci* (2006) 249(2):145–52. doi:10.1016/j.jns.2006.06.011
192. Wang HH, Dai YQ, Qiu W, Lu ZQ, Peng FH, Wang YG, et al. Interleukin-17-secreting T cells in neuromyelitis optica and multiple sclerosis during relapse. *J Clin Neurosci* (2011) 18(10):1313–7. doi:10.1016/j.jocn.2011.01.031
193. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* (2015) 16(5):448–57. doi:10.1038/ni.3153
194. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *Proc Natl Acad Sci U S A* (2008) 105(47):18460–5. doi:10.1073/pnas.0809850105
195. Uzawa A, Mori M, Arai K, Sato Y, Hayakawa S, Masuda S, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. *Mult Scler* (2010) 16(12):1443–52. doi:10.1177/1352458510379247
196. Ayzenberg I, Kleiter I, Schroder A, Hellwig K, Chan A, Yamamura T, et al. Interleukin 6 receptor blockade in patients with neuromyelitis optica nonresponsive to anti-CD20 therapy. *JAMA Neurol* (2013) 70(3):394–7. doi:10.1001/jamaneurol.2013.1246
197. Kieseier BC, Stuve O, Dehmel T, Goebels N, Leussink VI, Mausberg AK, et al. Disease amelioration with tocilizumab in a treatment-resistant patient with neuromyelitis optica: implication for cellular immune responses. *JAMA Neurol* (2013) 70(3):390–3. doi:10.1001/jamaneurol.2013.668
198. Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, et al. Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders. *Tissue Antigens* (2009) 73(2):171–6. doi:10.1111/j.1399-0039.2008.01172.x
199. Farkas AM, Panea C, Goto Y, Nakato G, Galan-Diez M, Narushima S, et al. Induction of Th17 cells by segmented filamentous bacteria in the murine intestine. *J Immunol Methods* (2015) 421:104–11. doi:10.1016/j.jim.2015.03.020
200. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG autoantibody-associated demyelinating diseases. *Nat Rev Neurol* (2013) 9(8):455–61. doi:10.1038/nrneurol.2013.118
201. Brilot F, Dale RC, Selter RC, Grummel V, Kalluri SR, Aslam M, et al. Antibodies to native myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. *Ann Neurol* (2009) 66(6):833–42. doi:10.1002/ana.21916
202. Dale RC, Tantsis EM, Merheb V, Kumaran RY, Sinmaz N, Pathmanandavel K, et al. Antibodies to MOG have a demyelination phenotype and affect oligodendrocyte cytoskeleton. *Neurol Neuroimmunol Neuroinflamm* (2014) 1(1):e12. doi:10.1212/NXI.0000000000000012
203. Mader S, Gredler V, Schanda K, Rostasy K, Dujmovic I, Pfaller K, et al. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. *J Neuroinflammation* (2011) 8:184. doi:10.1186/1742-2094-8-184
204. McLaughlin KA, Chitnis T, Newcombe J, Franz B, Kennedy J, McArdel S, et al. Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis. *J Immunol* (2009) 183(6):4067–76. doi:10.4049/jimmunol.0801888
205. O'Connor KC, McLaughlin KA, De Jager PL, Chitnis T, Bettelli E, Xu C, et al. Self-antigen tetramers discriminate between myelin autoantibodies to native or denatured protein. *Nat Med* (2007) 13(2):211–7. doi:10.1038/nm1488
206. Probstel AK, Dornmair K, Bittner R, Sperl P, Jenne D, Magalhaes S, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. *Neurology* (2011) 77(6):580–8. doi:10.1212/WNL.0b013e318228c0b1
207. Franciotta D, Zardini E, Ravaglia S, Piccolo G, Andreoni L, Bergamaschi R, et al. Cytokines and chemokines in cerebrospinal fluid and serum of adult patients with acute disseminated encephalomyelitis. *J Neurol Sci* (2006) 247(2):202–7. doi:10.1016/j.jns.2006.05.049
208. Kothur K, Wienholt L, Tantsis EM, Earl J, Bandodkar S, Prelog K, et al. B Cell, Th17, and neutrophil related cerebrospinal fluid cytokine/chemokines are elevated in MOG antibody associated demyelination. *PLoS One* (2016) 11(2):e0149411. doi:10.1371/journal.pone.0149411
209. Horellou P, Wang M, Keo V, Chretien P, Serguera C, Waters P, et al. Increased interleukin-6 correlates with myelin oligodendrocyte glycoprotein antibodies in pediatric monophasic demyelinating diseases and multiple sclerosis. *J Neuroimmunol* (2015) 289:1–7. doi:10.1016/j.jneuroim.2015.10.002
210. Lalive PH, Menge T, Delarasse C, Della Gaspera B, Pham-Dinh D, Villoslada P, et al. Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. *Proc Natl Acad Sci U S A* (2006) 103(7):2280–5. doi:10.1073/pnas.0510672103

211. Honnorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. *Arch Neurol* (2001) 58(2):225–30. doi:10.1001/archneur.58.2.225
212. Malter MP, Helmstaedter C, Urbach H, Vincent A, Bien CG. Antibodies to glutamic acid decarboxylase define a form of limbic encephalitis. *Ann Neurol* (2010) 67(4):470–8. doi:10.1002/ana.21917
213. Peltola J, Kulmala P, Isojarvi J, Saiz A, Latvala K, Palmio J, et al. Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. *Neurology* (2000) 55(1):46–50. doi:10.1212/WNL.55.1.46
214. Solimena M, Folli F, Denis-Donini S, Comi GC, Pozza G, De Camilli P, et al. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N Engl J Med* (1988) 318(16):1012–20. doi:10.1056/NEJM198804213181602
215. Dinkel K, Meinck HM, Jury KM, Karges W, Richter W. Inhibition of gamma-aminobutyric acid synthesis by glutamic acid decarboxylase autoantibodies in stiff-man syndrome. *Ann Neurol* (1998) 44(2):194–201. doi:10.1002/ana.410440209
216. Jarius S, Stich O, Speck J, Rasiah C, Wildemann B, Meinck HM, et al. Qualitative and quantitative evidence of anti-glutamic acid decarboxylase-specific intrathecal antibody synthesis in patients with stiff person syndrome. *J Neuroimmunol* (2010) 229(1–2):219–24. doi:10.1016/j.jneuroim.2010.07.019
217. Levy LM, Levy-Reis I, Fujii M, Dalakas MC. Brain gamma-aminobutyric acid changes in stiff-person syndrome. *Arch Neurol* (2005) 62(6):970–4. doi:10.1001/archneur.62.6.970
218. Vincent A. Stiff, twitchy or wobbly: are GAD antibodies pathogenic? *Brain* (2008) 131(Pt 10):2536–7. doi:10.1093/brain/awn221
219. Warren JD, Scott G, Blumbergs PC, Thompson PD. Pathological evidence of encephalomyelitis in the stiff man syndrome with anti-GAD antibodies. *J Clin Neurosci* (2002) 9(3):328–9. doi:10.1054/jocn.2001.1014
220. Lohmann T, Hawa M, Leslie RD, Lane R, Picard J, Londei M. Immune reactivity to glutamic acid decarboxylase 65 in stiffman syndrome and type 1 diabetes mellitus. *Lancet* (2000) 356(9223):31–5. doi:10.1016/S0140-6736(00)02431-4
221. Lohmann T, Londei M, Hawa M, Leslie RD. Humoral and cellular autoimmune responses in stiff person syndrome. *Ann N Y Acad Sci* (2003) 998:215–22. doi:10.1196/annals.1254.024
222. Raju R, Foote J, Banga JP, Hall TR, Padoa CJ, Dalakas MC, et al. Analysis of GAD65 autoantibodies in stiff-person syndrome patients. *J Immunol* (2005) 175(11):7755–62. doi:10.4049/jimmunol.175.11.7755
223. Sinmaz N, Nguyen T, Tea F, Dale RC, Brilot F. Mapping autoantigen epitopes: molecular insights into autoantibody-associated disorders of the nervous system. *J Neuroinflammation* (2016) 13(1):219. doi:10.1186/s12974-016-0678-4
224. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* (2008) 7(12):1091–8. doi:10.1016/S1474-4422(08)70224-2
225. Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias Lecture. *Neurology* (2016) 87(23):2471–82. doi:10.1212/WNL.0000000000003414
226. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* (2010) 30(17):5866–75. doi:10.1523/JNEUROSCI.0167-10.2010
227. Mikasova L, De Rossi P, Bouchet D, Georges F, Rogemond V, Didelot A, et al. Disrupted surface cross-talk between NMDA and ephrin-B2 receptors in anti-NMDA encephalitis. *Brain* (2012) 135(Pt 5):1606–21. doi:10.1093/brain/aws092
228. Planaguma J, Leypoldt F, Mannara F, Gutierrez-Cuesta J, Martin-Garcia E, Aguilar E, et al. Human *N*-methyl-D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* (2015) 138(Pt 1):94–109. doi:10.1093/brain/awu310
229. Irani SR, Bera K, Waters P, Zuliani L, Maxwell S, Zandi MS, et al. *N*-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* (2010) 133(Pt 6):1655–67. doi:10.1093/brain/awq113
230. Camdessanche JP, Streichenberger N, Cavillon G, Rogemond V, Jousserand G, Honnorat J, et al. Brain immunohistopathological study in a patient with anti-NMDAR encephalitis. *Eur J Neurol* (2011) 18(6):929–31. doi:10.1111/j.1468-1331.2010.03180.x
231. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. *Neurology* (2011) 77(6):589–93. doi:10.1212/WNL.0b013e318228c136
232. Tuzun E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J. Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. *Acta Neuropathol* (2009) 118(6):737–43. doi:10.1007/s00401-009-0582-4
233. Bacher P, Schink C, Teutschbein J, Kniemeyer O, Assenmacher M, Brakhage AA, et al. Antigen-reactive T cell enrichment for direct, high-resolution analysis of the human naive and memory Th cell repertoire. *J Immunol* (2013) 190(8):3967–76. doi:10.4049/jimmunol.1202221
234. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, et al. Phenotypic analysis of antigen-specific T lymphocytes. *Science* (1996) 274(5284):94–6. doi:10.1126/science.274.5284.94
235. Davis MM, Boniface JJ, Reich Z, Lyons D, Hampl J, Arden B, et al. Ligand recognition by alpha beta T cell receptors. *Annu Rev Immunol* (1998) 16:523–44. doi:10.1146/annurev.immunol.16.1.523
236. Bacher P, Scheffold A. Flow-cytometric analysis of rare antigen-specific T cells. *Cytometry A* (2013) 83(8):692–701. doi:10.1002/cyto.a.22317
237. Bill JR, Kotzin BL. Use of soluble MHC class II/peptide multimers to detect antigen-specific T cells in human disease. *Arthritis Res* (2002) 4(4):261–5. doi:10.1186/ar417
238. Davis MM, Altman JD, Newell EW. Interrogating the repertoire: broadening the scope of peptide-MHC multimer analysis. *Nat Rev Immunol* (2011) 11(8):551–8. doi:10.1038/nri3020
239. Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat Med* (1999) 5(6):677–85. doi:10.1038/9525
240. Laughlin EM, Miller JD, James E, Fillos D, Ibegbu CC, Mittler RS, et al. Antigen-specific CD4+ T cells recognize epitopes of protective antigen following vaccination with an anthrax vaccine. *Infect Immun* (2007) 75(4):1852–60. doi:10.1128/IAI.01814-06
241. Caruso A, Licenziati S, Corulli M, Canaris AD, De Francesco MA, Fiorentini S, et al. Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. *Cytometry* (1997) 27(1):71–6. doi:10.1002/(SICI)1097-0320(19970101)27:13.0.CO;2-O
242. Frentsch M, Arbach O, Kirchhoff D, Moewes B, Worm M, Rothe M, et al. Direct access to CD4+ T cells specific for defined antigens according to CD154 expression. *Nat Med* (2005) 11(10):1118–24. doi:10.1038/nm1292
243. Testi R, D'Ambrosio D, De Maria R, Santoni A. The CD69 receptor: a multipurpose cell-surface trigger for hematopoietic cells. *Immunol Today* (1994) 15(10):479–83. doi:10.1016/0167-5699(94)90193-7
244. Wolfl M, Kuball J, Ho WY, Nguyen H, Manley TJ, Bleakley M, et al. Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8+ T cells responding to antigen without requiring knowledge of epitope specificities. *Blood* (2007) 110(1):201–10. doi:10.1182/blood-2006-11-056168
245. Zaunders JJ, Munier ML, Seddiki N, Pett S, Ip S, Bailey M, et al. High levels of human antigen-specific CD4+ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40). *J Immunol* (2009) 183(4):2827–36. doi:10.4049/jimmunol.0803548
246. Madhavan H. Simple laboratory methods to measure cell proliferation using DNA synthesis property. *J Stem Cells Regen Med* (2007) 3(1):12–4.
247. Mannering SI, Morris JS, Jensen KP, Purcell AW, Honeyman MC, van Endert PM, et al. A sensitive method for detecting proliferation of rare autoantigen-specific human T cells. *J Immunol Methods* (2003) 283(1–2):173–83. doi:10.1016/j.jim.2003.09.004
248. De Clerck LS, Bridts CH, Mertens AM, Moens MM, Stevens WJ. Use of fluorescent dyes in the determination of adherence of human leucocytes to endothelial cells and the effect of fluorochromes on cellular function. *J Immunol Methods* (1994) 172(1):115–24. doi:10.1016/0022-1759(94)90384-0
249. Mobs C, Schmidt T. Research techniques made simple: monitoring of T-cell subsets using the ELISPOT assay. *J Invest Dermatol* (2016) 136(6):e55–9. doi:10.1016/j.jid.2016.04.009

250. Mease PJ, McInnes IB, Kirkham B, Kavanaugh A, Rahman P, van der Heijde D, et al. Secukinumab inhibition of interleukin-17A in patients with psoriatic arthritis. *N Engl J Med* (2015) 373(14):1329–39. doi:10.1056/NEJMoa1412679

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 23 March 2017
doi: 10.3389/fimmu.2017.00336

# Adaptive Immunity Is the Key to the Understanding of Autoimmune and Paraneoplastic Inflammatory Central Nervous System Disorders

*Robert Weissert**

*Department of Neurology, Neuroimmunology, University of Regensburg, Regensburg, Germany*

There are common aspects and mechanisms between different types of autoimmune diseases such as multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSDs), and autoimmune encephalitis (AE) as well as paraneoplastic inflammatory disorders of the central nervous system. To our present knowledge, depending on the disease, T and B cells as well as antibodies contribute to various aspects of the pathogenesis. Possibly the events leading to the breaking of tolerance between the different diseases are of great similarity and so far, only partially understood. Beside endogenous factors (genetics, genomics, epigenetics, malignancy) also exogenous factors (vitamin D, sun light exposure, smoking, gut microbiome, viral infections) contribute to susceptibility in such diseases. What differs between these disorders are the target molecules of the immune attack. For T cells, these target molecules are presented on major histocompatibility complex (MHC) molecules as MHC-bound ligands. B cells have an important role by amplifying the immune response of T cells by capturing antigen with their surface immunoglobulin and presenting it to T cells. Antibodies secreted by plasma cells that have differentiated from B cells are highly structure specific and can have important effector functions leading to functional impairment or/and lesion evolvement. In MS, the target molecules are mainly myelin- and neuron/axon-derived proteins; in NMOSD, mainly aquaporin-4 expressed on astrocytes; and in AE, various proteins that are expressed by neurons and axons.

**Keywords: T cell, B cell, major histocompatibility complex, human leukocyte antigen, multiple sclerosis, neuromyelitis optica spectrum disorders, autoimmune encephalitis, paraneoplastic disease**

OPEN ACCESS

***Edited by:***
*Björn Tackenberg, Philipps University of Marburg, Germany*

***Reviewed by:***
*Anne Kathrin Mausberg, Essen University Hospital, Germany*
*Pavan Bhargava, Johns Hopkins School of Medicine, USA*

***Correspondence:**
*Robert Weissert*
*robert.weissert@ukr.de,*
*robert.weissert@googlemail.com*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *22 January 2017*
***Accepted:*** *08 March 2017*
***Published:*** *23 March 2017*

***Citation:***
*Weissert R (2017) Adaptive Immunity Is the Key to the Understanding of Autoimmune and Paraneoplastic Inflammatory Central Nervous System Disorders. Front. Immunol. 8:336. doi: 10.3389/fimmu.2017.00336*

## INTRODUCTION

Various autoimmune and paraneoplastic disorders of the central nervous system (CNS) share many immunological similarities. In these disorders, immunologic tolerance to self-antigens is broken (1). This failure can be on the T cell as well as on the B cell side or on both sides. The reasons why tolerance is broken in autoimmune diseases are multiple and can differ from paraneoplastic diseases (2). In autoimmune disease, the initial trigger that leads to breaking of tolerance is not as well understood (3). Possibly viral, bacterial and fungal antigens that share antigenic properties with self-antigens can result in activation of T or/and B cells that also recognize self-antigens in the CNS (4). Another possibility could be that in certain autoimmune-prone individuals compared

with non-autoimmune-prone individuals, the T and B cell repertoires contain higher quantities of cells with a high avidity for self-antigens that can be activated and can gain access to the CNS in which they find relevant target structures. Such a scenario has been underscored in rodent models of CNS autoimmune diseases (5). In paraneoplastic diseases of the CNS, epitopes from a neoplasm are exposed on antigen-presenting cells to T cells which subsequently also recognize an epitope of similar structural appearance in the CNS (6).

Importantly, the T and B cell epitopes can differ depending on antigen processing in autoimmune disease and possibly also paraneoplastic disease (7, 8). In many autoimmune and paraneoplastic diseases of the CNS, the B cell response is much better characterized as compared with the T cell response (1, 9). T cell help is required for differentiation of B cells into plasma cells and affinity maturation of antibodies (10, 11). There are also B cells which do not require T cell help in autoimmunity, but these do not seem to be of major importance in CNS autoimmunity and CNS paraneoplastic diseases as far as one knows to date (12–14).

## ENDOGENOUS FACTORS

Endogenous factors that contribute to the induction of autoimmunity or paraneoplastic diseases are multiple. First, genetics is of paramount importance. Most autoimmune diseases are complex genetic diseases (15). This means that certain allelic variants of genes predispose to autoimmunity. There are also few examples of autoimmune diseases in which single mutated genes predispose to autoimmunity (16). For CNS-directed autoimmune diseases, no confirmed single genes with mutations have been discovered so far. Much work has been done in elucidating genes that contribute to the complex genetic etiologies (17). Most probably also in CNS immune-directed disorders with paraneoplastic origins, complex genetics are of importance. RNA expression levels have been shown to be altered in autoimmune and paraneoplastic diseases of the CNS (18–20). Tissue with altered RNA expression levels as compared with healthy tissue might predispose to autoimmunity and paraneoplastic diseases (21). There is increased understanding that epigenetics is very crucial in susceptibility to autoimmunity and paraneoplastic disorders (22, 23). Much will be learned in the next years regarding epigenetic regulation of immunity and autoimmunity.

## EXOGENOUS FACTORS

Much has been discovered regarding exogenous factors that affect autoimmune diseases of the CNS. These exogenous factors cooperate with endogenous factors in susceptibility to autoimmune diseases of the CNS (24). Low vitamin D levels as well as low sun light exposure have been shown to contribute to susceptibility to multiple sclerosis (MS) also independently of other factors (25–27). So far, the influence of vitamin D and sun light exposure has not been defined to the same degree for neuromyelitis optica spectrum disorders (NMOSDs) and autoimmune encephalitis (AE) (28). Smoking has a negative influence on MS (29, 30). This influence is controlled to some degree by human leukocyte antigen (HLA) genes underscoring that HLA-presented autoantigens are possibly modified and promote a more vigorous autoimmune response (29). It has been shown that in MS there is a change of the gut microbiome (31, 32). Also in NMOSD, changes in the gut microbiome have been observed with overrepresentation of *Clostridium perfringens* (33). In experimental autoimmune encephalomyelitis (EAE), it has been experimentally proven that the gut microbiome contributes to disease susceptibility (34). So far, in most types of diseases it is not well defined what specific bacteria of the gut microbiome drive autoimmune disease. It has been shown that Epstein–Barr virus (EBV) infection has an influence on MS susceptibility (35, 36). The influence is mainly mediated in childhood and most likely affects the T cell repertoire. Even though a direct role of EBV infection in MS lesion development was claimed, this could not be confirmed (37). Also, salt intake has been shown to influence EAE susceptibility (38). So far, it is not clear if levels of salt intake are influencing susceptibility or disease course in MS (39). The elucidation of the influence of nutritional factors in various autoimmune diseases of the CNS is presently investigated in more detail. Regarding paraneoplastic diseases, no such influence has been elucidated so far.

## MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)/HLA HAPLOTYPES

Most autoimmune diseases are associated with certain MHC/HLA haplotypes (40). Such associations also exist for some paraneoplastic diseases such as paraneoplastic pemphigus (41). So far, influences of HLA haplotypes on paraneoplastic diseases have not been investigated in much detail. The reason for the haplotype preferences of specific autoimmune diseases is not known.

The most likely scenario for influences of HLA haplotypes on autoimmune diseases indicates that during early tolerance development certain HLA haplotypes select for a T cell repertoire that can be self-biased to certain autoantigens and certain organs (42–44). In the emergence of tolerance, there is selection of a broad range of T cell receptors (TCRs) on various self-antigens. In a first step, only T cells are selected that recognize self MHC-peptide complexes (45–47). In the next step, T cells with TCRs with a too high affinity for such complexes are deleted from the repertoire (48, 49). MHC displayed peptide repertoires influence positive and negative selection (50). Based on the expressed HLA haplotypes, the predetermined T cell repertoire differs in individuals (51, 52). The TCR repertoire has a bias depending on the HLA haplotype in avidity for certain self-antigens (53, 54).

In MHC congenic rat strains, we have shown that there is an autoantigen preference that can result, depending on the expressed MHC alleles, in disease susceptibility or protection from certain diseases (5, 55). Interestingly with increasing complexity of the disease driving autoantigen, the MHC haplotype-dependent effects alleviate (56, 57). Also, we have shown that the amount of autoantigen that leads to disease induction can differ between different MHC haplotypes (5, 58). This means that in one MHC haplotype minute amounts of antigens are sufficient to induce severe disease, while in others much higher amounts would be necessary. These findings underscore the influence of the antigenic load in context with genetic factors. It has been shown that depending on the expressed MHC haplotype,

the cytokine preference of the selected T cell repertoire differs (44, 59). Recently in an experimental model of rheumatoid arthritis (RA), it has been shown that MHC alleles that drive disease are associated with a T helper cell type 1 (Th1) response with secretion of interferon-gamma (IFN-γ) (60). By contrast, protective MHC alleles promoted an interleukin-17 T helper (Th17) cell response. Such a predetermination of cytokine responses to disease-inducing factors is potentially also shaped early in tolerance development and can also contribute to the finding that certain HLA haplotypes predispose to certain autoimmune diseases while others protect from disease.

## NEOANTIGENS

Tolerance can be broken by presentation of neoantigens on MHC molecules to T cells recognizing antigens that share structural similarities to self-molecules (61). Recently, it has been shown that neoantigens for presentation on MHC I molecules can be generated by fusion of different fragments of degraded proteins during antigen processing (62). In addition, endogenous neoantigens could evolve by mutation or translational defects. So far, the experimental data that such novel antigens could play a role in the induction or maintenance of autoimmune disease of the CNS are still lacking but an interesting avenue of future research efforts.

Posttranslational modifications of antigens can also lead to induction of autoimmunity (63). This has been shown for RA in which citrullinated epitopes have been shown to be disease inducing (64, 65). Also for MS, a role for citrullination has been proposed but so far there is no proof for the relevance in the experimental or human setting (66, 67). Possibly transpeptidation could be of importance as has been shown in a model of diabetes (68). Changes in glycosylation can affect induction of autoimmunity (69). Also, other types of posttranslational modifications could be of great relevance but have not been investigated in much detail regarding CNS autoimmunity or CNS paraneoplastic diseases. We have shown that even the conformational state of an autoantigen can have different consequences on disease induction capacity (8). Therefore, different conformations of an antigen can be seen be the immune system in a "neoantigenic" fashion and lead to autoimmunity (70).

## SPECIFIC DISEASES

### Multiple Sclerosis

In MS, the target of the autoimmune response, which seems to be predominantly T cell driven, is mainly directed against proteins of the myelin sheath which is produced by oligodendrocytes (1) (**Table 1**). Myelin basic protein (MBP) is thought to be the major autoantigen which is involved (71, 72). Many researchers have addressed this topic and found additional myelin proteins that can be the target of the autoimmune response (1). There are strong indications that the humoral immune response is important as well (73). Nevertheless, the exact autoantigens driving this B cell response are not known to date in detail. Myelin oligodendrocyte glycoprotein (MOG) is a model antigen which has been shown to be of major importance driving the B cells response in rodent and primate models (74). This protein, which is expressed on the outer surface of the myelin sheath, seems to be involved in children but not to the same extent in older people with MS in the immune pathogenesis of MS (75). Especially young children with MS with an age under 10 years have a robust anti-MOG antibody response. This finding underscores that potentially early in life immunological events are taking place that predispose to development of MS later in life. CNS lesions of MS patients show antibody-dependent complement destruction underscoring the importance of the antibody response in MS (73). Moreover, proteins expressed on neurons and axons have also been discussed to be targets of the immune response in patients with MS based on work in EAE (76). Recently, in patients with MS, we have shown that peptides can be eluted from MHC molecules from CNS tissue that are recognized by T cells secreting IFN-γ (Th1) (72, 77). Importantly, the increased immune reactivity against such peptides is observed in patients with active MS, i.e., in patients with MS who have an acute bout- or/and contrast-enhancing lesions in the CNS indicating active inflammation. This finding underscores that the adaptive immune response against CNS-derived autoantigens is of significance in MS. Importantly, the T cell reactivity is directed not only against MBP but additional autoantigens and differs between individuals.

### Neuromyelitis Optica Spectrum Disorders

In NMOSD, it has been demonstrated that the immune response is targeting aquaporin-4 (AQP4), a water channel protein on astrocytes (127) (**Table 1**). Certain cases of NMOSD are associated with an immune response against MOG (128). In both types, antibody-dependent tissue destruction is of major importance (96). The role of T cells is presently analyzed in more detail (94). In rodent models, it has been delineated that direct injection of anti-AQP4 antibody in the CNS can lead to severe pathology without the presence of T cells (129). Also, antibody-dependent destruction of tissue by complement seems to be of paramount importance and dependent on the antigen conformation and the presence of antibodies (130). It is not excluded that also additional target molecules will be discovered, which are associated with seronegative forms of NMOSD in the future.

### Autoimmune Encephalitis

There are a high number of diseases in which the autoimmune response is directed against neuronal antigens (1) (**Table 1**). The target molecules can be localized intracellular or extracellular (9, 98). Some of the intracellular antigens are nuclear proteins. Most of the diseases in which the immune response is directed against intracellular neuronal targets are of paraneoplastic origin. Mainly, CD8+ T cells, which are MHC I restricted, are involved in the immune pathogenesis of these types of AE (131). In affected patients, most important is the search for the underlying neoplasm and its treatment. In addition, immunotherapy is meaningful (132).

In diseases in which the target structures are exposed extracellular as membrane proteins, more diseases are of autoimmune origin and less paraneoplastic. A prototype is the anti-*N*-methyl-D-aspartate receptor (NMDA) receptor encephalitis in which the NMDA receptor is the target molecule of the immune response

**TABLE 1 | Human diseases and autoantigens, main cellular expression, and cellular compartment of expression as well as involved immune responses as presently known.**

| Disease | (Auto)antigen | Target cell | Main cellular localization | Established role of | | |
|---|---|---|---|---|---|---|
| | | | | T cells | Antibodies | Complement |
| MS | Actin | U | C, CS, ES | + (72) | ND | ND |
| MS | Alpha-synuclein | N | U, not P | + (72) | ND | ND |
| MS | CNPase, 2′,3′-cyclic-nucleotide 3′-phosphodiesterase | O, N | ES, CS, N | + (78) | + (79) | ND |
| MS | GFAP, glial fibrillary acidic protein | A | C, CS | + (72) | – (80) | ND |
| MS | Glutamate dehydrogenase | U | M | + (72) | ND | ND |
| MS | MAG, myelin-associated glycoprotein | O | PM | + (81) | + (81) | ND |
| MS | MBP, myelin basic protein | O | PM, C, N | + (72, 82, 83) | + (79) | ND |
| MS | MOBP, myelin-associated oligodendrocyte basic protein | O | PM | + (84) | + (79) | ND |
| MS | MOG, myelin oligodendrocyte glycoprotein | O | PM | + (85, 86) | + (87) | + (88) |
| MS | Neurofilament-3 | N | CS, C, N | + (72) | + (89) | ND |
| MS | PLP, proteolipid protein | O | PM | + (90) | + (79) | ND |
| MS | S100β, S100 calcium-binding protein B | A | E, C, N | + (91) | – (80) | ND |
| MS | Survivin | U | C, CS, N | + (72) | ND | ND |
| MS | Transaldolase | U | E, C, N | + (92) | + (93) | ND |
| NMOSD | AQP4, aquaporin-4 | A | PM | + (94) | + (95) | + (96) |
| NMOSD | MOG, myelin oligodendrocyte glycoprotein | O | PM | ND | + (97) | + (88) |
| AE | AK5, adenylate kinase 5 | N | C, ES | ND | + (98) | ND |
| AE | AMPAR, glutamate ionotropic receptor AMPA type | N | PM | ND | + (99) | ND |
| AE | Amphiphysin | N | PM, C, CS, GA | ND | + (100) | ND |
| AE | CASPR2, contactin associated protein-like 2 | N | PM, E, GA | ND | + (101) | ND |
| AE | CRMP5, dihydropyrimidinase-like 5 | N | C | ND | + (102) | ND |
| AE | DNER (Tr), delta-/notch-like EGF repeat containing | N | PM, E | ND | + (103) | ND |
| AE | Dopamine receptor D2 | N | PM, C | ND | + (104) | ND |
| AE | DPPX, dipeptidyl peptidase | N | ES, L, PM, V | ND | + (105) | ND |
| AE | GABAaR, gamma-aminobutyric acid type A receptor | N | PM | ND | + (106) | ND |
| AE | GABAbR, gamma-aminobutyric acid type B receptor | N | PM | ND | + (107) | ND |
| AE | GAD65, glutamate decarboxylase 2 | N | C, PM | + (108) | + (109) | – (108) |
| AE | GlyR, glycine receptor | N | PM | ND | + (110) | ND |
| AE | Hu, ELAV-like RNA-binding protein 4 | N | C, N | + (108, 111) | + (112) | – (108) |
| AE | IgLON5, IgLON family member 5 | N | ES, PM | ND | + (113) | ND |
| AE | LGI1, leucine-rich glioma-inactivated 1 | N | ES, PM | + (108) | + (114, 115) | + (108) |
| AE | Ma1, paraneoplastic Ma antigen 1 | N | N | ND | + (116) | ND |
| AE | Ma2, paraneoplastic Ma antigen 2 | N | N | + (108) | + (117) | – (108) |
| AE | mGluR1, glutamate metabotropic receptor 1 | N | PM, C | ND | + (118) | ND |
| AE | mGluR5, glutamate metabotropic receptor 5 | N | ES, PM | ND | + (119) | ND |
| AE | Neurexin-3a | N | PM | ND | + (120) | ND |
| AE | NMDAR, glutamate ionotropic receptor NMDA type | N | PM | + (108) | + (121) | – (108) |
| AE | P/Q type VGCC, calcium voltage-gated channel | N | PM | ND | + (122) | ND |
| AE | Ri, NOVA alternative splicing regulator 1 | N | N | ND | + (123) | ND |
| AE | Yo, cerebellar degeneration-related protein 2 | N | N | ND | + (124, 125) | ND |
| AE | Zic4, Zic family member 4 | N | N | ND | + (126) | ND |

*A, astrocytes; AE, autoimmune encephalitis; C, cytosol; CS, cytoskeleton; E, endosome; ES, extracellular space; GA, Golgi apparatus; L, lysosome; M, mitochondria; MS, multiple sclerosis; N, neurons; N, nucleus; ND, not determined; NMOSD, neuromyelitis optica spectrum disorder; O, oligodendrocytes; P, peroxisome; PM, plasma membrane; U, ubiquitous; V, vacuoles; +, positive findings; –, negative findings.*

(121). It has been shown that antibodies are most important in this type of diseases and that these antibodies can lead to alteration of cellular function with consequences on behavior (104, 133, 134) or tissue destruction by complement (108). There is a requirement for these antibodies to access the CNS in order to be of disease relevance (135). Recently, a strong influence of a specific HLA haplotype has been shown in anti-LGI1 encephalitis in Koreans (136). So far, the role of T cells has not been assessed in detail in these illnesses but deserves much more attention in future efforts. These diseases are treated by immunotherapy (132, 137). In cases in which the origin is paraneoplastic, the neoplasm needs to be treated in addition to immunotherapy.

## Paraneoplastic Disease of the CNS

Why is there such a preference of paraneoplastic CNS disorders for neuronal antigens? Why are there no or only few cases of paraneoplastic MS or NMOSD? Possibly the answer lies in the antigen repertoires that are preferentially displayed by neoplasms. In NMOSD, cases with paraneoplastic origin have been reported (138, 139). In addition, certain brain neoplasms might result in paraneoplastic cases of MS even though such cases have not been interpreted as paraneoplastic diseases so far but rather in the opposite way that the molecular changes in the MS lesion have led to development of the neoplasms (140–142). As discussed in the section regarding HLA haplotypes, the

density of the presented disease-inducing antigen expressed by the neoplasms is potentially an important factor that can lead to paraneoplastic disease. Therefore, a higher density of the presented autoantigen would possibly more likely lead to disease induction.

## THERAPEUTIC CONSIDERATIONS

Since the adaptive immune response is of such great relevance in various immunologically mediated disorder of the CNS it is obvious that it should be targeted to halt and possibly cure autoimmune and paraneoplastic diseases of the CNS. Of course, in paraneoplastic diseases always the underlying malignancy should be treated by surgical, radiotherapeutic, and chemotherapeutic approaches, since the eradication of the malignancy with presence of the antigens that drive the disease can possibly lead to an improvement of the paraneoplastic disease condition affecting the CNS. It has been proposed that immunotherapeutic approaches should mainly affect the humoral immune response, since the cellular immune response by CD8+ T cells is of great importance in tumor rejection (143). This aspect needs to be investigated in more depth.

In autoimmune diseases of CNS depending on the dominance of the T or/and B cell response, a rational treatment approach should be used. In most diseases in which autoantibodies are of major importance, the depletion of B cells by rituximab a monoclonal antibody (mAb) that targets CD20 has been shown to be of great efficacy (144–146). This is the case for AE with membrane molecules as target antigens of the immune response (132, 137, 146). In NMOSD, depletion of B cells is well established as a very efficacious treatment approach (145). Also in MS, depletion of B cells has been shown to be of great therapeutic efficacy (144). This has been underscored by recent data with ocrelizumab a novel human mAb also targeting CD20 (147, 148). Since B cells are very potent professional antigen-presenting cells, the depletion of such cells leads also to reduced presentation of antigens to T cells (1, 149, 150). This reduction of antigen presentation in individuals that have been treated with B cell depleting agents is potentially one of the most important immunotherapeutic effects of such a therapeutic approach.

In MS, it has been demonstrated that decreasing numbers of T cells that enter the CNS can result in reduction of contrast enhancing lesions, numbers of new lesions, and improvement of clinical disease score as well (3). Also, modulating T cell responses regarding the way how these cells react in expression of certain immune mediators can affect disease.

The combined depletion of T and B cells by alemtuzumab by targeting CD52 has been shown to be very efficacious in MS (151–153). In a retrospective case series in NMOSD, this approach failed to be effective (154). The reasons are not clear so far, but the authors recommend caution. The approach has not been used in AE so far. This restricted use is most likely because potential side effects are dreaded. Nevertheless, such a therapeutic approach embodies a great potential for cure in selected patient populations.

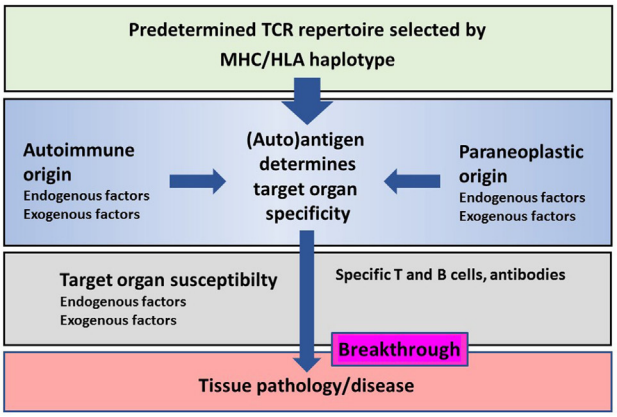



**FIGURE 1 | Schematic representation of the major histocompatibility complex (MHC)/human leukocyte antigen (HLA) haplotype and additional factors on disease breakthrough in autoimmune or/and paraneoplastic diseases.**

Another approach to affect autoimmune and paraneoplastic diseases would be the blockade of the terminal phase of inflammation which is partially mediated by antibodies and that leads to tissue destruction. In this aspect, the use of eculizumab, a mAb depleting the complement factor C5 holds great promise. There are trials ongoing in NMOSD that investigate the efficacy of eculizumab in disease arrest. Initial observations are very promising (155). The use of complement inhibitors could be of great therapeutic potential in MS as well as in certain types of AE in which complement is strongly involved in pathophysiology. So far, the use of eculizumab is restricted due to limited clinical development efforts because of its high cost.

## CONCLUSION

There is a great similarity in immune mechanisms of different autoimmune and paraneoplastic diseases of the CNS (**Figure 1**). The adaptive immunity seems to be the main driver of selected organ pathology in autoimmune and paraneoplastic diseases. Specific HLA haplotypes are associated with different autoimmune and paraneoplastic autoimmune disorders. HLA haplotypes predispose for selection of certain autoantigens that drive such diseases. The phenotype of autoimmune and paraneoplastic diseases of the CNS differs depending on the antigens that drive the immune responses. Neoantigens can possibly contribute to the development of these disorders. The pivotal role of the adaptive immunity in autoimmune and paraneoplastic diseases of the CNS allows directed immune interventions to modulate T and B cell responses.

## AUTHOR CONTRIBUTIONS

RW outlined the subject of the review; searched for, analyzed, and interpreted the literature; wrote the manuscript, and agreed to be accountable for all aspects of the work.

## REFERENCES


1. Riedhammer C, Weissert R. Antigen presentation, autoantigens, and immune regulation in multiple sclerosis and other autoimmune diseases. *Front Immunol* (2015) 6:322. doi:10.3389/fimmu.2015.00322
2. Maverakis E, Goodarzi H, Wehrli LN, Ono Y, Garcia MS. The etiology of paraneoplastic autoimmunity. *Clin Rev Allerg Immunol* (2012) 42(2):135–44. doi:10.1007/s12016-010-8248-5
3. Weissert R. The immune pathogenesis of multiple sclerosis. *J Neuroimmune Pharmacol* (2013) 8(4):857–66. doi:10.1007/s11481-013-9467-3
4. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* (1995) 80(5):695–705. doi:10.1016/0092-8674(95)90348-8
5. Weissert R, Wallstrom E, Storch MK, Stefferl A, Lorentzen J, Lassmann H, et al. MHC haplotype-dependent regulation of MOG-induced EAE in rats. *J Clin Invest* (1998) 102(6):1265–73. doi:10.1172/JCI3022
6. Posner JB. Pathogenesis of central nervous system paraneoplastic syndromes. *Rev Neurol (Paris)* (1992) 148(6–7):502–12.
7. Weissert R, de Graaf KL, Storch MK, Barth S, Linington C, Lassmann H, et al. MHC class II-regulated central nervous system autoaggression and T cell responses in peripheral lymphoid tissues are dissociated in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. *J Immunol* (2001) 166(12):7588–99. doi:10.4049/jimmunol.166.12.7588
8. de Graaf KL, Albert M, Weissert R. Autoantigen conformation influences both B- and T-cell responses and encephalitogenicity. *J Biol Chem* (2012) 287(21):17206–13. doi:10.1074/jbc.M111.304246
9. Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: the 2016 Cotzias Lecture. *Neurology* (2016) 87(23):2471–82. doi:10.1212/WNL.0000000000003414
10. Allen CD, Okada T, Tang HL, Cyster JG. Imaging of germinal center selection events during affinity maturation. *Science* (2007) 315(5811):528–31. doi:10.1126/science.1136736
11. Weinstein JS, Hernandez SG, Craft J. T cells that promote B-Cell maturation in systemic autoimmunity. *Immunol Rev* (2012) 247(1):160–71. doi:10.1111/j.1600-065X.2012.01122.x
12. Shlomchik MJ. Activating systemic autoimmunity: B's, T's, and tolls. *Curr Opin Immunol* (2009) 21(6):626–33. doi:10.1016/j.coi.2009.08.005
13. Claes N, Fraussen J, Stinissen P, Hupperts R, Somers V. B cells are multifunctional players in multiple sclerosis pathogenesis: insights from therapeutic interventions. *Front Immunol* (2015) 6:642. doi:10.3389/fimmu.2015.00642
14. von Budingen HC, Bar-Or A, Zamvil SS. B cells in multiple sclerosis: connecting the dots. *Curr Opin Immunol* (2011) 23(6):713–20. doi:10.1016/j.coi.2011.09.003
15. Richard-Miceli C, Criswell LA. Emerging patterns of genetic overlap across autoimmune disorders. *Genome Med* (2012) 4(1):6. doi:10.1186/gm305
16. Tadaki H, Saitsu H, Nishimura-Tadaki A, Imagawa T, Kikuchi M, Hara R, et al. De novo 19q13.42 duplications involving NLRP gene cluster in a patient with systemic-onset juvenile idiopathic arthritis. *J Hum Genet* (2011) 56(5):343–7. doi:10.1038/jhg.2011.16
17. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* (2011) 476(7359):214–9. doi:10.1038/nature10251
18. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* (2002) 8(5):500–8. doi:10.1038/nm0502-500
19. Herrmann MM, Barth S, Greve B, Schumann KM, Bartels A, Weissert R. Identification of gene expression patterns crucially involved in experimental autoimmune encephalomyelitis and multiple sclerosis. *Dis Model Mech* (2016) 9(10):1211–20. doi:10.1242/dmm.025536
20. Ince-Dunn G, Okano HJ, Jensen KB, Park WY, Zhong R, Ule J, et al. Neuronal Elav-like (Hu) proteins regulate RNA splicing and abundance to control glutamate levels and neuronal excitability. *Neuron* (2012) 75(6):1067–80. doi:10.1016/j.neuron.2012.07.009
21. Hathaway CK, Chang AS, Grant R, Kim HS, Madden VJ, Bagnell CR Jr, et al. High Elmo1 expression aggravates and low Elmo1 expression prevents diabetic nephropathy. *Proc Natl Acad Sci U S A* (2016) 113(8):2218–22. doi:10.1073/pnas.1600511113
22. Huynh JL, Garg P, Thin TH, Yoo S, Dutta R, Trapp BD, et al. Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. *Nat Neurosci* (2014) 17(1):121–30. doi:10.1038/nn.3588
23. Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, et al. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* (2014) 511(7508):246–50. doi:10.1038/nature13305
24. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* (2017) 13(1):25–36. doi:10.1038/nrneurol.2016.187
25. Becklund BR, Severson KS, Vang SV, DeLuca HF. UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. *Proc Natl Acad Sci U S A* (2010) 107(14):6418–23. doi:10.1073/pnas.1001119107
26. Rhead B, Baarnhielm M, Gianfrancesco M, Mok A, Shao X, Quach H, et al. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. *Neurol Genet* (2016) 2(5):e97. doi:10.1212/NXG.0000000000000097
27. Islam T, Gauderman WJ, Cozen W, Mack TM. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. *Neurology* (2007) 69(4):381–8. doi:10.1212/01.wnl.0000268266.50850.48
28. Min JH, Waters P, Vincent A, Cho HJ, Joo BE, Woo SY, et al. Low levels of vitamin D in neuromyelitis optica spectrum disorder: association with disease disability. *PLoS One* (2014) 9(9):e107274. doi:10.1371/journal.pone.0107274
29. Hedstrom AK, Sundqvist E, Baarnhielm M, Nordin N, Hillert J, Kockum I, et al. Smoking and two human leukocyte antigen genes interact to increase the risk for multiple sclerosis. *Brain* (2011) 134(Pt 3):653–64. doi:10.1093/brain/awq371
30. Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology* (2009) 73(9):696–701. doi:10.1212/WNL.0b013e3181b59c40
31. Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Soldan MM, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* (2016) 6:28484. doi:10.1038/srep28484
32. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. *Nat Commun* (2016) 7:12015. doi:10.1038/ncomms12015
33. Cree BA, Spencer CM, Varrin-Doyer M, Baranzini SE, Zamvil SS. Gut microbiome analysis in neuromyelitis optica reveals overabundance of *Clostridium perfringens*. *Ann Neurol* (2016) 80(3):443–7. doi:10.1002/ana.24718
34. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* (2011) 479(7374):538–41. doi:10.1038/nature10554
35. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernan MA, Olek MJ, et al. Epstein–Barr virus antibodies and risk of multiple sclerosis: a prospective study. *JAMA* (2001) 286(24):3083–8. doi:10.1001/jama.286.24.3083
36. Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the Epstein–Barr virus and risk of multiple sclerosis. *Ann Neurol* (2010) 67(6):824–30. doi:10.1002/ana.21978
37. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, et al. Epstein–Barr virus infection is not a characteristic feature of multiple sclerosis brain. *Brain* (2009) 132(Pt 12):3318–28. doi:10.1093/brain/awp200
38. Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature* (2013) 496(7446):518–22. doi:10.1038/nature11868
39. Nourbakhsh B, Graves J, Casper TC, Lulu S, Waldman A, Belman A, et al. Dietary salt intake and time to relapse in paediatric multiple sclerosis. *J Neurol Neurosurg Psychiatry* (2016) 87(12):1350–3. doi:10.1136/jnnp-2016-313410

40. Klein J, Sato A. The HLA system. First of two parts. *N Engl J Med* (2000) 343(10):702–9. doi:10.1056/NEJM200009073431006
41. Martel P, Loiseau P, Joly P, Busson M, Lepage V, Mouquet H, et al. Paraneoplastic pemphigus is associated with the DRB1*03 allele. *J Autoimmun* (2003) 20(1):91–5. doi:10.1016/S0896-8411(02)00092-6
42. Segurado OG, Arnaiz-Villena A, Wank R, Schendel DJ. The multifactorial nature of MHC-linked susceptibility to insulin-dependent diabetes. *Autoimmunity* (1993) 15(1):85–9. doi:10.3109/08916939309004844
43. Abraham RS, Kudva YC, Wilson SB, Strominger JL, David CS. Co-expression of HLA DR3 and DQ8 results in the development of spontaneous insulitis and loss of tolerance to GAD65 in transgenic mice. *Diabetes* (2000) 49(4):548–54. doi:10.2337/diabetes.49.4.548
44. Murray JS, Madri J, Tite J, Carding SR, Bottomly K. MHC control of CD4+ T cell subset activation. *J Exp Med* (1989) 170(6):2135–40. doi:10.1084/jem.170.6.2135
45. MacDonald HR, Lees RK, Schneider R, Zinkernagel RM, Hengartner H. Positive selection of CD4+ thymocytes controlled by MHC class II gene products. *Nature* (1988) 336(6198):471–3. doi:10.1038/336471a0
46. Kappler J, Kotzin B, Herron L, Gelfand EW, Bigler RD, Boylston A, et al. V beta-specific stimulation of human T cells by staphylococcal toxins. *Science* (1989) 244(4906):811–3. doi:10.1126/science.2524876
47. Sha WC, Nelson CA, Newberry RD, Kranz DM, Russell JH, Loh DY. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* (1988) 336(6194):73–6. doi:10.1038/336073a0
48. Hengartner H, Odermatt B, Schneider R, Schreyer M, Walle G, MacDonald HR, et al. Deletion of self-reactive T cells before entry into the thymus medulla. *Nature* (1988) 336(6197):388–90. doi:10.1038/336388a0
49. Gascoigne NR, Rybakin V, Acuto O, Brzostek J. TCR signal strength and T cell development. *Annu Rev Cell Dev Biol* (2016) 32:327–48. doi:10.1146/annurev-cellbio-111315-125324
50. Kincaid EZ, Murata S, Tanaka K, Rock KL. Specialized proteasome subunits have an essential role in the thymic selection of CD8(+) T cells. *Nat Immunol* (2016) 17(8):938–45. doi:10.1038/ni.3480
51. Gulwani-Akolkar B, Posnett DN, Janson CH, Grunewald J, Wigzell H, Akolkar P, et al. T cell receptor V-segment frequencies in peripheral blood T cells correlate with human leukocyte antigen type. *J Exp Med* (1991) 174(5):1139–46. doi:10.1084/jem.174.5.1139
52. Akolkar PN, Gulwani-Akolkar B, Pergolizzi R, Bigler RD, Silver J. Influence of HLA genes on T cell receptor V segment frequencies and expression levels in peripheral blood lymphocytes. *J Immunol* (1993) 150(7):2761–73.
53. Moudgil KD, Sercarz EE. Dominant determinants in hen eggwhite lysozyme correspond to the cryptic determinants within its self-homologue, mouse lysozyme: implications in shaping of the T cell repertoire and autoimmunity. *J Exp Med* (1993) 178(6):2131–8. doi:10.1084/jem.178.6.2131
54. Morgan DJ, Nugent CT, Raveney BJ, Sherman LA. In a transgenic model of spontaneous autoimmune diabetes, expression of a protective class II MHC molecule results in thymic deletion of diabetogenic CD8+ T cells. *J Immunol* (2004) 172(2):1000–8. doi:10.4049/jimmunol.172.2.1000
55. Weissert R, editor. Experimental autoimmune encephalomyelitis. *Models, Disease Biology and Experimental Therapy*. Rijeka: In Tech (2012). p. 1–19.
56. Gaertner S, de Graaf KL, Olsson T, Weissert R. Immunogenicity of Torpedo acetylcholine receptor in the context of different rat MHC class II haplotypes and non-MHC genomes. *Immunogenetics* (2004) 56(1):61–4. doi:10.1007/s00251-004-0656-3
57. Gaertner S, de Graaf KL, Wienhold W, Wiesmuller KH, Melms A, Weissert R. Lack of pathogenicity of immunodominant T and B cell determinants of the nicotinic acetylcholine receptor epsilon-chain. *J Neuroimmunol* (2004) 152(1–2):44–56. doi:10.1016/j.jneuroim.2004.03.019
58. de Graaf KL, Berne GP, Herrmann MM, Hansson GK, Olsson T, Weissert R. CDR3 sequence preference of TCRBV8S2+ T cells within the CNS does not reflect single amino acid dependent avidity expansion. *J Neuroimmunol* (2005) 166(1–2):47–54. doi:10.1016/j.jneuroim.2005.05.004
59. Mangalam AK, Taneja V, David CS. HLA class II molecules influence susceptibility versus protection in inflammatory diseases by determining the cytokine profile. *J Immunol* (2013) 190(2):513–8. doi:10.4049/jimmunol.1201891
60. Tuncel J, Haag S, Holmdahl R. MHC class II alleles associated with Th1 rather than Th17 type immunity drive the onset of early arthritis in a rat model of rheumatoid arthritis. *Eur J Immunol* (2016). doi:10.1002/eji.201646760
61. Lambe T, Leung JC, Bouriez-Jones T, Silver K, Makinen K, Crockford TL, et al. CD4 T cell-dependent autoimmunity against a melanocyte neoantigen induces spontaneous vitiligo and depends upon Fas-Fas ligand interactions. *J Immunol* (2006) 177(5):3055–62. doi:10.4049/jimmunol.177.5.3055
62. Liepe J, Marino F, Sidney J, Jeko A, Bunting DE, Sette A, et al. A large fraction of HLA class I ligands are proteasome-generated spliced peptides. *Science* (2016) 354(6310):354–8. doi:10.1126/science.aaf4384
63. Anderton SM. Post-translational modifications of self antigens: implications for autoimmunity. *Curr Opin Immunol* (2004) 16(6):753–8. doi:10.1016/j.coi.2004.09.001
64. Burkhardt H, Sehnert B, Bockermann R, Engstrom A, Kalden JR, Holmdahl R. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. *Eur J Immunol* (2005) 35(5):1643–52. doi:10.1002/eji.200526000
65. Uysal H, Bockermann R, Nandakumar KS, Sehnert B, Bajtner E, Engstrom A, et al. Structure and pathogenicity of antibodies specific for citrullinated collagen type II in experimental arthritis. *J Exp Med* (2009) 206(2):449–62. doi:10.1084/jem.20081862
66. Carrillo-Vico A, Leech MD, Anderton SM. Contribution of myelin autoantigen citrullination to T cell autoaggression in the central nervous system. *J Immunol* (2010) 184(6):2839–46. doi:10.4049/jimmunol.0903639
67. Bradford CM, Ramos I, Cross AK, Haddock G, McQuaid S, Nicholas AP, et al. Localisation of citrullinated proteins in normal appearing white matter and lesions in the central nervous system in multiple sclerosis. *J Neuroimmunol* (2014) 273(1–2):85–95. doi:10.1016/j.jneuroim.2014.05.007
68. Jin N, Wang Y, Crawford F, White J, Marrack P, Dai S, et al. N-terminal additions to the WE14 peptide of chromogranin A create strong autoantigen agonists in type 1 diabetes. *Proc Natl Acad Sci U S A* (2015) 112(43):13318–23. doi:10.1073/pnas.1517862112
69. Backlund J, Treschow A, Bockermann R, Holm B, Holm L, Issazadeh-Navikas S, et al. Glycosylation of type II collagen is of major importance for T cell tolerance and pathology in collagen-induced arthritis. *Eur J Immunol* (2002) 32(12):3776–84. doi:10.1002/1521-4141(200212)32:12<3776::AID-IMMU3776>3.0.CO;2-A
70. Levine SM, Raben N, Xie D, Askin FB, Tuder R, Mullins M, et al. Novel conformation of histidyl-transfer RNA synthetase in the lung: the target tissue in Jo-1 autoantibody-associated myositis. *Arthritis Rheum* (2007) 56(8):2729–39. doi:10.1002/art.22790
71. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, et al. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med* (2000) 6(10):1167–75. doi:10.1038/80516
72. Riedhammer C, Halbritter D, Weissert R. Increased immune reactivity to central nervous system-derived naturally presented peptides in patients with active multiple sclerosis. *J Allergy Clin Immunol* (2017) 139(2):694–6.e7. doi:10.1016/j.jaci.2016.08.015
73. Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. *Ann Neurol* (1998) 43(4):465–71. doi:10.1002/ana.410430409
74. Lassmann H, Bradl M. Multiple sclerosis: experimental models and reality. *Acta Neuropathol* (2017) 133(2):223–44. doi:10.1007/s00401-016-1631-4
75. McLaughlin KA, Chitnis T, Newcombe J, Franz B, Kennedy J, McArdel S, et al. Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis. *J Immunol* (2009) 183(6):4067–76. doi:10.4049/jimmunol.0801888
76. Krishnamoorthy G, Saxena A, Mars LT, Domingues HS, Mentele R, Ben-Nun A, et al. Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of multiple sclerosis. *Nat Med* (2009) 15(6):626–32. doi:10.1038/nm.1975
77. Fissolo N, Haag S, de Graaf KL, Drews O, Stevanovic S, Rammensee HG, et al. Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol Cell Proteomics* (2009) 8(9):2090–101. doi:10.1074/mcp.M900001-MCP200
78. Muraro PA, Kalbus M, Afshar G, McFarland HF, Martin R. T cell response to 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) in multiple

sclerosis patients. *J Neuroimmunol* (2002) 130(1–2):233–42. doi:10.1016/S0165-5728(02)00229-1
79. Quintana FJ, Farez MF, Viglietta V, Iglesias AH, Merbl Y, Izquierdo G, et al. Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. *Proc Natl Acad Sci U S A* (2008) 105(48):18889–94. doi:10.1073/pnas.0806310105
80. Terryberry JW, Thor G, Peter JB. Autoantibodies in neurodegenerative diseases: antigen-specific frequencies and intrathecal analysis. *Neurobiol Aging* (1998) 19(3):205–16. doi:10.1016/S0197-4580(98)00049-9
81. Andersson M, Yu M, Soderstrom M, Weerth S, Baig S, Solders G, et al. Multiple MAG peptides are recognized by circulating T and B lymphocytes in polyneuropathy and multiple sclerosis. *Eur J Neurol* (2002) 9(3):243–51. doi:10.1046/j.1468-1331.2002.00391.x
82. Bielekova B, Sung MH, Kadom N, Simon R, McFarland H, Martin R. Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. *J Immunol* (2004) 172(6):3893–904. doi:10.4049/jimmunol.172.6.3893
83. Olsson T, Sun J, Hillert J, Hojeberg B, Ekre HP, Andersson G, et al. Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. *Eur J Immunol* (1992) 22(4):1083–7. doi:10.1002/eji.1830220431
84. de Rosbo NK, Kaye JF, Eisenstein M, Mendel I, Hoeftberger R, Lassmann H, et al. The myelin-associated oligodendrocytic basic protein region MOBP15-36 encompasses the immunodominant major encephalitogenic epitope(s) for SJL/J mice and predicted epitope(s) for multiple sclerosis-associated HLA-DRB1*1501. *J Immunol* (2004) 173(2):1426–35. doi:10.4049/jimmunol.173.2.1426
85. Wallstrom E, Khademi M, Andersson M, Weissert R, Linington C, Olsson T. Increased reactivity to myelin oligodendrocyte glycoprotein peptides and epitope mapping in HLA DR2(15)+ multiple sclerosis. *Eur J Immunol* (1998) 28(10):3329–35. doi:10.1002/(SICI)1521-4141(199810)28:10<3329::AID-IMMU3329>3.3.CO;2-2
86. Weissert R, Kuhle J, de Graaf KL, Wienhold W, Herrmann MM, Muller C, et al. High immunogenicity of intracellular myelin oligodendrocyte glycoprotein epitopes. *J Immunol* (2002) 169(1):548–56. doi:10.4049/jimmunol.169.1.548
87. Probstel AK, Dornmair K, Bittner R, Sperl P, Jenne D, Magalhaes S, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. *Neurology* (2011) 77(6):580–8. doi:10.1212/WNL.0b013e318228c0b1
88. Johns TG, Bernard CC. Binding of complement component Clq to myelin oligodendrocyte glycoprotein: a novel mechanism for regulating CNS inflammation. *Mol Immunol* (1997) 34(1):33–8. doi:10.1016/S0161-5890(97)00005-9
89. Bartos A, Fialova L, Soukupova J, Kukal J, Malbohan I, Pit'ha J. Elevated intrathecal antibodies against the medium neurofilament subunit in multiple sclerosis. *J Neurol* (2007) 254(1):20–5. doi:10.1007/s00415-006-0185-0
90. Trotter JL, Hickey WF, van der Veen RC, Sulze L. Peripheral blood mononuclear cells from multiple sclerosis patients recognize myelin proteolipid protein and selected peptides. *J Neuroimmunol* (1991) 33(1):55–62. doi:10.1016/0165-5728(91)90034-5
91. Schmidt S, Linington C, Zipp F, Sotgiu S, de Waal Malefyt R, Wekerle H, et al. Multiple sclerosis: comparison of the human T-cell response to S100 beta and myelin basic protein reveals parallels to rat experimental autoimmune panencephalitis. *Brain* (1997) 120(Pt 8):1437–45. doi:10.1093/brain/120.8.1437
92. Banki K, Colombo E, Sia F, Halladay D, Mattson DH, Tatum AH, et al. Oligodendrocyte-specific expression and autoantigenicity of transaldolase in multiple sclerosis. *J Exp Med* (1994) 180(5):1649–63. doi:10.1084/jem.180.5.1649
93. Colombo E, Banki K, Tatum AH, Daucher J, Ferrante P, Murray RS, et al. Comparative analysis of antibody and cell-mediated autoimmunity to transaldolase and myelin basic protein in patients with multiple sclerosis. *J Clin Invest* (1997) 99(6):1238–50. doi:10.1172/JCI119281
94. Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, Nelson PA, Stroud RM, Cree BA, et al. Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter. *Ann Neurol* (2012) 72(1):53–64. doi:10.1002/ana.23651
95. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
96. Bruck W, Popescu B, Lucchinetti CF, Markovic-Plese S, Gold R, Thal DR, et al. Neuromyelitis optica lesions may inform multiple sclerosis heterogeneity debate. *Ann Neurol* (2012) 72(3):385–94. doi:10.1002/ana.23621
97. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, Takahashi T, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* (2014) 82(6):474–81. doi:10.1212/WNL.0000000000000101
98. Do LD, Chanson E, Desestret V, Joubert B, Ducray F, Brugiere S, et al. Characteristics in limbic encephalitis with anti-adenylate kinase 5 autoantibodies. *Neurology* (2017) 88(6):514–24. doi:10.1212/WNL.0000000000003586
99. Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol* (2009) 65(4):424–34. doi:10.1002/ana.21589
100. Antoine JC, Absi L, Honnorat J, Boulesteix JM, de Brouker T, Vial C, et al. Antiamphiphysin antibodies are associated with various paraneoplastic neurological syndromes and tumors. *Arch Neurol* (1999) 56(2):172–7. doi:10.1001/archneur.56.2.172
101. Lancaster E, Huijbers MG, Bar V, Boronat A, Wong A, Martinez-Hernandez E, et al. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. *Ann Neurol* (2011) 69(2):303–11. doi:10.1002/ana.22297
102. Monstad SE, Nostbakken JK, Vedeler CA. CRMP5 antibodies found in a patient with limbic encephalitis and myasthenia gravis. *J Neurol Neurosurg Psychiatry* (2009) 80(2):241–2. doi:10.1136/jnnp.2008.149336
103. Trotter JL, Hendin BA, Osterland CK. Cerebellar degeneration with Hodgkin disease. An immunological study. *Arch Neurol* (1976) 33(9):660–1. doi:10.1001/archneur.1976.00500090066014
104. Dale RC, Merheb V, Pillai S, Wang D, Cantrill L, Murphy TK, et al. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain* (2012) 135(Pt 11):3453–68. doi:10.1093/brain/aws256
105. Boronat A, Gelfand JM, Gresa-Arribas N, Jeong HY, Walsh M, Roberts K, et al. Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. *Ann Neurol* (2013) 73(1):120–8. doi:10.1002/ana.23756
106. Petit-Pedrol M, Armangue T, Peng X, Bataller L, Cellucci T, Davis R, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABAA receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. *Lancet Neurol* (2014) 13(3):276–86. doi:10.1016/S1474-4422(13)70299-0
107. Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, et al. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* (2010) 9(1):67–76. doi:10.1016/S1474-4422(09)70324-2
108. Bien CG, Vincent A, Barnett MH, Becker AJ, Blumcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain* (2012) 135(Pt 5):1622–38. doi:10.1093/brain/aws082
109. Kim J, Namchuk M, Bugawan T, Fu Q, Jaffe M, Shi Y, et al. Higher autoantibody levels and recognition of a linear NH2-terminal epitope in the autoantigen GAD65, distinguish stiff-man syndrome from insulin-dependent diabetes mellitus. *J Exp Med* (1994) 180(2):595–606. doi:10.1084/jem.180.2.595
110. Carvajal-Gonzalez A, Leite MI, Waters P, Woodhall M, Coutinho E, Balint B, et al. Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes. *Brain* (2014) 137(Pt 8):2178–92. doi:10.1093/brain/awu142
111. Tanaka M, Maruyama Y, Sugie M, Motizuki H, Kamakura K, Tanaka K. Cytotoxic T cell activity against peptides of Hu protein in anti-Hu syndrome. *J Neurol Sci* (2002) 201(1–2):9–12. doi:10.1016/S0022-510X(02)00157-0
112. Dalmau J, Graus F, Rosenblum MK, Posner JB. Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuronopathy. A clinical study of 71 patients. *Medicine* (1992) 71(2):59–72. doi:10.1097/00005792-199203000-00001

113. Sabater L, Gaig C, Gelpi E, Bataller L, Lewerenz J, Torres-Vega E, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. *Lancet Neurol* (2014) 13(6):575–86. doi:10.1016/S1474-4422(14)70051-1
114. Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* (2004) 127(Pt 3):701–12. doi:10.1093/brain/awh077
115. Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, et al. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. *Lancet Neurol* (2010) 9(8):776–85. doi:10.1016/S1474-4422(10)70137-X
116. Dalmau J, Gultekin SH, Voltz R, Hoard R, DesChamps T, Balmaceda C, et al. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. *Brain* (1999) 122(Pt 1):27–39. doi:10.1093/brain/122.1.27
117. Dalmau J, Graus F, Villarejo A, Posner JB, Blumenthal D, Thiessen B, et al. Clinical analysis of anti-Ma2-associated encephalitis. *Brain* (2004) 127(Pt 8):1831–44. doi:10.1093/brain/awh203
118. Sillevis Smitt P, Kinoshita A, De Leeuw B, Moll W, Coesmans M, Jaarsma D, et al. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *N Engl J Med* (2000) 342(1):21–7. doi:10.1056/NEJM200001063420104
119. Lancaster E, Martinez-Hernandez E, Titulaer MJ, Boulos M, Weaver S, Antoine JC, et al. Antibodies to metabotropic glutamate receptor 5 in the Ophelia syndrome. *Neurology* (2011) 77(18):1698–701. doi:10.1212/WNL.0b013e3182364a44
120. Gresa-Arribas N, Planaguma J, Petit-Pedrol M, Kawachi I, Katada S, Glaser CA, et al. Human neurexin-3alpha antibodies associate with encephalitis and alter synapse development. *Neurology* (2016) 86(24):2235–42. doi:10.1212/WNL.0000000000002775
121. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* (2007) 61(1):25–36. doi:10.1002/ana.21050
122. Hiasa Y, Kunishige M, Mitsui T, Kondo S, Kuriwaka R, Shigekiyo S, et al. Complicated paraneoplastic neurological syndromes: a report of two patients with small cell or non-small cell lung cancer. *Clin Neurol Neurosurg* (2003) 106(1):47–9. doi:10.1016/S0303-8467(03)00059-3
123. Luque FA, Furneaux HM, Ferziger R, Rosenblum MK, Wray SH, Schold SC Jr, et al. Anti-Ri: an antibody associated with paraneoplastic opsoclonus and breast cancer. *Ann Neurol* (1991) 29(3):241–51. doi:10.1002/ana.410290303
124. Greenlee JE, Brashear HR. Antibodies to cerebellar Purkinje cells in patients with paraneoplastic cerebellar degeneration and ovarian carcinoma. *Ann Neurol* (1983) 14(6):609–13. doi:10.1002/ana.410140603
125. Jaeckle KA, Graus F, Houghton A, Cardon-Cardo C, Nielsen SL, Posner JB. Autoimmune response of patients with paraneoplastic cerebellar degeneration to a Purkinje cell cytoplasmic protein antigen. *Ann Neurol* (1985) 18(5):592–600. doi:10.1002/ana.410180513
126. Bataller L, Wade DF, Graus F, Stacey HD, Rosenfeld MR, Dalmau J. Antibodies to Zic4 in paraneoplastic neurologic disorders and small-cell lung cancer. *Neurology* (2004) 62(5):778–82. doi:10.1212/01.WNL.0000113749.77217.01
127. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* (2005) 202(4):473–7. doi:10.1084/jem.20050304
128. Kitley J, Woodhall M, Waters P, Leite MI, Devenney E, Craig J, et al. Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. *Neurology* (2012) 79(12):1273–7. doi:10.1212/WNL.0b013e31826aac4e
129. Asavapanumas N, Ratelade J, Verkman AS. Unique neuromyelitis optica pathology produced in naive rats by intracerebral administration of NMO-IgG. *Acta Neuropathol* (2014) 127(4):539–51. doi:10.1007/s00401-013-1204-8
130. Phuan PW, Ratelade J, Rossi A, Tradtrantip L, Verkman AS. Complement-dependent cytotoxicity in neuromyelitis optica requires aquaporin-4 protein assembly in orthogonal arrays. *J Biol Chem* (2012) 287(17):13829–39. doi:10.1074/jbc.M112.344325
131. McKeon A, Pittock SJ. Paraneoplastic encephalomyelopathies: pathology and mechanisms. *Acta Neuropathol* (2011) 122(4):381–400. doi:10.1007/s00401-011-0876-1
132. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* (2016) 15(4):391–404. doi:10.1016/S1474-4422(15)00401-9
133. Planaguma J, Leypoldt F, Mannara F, Gutierrez-Cuesta J, Martin-Garcia E, Aguilar E, et al. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* (2015) 138(Pt 1):94–109. doi:10.1093/brain/awu310
134. Moscato EH, Peng X, Jain A, Parsons TD, Dalmau J, Balice-Gordon RJ. Acute mechanisms underlying antibody effects in anti-N-methyl-D-aspartate receptor encephalitis. *Ann Neurol* (2014) 76(1):108–19. doi:10.1002/ana.24195
135. Castillo-Gomez E, Kastner A, Steiner J, Schneider A, Hettling B, Poggi G, et al. The brain as immunoprecipitator of serum autoantibodies against N-methyl-D-aspartate receptor subunit NR1. *Ann Neurol* (2016) 79(1):144–51. doi:10.1002/ana.24545
136. Kim TJ, Lee ST, Moon J, Sunwoo JS, Byun JI, Lim JA, et al. Anti-LGI1 encephalitis is associated with unique HLA subtypes. *Ann Neurol* (2017) 81(2):183–92. doi:10.1002/ana.24860
137. Gastaldi M, Thouin A, Vincent A. Antibody-mediated autoimmune encephalopathies and immunotherapies. *Neurotherapeutics* (2016) 13(1):147–62. doi:10.1007/s13311-015-0410-6
138. De Santis G, Caniatti L, De Vito A, De Gennaro R, Granieri E, Tola MR. A possible paraneoplastic neuromyelitis optica associated with lung cancer. *Neurol Sci* (2009) 30(5):397–400. doi:10.1007/s10072-009-0112-0
139. Al-Harbi T, Al-Sarawi A, Binfalah M, Dermime S. Paraneoplastic neuromyelitis optica spectrum disorder associated with stomach carcinoid tumor. *Hematol Oncol Stem Cell Ther* (2014) 7(3):116–9. doi:10.1016/j.hemonc.2014.06.001
140. Reagan TJ, Freiman IS. Multiple cerebral gliomas in multiple sclerosis. *J Neurol Neurosurg Psychiatry* (1973) 36(4):523–8. doi:10.1136/jnnp.36.4.523
141. Currie S, Urich H. Concurrence of multiple sclerosis and glioma. *J Neurol Neurosurg Psychiatry* (1974) 37(5):598–605. doi:10.1136/jnnp.37.5.598
142. Paydarfar D, de la Monte SM. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 12-1997. A 50-year-old woman with multiple sclerosis and an enlarging frontal-lobe mass. *N Engl J Med* (1997) 336(16):1163–71. doi:10.1056/NEJM199704173361608
143. Blachere NE, Orange DE, Santomasso BD, Doerner J, Foo PK, Herre M, et al. T cells targeting a neuronal paraneoplastic antigen mediate tumor rejection and trigger CNS autoimmunity with humoral activation. *Eur J Immunol* (2014) 44(11):3240–51. doi:10.1002/eji.201444624
144. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* (2008) 358(7):676–88. doi:10.1056/NEJMoa0706383
145. Jarius S, Aboul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. *Brain* (2008) 131(Pt 11):3072–80. doi:10.1093/brain/awn240
146. Ishiura H, Matsuda S, Higashihara M, Hasegawa M, Hida A, Hanajima R, et al. Response of anti-NMDA receptor encephalitis without tumor to immunotherapy including rituximab. *Neurology* (2008) 71(23):1921–3. doi:10.1212/01.wnl.0000336648.43562.59
147. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med* (2017) 376(3):221–34. doi:10.1056/NEJMoa1601277
148. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N Engl J Med* (2017) 376(3):209–20. doi:10.1056/NEJMoa1606468
149. Molnarfi N, Schulze-Topphoff U, Weber MS, Patarroyo JC, Prod'homme T, Varrin-Doyer M, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* (2013) 210(13):2921–37. doi:10.1084/jem.20130699
150. Forsthuber TG, Shive CL, Wienhold W, de Graaf K, Spack EG, Sublett R, et al. T cell epitopes of human myelin oligodendrocyte glycoprotein identified in HLA-DR4 (DRB1*0401) transgenic mice are encephalitogenic and are

presented by human B cells. *J Immunol* (2001) 167(12):7119–25. doi:10.4049/jimmunol.167.12.7119

151. Investigators CT, Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med* (2008) 359(17):1786–801. doi:10.1056/NEJMoa0802670
152. Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP, et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* (2012) 380(9856):1819–28. doi:10.1016/S0140-6736(12)61769-3
153. Coles AJ, Twyman CL, Arnold DL, Cohen JA, Confavreux C, Fox EJ, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* (2012) 380(9856):1829–39. doi:10.1016/S0140-6736(12)61768-1
154. Azzopardi L, Cox AL, McCarthy CL, Jones JL, Coles AJ. Alemtuzumab use in neuromyelitis optica spectrum disorders: a brief case series. *J Neurol* (2016) 263(1):25–9. doi:10.1007/s00415-015-7925-y
155. Pittock SJ, Lennon VA, McKeon A, Mandrekar J, Weinshenker BG, Lucchinetti CF, et al. Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. *Lancet Neurol* (2013) 12(6):554–62. doi:10.1016/S1474-4422(13)70076-0

**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 18 April 2017
doi: 10.3389/fimmu.2017.00425

# The Multiple Sclerosis (MS) Genetic Risk Factors Indicate both Acquired and Innate Immune Cell Subsets Contribute to MS Pathogenesis and Identify Novel Therapeutic Opportunities

*Grant P. Parnell and David R. Booth**

*Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, University of Sydney, Westmead, NSW, Australia*

OPEN ACCESS

**Edited by:**
*Robert Weissert, University of Regensburg, Germany*

**Reviewed by:**
*Jun-ichi Kira, Kyushu University, Japan*
*Anna Fogdell-Hahn, Karolinska Institutet, Sweden*

***Correspondence:**
*David R. Booth*
*david.booth@sydney.edu.au*

**Specialty section:**
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

**Received:** *30 January 2017*
**Accepted:** *27 March 2017*
**Published:** *18 April 2017*

**Citation:**
*Parnell GP and Booth DR (2017) The Multiple Sclerosis (MS) Genetic Risk Factors Indicate both Acquired and Innate Immune Cell Subsets Contribute to MS Pathogenesis and Identify Novel Therapeutic Opportunities. Front. Immunol. 8:425. doi: 10.3389/fimmu.2017.00425*

Multiple sclerosis (MS) is known to be a partially heritable autoimmune disease. The risk of developing MS increases from typically 1 in 1,000 in the normal population to 1 in 4 or so for identical twins where one twin is affected. Much of this heritability is now explained and is due almost entirely to genes affecting the immune response. The largest and first identified genetic risk factor is an allele from the MHC class II HLA-DRB1 gene, HLA-DRB1*15:01, which increases risk about threefold. The HLA-DRB1 gene is expressed in antigen-presenting cells, and its protein functions in presenting particular types of antigen to CD4 T cells. This discovery supported the development of the first successful immunomodulatory therapies: glatiramer acetate, which mimics the antigen presentation process, and interferon beta, which targets CD4 T cell activation. Over 200 genetic risk variants, all single nucleotide polymorphisms (SNPs), have now been described. The SNPs are located within, or close to, genes expressed predominantly in acquired and innate immune cell subsets, indicating that both contribute to MS pathogenesis. The risk alleles indicate variation in the regulation of gene expression, rather than protein variation, underpins genetic susceptibility. In this review, we discuss how the expression and function of the risk genes, as well as the effect on these of the risk SNPs, indicate specific acquired immune cell processes that are the target of current successful therapies, and also point to novel therapeutic approaches.

**Keywords: multiple sclerosis, genes, vitamin D, Epstein–Barr virus, immune tolerance**

## INTRODUCTION

Multiple sclerosis (MS) is an autoinflammatory disease in which the oligodendrocytes are destroyed and neuronal function is progressively lost (1). Risk is greatly increased with increasing relatedness to someone who has MS. The genetic basis for this increase in risk has been largely determined by genome-wide association studies (GWAS), which indicates that common variation in the regulatory regions of immune genes largely drives variation in susceptibility to MS (2). Over 200 genes have

now been identified (3) and, of these, 110 non-MHC genetic loci have been detailed (2) and 13 MHC loci identified (4).

The heritability of a disease is the proportion of total variance in disease risk that is explained by genetic variance (5). A recent meta-analysis of twins concluded that genetic variation may be responsible for about half of the individual differences in susceptibility to MS (6), further supported by a large national study (7). This is similar for many common autoimmune diseases (8). The c.200 risk genes identified from GWAS, using genotyping from more than 100,000 cases and controls, is estimated to account for toward half of MS heritability (3).

These MS risk variants are expressed in a wide range of immune cell types (9), indicating that multiple immune cell types contribute to the immune dysregulation that alters susceptibility to MS. This is consistent with successful MS therapies targeting highly different immune cell types: a monoclonal antibody to CD20 is B cell specific (10); to CD25 is T/NK/MP cell specific (11); and others are pan immune [CD52 (12); CD49d (13)]. Of these therapeutic targets, both CD25 and the ligand for CD49d (VCAM1) are MS risk genes. This suggests that many therapeutics could be employed targeting the other 200+ MS risk variants, and that novel therapies targeting specific immune cell types and states identified by the risk genes should be possible. Other therapies alter migration to the central nervous system (CNS) by retaining multiple immune cell types in secondary lymphoid organs [S1PR agonists; (14)], or by altering immune cell physiological state [Tekfidera; (15); Teriflunomide; (16)]. All therapies fail in a proportion of patients, with resulting CNS damage and significant economic cost (17).

Environment also contributes to MS susceptibility. These include latitude of childhood, age at Epstein–Barr virus (EBV) infection, salt, and smoking (18). Each of the environmental risk factors can be manipulated or their effects modified through understanding how they affect immune response. Similarly, although an individual's genetic risk factors cannot yet be altered, their consequences on immune response can be manipulated through an understanding of how they affect MS risk, including how they interact with environmental risk factors.

In this review, we discuss how the known MS genetic risk factors may affect the acquired immune response, and how this points to novel therapeutic strategies. Unlike Mendelian diseases, single genetic effects are small in MS. However, they point to processes and cell subsets necessary for MS pathogenesis, and as mentioned above, targeting single genes tagged by their albeit small risk factors has proved highly effective in reducing disease. Defining the gene, and the cell subset and state it in turn tags, should be beneficial in improving therapy. This is particularly likely given the recent discoveries that many key immune cell populations are highly heritable (19–21).

Our approach in this review is to consider the immune cell subsets in which the MS risk genes are most highly expressed, as these are the most likely to underpin the risk genes' contribution to pathogenesis. The risk genes typically control differentiation and state of the immune cells and act through their function on particular cellular processes in these cells. These cellular genetic effects are modulated through the effect of the risk single nucleotide polymorphisms (SNPs). The context and consequences in which these SNPs exert their effects are often difficult to determine, especially for pleiotropic molecules. The SNP effect may be within the major immune cell type in which the risk gene functions, or be due to the balance of many risk gene effects on the immune response, and may be highly context specific, such as on infection at a particular tissue location and time.

A consilient approach, where the more genetic factors that point to a particular process as being pathogenic the more likely it is to be true, can be facilitated by considering how these genetic risk factors might function to mediate environmental risk. Consequently, in this review, we have focused on how the effects of the genes on the acquired immune cell state, function, and differentiation might be shared with the effects of environmental risk factors; and how this might contribute to the development of novel therapeutic approaches.

## THE FIRST RISK GENE, HLA-DRB1*15:01

The first MS risk gene variant, HLA-DRB1*15:01, increases risk by threefold (22). The others confer an increased risk of less than 1.2-fold. Although the increased risk of the gene variant does not necessarily indicate the relative importance of the risk gene in pathogenesis, it does indicate the relative effect of the genetic variant haplotype. The HLA-DRB1 variant 15:01 therefore, affects pathogenesis more than the other known risk variants, but the relative importance of other risk genes to pathogenesis is unknown. HLA-DRB1 has limited and well-known roles: it is expressed in antigen-presenting cells, and it presents peptides to CD4 T cells in the process of their regulation, both activation and inactivation. Consequently, we can conclude CD4 T cells are important in pathogenesis, and the peptide presented by DRB1*15:01, and/or the regulation of this variant, are highly important to pathogenesis. From protein prediction studies, these peptides are hydrophobic. Many myelin sheath proteins have highly hydrophobic peptides. Unlike other DRB1 alleles, the structure of the 15:01-binding groove has been shown to present both myelin and EBV peptides to T cells (23). Molecular mimicry (where non-myelin peptides select the same T cell clones) and epitope spreading (where related T cell clones are selected by peptides similar to the one initiating an immune response) could contribute to T cell activation through DRB1*15:01. A pathogen such as EBV could drive such immune activation. Tschochner et al. (24) have recently demonstrated a number of potential cross-reactive targets of major myelin antigens and EBV proteins. Antigen presentation occurs in the thymus, where the CD4 T cell repertoire is restricted, and where regulatory T cells are selected. It also occurs in the secondary lymphoid organs, notably, the draining cervical lymph nodes, which present antigen from the CNS to naïve T cells, resulting in their activation or inactivation (tolerance, cell death, and regulatory T cells). CD4 T cells will also recognize antigen in the CNS, including that presented by antigen presenting cells (APCs) there. Finally, the B cell arm of the immune response will be activated by CD4 Th2 cells in germinal centers of the secondary lymphoid organs, elsewhere, and in the tissues. B cells can also act as antigen-presenting cells. The *HLA-DRB1*15:01* allele is associated with phenotypic features of the disease including female sex and presence of cerebrospinal

fluid-restricted oligoclonal bands, and the HLA genetic burden has now been associated with several MRI traits (25). Other HLA *DRB1* loci are also independently associated with MS risk (4).

Several MHC class I alleles have also been identified, all with protective effects (4). These present antigen to CD8 T cells, or interact with natural killer (NK) cells. CD8 T cells responding to antigens presented by these protective alleles may be more effectively activated to kill EBV infected cells. Or, these alleles could facilitate superior regulation by NK cells of target cells, such as infected cells, or autoreactive T cells. These processes might be augmented by therapies or vaccines. It is interesting that although HLA A/B/C genes might be expected to be expressed in a wide range of cell types, of immune cells *ex vivo*, we found that their expression was highest in NK cells (**Figure 1**). Higher expression of these risk alleles in NK cells might indicate their importance in regulating the NK cells response, at least in the homeostatic conditions tested, and suggests an interesting area for further investigation.

Risk gene effects, both MHC and non-MHC, appear to be additive, with little evidence for interactive effects (2, 4). An exception is two interactions between pairs of class II alleles: HLA-DQA1*01:01–HLA-DRB1*15:01 and HLA-DQB1*03:01–HLA-DQB1*03:02 (4). These authors found no evidence for interactions between classical HLA alleles and non-HLA risk-associated variants. Genetic load, including just the MHC arm (27) can predict altered MS susceptibility and phenotypes, but have little diagnostic utility, since even the few people with unusually high MS genetic loads are unlikely to develop MS.

## RISK GENE IMMUNE CELL PHENOTYPES

The possibility that MS risk genes define immune cell population differences that contribute to pathogenesis has been supported by the recent finding that many immune cell populations are highly heritable (19–21). Differences in differentiation of immune cells between individuals are controlled by genetic variation, e.g., of transcription factors, cytokine receptors, and signaling molecules. MS risk genes such as TYK2 (28, 29), IL2Ra (30), EOMES (31), NFKB1 (32), and ZMIZ1 (33) are associated with immune cell population differences in MS. Increased understanding of immune dysregulation in MS, the nature of control of this dysregulation, and identification of points for therapeutic intervention will come from further investigation of how risk genes and alleles affect heritable immune cell populations. For example, Hartmann et al. found a significantly increased propensity of $T_H$ cells from individuals carrying *IL2RA* risk alleles to secrete GM-CSF, and that such cells were more abundant in MS (30). GM-CSF neutralization trials are also currently ongoing in MS patients (NCT01517282). GM-CSF B cells (34) may yet prove to be driven by MS risk factors. These populations are therapeutic targets, and agents exist that can modulate each of them: novel agents may prove to be more specific to the MS-promoting aspect of these populations. The latter needs to be determined by further study.

Brodin et al. (20) also demonstrated that the difference in response of immune cell subsets to the cytokines IL7 and IL2 could be highly heritable. These genes and/or their receptors are MS genetic risk factors (35). Genes such as these defining heritable immune cell subsets and responses are good candidates for drug targets and biomarkers. It is notable that the percentage of lymphocytes in peripheral blood-expressing CD4 is highly heritable and predicts response to fingolimod (36).

## CELL SUBSET AND STATE

Antigen-presenting cells and CD4 T cells are implicated by the HLA-DRB1 genetic association; CD8 and NK cells by the MHC class I associations. These and other cell types are also implicated by the non-HLA MS risk genes (**Figure 2**) (4, 9, 35). The general immune cell pattern of cells expressing risk factor genes indicate that a wide range of subsets and contexts are likely to contribute to disease development. For example, pathogenic studies have implicated autoreactive CD4, CD8, and B cells; and the cells that regulate them including regulatory T (many types), regulatory B (many types), the cells that regulate all of these [collectively the

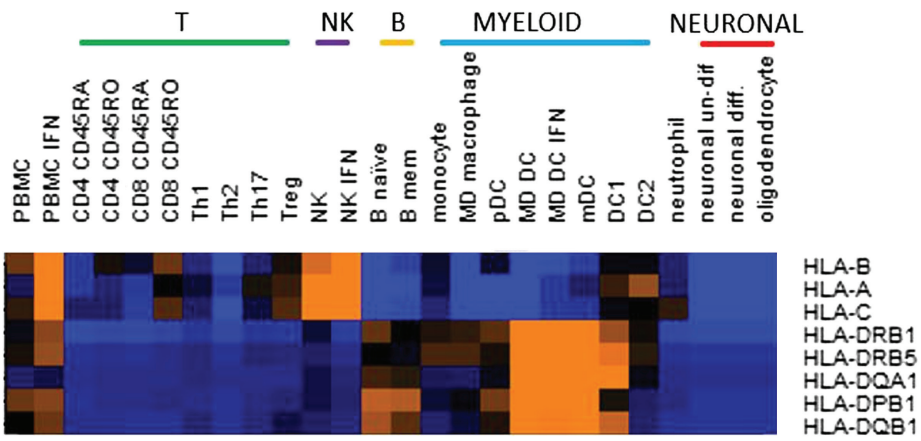



**FIGURE 1 | Relative expression in cell subsets of the multiple sclerosis risk genes located in the major histocompatibility complex region**. Expression was by RNASeq and color on heatmap indicates relative expression level: orange is high, blue is low. MHC risk genes are identified in Ref. (4). Cell subsets were *ex vivo* or *in vitro* generated as previously described (26).

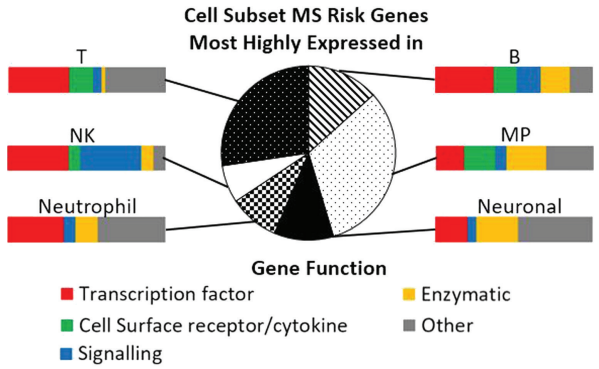



**FIGURE 2 | Cell subset expression and function of multiple sclerosis (MS) risk genes**. Pie chart indicates the proportion of MS risk genes that are most highly expressed in each of the cell subsets, as measured by RNAseq. Details of cell isolation and RNAseq workflow are described in Ref. (26). Bar charts illustrate the function of the genes most highly expressed in each of the cell subsets. MP, mononuclear phagocytic; NK, natural killer. Gene function was categorized using the Entrez gene summary and gene ontology annotations for each gene.

**TABLE 1 | Gene ontology (GO) pathways overrepresented in the lists of multiple sclerosis risk genes most highly expressed in T, B, mononuclear phagocytic (MP), and natural killer (NK) cells, respectively.**

| Cell type | GO pathways overrepresented (*p*-value) |
|---|---|
| T | Differentiation (1E-18), activation (1E-17), cell adhesion (1E-16) |
| B | Chemokine secretion (1E-9), activation (1E-8), migration (1E-8) |
| MP | Activation (1E-8), adhesion (1E-7), differentiation (1E-6) |
| NK | Activation (1E-8), STAT cascade (1E-7) |

*p-Values were calculated using GeneGo MetaCore.*

mononuclear phagocytic (MP) cells]; as well as NK cells (37). Each individual may have dysregulation in a particular combination of these, explaining at least some of the variation in therapeutic response. The risk genes dominantly expressed in these major subsets include transcription factors, chemokines, receptors, and intracellular enzymes and signaling factors (**Figure 2**), which would be expected to not only control the proportion, differentiation, and state of these subsets (**Table 1**) but also provide therapeutic targets for manipulating them. By identifying how these risk variants affect gene function, off-the-shelf therapeutic agents already available for the risk genes could be potentially repurposed to MS, especially for cell surface receptors.

As well as those immune populations described in Section "Risk Gene Immune Cell Phenotypes," some progress has been made in determining the effects of particular SNPs and genes. The IL2R risk variants control splicing and affect the proportion of GM-CSF-producing T helper cells (30). The IL7R risk variant affects splicing, affects T cell repopulation after lymphopenia in transplants (38) and HIV (39), and could also affect T regulatory cell proportion and function (40). Several other genes support dysregulation of Tregs as pathogenic, notably the transcription factors BACH2, IKZF1, and IKZF3.

Immune effects of risk genes are likely more easy to identify than the specific effects of SNPS, but the latter has indicated why therapies are useful in some autoimmune diseases, but exacerbate them in others. For example, the MS CD40 risk variant of SNP rs6074022 is protective for rheumatoid arthritis, and monoclonal antibodies to CD40 are effective for the latter, but exacerbate disease in the former (41). Similarly, opposite associations of the TNFRSF1A allele rs1800693 correspond to opposite outcomes of anti-TNF in treatment for MS and other autoimmune diseases (42).

Although it is been widely considered that the genetic associations with MS provide a roadmap to understand pathogenesis and devise new therapeutic strategies, most of that map has not been exploited for the investigation of how the genes and their variants affect pathogenesis.

## INTERACTION WITH ENVIRONMENTAL RISK FACTORS: THERAPEUTIC IMPLICATIONS

Multiple sclerosis risk is greatly reduced in low latitudes, and this has been attributed to the effects of ultraviolet light, including the production of vitamin D, leading to clinical applications (43). An immediate benefit of the identification of the first 110 MS risk genes was the compelling evidence that vitamin D regulation contributes to MS susceptibility: the risk gene CYP24A1 inactivates vitamin D, and the risk variant increases this inactivation in dendritic cells (26). The vitamin D-activating gene CYP27B1 is also implicated, and its risk variant is less active in tolerizing dendritic cells. Other vitamin D-regulated genes in MP cells have been identified (44), and there is an overrepresentation of genes associated with MS and other latitude-dependent autoimmune diseases. These data have supported the now widespread use of vitamin D supplementation in clinical management of MS. By indicating the immune cells mediating the vitamin D protection, and the genes regulated by vitamin D in these immune cells, this genetic finding also provides a tool to dissect out the molecular architecture of vitamin D control of tolerance. Vitamin D supplementation can be implemented in a variety of ways. Optimal and improved methods to stimulate tolerance, and to monitor vitamin D sufficiency by immune cell readout, may result from further investigations of the regulation of the vitamin D pathway.

Common disease-associated variants affect expression of pathogen-sensing genes in dendritic cells, highlighting the importance of infection on driving functional variation that also affects disease (45). There is a strong evidence that the EBV is necessary for development of MS (46), with compelling evidence for causation (47). However, most of those infected never develop MS. Some MS risk genes would be expected to indicate differences in the immune response to EBV that contribute to MS risk. The paradox of the high expression of the T cell-activating gene CD40 being protective in MS may be explained by its role in EBV proliferation in B cells (9). The risk gene TRAF3 also functions on this signaling pathway. The risk genes ZMIZ1 (33) and EOMES (48) are associated with levels of antibodies to EBV. Given that the recent dramatic success of B cell therapies may be due to their effect on EBV-infected B cells (49), identifying host genetic factors affecting immune control of EBV and MS risk may prove particularly promising areas of investigation.

Further, environmental risk factors (EBV, smoking, and obesity) increase the risk of MS with combinations of the MHC risk factors (HLA-A*2 protective, DRB1*15:01 risk) greatly beyond the additive effects of these genetic risk factors: additive risk 5.0, interactive risk 14 (50). This suggests that there is an increased risk of MS due to the antigen-presentation pathways when environmental factors increase the effects of an immune response and highlight the value of interventions based on reducing these environmental risk factors.

## CONCLUSION AND FUTURE WORK

Although much of the heritability of MS has been discovered, the current findings have not yet been sufficiently exploited. Some progress has been made in the identification of pathogenically significant changes to immune cell types, state, and differentiation; and interaction with UV light and vitamin D. Design of new therapies for risk gene-guided immunomodulation, repurposing of existing drugs, better use of vitamin D analogs and methods of use, and guided use of current drugs should be facilitated by knowledge of the pathogenic processes tagged by the risk factors.

## AUTHOR CONTRIBUTIONS

DB drafted the manuscript, GP the figures, and both edited the final version.

## FUNDING

This work was funded by grants from the Australian National Health and Medical Research Council (NHMRC) (GNTs 1006759, 1049936, and 1050074), MS Research Australia, and the Trish MS Research Foundation. Funding bodies had no role in study design, in collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

## REFERENCES

1. Compston A, Coles A. Multiple sclerosis. *Lancet* (2008) 372(9648):1502–17. doi:10.1016/s0140-6736(08)61620-7
2. IMSGC, Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kemppinen A, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet* (2013) 45(11):1353–60. doi:10.1038/ng.2770
3. IMSGC, Patsopoulos N. 200 loci complete the genetic puzzle of multiple sclerosis. *Presented at the American Society of Human Genetics 2016 Annual Meeting*. Vancouver, BC, Canada (2016).
4. Moutsianas L, Jostins L, Beecham AH, Dilthey AT, Xifara DK, Ban M, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet* (2015) 47(10):1107–13. doi:10.1038/ng.3395
5. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* (2002) 3(11):872–82. doi:10.1038/nrg932
6. Fagnani C, Neale MC, Nistico L, Stazi MA, Ricigliano VA, Buscarinu MC, et al. Twin studies in multiple sclerosis: a meta-estimation of heritability and environmentality. *Mult Scler* (2015) 21(11):1404–13. doi:10.1177/1352458514564492
7. Westerlind H, Ramanujam R, Uvehag D, Kuja-Halkola R, Boman M, Bottai M, et al. Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden. *Brain* (2014) 137(Pt 3):770–8. doi:10.1093/brain/awt356
8. Selmi C, Lu Q, Humble MC. Heritability versus the role of the environment in autoimmunity. *J Autoimmun* (2012) 39(4):249–52. doi:10.1016/j.jaut.2012.07.011
9. Booth DR. The clinical implications from the first hundred known MS susceptibility genes. *Adv Clin Neurosci Rehabil* (2014) 14(2):10–3.
10. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N Engl J Med* (2017) 376(3):221–34. doi:10.1056/NEJMoa1601277
11. Gross CC, Schulte-Mecklenbeck A, Runzi A, Kuhlmann T, Posevitz-Fejfar A, Schwab N, et al. Impaired NK-mediated regulation of T-cell activity in multiple sclerosis is reconstituted by IL-2 receptor modulation. *Proc Natl Acad Sci U S A* (2016) 113(21):E2973–82. doi:10.1073/pnas.1524924113
12. Jones JL, Coles AJ. Mode of action and clinical studies with alemtuzumab. *Exp Neurol* (2014) 262(Pt A):37–43. doi:10.1016/j.expneurol.2014.04.018
13. Marousi S, Karkanis I, Kalamatas T, Travasarou M, Paterakis G, Karageorgiou CE. Immune cells after prolonged natalizumab therapy: implications for effectiveness and safety. *Acta Neurol Scand* (2013) 128(1):e1–5. doi:10.1111/ane.12080
14. Mehling M, Kappos L, Derfuss T. Fingolimod for multiple sclerosis: mechanism of action, clinical outcomes, and future directions. *Curr Neurol Neurosci Rep* (2011) 11(5):492–7. doi:10.1007/s11910-011-0216-9
15. Li R, Rezk A, Ghadiri M, Luessi F, Zipp F, Li H, et al. Dimethyl fumarate treatment mediates an anti-inflammatory shift in B cell subsets of patients with multiple sclerosis. *J Immunol* (2017) 198(2):691–8. doi:10.4049/jimmunol.1601649
16. Fragoso YD, Brooks JB. Leflunomide and teriflunomide: altering the metabolism of pyrimidines for the treatment of autoimmune diseases. *Expert Rev Clin Pharmacol* (2015) 8(3):315–20. doi:10.1586/17512433.2015.1019343
17. O'Day K, Meyer K, Stafkey-Mailey D, Watson C. Cost-effectiveness of natalizumab vs fingolimod for the treatment of relapsing-remitting multiple sclerosis: analyses in Sweden. *J Med Econ* (2015) 18(4):295–302. doi:10.3111/13696998.2014.991786
18. Simpson S Jr, Taylor BV, van der Mei I. The role of epidemiology in MS research: past successes, current challenges and future potential. *Mult Scler* (2015) 21(8):969–77. doi:10.1177/1352458515574896
19. Orru V, Steri M, Sole G, Sidore C, Virdis F, Dei M, et al. Genetic variants regulating immune cell levels in health and disease. *Cell* (2013) 155(1):242–56. doi:10.1016/j.cell.2013.08.041
20. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, et al. Variation in the human immune system is largely driven by non-heritable influences. *Cell* (2015) 160(1–2):37–47. doi:10.1016/j.cell.2014.12.020
21. Roederer M, Quaye L, Mangino M, Beddall MH, Mahnke Y, Chattopadhyay P, et al. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell* (2015) 161(2):387–403. doi:10.1016/j.cell.2015.02.046
22. Olerup O, Hillert J. HLA class II-associated genetic susceptibility in multiple sclerosis: a critical evaluation. *Tissue Antigens* (1991) 38(1):1–15. doi:10.1111/j.1399-0039.1991.tb02029.x
23. Kumar A, Cocco E, Atzori L, Marrosu MG, Pieroni E. Structural and dynamical insights on HLA-DR2 complexes that confer susceptibility to multiple sclerosis in Sardinia: a molecular dynamics simulation study. *PLoS One* (2013) 8(3):e59711. doi:10.1371/journal.pone.0059711
24. Tschochner M, Leary S, Cooper D, Strautins K, Chopra A, Clark H, et al. Identifying patient-specific Epstein-Barr nuclear antigen-1 genetic variation and potential autoreactive targets relevant to multiple sclerosis pathogenesis. *PLoS One* (2016) 11(2):e0147567. doi:10.1371/journal.pone.0147567
25. Isobe N, Keshavan A, Gourraud PA, Zhu AH, Datta E, Schlaeger R, et al. Association of HLA genetic risk burden with disease phenotypes in multiple sclerosis. *JAMA Neurol* (2016) 73(7):795–802. doi:10.1001/jamaneurol.2016.0980
26. Shahijanian F, Parnell GP, McKay FC, Gatt PN, Shojoei M, O'Connor KS, et al. The CYP27B1 variant associated with an increased risk of autoimmune disease is underexpressed in tolerizing dendritic cells. *Hum Mol Genet* (2014) 23(6):1425–34. doi:10.1093/hmg/ddt529
27. Isobe N, Damotte V, Lo Re V, Ban M, Pappas D, Guillot-Noel L, et al. Genetic burden in multiple sclerosis families. *Genes Immun* (2013) 14(7):434–40. doi:10.1038/gene.2013.37

28. Couturier N, Bucciarelli F, Nurtdinov RN, Debouverie M, Lebrun-Frenay C, Defer G, et al. Tyrosine kinase 2 variant influences T lymphocyte polarization and multiple sclerosis susceptibility. *Brain* (2011) 134(Pt 3):693–703. doi:10.1093/brain/awr010
29. Dendrou CA, Cortes A, Shipman L, Evans HG, Attfield KE, Jostins L, et al. Resolving TYK2 locus genotype-to-phenotype differences in autoimmunity. *Sci Transl Med* (2016) 8(363):363ra149. doi:10.1126/scitranslmed.aag1974
30. Hartmann FJ, Khademi M, Aram J, Ammann S, Kockum I, Constantinescu C, et al. Multiple sclerosis-associated IL2RA polymorphism controls GM-CSF production in human TH cells. *Nat Commun* (2014) 5:5056. doi:10.1038/ncomms6056
31. McKay FC, Gatt PN, Fewings N, Parnell GP, Schibeci SD, Basuki MA, et al. The low EOMES/TBX21 molecular phenotype in multiple sclerosis reflects CD56+ cell dysregulation and is affected by immunomodulatory therapies. *Clin Immunol* (2016) 163:96–107. doi:10.1016/j.clim.2015.12.015
32. Housley WJ, Fernandez SD, Vera K, Murikinati SR, Grutzendler J, Cuerdon N, et al. Genetic variants associated with autoimmunity drive NFkappaB signaling and responses to inflammatory stimuli. *Sci Transl Med* (2015) 7(291):291ra293. doi:10.1126/scitranslmed.aaa9223
33. Fewings NL, Gatt PN, McKay FC, Parnell GP, Schibeci SD, Edwards J, et al. The autoimmune risk gene ZMIZ1 is a vitamin D responsive marker of a molecular phenotype of multiple sclerosis. *J Autoimmun* (2017) 78:57–69. doi:10.1016/j.jaut.2016.12.006
34. Li R, Rezk A, Miyazaki Y, Hilgenberg E, Touil H, Shen P, et al. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Sci Transl Med* (2015) 7(310):310ra166. doi:10.1126/scitranslmed.aab4176
35. IMSGC, WTCCC2, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* (2011) 476(7359):214–9. doi:10.1038/nature10251
36. Song ZY, Yamasaki R, Kawano Y, Sato S, Masaki K, Yoshimura S, et al. Peripheral blood T cell dynamics predict relapse in multiple sclerosis patients on fingolimod. *PLoS One* (2014) 10(4):e0124923. doi:10.1371/journal.pone.0124923
37. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* (2015) 15(9):545–58. doi:10.1038/nri3871
38. Broux B, Shamim Z, Wang T, Spellman S, Haagenson M, Stinissen P, et al. The influence of interleukin-7 receptor alpha-chain haplotypes on outcome after allogeneic hematopoietic cell transplantation. *Int J Immunogenet* (2014) 41(6):521–7. doi:10.1111/iji.12158
39. Rajasuriar R, Booth D, Solomon A, Chua K, Spelman T, Gouillou M, et al. Biological determinants of immune reconstitution in HIV-infected patients receiving antiretroviral therapy: the role of interleukin 7 and interleukin 7 receptor alpha and microbial translocation. *J Infect Dis* (2010) 202(8):1254–64. doi:10.1086/656369
40. McKay FC, Hoe E, Parnell G, Gatt P, Schibeci SD, Stewart GJ, et al. IL7Ralpha expression and upregulation by IFNbeta in dendritic cell subsets is haplotype-dependent. *PLoS One* (2013) 8(10):e77508. doi:10.1371/journal.pone.0077508
41. Gandhi KS, McKay FC, Cox M, Riveros C, Armstrong N, Heard RN, et al. The multiple sclerosis whole blood mRNA transcriptome and genetic associations indicate dysregulation of specific T cell pathways in pathogenesis. *Hum Mol Genet* (2010) 19(11):2134–43. doi:10.1093/hmg/ddq090
42. Gregory AP, Dendrou CA, Attfield KE, Haghikia A, Xifara DK, Butter F, et al. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* (2012) 488(7412):508–11. doi:10.1038/nature11307
43. Hart PH, Lucas RM, Booth DR, Carroll WM, Nolan D, Cole JM, et al. Narrowband UVB phototherapy for clinically isolated syndrome: a trial to deliver the benefits of vitamin D and other UVB-induced molecules. *Front Immunol* (2017) 8:3. doi:10.3389/fimmu.2017.00003
44. Booth DR, Ding N, Parnell GP, Shahijanian F, Coulter S, Schibeci SD, et al. Cistromic and genetic evidence that the vitamin D receptor mediates susceptibility to latitude-dependent autoimmune diseases. *Genes Immun* (2016) 17(4):213–9. doi:10.1038/gene.2016.12
45. Lee MN, Ye C, Villani AC, Raj T, Li W, Eisenhaure TM, et al. Common genetic variants modulate pathogen-sensing responses in human dendritic cells. *Science* (2014) 343(6175):1246980. doi:10.1126/science.1246980
46. Pender MP. CD8+ T-cell deficiency, Epstein-Barr virus infection, vitamin D deficiency, and steps to autoimmunity: a unifying hypothesis. *Autoimmune Dis* (2012) 2012:189096. doi:10.1155/2012/189096
47. Ascherio A, Munger KL. EBV and autoimmunity. *Curr Top Microbiol Immunol* (2015) 390(Pt 1):365–85. doi:10.1007/978-3-319-22822-8_15
48. Zhou Y, Zhu G, Charlesworth JC, Simpson S Jr, Rubicz R, Goring HH, et al. Genetic loci for Epstein-Barr virus nuclear antigen-1 are associated with risk of multiple sclerosis. *Mult Scler* (2016) 22(13):1655–64. doi:10.1177/1352458515626598
49. Calabresi PA. B-cell depletion – a frontier in monoclonal antibodies for multiple sclerosis. *N Engl J Med* (2017) 376(3):280–2. doi:10.1056/NEJMe1614717
50. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* (2017) 13(1):25–36. doi:10.1038/nrneurol.2016.187


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 28 February 2017
doi: 10.3389/fneur.2017.00062

# Different Phenotypes at Onset in Neuromyelitis Optica Spectrum Disorder Patients with Aquaporin-4 Autoimmunity

*Youming Long[1,2], Junyan Liang[1,2], Linzhan Wu[1,2], Shaopeng Lin[3], Cong Gao[1,2]*, Xiaohui Chen[3], Wei Qiu[4], Yu Yang[4], Xueping Zheng[5], Ning Yang[6], Min Gao[7], Yaotang Chen[1,2], Zhanhang Wang[8] and Quanxi Su[9]*

*[1] Department of Neurology, the Second Affiliated Hospital of GuangZhou Medical University, Guangzhou, Guangdong Province, China, [2] Institute of Neuroscience and The Second Affiliated Hospital of Guangzhou Medical University, Key Laboratory of Neurogenetics and Channelopathies of Guangdong Province and the Ministry of Education of China, Collaborative Innovation Center for Neurogenetics and Channelopathies, Guangzhou, China, [3] Department of Emergency, The Second Affiliated Hospital of GuangZhou Medical University, Guangzhou, Guangdong Province, China, [4] Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong Province, China, [5] Department of Neurology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China, [6] Department of Neurology, The Fifth Affiliated Hospital of GuangZhou Medical University, Guangzhou, Guangdong Province, China, [7] Department of Neurology, The Second Chinese Medicine Hospital of Guangdong Province, Guangzhou, Guangdong Province, China, [8] Department of Neurology, Guangdong 999 Brain Hospital, Guangzhou, Guangdong Province, China, [9] Department of Neurology, Yunfu City People's Hospital, Yunfu, Guangdong Province, China*

OPEN ACCESS

**Edited by:**
*Fabienne Brilot, University of Sydney, Australia*

**Reviewed by:**
*Scott S. Zamvil, University of California San Francisco, USA*
*Samar S. Ayache, Paris Est University Creteil, France*

***Correspondence:**
*Cong Gao*
*smilegaocong@126.com*

**Specialty section:**
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology*

**Received:** *16 July 2016*
**Accepted:** *13 February 2017*
**Published:** *28 February 2017*

**Citation:**
*Long Y, Liang J, Wu L, Lin S, Gao C, Chen X, Qiu W, Yang Y, Zheng X, Yang N, Gao M, Chen Y, Wang Z and Su Q (2017) Different Phenotypes at Onset in Neuromyelitis Optica Spectrum Disorder Patients with Aquaporin-4 Autoimmunity. Front. Neurol. 8:62. doi: 10.3389/fneur.2017.00062*

**Background:** Although rare, brain abnormalities without optic neuritis (ON) or transverse myelitis (TM) diagnosed with neuromyelitis optica spectrum disorder (NMOSD) have been reported in patients positive for the aquaporin-4 (AQP4) antibody.

**Objective:** To analyze demographic and clinical differences among NMOSD patients without ON or TM, those with either ON or TM, and patients with simultaneous ON and TM at disease onset.

**Methods:** In this retrospective study, patients who were positive for the AQP4 antibody, as detected using a cell-based assay, at the Second Affiliated Hospital of Guangzhou Medical University in China were recruited. Demographic and clinical data were obtained from each patient's medical record.

**Results:** A total of 292 patients were included in this study and were divided into four subgroups based on their initial manifestations: (i) NMOSD without ON or TM (NMOSD-ON$^-$TM$^-$, $n$ = 70); (ii) NMOSD with ON (NMOSD-ON$^+$, $n$ = 95); (iii) NMOSD with TM (NMOSD-TM$^+$, $n$ = 116); and (iv) simultaneous ON and TM [neuromyelitis optica (NMO), $n$ = 11]. We found that age at onset was lower in the NMOSD-ON$^-$TM$^-$ group than that in the other groups. The interval from the first episode to relapse was shorter in the NMOSD-ON$^-$TM$^-$ group than that in NMOSD-TM$^+$ group. Cerebral spinal fluid white cell counts and protein levels were significantly higher in the NMOSD-ON$^-$TM$^-$ group than those in the other groups. Lower Expanded Disability Status Scale scores were observed in the NMOSD-ON$^-$TM$^-$ group. Brain abnormalities, including in area postrema and hemisphere lesions, were more frequent in the NMOSD-ON$^-$TM$^-$ group. Kaplan–Meier

analysis showed that patients in the NMOSD-ON$^{-}$TM$^{-}$ group experienced earlier relapse than those in other groups. Conversion to NMO in the NMOSD-ON$^{+}$ group was greater than that in the other groups. Only 14 patients (4.8%, 14/292) had pure brain abnormalities, of which 12 had disease duration of several more years and 8 (57.1%) experienced relapses.

**Conclusion:** NMOSD patients with different initial manifestations present with significant differences in clinical features during follow-up. Patients with long-term AQP4 autoimmunity in the brain in the absence of ON or TM are not common.

**Keywords: neuromyelitis optica, aquaporin-4, optic neuritis, myelitis, brain**

## INTRODUCTION

Neuromyelitis optica (NMO) is generally a severe, idiopathic, immune-mediated inflammatory, demyelinating, and necrotizing disease that mainly involves the optic nerve and spinal cord, but rarely the brain. The presence of aquaporin-4 (AQP4) antibody in NMO (1) facilitates its distinction from multiple sclerosis, and many studies have shown that AQP4 autoimmune lesions outside the optic nerve and spinal cord are common (2, 3).

Limited forms of NMO, optic neuritis (ON) or transverse myelitis (TM), positive for the anti-AQP4 antibody are diagnosed as NMO spectrum disorder (NMOSD) (4, 5). Furthermore, increasing numbers of positive cases without optic nerve and spinal cord involvement have been reported, indicating that the requirement for the presence of either ON or TM may confound the definition of NMOSD (6). Previous reports have shown that many manifestations outside the optic nerve and spinal cord in patients with NMOSD occur frequently during the disease and may precede ON or TM by months or years (7–9). Thus, the international panel for NMO diagnosis has updated the definition of NMOSD to include the presence or absence of anti-AQP4 antibody (2). A diagnosis of NMOSD with anti-AQP4 antibody requires several core clinical characteristics, including clinical syndromes or magnetic resonance imaging (MRI) findings related to optic nerve, spinal cord, area postrema, other brainstem locations, diencephalic, or cerebral presentations (2). Recently, some cases have been reported with abnormalities in skeletal muscle and retinal cells expressing AQP4, characterized by the presence of immune complex deposition and AQP4 loss (10, 11). Therefore, manifestations in patients positive for AQP4 antibody are heterogeneous.

Surprisingly, despite the interest in brain abnormalities of patients with NMO/NMOSD, patients displaying pure brain symptoms have been rarely reported (7, 9, 12). Here, we describe the initial and follow-up clinical manifestations of patients with NMOSD who initially presented with different phenotypes, especially those with pure brain symptoms.

## PATIENTS AND METHODS

### Patients

This retrospective study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University, China. All patients provided informed consent in the present study. Data analysis was performed based on the Chinese laws for data protection.

Consecutive patients positive for AQP4 antibodies, as detected retrospectively using a cell-based assay at the Second Affiliated Hospital of Guangzhou Medical University, were recruited until August 2015. The following data were acquired from each patient's medical record: age, sex, medication, number of demyelinating events, clinical characteristics, and cerebral spinal fluid (CSF) protein levels and white cell counts. The Expanded Disability Status Scale (EDSS) (13) was conducted in these patients during follow-up at their most recent interview. Relapse was defined as objective worsening of new neurological symptoms that lasted at least 24 h and was preceded by disease stability for at least 1 month.

The patients were diagnosed as NMO/NMOSD based on the 2006 NMO diagnostic criteria (14) and the recent international panel guidelines (2). Cases of longitudinally extensive transverse myelitis (LETM) and acute partial transverse myelitis (APTM) were confirmed using MRI (15, 16). Cases of ON were defined by acute or subacute, unilateral or bilateral vision loss.

The recruited patients were divided into the following four groups based on initial disease manifestation: (i) with ON (NMOSD-ON$^{+}$); (ii) with TM (NMOSD-TM$^{+}$); (iii) without ON and TM (NMOSD-ON$^{-}$TM$^{-}$); and (iv) with simultaneous ON and TM NMO.

### AQP4 Antibody Testing

All CSF and serum samples were stored at −80°C. AQP4 antibodies were detected with a cell-based assay using a commercially available kit (Euroimmun, Luebeck, Germany) or by transfection of HEK293T cells with a construct containing human AQP4-M1 and AQP4-M23 genes.

### Statistical Analysis

All statistical analyses were conducted using Statistical Program for Social Sciences version 11.0 (SPSS, Chicago, IL, USA) software. The $\chi^2$-test was used for binary and categorical data. One-way ANOVA and Mann–Whitney *U* tests were used for continuous variables. A Kaplan–Meier analysis was performed to evaluate survival (time to relapse, conversion to NMO). The Kaplan–Meier analysis was compared between groups using log-rank tests. Values of $p$ less than 0.05 were considered significant.

# RESULTS

## Patient Demographics

A total of 292 patients with positive AQP4 antibodies were included in this retrospective study. This cohort comprised 253 females and 49 males (a female to male ratio of 6.49). Among these 292 participants, 178 (61%) were diagnosed with NMO and 114 (39%) with NMOSD based on their most recent follow-up (2) (**Table 1**). Their mean age at onset was 38.1 ± 14.5 years (range, 4–79 years); 22 of the 292 patients (7.53%) were older than 60 years at disease onset, and 10 (3.42%) were under 18 years old.

The initial symptoms of the four groups are shown in **Figure 1**. Group (i) comprised 95 patients (32.5%, 95/292) diagnosed with ON (NMOSD-ON$^+$) at onset. The disease started with isolated left ON in 60/95 cases (63.2%), isolated right ON in 19 (20%), and simultaneous bilateral ON in 16 (16.8%). Group (ii) consisted of 116 patients (39.7%, 116/292) diagnosed with TM (NMOSD-TM$^+$) at onset. The disease started with LETM in 113/116 cases (97.4%) and APTM in 3/116 (2.6%) cases. Cervical lesions were found in 46/116 (39.7%), thoracic lesions in 35/116 (30.2%), and simultaneous cervical and thoracic lesions in 35/116 (30.2%) patients. Group (iii) was composed of 70 patients (24%, 70/292) without ON and TM (NMOSD-ON$^-$TM$^-$) at onset. The main brain symptoms observed included area postrema syndrome with hiccups or nausea and vomiting (44.2%, 31/70), acute brainstem syndrome (22.9%, 16/70), acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions (17.1%, 12/70), and symptomatic cerebral syndrome (15.7%, 11/70). At the most recent interview, 14 patients (4.8%, 14/292) had confirmed episodes involving brain abnormalities only, without ON or TM, of which the duration was >12 months for 12 patients, 8 (57.1%) of whom experienced relapse. None of these patients were diagnosed as having NMO or NMOSD prior to the AQP4 antibody test. Two patients were from the same family, and their younger sister had been diagnosed with typical NMO with bilateral ON and LETM (**Figure 2**). Two patients had comorbidity of autoimmune nephritis. One case had comorbidity of anti-*N*-methyl-D-aspartate receptor encephalitis. Group 4 was composed of 11 patients (3.8%) diagnosed with simultaneous ON and TM (NMO) at onset.

**TABLE 1 | Final diagnosis and distribution of patients in three subgroups.**

| Groups | *N* (%) of patients | Age at onset (median) | Female/ male | No. (%) of immunosuppressive therapy[a] |
|---|---|---|---|---|
| (NMOSD)-ON$^-$TM$^-$ | 70 (100) | 30 | 60/10 | 7 (10) |
| NMO* | 38 (54.3) | 30 | 35/3 | 3 (7.9) |
| RLETM | 8 (11.4) | 39 | 7/1 | 1 (12.5) |
| APTM | 3 (4.3) | 28 | 2/1 | 0 |
| MON | 3 (4.3) | 30 | 3/0 | 0 |
| RON | 2 (2.9) | 28.43 | 1/1 | 0 |
| Others[b] | 16 (22.9) | 26 | 12/4 | 3 (18.8%) |
| NMOSD-ON$^+$ | 95 (100) | 37 | 83/12 | 22 (23.2%) |
| NMO* | 78 (82.1) | 38 | 69/9 | 20 (25.6%) |
| RON | 12 (12.6) | 37 | 9/3 | 2 (16.7%) |
| MON | 5 (5.3) | 25 | 5/0 | 0 |
| NMOSD-TM$^+$ | 116 (100) | 40 | 98/18 | 10 (8.6) |
| NMO* | 51 (44.00) | 42 | 44/7 | 5 (9.8) |
| RLETM | 40 (34.5) | 40 | 34/6 | 5 (12.5) |
| MLETM | 22 (19.0) | 43 | 17/5 | 0 |
| APTM | 3 (2.6) | 36 | 3/0 | 0 (0) |
| NMO | 11 (100) | 49 | 11/0 | 2 (6.8) |

*NMOSD, neuromyelitis optica spectrum disorder; NMO, neuromyelitis optica; RLETM, recurrent longitudinal extensive transverse myelitis; MLETM, monophasic LETM; APTM, acute partial transverse myelitis; RON, recurrent optic neuritis; MON, monophasic ON.*

[a]*Long therapy with azathioprine, cyclophosphamide, methotrexate, and mycophenolate mofetil.*

[b]*Patients without optic neuritis (ON) and transverse myelitis (TM).*

**Significantly different among the three groups ($p < 0.0001$).*

## Comparison of NMOSD Phenotypes at Onset

The characteristics and phenotypes of the patients ($n = 174$) in the three NMOSD groups (NMOSD-ON$^+$, NMOSD-TM$^+$, and NMOSD-ON$^-$TM$^-$) who had complete MRI, CSF, demographic, and clinical data were compared. These three groups showed no significant differences in the sex ratio, disease duration, and number of relapsing cases ($p > 0.05$). However, the age at onset was lower in the NMOSD-ON$^-$TM$^-$ group than that in the other two groups ($p < 0.005$). The interval from the first episode to relapse (relapse-free time) was shorter in the NMOSD-ON$^-$TM$^-$ group than that in the NMOSD-TM$^+$ group ($p = 0.027$). In addition, a significant difference was found among these groups for the NMO conversion ($p < 0.0001$). The CSF white cell count and protein level were significantly higher in the NMOSD-ON$^-$TM$^-$ group than those in the other two groups. The NMOSD-ON$^-$TM$^-$ group had lower EDSS scores than the other two groups in the most recent follow-up. Brain MRI abnormalities in area postrema and hemisphere lesions were more frequent in the NMOSD-ON$^-$TM$^-$ group ($p < 0.005$; **Table 2**), whereas brain MRI abnormalities were similar between the NMOSD-ON$^+$ and NMOSD-TM$^+$ groups. Pure cervical cord lesions were more frequent in the NMOSD-ON$^-$TM$^-$ group ($p < 0.01$).

## Follow-up and Kaplan–Meier Analysis

Patients were included in this analysis if they had validated relapsing events that occurred from the time of the initial incident to the most recent interview and the duration of those symptoms was greater than 12 months. Thus, follow-up data were analyzed for 226 patients in the three NMOSD groups (NMOSD-ON$^+$, NMOSD-TM$^+$, and NMOSD-ON$^-$TM$^-$).

Among the 61 patients analyzed in the NMOSD-ON$^-$TM$^-$ group, 60 (98.4%) experienced relapse, and 31 patients (50.8%) met the NMO diagnostic criteria during follow-up (12–268 months). Among the 80 patients analyzed in the NMOSD-TM$^+$ group, 72 patients (90%) experienced relapse, and 34 (42.5%) were diagnosed with NMO during follow-up (12–324 months). Among the 85 patients analyzed in the NMOSD-ON$^+$ group, 80 patients (94.1%) experienced relapse, and 71 (83.5%) met the NMO diagnostic criteria during follow-up (12–346 months). The conversion to NMO in the NMOSD-ON$^+$ group was greater than that in the other tow groups ($p < 0.0001$).

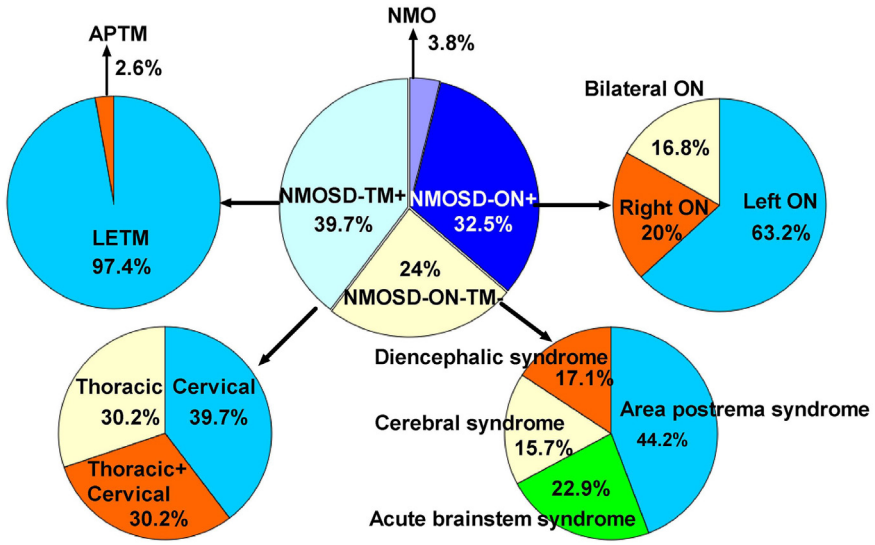



**FIGURE 1 | Distribution of initial manifestations status in 292 patients**. NMOSD, neuromyelitis optica spectrum disorder; ON, optic neuritis; TM, transverse myelitis; APTM, acute partial transverse myelitis; LETM, longitudinally extensive transverse myelitis; NMOSD-ON−TM−, patient initial manifestation without ON and TM; NMOSD-ON+, patient initial manifestation with ON; NMOSD-TM+, patient initial manifestation with TM; neuromyelitis optica (NMO), patient initial manifestation with simultaneous ON and TM.

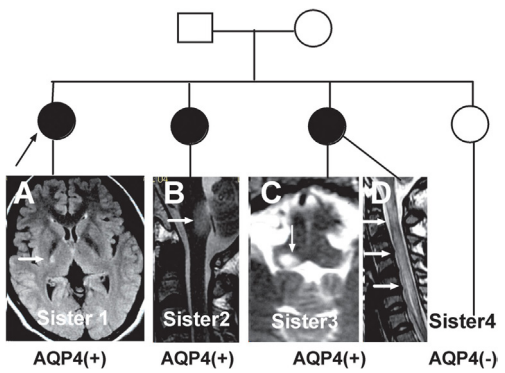



**FIGURE 2 | Three neuromyelitis optica/neuromyelitis optica spectrum disorder cases from the same family**. **(A)** Case one is the oldest sister with autoimmune nephritis and the proband in this family. She experienced left limb weakness in 2004. A recent magnetic resonance imaging (MRI) scan showed a residual lesion in the posterior limb of internal capsule (arrow). **(B)** Case two is the second sister who experienced intractable hiccups and nausea and inappropriate antidiuretic hormone secretion in 2011. Her MRI showed a lesion in the dorsal medulla (arrow). **(C,D)** Case three, the third sister, had typical optic neuritis and transverse myelitis. Her MRI scan showed a lesion in the dorsal medulla and longitudinally extensive transverse myelitis (arrow).

A Kaplan–Meier analysis revealed that compared with NMOSD-TM$^+$ cases, NMOSD-ON$^-$TM$^-$ patients experienced significantly earlier relapses after the first attack ($p = 0.002$). However, these two groups showed a similar relapse rate at late follow-up (>100 months) (**Figure 3**). Furthermore, compared with NMOSD-ON$^+$ cases, NMOSD-ON$^-$TM$^-$ cases also experienced significantly earlier relapses ($p = 0.023$), although both of these groups had similar relapse rates at >50 months. The Kaplan–Meier analysis also revealed that the interval from first attack to NMO conversion differed among the groups (**Figure 4**). The median time of 120 months [95% confidence interval (CI): 40.6–199.4 months] in NMOSD-TM$^+$ cases was significantly longer than that of 49.0 months for the NMOSD-ON$^-$TM$^-$ cases (95% CI: 26.8–71.2 months, $p = 0.012$) and 36.0 months for the NMOSD-ON$^+$ cases (95% CI: 25.3–46.7 months, $p < 0.0001$).

## DISCUSSION

The present study found that initial manifestations without TM or ON were not uncommon in NMOSD patients. In all the patients examined, the onset of brain/brainstem lesions was more frequent than that previously shown in a large study (12). We observed that 19.5% (57/292) of patients had involvement in area postrema/brainstem, including displaying intractable hiccups and nausea (IHN) (10.6%, 31/29). Although increasing numbers of NMOSD patients with brain/brainstem-onset manifestations have been reported, such patients without ON and TM in NMOSD have been rarely reported in previously conducted large studies. For example, a relatively large study (12) reported 18% of patients positive for AQP4 antibodies without TM and ON presented with brain symptoms as their initial manifestation. In Japan, 28.6% (10/35) of cases showed IHN preceding ON and TM, and IHN was preceded by an episode of viral infection (17). Apiwattanakul et al. reported that the initial presenting symptom of NMO was intractable vomiting in 12% of AQP4 antibody-positive patients (8). In addition, hiccup and nausea often preceded neurological symptoms such as ON and TM,

**TABLE 2 | Demographic and paraclinical characteristics in three subgroups with valid data.**

| Characteristics | NMOSD-ON$^-$TM$^-$ | NMOSD-TM$^+$ | NMOSD-ON$^+$ | p1 | p2 | p3 |
|---|---|---|---|---|---|---|
| | (*n* = 53) | (*n* = 57) | (*n* = 64) | | | |
| Age onset (years) | 31.6 ± 17.8 | 41.6 ± 14.2 | 37.0 ± 14.2 | <0.0001 | 0.012 | NS |
| Age onset < 30 years, *n* (%) | 31 (58.5) | 10 (17.5) | 18 (28.1) | <0.0001 | 0.001 | NS |
| Age onset >40 years, *n* (%) | 16 (30.2) | 30 (52.6) | 25 (39.1) | 0.017 | NS | NS |
| Age onset >50 years, *n* (%) | 5 (9.4) | 16 (28.1) | 13 (20.3) | 0.013 | NS | NS |
| Female/male | 47/6 | 52/5 | 55/9 | NS | NS | NS |
| Duration (months) | 68.8 ± 58.8 | 76.6 ± 65.6 | 79.7 ± 68.4 | NS | NS | NS |
| Relapsing cases, *n* (%) | 48 (90.6) | 48 (84.2) | 62 (96.9) | NS | NS | NS |
| Relapse-free time (months)[a] | 4 (1–96) | 14 (2–312) | 8 (1–120) | 0.027 | NS | NS |
| Neuromyelitis optica (NMO)-free time (months)[b] | 24 (1–156) | 24 (2–156) | 16 (2–223) | NS | NS | NS |
| Meeting 2006 NMO criteria, *n* (%) | 31 (57.4%) | 21 (36.8%) | 58 (90.6) | <0.0001 | <0.0001 | <0.0001 |
| CSF protein (g/L) | 0.44 ± 0.28 | 0.37 ± 0.20 | 0.27 ± 0.15 | 0.041 | 0.012 | NS |
| CSF pleocytosis, *n* (%) | 31 (58.5) | 22 (38.6) | 31 (48.4) | NS | NS | NS |
| CSF cells (no./mm$^3$) | 8 (0–325) | 5 (0–161) | 5 (0–98) | 0.005 | 0.050 | NS |
| Median EDSS (range) | 3 (1–10) | 5 (1–10) | 5 (1–10) | 0.008 | 0.010 | NS |
| EDSS ≤ 3, *n* (%) | 27 (50.9%) | 10 (17.5) | 16 (25) | <0.0001 | <0.0001 | <0.0001 |
| EDSS ≥ 6, *n* (%) | 10 (18.9%) | 17 (29.8) | 20 (31.3) | NS | NS | NS |
| Death, *n* (%) | 2 (3.8) | 2 (3.5) | 2 (3.1) | NS | NS | NS |
| Brain NMO lesions in history | | | | | | |
| Area postrema lesions, *n* (%) | 32 (60.4) | 11 (19.3) | 14 (21.9) | <0.0001 | <0.0001 | NS |
| Brain stem lesions, *n* (%) | 9 (17.0) | 5 (8.8) | 4 (6.3) | NS | NS | NS |
| Diencephalic lesion, *n* (%) | 25 (47.2) | 3 (5.3) | 9 (14.1) | <0.0001 | <0.0001 | NS |
| Cerebral lesion, *n* (%) | 15 (28.3) | 2 (3.5) | 4 (6.3) | 0.001 | 0.001 | NS |
| Spinal cord lesions in history | 37 (69.8) | 57 (100) | 57 (89.1) | <0.0001 | 0.009 | 0.010 |
| LETM, *n* (%) | 30/37 (81.1) | 55/57 (96.5) | 47/57 (82.5) | 0.013 | NS | 0.015 |
| Cervical lesions, *n* (%) | 24/37 (64.9) | 21/57 (36.8) | 20/57 (35.1) | 0.008 | 0.005 | NS |
| Thoracic lesions, *n* (%) | 6/37 (16.2) | 16/57 (28.1) | 25/57 (43.9) | NS | 0.005 | NS |
| Cervical + thoracic lesions, *n* (%) | 7/37 (18.9) | 20/57 (35.1) | 12/57 (21.1) | NS | NS | NS |

*NMOSD, neuromyelitis optica spectrum disease; LETM, longitudinal extensive transverse myelitis; EDSS, Expanded Disability Status Scale; NMOSD-ON$^+$, patient initial manifestation with ON; NMOSD-TM$^+$, patient initial manifestation with TM; NMOSD-ON$^+$TM$^+$, patient initial manifestation with simultaneous optic neuritis (ON) and transverse myelitis (TM); CSF, cerebral spinal fluid; p1, comparison between NMOSD-ON$^-$TM$^-$ and NMOSD-TM$^+$ patients; p2, comparison between NMOSD-ON$^-$TM$^-$ and NMOSD-ON$^+$ patients; p3, comparison between NMOSD-ON$^+$ and NMOSD-TM$^+$ patients.*

[a]*Duration from the first attack to the first relapse.*

[b]*Duration from the first attack to diagnosis of NMO.*

and 14% (10/70) of newly identified AQP4-IgG-positive patients had nausea and vomiting as the initial presenting symptoms of NMOSD (18). Most brainstem attacks were first events and were regarded as monophasic brainstem symptoms until follow-up (19, 20). However, a large study of 106 NMOSD patients seropositive for AQP4 antibodies reported that 4.7% (5/106) of patients had initial brain/brainstem manifestations without TM and ON (21). Another study from multiple centers showed only 2.3% (4/175) of NMOSD patients had brainstem-onset involvement (22). Our study found that 10.6% of patients had IHN, which is comparable to that found in a recent large study (8, 18). Therefore, IHN may have been underestimated in some previous studies because acute clinical events without TM or ON may have been overlooked, and some patients may have experienced lesion resolution. Although these patients had brain disease involvement before an ON or TM episode, most brain attacks were first events and almost all developed into NMO/NMOSD with ON and/or TM at follow-up (19, 20).

All groups in our cohort consisted of more females than males, consistent with observations in previous studies. Furthermore, the mean age at disease onset in patients presenting with ON was significantly lower than that in patients presenting with TM (9, 14, 21, 22). In our study, patients with disease onset were >40 years of age; however, similar numbers presented with TM (52.6%) or ON (39.1%) ($p > 0.005$), indicating a predominance of disease onset in young patients without TM and ON. Their mean age at disease onset was similar to that reported in previous studies showing a younger mean age at disease onset in patients with only brain/brainstem manifestations (9, 12). Thus, AQP4-mediated brain/brainstem disease may occur in patients younger than those with ON and TM, supporting age-dependent anatomical susceptibility differences or differences in AQP4 antibody accessibility of the target organs (23). However, Afro-Caribbean patients (9) reportedly have a younger age of disease onset, indicating ethnicity may be an important factor. Studies in China examining such differences would also be warranted.

The Kaplan–Meier analysis of our cohort demonstrated that >50% of patients experienced a relapse within 1.5 years of disease onset, and almost all experienced relapse within 10 years (**Figure 3**). Patients presenting with only brain/brainstem lesions had a shorter relapse-free time than those with ON or TM, a result similar to previous findings (22). There was a trend toward ON-onset patients relapsing sooner than TM-onset patients, which is in contrast to other studies in which AQP4 antibody-positive patients with TM-onset had earlier relapse than ON-onset patients (21, 22). Patients without ON or TM at

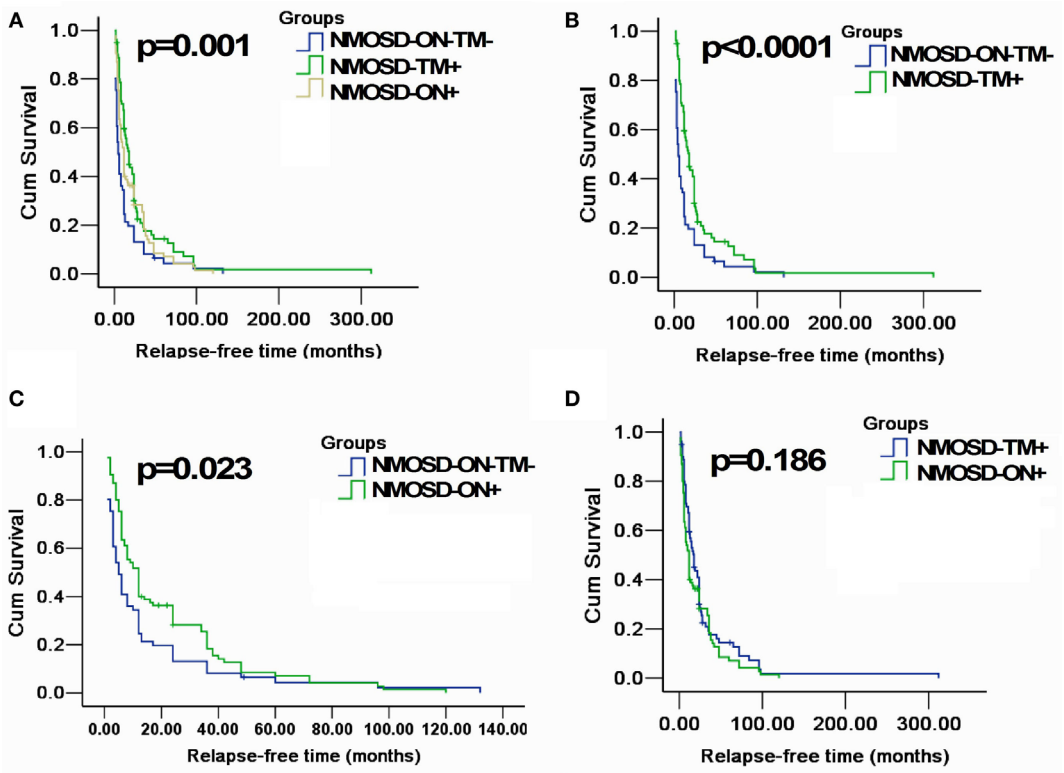



**FIGURE 3 | Kaplan–Meier analyses stratified by different groups: considering the end point is the first relapse. (A)** Kaplan–Meier analysis revealed that patients in three groups would experience different relapsing time after the first attack ($p = 0.001$); **(B,C)** Kaplan–Meier analysis revealed that patients in MOSD-ON$^-$TM$^-$ group would experience earlier relapse after the first attack and was significantly different neuromyelitis optica spectrum disorder (NMOSD)-TM$^+$ group and NMOSD-ON$^+$ ($p < 0.05$); and **(D)** Kaplan–Meier analysis revealed no significant differences in time to the first relapse between NMOSD-ON$^+$ and NMOSD-TM$^+$ group ($p = 0.186$).

onset had more lesions in areas without an intact blood–brain barrier, indicating that AQP4-mediated relapsing episodes may precede other neurological symptoms. However, it is unclear why lesions in these areas are less common than ON and spinal cord lesions (17).

We observed a different median time for NMO to develop in the three subgroups, as reported previously (21); however, the median time was relatively short in our cohort. Additionally, patients presenting with ON had a higher probability of developing NMO over time than those presenting with TM or brain/brainstem episodes. This may be because AQP4-mediated monophasic or relapsing ON is underrecognized, and prophylactic immunosuppressant therapy is not readily available (21). However, in our cohort, >60% of patients with TM did not develop NMO based on the 2006 diagnostic criteria (14), and even relapsing patients experienced a delay of >20 years. Therefore, our results support the ideas that AQP4-mediated disease is not synonymous with the classical description of NMO and that the first manifestations reflect different NMO phenotypes.

In our cohort, fewer patients presenting with brain disease progressed to NMO (31/61, 50.8%) compared with those in a study examining Korean patients (10/15, 66.7%) (12), which may be associated with the small sample size used in their study. Interestingly, some of our patients lacked typical ON and TM at onset attack, with an interval of years between onset attack and first episodes of ON or TM. However, AQP4 antibody-positive patients with manifestations suggesting long-term brain/brainstem involvement without ON and TM, especially those with a relapsing course, have been rarely reported (7, 9, 24–27). We found 12 patients (4.1%, 12/292) with episodes involving the brain without ON or TM over years, which is higher than the 2.4% (7/289) detected in Japan (9). In the present retrospective analysis, all patients with manifestations suggestive of brain involvement at onset were misdiagnosed with other diseases because of their atypical and complicated manifestations. Isolated lesions in the supratentorial region of the brain were rarely observed in the present study and have only been described in single case reports (7, 24–27). Atypical manifestations make diseases more difficult to diagnose, indicating AQP4 antibody-positive patients with long-term relapsing symptoms other than ON or TM may be easily misdiagnosed without the detection of AQP4 antibodies. Additionally, some patients with phenotypic presentations suggestive of brain disease presented concomitantly with an immune disorder involving another organ; for example, two patients had immunological disorders of the kidney. Previously, recurrent hyperCKemia accompanying AQP4-IgG seropositivity reflected

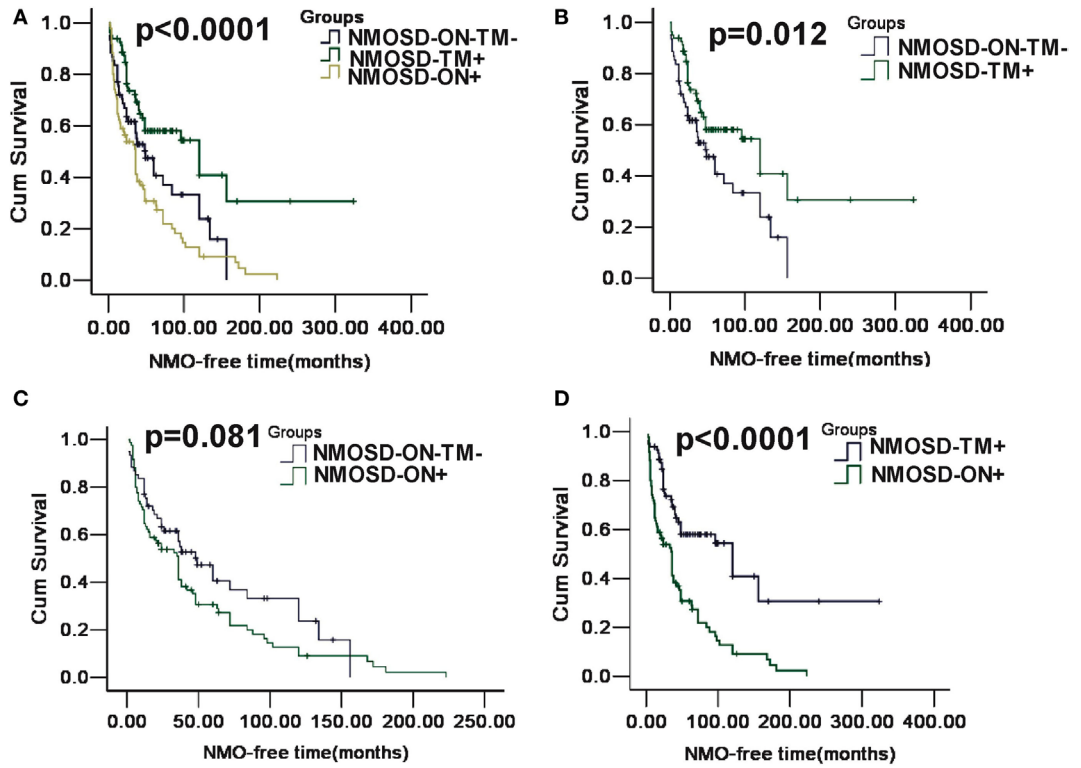



**FIGURE 4 | Kaplan–Meier analyses stratified by different groups: considering the conversion to neuromyelitis optica (NMO)**. **(A)** Kaplan–Meier analysis revealed that patients in three groups would experience different time of NMO conversion after the first attack ($p < 0.0001$); **(B)** Kaplan–Meier analysis revealed that patients in MOSD-ON$^-$TM$^-$ group would experience earlier NMO event after the first attack and was significantly different neuromyelitis optica spectrum disorder (NMOSD)-TM$^+$ group ($p = 0.012$); **(C)** Kaplan–Meier analysis revealed no significant differences in time to the NMO event between NMOSD-ON$^+$ and MOSD-ON$^-$TM$^-$ group ($p = 0.081$); and **(D)** Kaplan–Meier analysis revealed patients in NMOSD-ON$^+$ group would experienced earlier NMO event after the first attack and was significantly different NMOSD-TM$^+$ group ($p < 0.0001$).

pathogenic IgG targeting of skeletal muscle AQP4 (10). However, no typical AQP4 loss in kidney could be found with biopsy in our cases, although the AQP4 expression was relatively weaker than that in the control (not shown). Therefore, whether autoimmune AQP4 in the kidney is associated with NMOSD should be examined further, because AQP4-IgG seropositive cases do not meet the present definition of NMOSD, indicating that they may be autoimmune AQP4 channelopathies (3).

Mortality was significantly different in the three subgroups. First, the most recent median EDSS scores were lower in patients with brain/brainstem manifestations compared with those with ON or TM attacks at onset. Second, further analysis showed that compared with patients with ON, more patients with brain/brainstem manifestations had EDSS scores <3.0, and there was a trend toward fewer brain/brainstem-onset patients having EDSS scores >6.0. The prognosis for ON-onset patients was worse, and this may be related to a high proportion of these patients with NMO development, resulting in visual and motor disabilities. Although a low proportion of TM-onset patients developed NMO, their older age and increased proportion with LETM may be important factors for mortality. Older-onset patients are reportedly more likely to present with LETM and have a high risk of developing motor disability (21). It was previously shown that brain lesions with AQP4 autoimmunity in patients with NMOSD are accompanied by discontinued vasogenic edema (14). Thus, brain/brainstem-onset patients with recurrent brain/brainstem lesions may be predisposed to revisable edema without axonal injury resulting in slight persistent disability. Effective immunosuppressive treatment with good tolerance may prevent relapse or conversion to NMO. However, although some patients followed immunosuppressive regimes, >80% of these patients started treatment with only high-dose corticosteroids, tapering over several months to low-dosage steroids. The potential benefit of immunosuppressive therapy was not observed in the present cohort because of low use. In China, long-term immunosuppressive treatment is limited by adverse effects, patient compliance, and poor doctor–patient relationships (28). We believe that using recommended first-line long-term immunosuppression would provide a better prognosis.

The present study had some limitations. First, there was a selection bias because of the retrospective nature of the study and because some patients had incomplete data. Furthermore, a recall bias might result from data collection acquired from a patient's medical record or interview. Second, detection of AQP4 was retrospective; therefore, AQP4 status at the initial episode was unknown. Although prospective studies are

important, the current study is valid and will help clinicians treat different phenotypes of NMOSD. Third, our study focus is limited on the AQP4-positive NMOSD patients, which allows for a clear disease population, but leaves out AQP4-negative NMOSD. In particular in the latter disease group, would an analysis of the different phenotypes be of diagnostic importance and guidance for treatment decision. Given the recent insights into the role of antibodies against native conformational myelin oligodendrocyte glycoprotein (MOG) (29), embedding the data on NMOSD with mere brain involvement in this context would give a more comprehensive and up-to date picture of the patients with an "NMOSD phenotype" and brain involvement. Therefore, detection of MOG antibody is necessary in our further study.

In summary, we observed significant differences in clinical features during follow-up among NMOSD patients who presented with different initial manifestations. Patients who presented without ON and TM as their initial manifestations were younger at onset and had earlier relapses and more brain abnormalities but better prognosis than those who presented with ON and TM. The conversion to NMO in patients with TM at onset was lower than that in the other patient groups. Furthermore, the conversion to NMO was more frequent in patients with ON at onset than that in patients with brain/brainstem manifestations at onset. Patients with long-term AQP4 autoimmunity in the brain in the absence of ON or TM were not common.

## AUTHOR CONTRIBUTIONS

Study concept and design: YL, XC, and CG. Acquisition of data: YL, JL, LW, SL, XC, WQ, YY, XZ, NY, MG, YC, ZW, and QS. Analysis and interpretation of data: YL and CG. Drafting of the manuscript: YL, LW, and JL. Critical revision of the manuscript for important intellectual content: YL, JL, LW, Zhong, and CG. Obtained funding: YL and CG. Administrative, technical, and material support: YL, JL, LW, SL, XC, WQ, YY, XZ, NY, MG, and YC.

## ACKNOWLEDGMENTS

The authors also are very grateful to the following departments for data collection, which include Department of Neurology, The Chinese Medicine Hospital of Nanhai city; Department of Neurology, The Chinese Medicine Hospital of Zhongshan city; Department of Neurology, The Zhongshan People's Hospital; Department of Neurology, The First Affiliated Hospital of GuangDong Pharmaceutical University; Department of Neurology, The Eighth People's Hospital of Guangzhou; Department of Neurology, The First Affiliated Hospital of GuangZhou Medical University; Department of Neurology, The Guangzhou General Hospital of Guangzhou Military Command; Department of Neurology, The Guangzhou Women and Children Medical Center; Department of Neurology, The Guangzhou Brain Hospital; Department of Neurology, The Chinese Medicine Hospital of Guangdong Province; Department of Neurology, The Fourth Affiliated Hospital of GuangZhou Medical University; Department of Neurology, The Guangzhou Red Cross Hospital; and Department of Neurology, The Boai Hospital of Zhongshan city.

## FUNDING

This study was supported by the Natural Science Foundation of Guangdong Province (2014A030313499) and the Science and Technology plan project of Guangdong Province (2014A020212332).

## REFERENCES

1. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
2. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* (2015) 85(2):177–89. doi:10.1212/WNL.0000000000001729
3. Pittock SJ, Lucchinetti CF. Neuromyelitis optica and the evolving spectrum of autoimmune aquaporin-4 channelopathies: a decade later. *Ann N Y Acad Sci* (2016) 1366(1):20–39. doi:10.1111/nyas.12794
4. Fujihara K, Sato DK. AQP4 antibody serostatus: is its luster being lost in the management and pathogenesis of NMO. *Neurology* (2013) 81(14):1186–8. doi:10.1212/WNL.0b013e3182a6cc23
5. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. *Lancet Neurol* (2007) 6(9):805–15. doi:10.1016/S1474-4422(07)70216-8
6. Lana-Peixoto MA, Callegaro D. The expanded spectrum of neuromyelitis optica: evidences for a new definition. *Arq Neuropsiquiatr* (2012) 70(10):807–13. doi:10.1590/S0004-282X2012001000010
7. Amemiya S, Hamamoto M, Kumagai T, Ueda M, Katayama Y, Tanaka K. Neuromyelitis optica preceded by brain demyelinating episode. *J Neuroimaging* (2009) 19(3):263–5. doi:10.1111/j.1552-6569.2008.00288.x
8. Apiwattanakul M, Popescu BF, Matiello M, Weinshenker BG, Lucchinetti CF, Lennon VA, et al. Intractable vomiting as the initial presentation of neuromyelitis optica. *Ann Neurol* (2010) 68(5):757–61. doi:10.1002/ana.22121
9. Nagaishi A, Takagi M, Umemura A, Tanaka M, Kitagawa Y, Matsui M, et al. Clinical features of neuromyelitis optica in a large Japanese cohort: comparison between phenotypes. *J Neurol Neurosurg Psychiatry* (2011) 82(12):1360–4. doi:10.1136/jnnp-2011-300403
10. Guo Y, Lennon VA, Popescu BF, Grouse CK, Topel J, Milone M, et al. Autoimmune aquaporin-4 myopathy in neuromyelitis optica spectrum. *JAMA Neurol* (2014) 71(8):1025–9. doi:10.1001/jamaneurol.2014.775
11. Borruat FX. In vivo identification of morphologic retinal abnormalities in neuromyelitis optica. *Neurology* (2014) 82(2):188. doi:10.1212/WNL.0000000000000003
12. Kim W, Kim SH, Lee SH, Li XF, Kim HJ. Brain abnormalities as an initial manifestation of neuromyelitis optica spectrum disorder. *Mult Scler* (2011) 17(9):1107–12. doi:10.1177/1352458511404917
13. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* (1983) 33(11):1444–52. doi:10.1212/WNL.33.11.1444
14. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* (2006) 66(10):1485–9. doi:10.1212/01.wnl.0000216139.44259.74
15. Roman GC. Proposed diagnostic criteria and nosology of acute transverse myelitis. *Neurology* (2003) 60(4):730–1; author reply 730–1. doi:10.1212/WNL.60.4.730
16. Bourre B, Zéphir H, Ongagna JC, Cordonnier C, Collongues N, Debette S, et al. Long-term follow-up of acute partial transverse myelitis. *Arch Neurol* (2012) 69(3):357–62. doi:10.1001/archneurol.2011.949

17. Takahashi T, Miyazawa I, Misu T, Takano R, Nakashima I, Fujihara K, et al. Intractable hiccup and nausea in neuromyelitis optica with anti-aquaporin-4 antibody: a herald of acute exacerbations. *J Neurol Neurosurg Psychiatry* (2008) 79(9):1075–8. doi:10.1136/jnnp.2008.145391
18. Iorio R, Lucchinetti CF, Lennon VA, Farrugia G, Pasricha PJ, Weinshenker BG, et al. Intractable nausea and vomiting from autoantibodies against a brain water channel. *Clin Gastroenterol Hepatol* (2013) 11(3):240–5. doi:10.1016/j.cgh.2012.11.021
19. Wang KC, Lee CL, Chen SY, Lin KH, Tsai CP. Prominent brainstem symptoms/signs in patients with neuromyelitis optica in a Taiwanese population. *J Clin Neurosci* (2011) 18(9):1197–200. doi:10.1016/j.jocn.2010.12.052
20. Min JH, Waters P, Vincent A, Kang ES, Lee S, Lee DK, et al. Symptomatic brain involvement as the initial manifestation of neuromyelitis optica. *J Clin Neurosci* (2013) 20(7):938–42. doi:10.1016/j.jocn.2012.08.007
21. Kitley J, Leite MI, Nakashima I, Waters P, McNeillis B, Brown R, et al. Prognostic factors and disease course in aquaporin-4 antibody-positive patients with neuromyelitis optica spectrum disorder from the United Kingdom and Japan. *Brain* (2012) 135(Pt 6):1834–49. doi:10.1093/brain/aws109
22. Jarius S, Ruprecht K, Wildemann B, Kuempfel T, Ringelstein M, Geis C, et al. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: a multicentre study of 175 patients. *J Neuroinflammation* (2012) 9:14. doi:10.1186/1742-2094-9-14
23. McKeon A, Lennon VA, Lotze T, Tenenbaum S, Ness JM, Rensel M, et al. CNS aquaporin-4 autoimmunity in children. *Neurology* (2008) 71(2):93–100. doi:10.1212/01.wnl.0000314832.24682.c6
24. Kim SH, Kim W, Kook MC, Hong EK, Kim HJ. Central nervous system aquaporin-4 autoimmunity presenting with an isolated cerebral abnormality. *Mult Scler* (2012) 18(9):1340–3. doi:10.1177/1352458512441271
25. Ikeda K, Ito H, Hidaka T, Takazawa T, Sekine T, Yoshii Y, et al. Repeated non-enhancing tumefactive lesions in a patient with a neuromyelitis optica spectrum disorder. *Intern Med* (2011) 50(9):1061–4. doi:10.2169/internalmedicine.50.4295
26. Nishiyama S, Ito T, Misu T, Takahashi T, Kikuchi A, Suzuki N, et al. A case of NMO seropositive for aquaporin-4 antibody more than 10 years before onset. *Neurology* (2009) 72(22):1960–1. doi:10.1212/WNL.0b013e3181a82621
27. Numata Y, Uematsu M, Suzuki S, Miyabayashi T, Oyama T, Kubota S, et al. Aquaporin-4 autoimmunity in a child without optic neuritis and myelitis. *Brain Dev* (2015) 37(1):149–52. doi:10.1016/j.braindev.2014.03.015
28. Chinese doctors are under threat. *Lancet* (2010) 376(9742):657. doi:10.1016/S0140-6736(10)61315-3
29. Zamvil SS, Slavin AJ. Does MOG Ig-positive AQP4-seronegative opticospinal inflammatory disease justify a diagnosis of NMO spectrum disorder? *Neurol Neuroimmunol Neuroinflamm* (2015) 2(1):e62. doi:10.1212/NXI.0000000000000062

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 15 March 2017
doi: 10.3389/fneur.2017.00104

# Increased Circulating T Follicular Helper Cells Are Inhibited by Rituximab in Neuromyelitis Optica Spectrum Disorder

*Cong Zhao[1†], Hong-Zeng Li[1†], Dai-Di Zhao[1†], Chao Ma[2], Fang Wu[1,3], Ya-Nan Bai[1], Min Zhang[1], Zhu-Yi Li[1]* and Jun Guo[1]**

[1] Department of Neurology, Tangdu Hospital, Fourth Military Medical University, Xi'an, China, [2] Department of Cardiology, Tangdu Hospital, Fourth Military Medical University, Xi'an, China, [3] Department of Neurology, Xi'an Children's Hospital, Xi'an, China

Neuromyelitis optica spectrum disorder (NMOSD) is a severe autoimmune disease of the central nervous system. The existence of autoantibody targeting aquaporin-4 (AQP4-Ab) indicates the involvement of humoral immunity in the pathogenesis of this disease. Rituximab (RTX), a monoclonal antibody against CD20, has been used to treat NMOSD by depleting circulating B cells and overall satisfactory outcome has been achieved. Although T follicular helper cells have been proved to regulate B cell activation and antibody production, the role of these cells in NMOSD and the impact of RTX treatment on these cells remain less understood. In this study, we found that frequencies of circulating T follicular helper (cTfh) cells and B cells together with the related cytokines, IL-21 and IL-6, were closely correlated with disease activity of NMOSD. Furthermore, B cell depletion with RTX treatment inhibited the expansion of cTfh cells, and these effects were achieved through eliminating IL-6-producing B cells and blocking the direct contact between cTfh cells and B cells. These findings imply the complicated cross talk between cTfh cells and B cells and may provide a novel therapeutic target for NMOSD.

**Keywords: neuromyelitis optica spectrum disorder, circulating T follicular helper cells, rituximab, B cells, interleukin-6**

OPEN ACCESS

***Edited by:***
*Fabienne Brilot, University of Sydney, Australia*

***Reviewed by:***
*Gerd Meyer Zu Horste, Westfälische Wilhelms-University Münster, Germany*
*Jorge Correale, Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, Argentina*
*Yaping Yan, Tianjin Medical University General Hospital, China*

***Correspondence:**
*Jun Guo*
*guojun_81@163.com;*
*Zhu-Yi Li*
*lizhuyi@fmmu.edu.cn*

*[†]These authors have contributed equally to this work.*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology*

***Received:*** *16 October 2016*
***Accepted:*** *02 March 2017*
***Published:*** *15 March 2017*

***Citation:***
*Zhao C, Li H-Z, Zhao D-D, Ma C, Wu F, Bai Y-N, Zhang M, Li Z-Y and Guo J (2017) Increased Circulating T Follicular Helper Cells Are Inhibited by Rituximab in Neuromyelitis Optica Spectrum Disorder. Front. Neurol. 8:104. doi: 10.3389/fneur.2017.00104*

## INTRODUCTION

Neuromyelitis optica spectrum disorder (NMOSD) is a rare inflammatory demyelinating disease of the central nervous system (CNS) characterized by recurrent attacks of optic neuritis and transverse myelitis (1). In the past decades, NMOSD was believed to be a variant of multiple sclerosis (MS) based on the overlapping clinical and magnetic resonance imaging phenotypes. The identification of an autoantibody targeting aquaporin-4 (AQP4-Ab) has differentiated NMOSD from MS as an independent disease entity (2). AQP4-Ab is positive in more than half of patients with NMOSD, and its pathogenic role has been well demonstrated (3–5). Other antibodies against myelin antigens have also been reported in NMOSD, such as anti-MOG (6) and anti-MBP (7). Although the concrete role of these autoantibodies has not been clearly identified, recent evidence strongly suggested that humoral immunity would contribute to the pathology of NMOSD (8).

T follicular helper (Tfh) cells, as a newly defined $CD4^+$ T cell subset, are critical for B cell activation and differentiation (9, 10). Tfh cells constitutively express the chemokine receptor CXCR5, which allows them to migrate into germinal center (GC) (11) and provide costimulation signals, such as CD40L and IL-21, to B cells (12). Recently, circulating Tfh (cTfh) cells were identified to be the peripheral subsets of their GC counterparts (13). And an increased frequency of cTfh cells was observed in multiple antibody-mediated diseases (14–17).

Rituximab (RTX), which depletes circulating $CD20^+$ B cells, has emerged as the first-line immunosuppressant for treating NMOSD, though the precise mechanism still remains uncovered (18). In patients with MS, RTX treatment resulted in a significant decline of CNS-infiltrated T cells, which suggested this agent may also modulate T cell immune response besides depleting B cells (19). However in NMOSD, whether RTX exerts its therapeutic potential by regulating T cells, especially cTfh cells, still remains unclear.

In this study, we found that frequencies of cTfh cells and circulating B cells together with the related molecules were closely associated with the disease activity of NMOSD. Next, we firstly demonstrated that B cell depletion with RTX reduced the frequency of cTfh cells through ablation of IL-6 signaling and blockade of direct B–cTfh cell contact. The results strongly suggest the existence of B–cTfh cell interaction in NMOSD, which might provide a possible therapeutic target for this disease.

# PATIENTS AND METHODS

## Study Population

We enrolled patients with NMOSD from June 2015 to March 2016 who fulfilled the 2015 revised NMOSD diagnostic criteria (20) in our department. Qualitative serum AQP4 antibody (AQP4-Ab) assay was done by cell-based indirect immunofluorescence [EUROIMMUN Medical Diagnostics (China) Co., Ltd.]. Patients with new neurologic symptoms and signs or deterioration of residual disability lasting for at least 24 h with new lesions on MRI were determined as relapse. Patients with a stable clinical status for at least 30 days since the last relapse were considered as remission and enrolled in this study. Meanwhile, gender- and age-matched healthy volunteers were included as controls (healthy controls). The study was approved by the Tangdu Hospital Ethical Review Board of Fourth Military Medical University, and written informed consent was obtained from all the subjects.

## Sampling and Treatment

Blood samples were drawn from all the patients and HCs to collect peripheral blood mononuclear cells (PBMCs) and plasma for detecting cell frequencies and cytokine concentrations, respectively. Rituximab (RTX) treatment was carried out in the patients based on clinical status and patient's preference. Intravenous infusion of RTX at a fixed dose of 100 mg was performed once weekly for three consecutive weeks, as previously described (21). Blood samples were collected again 1 month after RTX treatment. Meanwhile, plasma AQP4-Ab levels were measured, respectively, before and 1 month after RTX treatment in seropositive patients.

## Flow Cytometry

Peripheral blood mononuclear cells were isolated by density gradient centrifugation as previously reported (17). After washed twice with phosphate-buffered saline (PBS), PBMCs were incubated with the following fluorochrome-conjugated monoclonal Abs: FITC-CD3, PerCP-Cy5.5-CD4, APC-CXCR5, PE-PD-1, PE-CD19, and relevant isotype controls (Biolegend, San Diego, CA, USA). After staining at 4°C for 30 min, PBMCs were washed twice with PBS containing 2% fetal bovine serum and then measured on a BD FACS Calibur instrument. cTfh cells were defined as $CD3^+CD4^+CXCR5^+PD\text{-}1^+$. Data were analyzed using the FlowJo 7.6 software.

## Detection of Cytokines and AQP4-Ab

Plasma levels of IL-21, IL-6, and IL-10 were measured with enzyme-linked immunosorbent assay (ELISA) (Biolegend for IL-21, and Dakewe for IL-6 and IL-10) according to the manufacturers' instructions. For seropositive patients, plasma AQP4-Ab levels were also measured by ELISA (Cusabio, Wuhan, China).

## Cell Sorting and Culturing

Circulating $CD4^+$ T cells and $CD19^+$ B cells were isolated from PBMCs by using specific magnetic beads (Miltenyi Biotec, Bergisch, Germany). To explore the role of B cells in maintenance of cTfh cells and the underlying mechanisms, $2 \times 10^5$ of whole or B cell-depleted PBMCs were stimulated with 1 μg/ml plate-bounded anti-CD3 (Biolegend, San Diego, CA, USA) and 1 μg/ml soluble anti-CD28 (Biolegend, San Diego, CA, USA) for 72 h, in presence or absence of IL-6-neutralizing mAb (5 μg/ml; Biolegend, San Diego, CA, USA), in a 96-well flat-bottom plate. In the transwell culture system, $2 \times 10^5$ of B cell-depleted PBMCs were cultured and stimulated with anti-CD3/CD28 in the lower chamber and autologous B cells were seeded into the inner chamber of 0.4 μm pore size (Merck Millipore, Billerica, MA, USA). Each coculture of cells was carried out in triplicate in the X-VIVO serum-free medium (Lonza, Basel, Switzerland) containing 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA).

## Coculture of Circulating $CD4^+$ T Cells and $CD19^+$ B Cells

To determine the potency of B cells in the maintenance of cTfh cells, $2 \times 10^5$ of $CD4^+$ T cells were cocultured with autologous $CD19^+$ B cells at different ratios (as indicated in **Figure 5**) for 72h in the presence of anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml). Frequencies of cTfh cells were measured with the markers CD3, CD4, CXCR5, and PD-1 on a BD FACS Calibur instrument.

## Statistical Analysis

Quantitative data are shown as means ± SEM, and categorical data are presented as number with percentage. Statistical analysis was performed using the SPSS19.0 software. Demographic and clinical characteristics among the relapsing patients, remitting patients, and HCs were compared with Fisher's exact test (gender, AQP4-Ab positive) and ANOVA (age, duration of disease). Multiple comparisons among the different groups were carried out with ANOVA for normally distributed data and with Kruskal–Wallis *H* non-parametric test for non-normally

distributed data. Comparison between pre- and post-RTX treatment was performed with Wilcoxon matched-pairs signed-rank test. Pearson's correlation test was used to measure the possible relationship between two variables of interest. A *P* value of less than 0.05 was considered as statistically significant.

# RESULTS

## Demographic and Clinical Characteristics of Patients with NMOSD and HCs

A total of 31 patients and 18 gender- and age-matched HCs were enrolled in this study, where NMOSD patients consisted of 15 relapsing and 16 remitting individuals. There were no difference found in the gender ratio and mean age among the relapsing patients, remitting patients, and HCs. A predominance of female was observed in both relapsing (93.3%) and remitting patients (93.8%) with a similar mean duration of disease (3.19 vs 4.00 months). Serum AQP4-Ab was positive in 24/31 (77.4%) patients. There were 11/15 (73.3%) relapsing patients and 13/16 (81.3%) remitting patients, respectively, positive for AQP4-Ab, with no significant intergroup difference seen (**Table 1**).

## Frequencies of cTfh Cells and B Cells Correlate with Disease Activity of NMOSD

Flow cytometry results showed that the frequency of cTfh cells in the relapsing patients with NMOSD was significantly higher than those in the remitting patients and HCs, while no difference existed between the latter groups, suggesting a correlation with disease activity of NMOSD (**Figures 1A,B**). Moreover, there was a similar tendency on the change of frequency of peripheral B cells (**Figures 1C,D**). A positive correlation was found between frequencies of cTfh cells and B cells among the patients with NMOSD (**Figure 1E**). Subsequently, we detected plasma AQP4-Ab levels in seropositive patients. No difference was found between the relapsing and remitting patients (**Figure 1F**). In addition, frequencies of both cTfh cells and B cells had no correlations with plasma AQP4-Ab level (**Figures 1G,H**).

**TABLE 1 | Demographic and clinical characteristics of patients with NMOSD and HCs.**

| | NMOSD | | HCs | *P* value |
|---|---|---|---|---|
| | Relapse | Remission | | |
| *n* | 15 | 16 | 18 | |
| Female, no. (%) | 14 (93.3) | 15 (93.8) | 16 (88.9) | 1.000 |
| Age (years) | 42.53 ± 3.16 | 47.19 ± 2.63 | 41.61 ± 2.81 | 0.343 |
| Duration of disease (months) | 3.19 ± 0.72 | 4.00 ± 0.79 | NA | 0.457 |
| AQP4-Ab positive, no. (%) | 11 (73.3) | 13 (81.3) | NA | 0.685 |

*Ab, antibody; AQP4, aquaporin-4; NMOSD, neuromyelitis optica spectrum disorders; HCs, healthy controls; NA, not applicable. Quantitative variables are presented as means ± SEM and categorical variables as number with percentages. Comparisons among the relapsing patients, remitting patients, and HCs were analyzed by ANOVA (age, duration of disease) and Fisher's exact test (gender ratio, AQP4-Ab positive). A P value of <0.05 was assumed as statistically significant.*

## Cytokines Concentration in Patients with NMOSD and HCs

Given the fact that IL-21 and IL-6 are pivotal regulators of humoral immune response and play a crucial role in Tfh cell differentiation, we evaluated the plasma levels of IL-21 and IL-6 by ELISA. There was a significant increase of plasma IL-21 and IL-6 levels in the relapsing patients with NMOSD compared with the remitting patients and HCs (**Figures 2A,B**), which was consistent with the changes of cTfh cells and B cells. Meanwhile, plasma level of IL-10, an anti-inflammatory cytokine, was also detected and a significant increase was found in the relapsing patients. Although there was a tendency of higher IL-10 levels in plasma of remitting patients than HCs, no significant difference was observed (Figure S1A in Supplementary Material). Correlation analysis revealed that plasma IL-21 level positively correlated with frequencies of both cTfh cells and B cells (**Figures 2C,D**). The same phenomenon was observed for IL-6 (**Figures 2F,G**) but not for IL-10 (Figures S1B,C in Supplementary Material). In addition, no correlation was found between plasma levels of IL-21, IL-6, and IL-10, respectively, and plasma AQP4-Ab levels (**Figures 2E,H**; Figure S1D in Supplementary Material).

## RTX Treatment Reduced cTfh Cells in Patients with NMOSD

RTX specifically depletes peripheral B cells and has been used as a first-line immunosuppressant for NMOSD. To further explore the possible effects of RTX on cTfh cells, eight seropositive patients with relapsing NMOSD enrolled in this study were treated with RTX in our center. The total lymphocyte counts in peripheral blood remained almost unchanged during RTX treatment (**Figure 3A**). All the patients responded well to RTX and circulating B cells were successfully depleted (**Figures 3B,C**; Table S1 in Supplementary Material). Furthermore, $CD4^+$ T cells decreased after RTX treatment (Figure S2 in Supplementary Material). Notably, the frequency of cTfh cells was significantly declined with RTX treatment (**Figure 3D**). Furthermore, a decreased tendency of plasma AQP4-Ab level was observed after RTX treatment but with no statistical significance (**Figure 3E**). Consistent with the change of cTfh cells, plasma levels of IL-21 and IL-6 were obviously decreased with RTX treatment (**Figures 3F,G**), while no significant alteration of plasma IL-10 level was observed between pre- and post-RTX treatment (**Figure 3H**). Meanwhile, we did *ex vivo* experiments which showed that B cells depletion could significantly reduce the frequency of IL-21-secreting $CD4^+$ T cells (i.e., Tfh cells). And the frequency of IL-17-secreting cells (i.e., Th17 cells) slightly decreased, whereas the frequency of the other $CD4^+$ T cell subsets was almost unchanged. This finding suggested that Tfh cells may be more sensitive to B cells depletion (Figure S3 in Supplementary Material).

## Both IL-6 and Direct B/cTfh Contact Were Essential for the Maintenance of cTfh Cells

It is known that Tfh cells promote B cell proliferation, activation, and differentiation. However, B-cell depletion with RTX in this study significantly decreased the frequency of cTfh cells. This

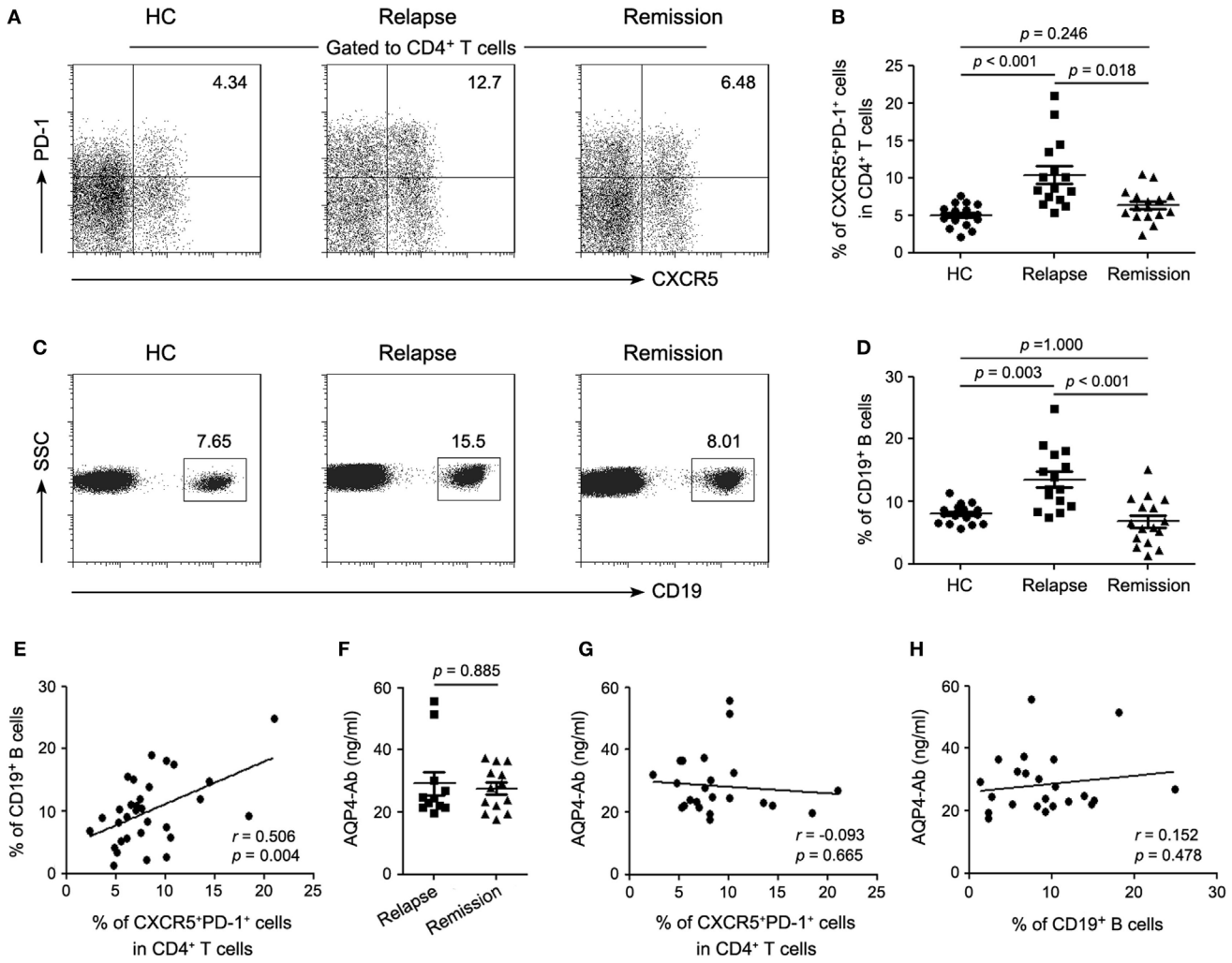



**FIGURE 1 | Frequencies of circulating T follicular helper (cTfh) cells and circulating CD19⁺ B cells correlate with disease activity of neuromyelitis optica spectrum disorder (NMOSD).** **(A)** Representative flow cytometry plots showing the frequency of CD4⁺CXCR5⁺PD-1⁺ cTfh cells in healthy controls (HCs), relapsing and remitting patients with NMOSD. **(B)** Comparison of the frequency of cTfh cells. **(C)** Representative flow cytometry plots gated on lymphocytes showing frequency of circulating CD19⁺ B cells. **(D)** Comparison of the frequency of circulating CD19⁺ B cells. **(E)** Correlation between frequencies of cTfh cells and circulating CD19⁺ B cells. **(F)** Comparison of plasma AQP4-Ab level between the relapsing and remitting seropositive patients with NMOSD. **(G)** Correlation between the frequency of cTfh cells and plasma AQP4-Ab level. **(H)** Correlation between the frequency of circulating CD19⁺ B cells and plasma AQP4-Ab level. Each symbol, including solid circle, square, and triangle, represents one subject's result. Horizontal lines in panels **(B,D,F)** illustrate the mean frequencies or levels with SEM. *P* values are shown.

suggested that B cells might contribute to the maintenance of cTfh cells reciprocally. To verify this hypothesis, PBMCs from patients with NMOSD were cultured *ex vivo* upon stimulation with anti-CD3/CD28, in parallel, B cell-depleted PBMCs were cultured to mimic RTX treatment. We observed a significantly reduced frequency of cTfh cells in B cell-depleted group (**Figures 4A,C**). To verify whether B cells directly affect the frequency of cTfh cells, we cocultured CD4⁺ T cells with B cells in different ratios under anti-CD3/CD28 stimulation and found that cTfh cells were maintained by B cells in a ratio-dependent manner (**Figure 5**), which suggested the direct supporting function of B cells.

In addition to the rise of plasma IL-6 level in relapsing patients (**Figure 2B**) and its positive correlation with circulating B cells in NMOSD (**Figure 2G**), we further observed an elevated mRNA level of IL-6 in B cells from the relapsing patients (Figure S4 in Supplementary Material), suggesting that B cells might maintain cTfh population through secretion of IL-6. As expected, IL-6 blockade indeed obviously reduced the frequency of cTfh cells in the culture system of PBMCs even though B cells were not depleted (**Figures 4A,C**). Besides, lack of direct contact between B cells and cTfh cells in a transwell culture system also led to a significant reduction of the frequency of cTfh cells (**Figures 4A,C**). Furthermore, all of the B-cell depletion, IL-6 blockade, and transwell experiments significantly reduced the protein level of CXCR5 and PD-1 on cTfh cells, as shown by the mean fluorescence intensity (MFI) in **Figure 4B**.

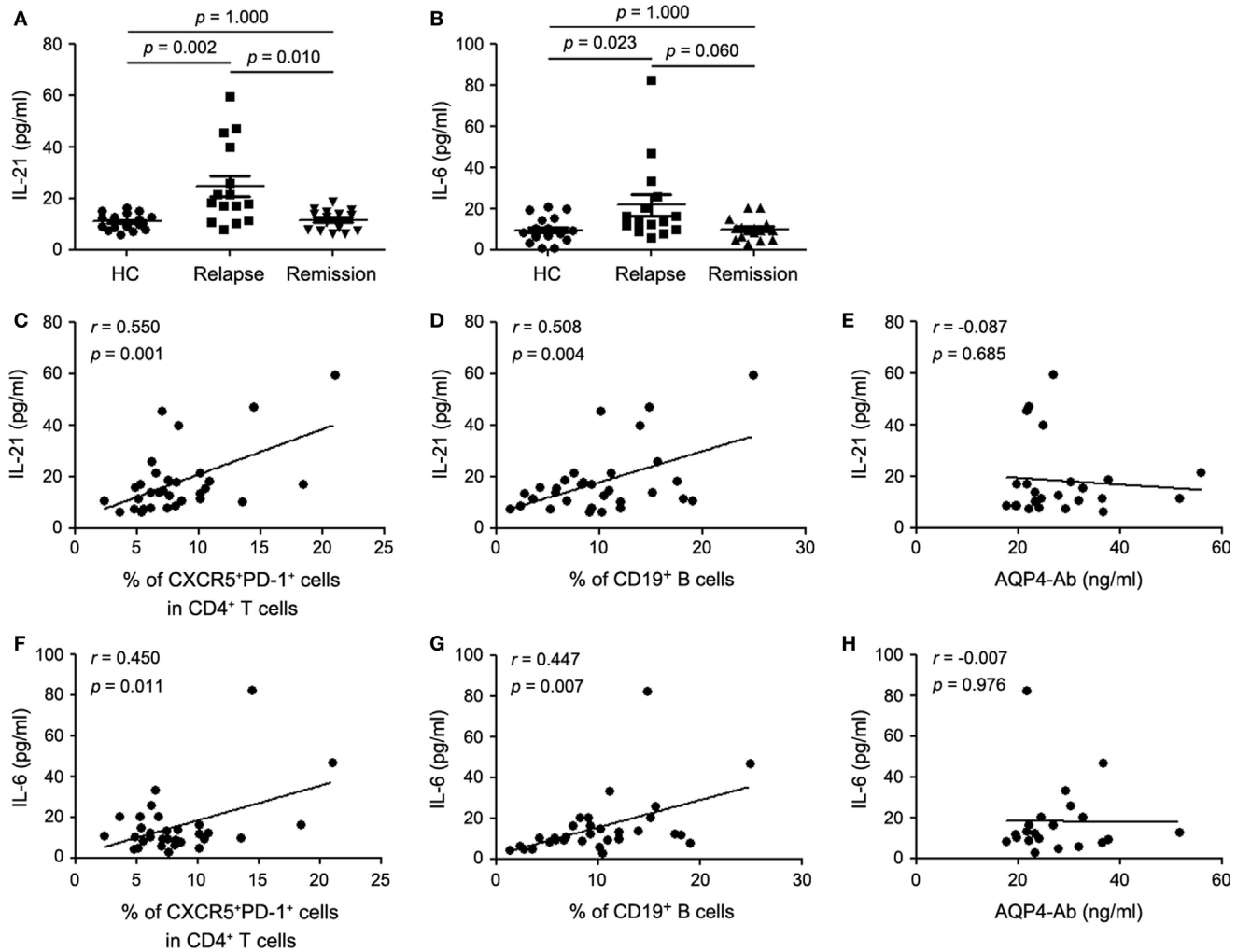



**FIGURE 2 | Plasma cytokine levels in healthy controls (HCs), the relapsing and remitting patients with neuromyelitis optica spectrum disorder (NMOSD)**. **(A)** Comparison of plasma IL-21 level. **(B)** Comparison of plasma IL-6 level. **(C)** Correlation between plasma IL-21 level and the frequency of circulating T follicular helper (cTfh) cells in all enrolled patients with NMOSD. **(D)** Correlation between plasma IL-21 level and the frequency of circulating $CD19^+$ B cells in all enrolled patients with NMOSD. **(E)** Correlation between plasma IL-21 level and AQP4-Ab in seropositive patients with NMOSD. **(F)** Correlation between plasma IL-6 level and the frequency of cTfh cells in all enrolled patients with NMOSD. **(G)** Correlation between plasma IL-6 level and the frequency of circulating $CD19^+$ B cells in all enrolled patients with NMOSD. **(H)** Correlation between plasma level of IL-6 AQP4-Ab in seropositive patients with NMOSD. Each symbol represents one subject's result. Horizontal lines in panel **(C)** illustrate the mean frequencies with SEM. *P* values are shown.

## DISCUSSION

T follicular helper cells have been identified as the most potent regulator of humoral immunity. Given the location of Tfh cells in the GC of secondary lymphoid organs, it is extremely hard to be obtained from patients routinely. Morita and colleagues have demonstrated that circulating $CD4^+CXCR5^+$ T cells appeared to be the memory subset of GC Tfh pool and had the same capacity to regulate B cells (13), which facilitated researchers to explore the role of these cells in human diseases. IL-21 and IL-6 are two major cytokines in regulating GC response, including Tfh cell proliferation and differentiation, B cell activation, and antibody production (22). A lack of both IL-6 and IL-21 fails to induce Tfh cell-dependent immune response; moreover, activated Tfh cells can produce a considerable amount of IL-21 (22). In accordance with a previous report (23), herein, we found that the frequency of cTfh cells as well as plasma levels of IL-21 and IL-6 were significantly upregulated in relapsing patients with NMOSD but not in remitting patients, compared with those in HCs. Both IL-21 and IL-6 were positively correlated with the frequency of cTfh cells. These results suggested that cTfh cells might be involved in the pathogenesis of NMOSD and the frequency of cTfh cells, together with plasma levels of IL-21 and IL-6, could be used as biomarkers for monitoring disease activity of NMOSD.

T follicular helper cells play the central role in helping B cells activation and differentiation, which is protective against infection. However, overactivity of Tfh response could manifest as many immune-related disorders, such as autoimmunity (24). The

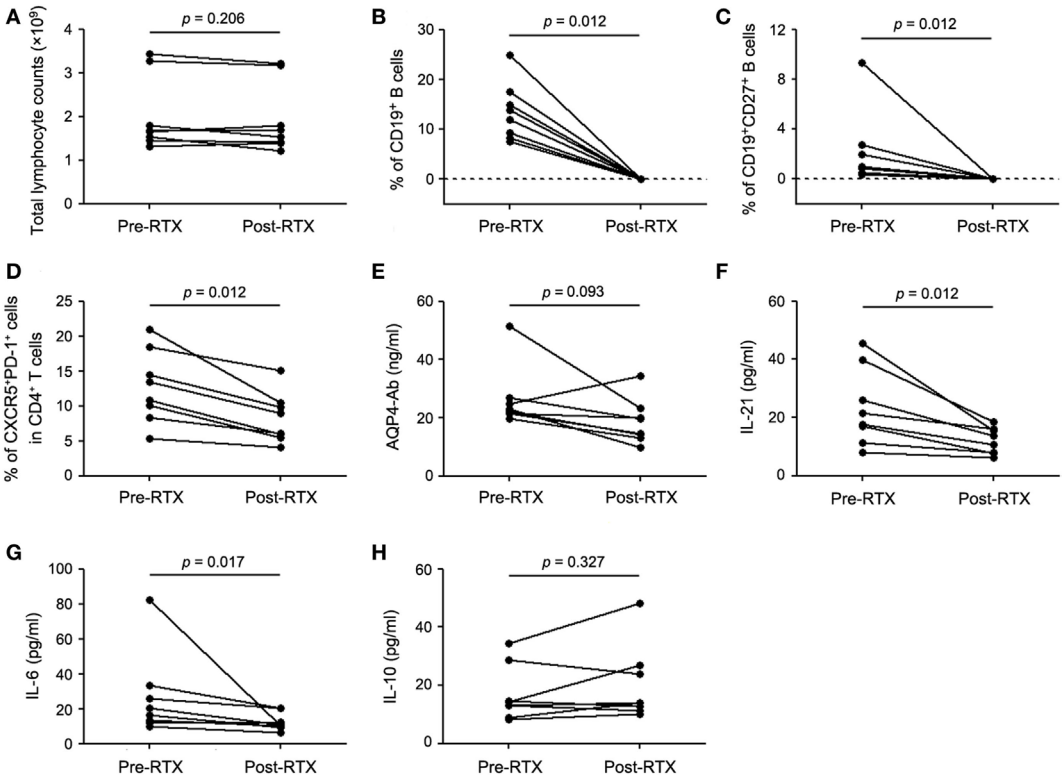



**FIGURE 3 | B-cell depletion with RTX decreases the frequency of circulating T follicular helper (cTfh) cells and plasma levels of IL-21 and IL-6.** **(A)** Comparison of total lymphocyte counts between pre- and post-RTX treatment. **(B)** Comparison of the frequency of circulating CD19⁺ B cells. **(C)** Comparison of the frequency of CD19⁺CD27⁺ memory B cells. **(D)** Comparison of the frequency of cTfh cells. **(E)** Comparison of plasma AQP4-Ab level. **(F)** Comparison of plasma IL-21 level. **(G)** Comparison of plasma IL-6 level. **(H)** Comparison of plasma IL-10 level. Line represents the changes of the frequency of specific cells, plasma AQP4-Ab, and cytokine levels before and after RTX treatment. *P* values are shown.

frequency of Tfh cells, together with the titer of autoantibodies, was found to elevate in many autoimmune disorders, both in animal models and human (14–17, 25–28). In this study, although no correlation between plasma AQP4-Ab level and disease activity of NMOSD was observed, the frequencies of circulating B cells and cTfh cells were synchronously upregulated in relapsing patients and positively correlated with each other. However, in our study, no correlation of AQP4-Ab titers with disease activity is observed, which is consistent with previous studies (29). This implies that cTfh cells might promote NMOSD by activating B cells to secrete cytokines other than producing antibodies, a hypothesis that needs further investigation.

B cells might contribute to autoimmunity *via* multiple ways, including antigen presentation, cytokine secretion, and antibody production. Depleting B cells with RTX is an effective approach to treat autoimmune disease, though the underlying mechanisms still remain debatable. In this study, circulating B cells were successfully depleted even by a reduced dose of RTX, which was consistent with a previous study (21). However, RTX treatment could only moderately reduce plasma level of AQP4-Ab, probably attributed to the fact that RTX removed CD20⁺ B cells but spared CD20⁻ antibody-producing plasma cells. Likewise, several studies reported that RTX treatment was effective for NMOSD (29) and other autoimmune diseases even in patients who showed no decline of autoantibody titers (30, 31), or the benefit of RTX treatment usually preceded the drops of antibody levels (30). All the findings suggested that B cells could contribute to the pathology through mechanisms other than antibody production.

Interestingly, we found that CD4⁺ T cells declined after RTX treatment. Given the multiple effects of B cells on T cell function, we think that RTX treatment may indeed have a non-specific effect on the frequency of the entire CD4⁺ T cells. Due to the fact that Tfh cells are the true activator of humoral immunity, our study focused on the impact of RTX treatment on cTfh cells and its related mechanism. We found that RTX treatment also significantly reduced the frequency of cTfh cells in patients with NMOSD. Other studies have also reported this phenomenon in some other autoimmune diseases (32, 33), but they did not elaborate the concrete mechanisms involved. We then further tried to explore the potential mechanisms *via* which B cells regulate Tfh response. A candidate factor may be IL-6, since it has been proved to play a vital role in the differentiation of Tfh cells (34). A study showed that B cells could secret an abundance of IL-6 and exhibited pathogenic effects in experimental autoimmune encephalomyelitis, an animal model for MS (35). Furthermore, circulating plasmablasts could induce the differentiation of Tfh cells *via* producing IL-6 in patients with rheumatoid arthritis, and

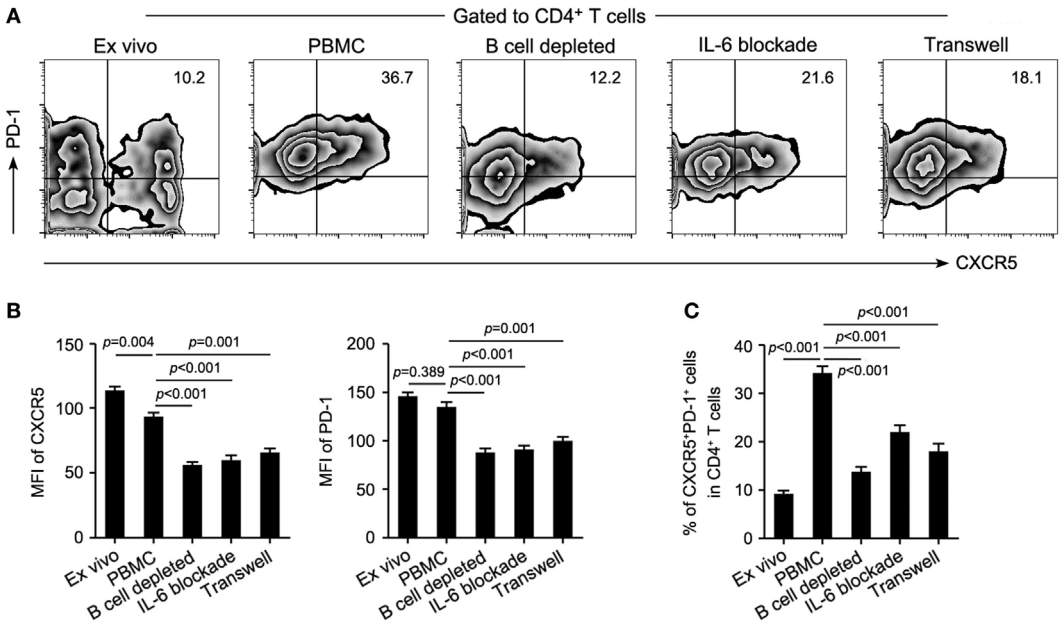



**FIGURE 4** | **Both IL-6 production and direct cell–cell contact are necessary for the maintenance of circulating T follicular helper (cTfh) cells by B cells**. B cell-depleted and whole peripheral blood mononuclear cells (PBMCs) from patients with neuromyelitis optica spectrum disorder were cultured upon stimulation with anti-CD3/CD28 for 72 h ($n = 4$), then the frequency of cTfh cells in the coculture system was measured. **(A)** Representative flow cytometry plots showing the frequency of T follicular helper cells of indicated cultured conditions. **(B)** Mean fluorescence intensity (MFI) of CXCR5 (left) and PD-1 (right) of $CD4^+CXCR5^+PD\text{-}1^+$ T cells in each group. **(C)** Comparison of the frequency of cTfh cells in panel **(A)**. *P* values are shown.

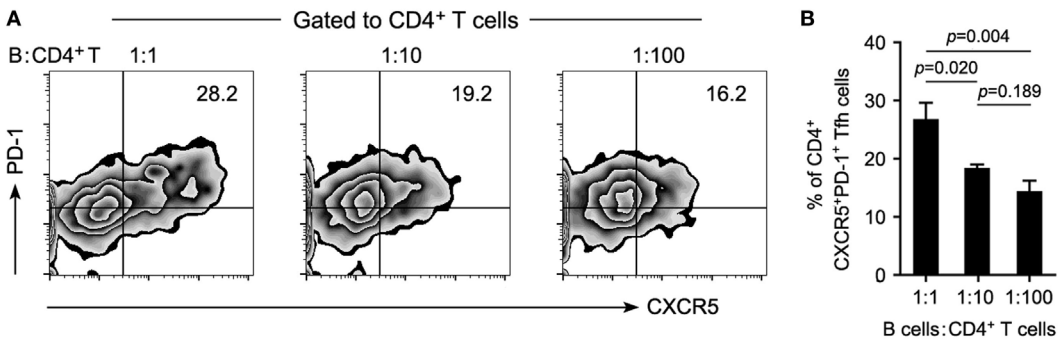



**FIGURE 5** | **Reduced B cells attenuate the maintenance of T follicular helper (Tfh) cells in a ratio-dependent manner**. Circulating $CD19^+$ B cells were cultured with $CD4^+$ T cells on different ratios of 1:1, 1:10, and 1:100 and stimulated with anti-CD3/CD28 for 72 h. Maintenance of Tfh cells by B cells was estimated by flow cytometry. **(A)** Representative flow cytometry plots showing the frequency of Tfh cells. **(B)** Cumulative data showing the frequency of Tfh cells ($n = 4$ for each group). A reduced ratio of B cells in the coculture system was accompanied by the gradually attenuated maintenance of Tfh cells. *P* values are shown. Representative data are from three independent experiments.

IL-6 blockade reduced the population of Tfh cells (36). Indeed, we observed a significant increase of plasma IL-6 level in patients with NMOSD and further demonstrated an elevated mRNA level of IL-6 in B cells, which suggested that B cells might be an important origin of IL-6. B cell depletion, both *ex vivo* and *in vivo*, decreased cTfh cells from patients with NMOSD, and this effect was achieved in a ratio-dependent manner *ex vivo*. Meanwhile, plasma IL-6 level was markedly decreased with RTX treatment in parallel with the change of frequency of B cells. Further study showed that IL-6 was required for the maintenance of cTfh cells by B cells, since blockade of IL-6 reduced the frequency of cTfh cells in the coculture system. Moreover, the intimate contact between B cells and cTfh cells was also essential for the survival of cTfh cells as proved by a transwell culture system. This contact-dependent maintenance of Tfh cells by B cells in this disease could be achieved by the crosslink of ICOS–ICOSL (37), OX40–OX40L (38), and MHC-II–TCR (39, 40) on the surface of these cells. But the central molecule involved in the cell–cell contact in this disease still needs a further research. Previous studies have reported that IL-6 and these costimulatory molecules were able to stimulate the

expression of CXCR5 and PD-1 (12, 41), and we also observed the reduction of MFI on CXCR5 and PD-1 in the *ex vivo* experiments. This suggested that the decrease of cTfh frequency might be achieved gradually by reducing the expression level of CXCR5 and PD-1. Taken together, our data raised a potential positive feedback loop between B and cTfh cells, namely, that circulating B cells maintain cTfh cells, and cTfh cells in turn activate B cells to persistently produce more IL-6 and costimulation molecules. This loop could be interrupted by RTX treatment, which revealed a new mechanism of RTX in treating NMOSD.

In conclusion, this study demonstrated that cTfh cells, circulating B cells, and associated molecules might play an important role in the pathogenesis of NMOSD. B cell depletion with RTX could reduce the frequency of cTfh cells through eliminating IL-6 signaling and blocking the direct B–cTfh cell contact. All the findings may provide an insight into the complicated cross talk between B cells and cTfh cells in NMOSD.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "the Biomedical Research Guideline involving Human Participants, National Health and Family Planning Commission of China" with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the "Tangdu Hospital Ethical Review Board of Fourth Military Medical University."

## AUTHOR CONTRIBUTIONS

JG and Z-YL designed the research. CZ performed the flow cytometry, cell cultures, and ELISA and drafted the manuscript. H-ZL and D-DZ took care of and followed up the patients, and helped revise the manuscript. FW and CM did the cell sorting. Y-NB and MZ performed the quantitative PCR. All of the authors read and approved the publication.

## ACKNOWLEDGMENTS

The authors thank Ms. Yongri Ouyang and Wen Fan for their excellent technique assistant. The authors gratefully thank Dr. Hui Liu and Zi-Peng Cao for their helps in revising and polishing this article.

## FUNDING

This study was supported by the Natural Science Basic Research Plan in Shaanxi Province of China (program no. 2016JM8033) and the National Natural Science Foundation of China (program no. 81301022).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00104/full#supplementary-material.

## REFERENCES

1. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. *Lancet Neurol* (2007) 6(9):805–15. doi:10.1016/S1474-4422(07)70216-8
2. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
3. Kinoshita M, Nakatsuji Y, Moriya M, Okuno T, Kumanogoh A, Nakano M, et al. Astrocytic necrosis is induced by anti-aquaporin-4 antibody-positive serum. *Neuroreport* (2009) 20(5):508–12. doi:10.1097/WNR.0b013e32832776f4
4. Saadoun S, Waters P, Bell BA, Vincent A, Verkman AS, Papadopoulos MC. Intra-cerebral injection of neuromyelitis optica immunoglobulin G and human complement produces neuromyelitis optica lesions in mice. *Brain* (2010) 133(Pt 2):349–61. doi:10.1093/brain/awp309
5. Uzawa A, Mori M, Arai K, Sato Y, Hayakawa S, Masuda S, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. *Mult Scler* (2010) 16(12):1443–52. doi:10.1177/1352458510379247
6. Kitley J, Woodhall M, Waters P, Leite MI, Devenney E, Craig J, et al. Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. *Neurology* (2012) 79(12):1273–7. doi:10.1212/WNL.0b013e31826aac4e
7. Haase CG, Schmidt S. Detection of brain-specific autoantibodies to myelin oligodendrocyte glycoprotein, S100beta and myelin basic protein in patients with Devic's neuromyelitis optica. *Neurosci Lett* (2001) 307(2):131–3. doi:10.1016/S0304-3940(01)01949-8
8. Krumbholz M, Meinl E. B cells in MS and NMO: pathogenesis and therapy. *Semin Immunopathol* (2014) 36(3):339–50. doi:10.1007/s00281-014-0424-x
9. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* (2000) 192(11):1545–52. doi:10.1084/jem.192.11.1545
10. Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. *J Exp Med* (2001) 193(12):1373–81. doi:10.1084/jem.193.12.1373
11. Rasheed AU, Rahn HP, Sallusto F, Lipp M, Muller G. Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression. *Eur J Immunol* (2006) 36(7):1892–903. doi:10.1002/eji.200636136
12. Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* (2011) 29:621–63. doi:10.1146/annurev-immunol-031210-101400
13. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* (2011) 34(1):108–21. doi:10.1016/j.immuni.2011.01.009
14. Ma J, Zhu C, Ma B, Tian J, Baidoo SE, Mao C, et al. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clin Dev Immunol* (2012) 2012:827480. doi:10.1155/2012/827480
15. Zhu C, Ma J, Liu Y, Tong J, Tian J, Chen J, et al. Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. *J Clin Endocr Metab* (2012) 97(3):943–50. doi:10.1210/jc.2011-2003
16. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum* (2010) 62(1):234–44. doi:10.1002/art.25032
17. Zhang X, Liu S, Chang T, Xu J, Zhang C, Tian F, et al. Intrathymic Tfh/B cells interaction leads to ectopic GCs formation and anti-AChR antibody production: central role in triggering MG occurrence. *Mol Neurobiol* (2016) 53(1):120–31. doi:10.1007/s12035-014-8985-1
18. Papadopoulos MC, Bennett JL, Verkman AS. Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. *Nat Rev Neurol* (2014) 10(9):493–506. doi:10.1038/nrneurol.2014.141

19. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol* (2006) 180(1–2):63–70. doi:10.1016/j.jneuroim.2006.06.029
20. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* (2015) 85(2):177–89. doi:10.1212/WNL.0000000000001729
21. Yang CS, Yang L, Li T, Zhang DQ, Jin WN, Li MS, et al. Responsiveness to reduced dosage of rituximab in Chinese patients with neuromyelitis optica. *Neurology* (2013) 81(8):710–3. doi:10.1212/WNL.0b013e3182a1aac7
22. Eto D, Lao C, DiToro D, Barnett B, Escobar TC, Kageyama R, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. *PLoS One* (2011) 6(3):e17739. doi:10.1371/journal.pone.0017739
23. Li YJ, Zhang F, Qi Y, Chang GQ, Fu Y, Su L, et al. Association of circulating follicular helper T cells with disease course of NMO spectrum disorders. *J Neuroimmunol* (2015) 278:239–46. doi:10.1016/j.jneuroim.2014.11.011
24. Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly – TFH cells in human health and disease. *Nat Rev Immunol* (2013) 13(6):412–26. doi:10.1038/nri3447
25. Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C, et al. ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. *J Exp Med* (2008) 205(12):2873–86. doi:10.1084/jem.20080840
26. Kenefeck R, Wang CJ, Kapadi T, Wardzinski L, Attridge K, Clough LE, et al. Follicular helper T cell signature in type 1 diabetes. *J Clin Invest* (2015) 125(1):292–303. doi:10.1172/JCI76238
27. Silva DG, Daley SR, Hogan J, Lee SK, Teh CE, Hu DY, et al. Anti-islet autoantibodies trigger autoimmune diabetes in the presence of an increased frequency of islet-reactive CD4 T cells. *Diabetes* (2011) 60(8):2102–11. doi:10.2337/db10-1344
28. Romme Christensen J, Bornsen L, Ratzer R, Piehl F, Khademi M, Olsson T, et al. Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. *PLoS One* (2013) 8(3):e57820. doi:10.1371/journal.pone.0057820
29. Pellkofer HL, Krumbholz M, Berthele A, Hemmer B, Gerdes LA, Havla J, et al. Long-term follow-up of patients with neuromyelitis optica after repeated therapy with rituximab. *Neurology* (2011) 76(15):1310–5. doi:10.1212/WNL.0b013e3182152881
30. Levesque MC, St Clair EW. B cell-directed therapies for autoimmune disease and correlates of disease response and relapse. *J Allergy Clin Immunol* (2008) 121(1):13–21; quiz 2–3. doi:10.1016/j.jaci.2007.11.030
31. Liossis SN, Sfikakis PP. Rituximab-induced B cell depletion in autoimmune diseases: potential effects on T cells. *Clin Immunol* (2008) 127(3):280–5. doi:10.1016/j.clim.2008.01.011
32. Zhao Y, Lutalo PM, Thomas JE, Sangle S, Choong LM, Tyler JR, et al. Circulating T follicular helper cell and regulatory T cell frequencies are influenced by B cell depletion in patients with granulomatosis with polyangiitis. *Rheumatology* (2014) 53(4):621–30. doi:10.1093/rheumatology/ket406
33. Xu X, Shi Y, Cai Y, Zhang Q, Yang F, Chen H, et al. Inhibition of increased circulating Tfh cell by anti-CD20 monoclonal antibody in patients with type 1 diabetes. *PLoS One* (2013) 8(11):e79858. doi:10.1371/journal.pone.0079858
34. Nish SA, Schenten D, Wunderlich FT, Pope SD, Gao Y, Hoshi N, et al. T cell-intrinsic role of IL-6 signaling in primary and memory responses. *Elife* (2014) 3:e01949. doi:10.7554/eLife.01949
35. Barr TA, Shen P, Brown S, Lampropoulou V, Roch T, Lawrie S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J Exp Med* (2012) 209(5):1001–10. doi:10.1084/jem.20111675
36. Chavele KM, Merry E, Ehrenstein MR. Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. *J Immunol* (2015) 194(6):2482–5. doi:10.4049/jimmunol.1401190
37. Stone EL, Pepper M, Katayama CD, Kerdiles YM, Lai CY, Emslie E, et al. ICOS coreceptor signaling inactivates the transcription factor FOXO1 to promote Tfh cell differentiation. *Immunity* (2015) 42(2):239–51. doi:10.1016/j.immuni.2015.01.017
38. Jacquemin C, Schmitt N, Contin-Bordes C, Liu Y, Narayanan P, Seneschal J, et al. OX40 ligand contributes to human lupus pathogenesis by promoting T follicular helper response. *Immunity* (2015) 42(6):1159–70. doi:10.1016/j.immuni.2015.05.012
39. Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, et al. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* (2010) 33(2):241–53. doi:10.1016/j.immuni.2010.07.015
40. Molnarfi N, Schulze-Topphoff U, Weber MS, Patarroyo JC, Prod'homme T, Varrin-Doyer M, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* (2013) 210(13):2921–37. doi:10.1084/jem.20130699
41. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, et al. Bcl6 mediates the development of T follicular helper cells. *Science* (2009) 325(5943):1001–5. doi:10.1126/science.1176676


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 08 May 2017
doi: 10.3389/fimmu.2017.00529

# Myelin Oligodendrocyte Glycoprotein: Deciphering a Target in Inflammatory Demyelinating Diseases

*Patrick Peschl[1], Monika Bradl[2], Romana Höftberger[3], Thomas Berger[1] and Markus Reindl[1]**

[1] Clinical Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria, [2] Department of Neuroimmunology, Center for Brain Research, Medical University of Vienna, Vienna, Austria, [3] Institute of Neurology, Medical University of Vienna, Vienna, Austria

Myelin oligodendrocyte glycoprotein (MOG), a member of the immunoglobulin (Ig) superfamily, is a myelin protein solely expressed at the outermost surface of myelin sheaths and oligodendrocyte membranes. This makes MOG a potential target of cellular and humoral immune responses in inflammatory demyelinating diseases. Due to its late postnatal developmental expression, MOG is an important marker for oligodendrocyte maturation. Discovered about 30 years ago, it is one of the best-studied autoantigens for experimental autoimmune models for multiple sclerosis (MS). Human studies, however, have yielded controversial results on the role of MOG, especially MOG antibodies (Abs), as a biomarker in MS. But with improved detection methods using different expression systems to detect Abs in patients' samples, this is meanwhile no longer the case. Using cell-based assays with recombinant full-length, conformationally intact MOG, several recent studies have revealed that MOG Abs can be found in a subset of predominantly pediatric patients with acute disseminated encephalomyelitis (ADEM), aquaporin-4 (AQP4) seronegative neuromyelitis optica spectrum disorders (NMOSD), monophasic or recurrent isolated optic neuritis (ON), or transverse myelitis, in atypical MS and in *N*-methyl-D-aspartate receptor-encephalitis with overlapping demyelinating syndromes. Whereas MOG Abs are only transiently observed in monophasic diseases such as ADEM and their decline is associated with a favorable outcome, they are persistent in multiphasic ADEM, NMOSD, recurrent ON, or myelitis. Due to distinct clinical features within these diseases it is controversially disputed to classify MOG Ab-positive cases as a new disease entity. Neuropathologically, the presence of MOG Abs is characterized by MS-typical demyelination and oligodendrocyte pathology associated with Abs and complement. However, it remains unclear whether MOG Abs are a mere inflammatory

OPEN ACCESS

**Edited by:**
*Robert Weissert, University of Regensburg, Germany*

**Reviewed by:**
*Tomas Per Olsson, Karolinska Institutet, Sweden*
*H.-Christian Von Büdingen, University of California San Francisco, USA*

***Correspondence:**
*Markus Reindl*
*markus.reindl@i-med.ac.at*

**Specialty section:**
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

**Received:** *12 December 2016*
**Accepted:** *19 April 2017*
**Published:** *08 May 2017*

**Citation:**
*Peschl P, Bradl M, Höftberger R, Berger T and Reindl M (2017) Myelin Oligodendrocyte Glycoprotein: Deciphering a Target in Inflammatory Demyelinating Diseases. Front. Immunol. 8:529. doi: 10.3389/fimmu.2017.00529*

**Abbreviations:** AA, amino acids; Ab, antibody; ADEM, acute disseminated encephalomyelitis; AQP4, aquaporin-4; BN, brown Norway; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dark agouti; EAE, experimental autoimmune encephalomyelitis; HLA, human leukocyte antigen; Ig, immunoglobulin; MBP, myelin basic protein; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorders; ON, optic neuritis; TM, transverse myelitis.

bystander effect or truly pathogenetic. This article provides deeper insight into recent developments, the clinical relevance of MOG Abs and their role in the immunpathogenesis of inflammatory demyelinating disorders.

**Keywords: myelin oligodendrocyte glycoprotein, demyelination, autoantibodies, inflammation, MOG**

## MOLECULAR STRUCTURE AND FUNCTION OF MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG)

Myelin oligodendrocyte glycoprotein is a minor myelin component, with a length of 245 amino acids (AA) and a molecular weight of 26–28 kDa. It is only present in mammals and has a highly conserved nucleotide and AA structure within different species (1). The human MOG gene is located at chromosome 6 within the human leukocyte antigen (HLA) gene locus, whereas the mouse MOG gene is located on chromosome 17 within the major histocompatibility complex (MHC) gene locus (2). MOG is exclusively expressed in the central nervous system (CNS) on the surface of myelin sheaths and oligodendrocyte processes (1–3). MOG expression starts at the onset of myelination and is therefore a potential differentiation marker for oligodendrocyte maturation (4). The function of MOG is not yet fully understood, but its molecular structure and its extracellular immunoglobulin (Ig) domain indicate a possible function as a cell surface receptor or cell adhesion molecule (5). MOG belongs to the Ig superfamily, with a single extracellular immunoglobuline variable (IgV) domain, one transmembrane domain, one cytoplasmic loop, a membrane-associated region, and a cytoplasmic tail (6). Fifteen different alternatively spliced isoforms have been detected in humans. Full-length variants alpha 1 and beta 1 are found in fetal stages, whereas alternative variants are expressed in later postnatal stages (1, 6). It has been shown, that these isoforms are localized on the cell surface, in the endoplasmic reticulum, in the endocytic system, or can be found in a secreted form. The secreted form could have important effects triggering autoimmunity if released into the cerebrospinal fluid (CSF) and then drained into the periphery. The cytoplasmic tail of MOG determines the intracellular localization of the various splice forms and could play a role in intracellular signaling (6). The cross-linking of antibodies (Abs) reactive with the extracellular domain of MOG resulted in the activation of intracellular signaling cascades resulting in survival signals, changes of cytoskeletal stability, and cellular stress responses (7). MOG is highly homologous to butyrophilins which are expressed in mammary glands (8) and might cause autoimmunity by molecular mimicry (9). Furthermore, a sequence homology of MOG AA 35–55 ($MOG_{35–55}$) to medium-sized neurofilament leads to the activation of $MOG_{35–55}$ specific T cells (10).

Myelin oligodendrocyte glycoprotein has been implicated to be the cellular receptor for Rubella virus (11), as a ligand for DC-SIGN on antigen-expressing cells (12), and as a receptor for nerve growth factor (13). The interaction of DC-SIGN and MOG along with its correct glycosylation might keep myeloid antigen-presenting cells (APC) in an immature and tolerogenic state and thereby prevent autoimmunity (12). However, the inactivation of mouse MOG by gene targeting resulted in no clinical or histological abnormalities (14, 15).

Whereas the biological function of MOG is still not clear, its topology at the surface of myelin and oligodendrocytes and its special characteristics predict MOG to be a very important target of autoantibodies and cell-mediated immune responses in inflammatory demyelinating diseases. Initially, MOG was discovered as a dominant target of autoantibodies (they so called it M2 antigen) after immunization of guinea pigs with CNS tissue (16, 17). Numerous studies have then established an important role of MOG as autoantigen for T and B cell responses in experimental models and inflammatory demyelinating diseases.

## AUTOIMMUNE RESPONSES AGAINST MOG IN ANIMAL MODELS

The first indications that humoral factors also contribute to demyelination have been described in 1947 by Kabat et al. who observed a demyelinating effect after immunization of rhesus monkeys with heterologous rabbit or homologous brain tissue (18). In 1968, it was noted that sera from guinea pigs sensitized with whole CNS preparations have a demyelinating effect *in vitro* (19). The first indication that MOG Abs might be pathogenic followed about 10 years later when it was observed that guinea pigs immunized with the M2 protein developed Abs with demyelinating activity *in vitro* (20, 21). Then, it was shown that the monoclonal MOG-specific Ab 8–18C5 induces demyelination in Lewis (LEW) rats with experimental autoimmune encephalomyelitis (EAE) (4, 22), that guinea pigs immunized with M2 show demyelinated lesions in their CNS, and that the M2 protein is identical to MOG (16). It soon became clear that MOG Abs may be pathogenic in a large number of additional species (**Table 1**) (23). Further characterizations of MOG revealed that this protein is found in the oligodendrocyte membrane with a large N-terminal extracellular IgG V-like domain (8) and that N-terminal domain (AA 1–125) is responsible for the formation of demyelinating Abs (23, 24). Studies in marmoset monkeys and mice clarified that pathogenic Abs recognize conformational epitopes on the extracellularly exposed MOG domain (25–27) and that strain specific differences in mounting such anti-conformational Ab responses correlate with exacerbation of diseases (28, 29). Epitopes for encephalitogenic T cells for many different strains of mice and for LEW rats are found on the extracellular domain of MOG (30–33), but also in its transmembrane region (34, 35) (**Table 2**).

Immunizations of LEW rats with MOG activates $MOG_{1–20}$- and $MOG_{35–55}$- specific T cells which are only poorly encephalitogenic (24) and induces MOG-specific Abs which cause formation of

**TABLE 1 | The effects of myelin oligodendrocyte glycoprotein (MOG)-specific antibodies (Abs).**

| Reference | Year | Findings |
|---|---|---|
| Trotter et al. (36) | 1986 | Myelin-specific Abs trigger macrophage-mediated demyelination |
| Linington and Lassmann (17) | 1987 | Ab-mediated demyelination in a chronic relapsing experimental autoimmune encephalomyelitis (EAE) in guinea pigs |
| Schluesener et al. (37) | 1887 | Monoclonal MOG Abs induced fatal relapses in a model of chronic relapsing-remitting EAE in SJL mice and enhanced acute EAE in Lewis (LEW) rats with increased inflammation and demyelination |
| Lassmann et al. (22) | 1988 | Demyelination occurs in a synergistic way between cellular (T cells) and humoral immune mechanisms |
| Linington et al. (4) | 1988 | MOG Abs augment demyelination in a myelin basic protein (MBP) T cell-mediated EAE model in LEW rats |
| Kerlero de Rosbo et al. (38) | 1990 | Monoclonal MOG Abs together with complement lead to demyelination and MBP loss in brain cells |
| Scolding and Compston (39) | 1991 | Abs mediate macrophage-dependent phagocytosis of oligodendrocytes *in vitro* |
| Vass et al. (40) | 1992 | MOG Ab-mediated demyelination is intensified by interferon-gamma |
| Linington et al. (41) | 1992 | Abs prevent tolarization effect of repeatedly induced MBP-T cell-mediated EAE and enhances demyelination |
| Piddlesden et al. (42) | 1993 | Ab-mediated demyelination is dependent on complement recruiting ability and independent on its epitope recognition |
| Genain et al. (43) | 1995 | MOG Abs facilitate demyelination in MOG-induced EAE in common marmosets |
| Johns et al. (44) | 1995 | MOG Abs lead to degradation of MBP and increased myelin protease activity |
| Ichikawa et al. (45) | 1996 | $MOG_{35–55}$ encephalitogenic in LEW rats and a potential target for Ab-mediated demyelination |
| Menon et al. (46) | 1997 | Ab induced MBP loss and myelin destabilization by neutral proteases in human myelin |
| Van der Goes et al. (47) | 1999 | Abs to MOG play a crucial role for the phagocytosis of myelin by macrophages *in vitro* |
| Von Budingen et al. (25) | 2002 | Ab pathogenicity in marmosets is dependent on their ability to bind on conformational epitopes |
| Marta et al. (48) | 2003 | Ab cross-linking on oligodendrocyte cultures leads to the formation of lipid rafts and to a reconstitution of MOG |
| Bourquin et al. (28) | 2003 | Generation of pathogenic Abs to conformational MOG in H-2b mice is dependent on genes encoded within the major histocompatibility complex |
| Von Budingen et al. (49) | 2004 | EAE phenotype in marmosets correlates with the availability of conformational MOG Abs resulting in typical multiple sclerosis-like disease pattern. In addition Abs to MOG peptides lead to focal disease pattern in brain stem and spinal cord. MBP T cell-mediated EAE animals showed no demyelination when injected with MOG peptides. By contrast, conformational MOG Abs were more pathologic as controls |
| Marta et al. (26) | 2005 | Human but not rat MOG-induced B cell-dependent EAE in MOG primed C57BL/6 mice and Abs of hMOG immunized mice only lead to EAE formation in B cell-deficient mice. Pathogenic Abs react to conformational intact and glycosylated antigen only |
| Zhou et al. (50) | 2006 | Patient-derived MOG Abs enhance demyelination in rat EAE models |
| Urich et al. (51) | 2006 | Ab-mediated demyelination is FcR independent but completely relies on complement activation |
| Jagessar et al. (52) | 2008 | Increased Ab-dependent demyelination in marmosets immunized with murine myelin compared to myelin lacking MOG |
| Harrer et al. (53) | 2009 | Complement induced demyelination in a murine *ex vivo* model |
| Ohtani et al. (54) | 2011 | Ab titer against conformational MOG are directly associated with EAE activity and demyelination in EAE rats |
| Mader et al. (55) | 2011 | Human MOG Abs lead to complement activated cytotoxicity in HEK293A cells |
| de Graaf et al. (27) | 2012 | Correct refolding of MOG increases its pathogenicity by generating conformation-dependent MOG Abs |
| Dale et al. (56) | 2014 | Oligodendrocytes incubated with purified human MOG IgG lead to organizational disturbances of the thin filaments and microtubule cytoskeleton |
| Saadoun et al. (57) | 2014 | Patient-derived MOG IgG lead to complement-independent myelin changes and altered expression of axonal proteins, but did not trigger inflammation or cellular death |
| Flach et al. (58) | 2016 | MOG Abs boost EAE by activation of effector T cells |
| Kinzel et al. (59) | 2016 | MOG Abs are able to trigger spontaneous EAE in mice harboring endogenous MOG-specific T cells in the absence of B cells |

focal small demyelinating lesions (24). In contrast to LEW rats are brown Norway and dark agouti rat strains highly susceptible to MOG-induced EAE (87). Different MHC haplotypes and non-MHC background genes modify the anti-MOG immune response (70, 75). This important information derived from EAE studies in MHC congenic LEW rats, i.e., in rats with different MHC class II alleles on the genetic background of LEW rats. Upon immunization with MOG, these animals either develop early onset acute lethal disease with extensive demyelinating plaques, chronic and/or relapsing types of disease, or do not show any evidence of clinical and histological disease, depending on the MHC class II haplotype present (62). Moreover, the MOG-induced T cell proliferation and interferon-gamma production, and the degree of MOG-specific B cell responses and Ab titers correlated with the severity of clinical disease (62). For further experiments, rats were selected which carried the most permissive MHC class II haplotype for the induction of MOG-specific autoimmune reactions, but differed in their non-MHC background genes. When these animals were sensitized with MOG, they mounted anti-MOG T cell and B cell responses, but showed differences in the maturation of these responses (62). Cumulatively, these data suggested that the MHC haplotype influences the degree of disease susceptibility, the clinical course, the recruitment of MOG-specific immunocompetent cells, and the CNS pathology, while non-MHC genes strongly influence the maturation of the anti-MOG response (62). A similar effect was also seen in human HLA DR4 transgenic mice which indicated that HLA DR shaped the anti-MOG response in both, humans and mice (88).

**TABLE 2 | T cell responses against myelin oligodendrocyte glycoprotein (MOG) in experimental autoimmune encephalomyelitis (EAE) animal models.**

| Reference | Year | Finding |
|---|---|---|
| Linington et al. (33) | 1993 | MOG peptide ($MOG_{44–53}$) specific T cells induce atypical EAE in Lewis (LEW) rats |
| Amor et al. (30) | 1994 | Epitope $MOG_{1–22}$, $MOG_{43–57}$, and $MOG_{134–148}$ induce clinically and pathological relevant EAE, however, mild effects in AB/H mice. Epitope $MOG_{92–106}$ is highly encephalitogenic in SJL mice |
| Adelmann et al. (24) | 1995 | N-terminal domain ($MOG_{1–125}$) leads to demyelination in LEW rats, T cells reactive to epitope $MOG_{1–20}$ and $MOG_{35–55}$ are only weakly encephalitogenic in EAE model |
| Kerlero de Rosbo et al. (31) | 1995 | Mild pathological signs were detected by inducing $MOG_{35–55}$ in PL/J mice |
| Mendel et al. (32) | 1995 | $MOG_{35–55}$ induces highly reproducible EAE in C57BL/6J and C3H.SW (H-2b) mice |
| Devaux et al. (60) | 1997 | Severe EAE with truncated human MOG (1–120) in SJL and (PLJ × SJL) F1 mice, encephalitogenic T cell proliferation against epitope $MOG_{92–106}$ |
| Slavin et al. (61) | 1998 | Relapsing-remitting disease course in NOD/Lt mice (H-2g7) and chronic paralytic disease course in C57BL/6 mice after injection of $MOG_{35–55}$ |
| Weissert et al. (62) | 1998 | Major histocompatibility complex (MHC) haplotype influences the degree of disease susceptibility, recruitment of MOG-specific immune cells, and pathology in MOG-induced EAE rats |
| Storch et al. (63) | 1998 | Immunization with MOG antigen in rats is able to mimic classical multiple sclerosis (MS) as well and variants such as optic neuritis (ON), Devic's and Marburg's disease |
| Encinas et al. (64) | 1999 | Active immunization with $MOG_{35–55}$ induces relapsing-remitting EAE followed by a secondary progression in NOD mice |
| Raine et al. (65) | 1999 | MOG-induced EAE in marmosets lead to vesicular disruption and production of antigen-specific autoantibodies similar to MS |
| Abdul-Majid et al. (66) | 2000 | $MOG_{79–96}$ is highly encephalitogenic in DBA/1 mice, including macrophage infiltration and demyelination |
| Kerlero de Rosbo et al. (67) | 2000 | rhMOG-EAE induced marmosets with different MHC background showed proliferative T cell responses against epitopes $MOG_{4–20}$, $MOG_{35–50}$, and $MOG_{94–116}$ |
| Bourquin et al. (68) | 2000 | MOG-DNA vaccination lead to severe EAE |
| Brok et al. (69) | 2000 | Human MOG peptide $MOG_{14–36}$ is highly encephalitogenic in marmosets (presented by a common class II Caja-DRB*W1201 molecule) |
| Weissert et al. (70) | 2001 | $MOG_{91–114}$ immunization lead to clinical and histopathological EAE signs in LEW.1AV1 and LEW.1N rats |
| Bettelli et al. (71) | 2003 | Development of spontaneous ON in T cell receptor ($MOG_{35–55}$) transgenic C57BL/6 mice |
| Delarasse et al. (14) | 2003 | MOG-deficient mice are resistant to rat MOG-induced EAE and developed a mild pathological phenotype after immunization of whole myelin. However, B- and T cell responses against the extracellular domain and peptides of MOG were not altered compared to wild-type mice, indicating MOG being resistant to the induction of immune tolerance |
| Sun et al. (72) | 2003 | $CD8^+$ MOG-specific T cells recognize H-2Db dimers coupled with encephalitogenic peptide $MOG_{40–54}$ |
| Smith et al. (73) | 2005 | Injection of full-length conformational MOG leads to chronic progressive EAE, but released MOG does not induce immunity during an ongoing disease in Biozzi ABH mice |
| Krishnamoorthy et al. (74) | 2006 | $MOG_{35–55}$ leads to paralytic EAE and ON in a double-transgenic ($IgH^{MOG}$ and $TCR^{MOG}$) C57BL/6 line |
| de Graaf et al. (75) | 2008 | In LEW.1N, LEW.1AV1, and dark agouti rats, MS-like pathology is mainly determined by presentation of MOG peptides on MHC class II molecules |
| Kap et al. (76) | 2008 | Cytotoxic T cells specific to epitope $MOG_{34–56}$ trigger fast progression of rhMOG-induced EAE in marmosets |
| Matsumoto et al. (77) | 2009 | $MOG_{91–108}$ is an encephalitogenic epitope able to induce mild T cell-mediated EAE but does not elicit Abs against the epitope or MOG in LEW.1AV1 rats |
| Pollinger et al. (78) | 2009 | Development of relapsing-remitting EAE in TCR ($MOG_{92–106}$) transgenic SJL/J mice |
| Bettini et al. (79) | 2009 | $CD8^+$ T cell dominant epitope $MOG_{37–46}$ lead to mild form of EAE |
| York et al. (80) | 2010 | MOG-specific $CD8^+$ T cells are able to ameliorate $CD4^+$ driven EAE |
| Anderson et al. (81) | 2012 | $CD4^+$ and $CD8^+$ T cell driven EAE in transgenic $MOG_{35–55}$ specific T cell mouse line (1C6) |
| de Graaf et al. (27) | 2012 | Correct refolding of MOG increases its encephalogenicity by enhancing its processing or/and presentation on MHC molecules |
| Jagessar et al. (82) | 2012 | $MOG_{34–56}$ specific cytotoxic T cells are key regulators for gray and white matter demyelination in marmosets |
| Delarasse et al. (34) | 2013 | Transmembrane regions $MOG_{113–127}$ and $MOG_{120–134}$ and second hydrophobic domain $MOG_{183–197}$ are found to be immunogenic and pathogenic in C57BL/6 (H-2b) |
| Ortega et al. (83) | 2013 | $CD8^+$ cells reactive to $MOG_{35–55}$ attenuate EAE severity in an adaptive CD4 T cell-mediated EAE model in C57BL/6 mice |
| Haanstra et al. (84) | 2013 | rhMOG (1–125) induces EAE in non-human primates |
| Shetty et al. (35) | 2014 | T cells directed to an encephalitogenic transmembrane domain ($MOG_{110–132}$) induced clinical EAE, inflammation, and demyelination |
| Curtis et al. (85) | 2014 | Injection of rat immunoglobuline variable of MOG together with incomplete Freud's adjuvant lead to atypical EAE in LEW rats and Macaca species |
| Herrera et al. (86) | 2014 | $MOG_{35–55}$ induced EAE in C57BL/6 mice lead to lesions along the optic chiasm |

Further knowledge about the role of B cells in MOG-induced CNS inflammation derived from transgenic mice (**Table 3**). Mice were genetically engineered to express the heavy chain from the monoclonal anti-MOG Ab 8–18C5 described above, paired with endogenous Ig light chains (89). These animals had many MOG-reactive B cells in their immune repertoire and had titers of anti-MOG Abs in their circulation. And yet, they remained completely healthy until they were challenged with MOG. Then, they developed EAE with higher incidence, severity, and earlier onset compared to their non-transgenic counterparts (89). Further studies using B cell-deficient mice showed that B cells are required for EAE induction using the MOG protein, but are dispensable when the encephalitogenic MOG peptide is used for EAE induction (90–92). These studies also revealed that B cells are needed for the recovery from EAE, by the production of IL-10 and expression of CD40 (93). The role of B cells in promoting EAE was further confirmed by using transgenic mouse lines in which MHC class II products were knocked-out in B cells, or in which B cells were able to express MOG-specific B cell receptors on their surface, but were unable to secrete MOG-specific Abs (94). This and several other studies (see **Table 3**) revealed that B cells can act as APC, and that they can sufficiently promote pro-inflammatory T cell activation and spontaneous EAE onset (91, 94, 95). In another study, in which MOG-specific B cells and T cells were actively transferred into an intact immune repertoire of C57BL/6J mice, MOG-specific B cells were shown to aggravate CNS inflammation and EAE disease course. These results were further confirmed by using human MOG positive serum Abs, reproducing the same disease accelerating effects (58). Hence, both B cells and myelin-specific Abs can independently activate T cells and thus increase the risk of an autoimmune mediated inflammation of the CNS (59).

Also spontaneous models of MOG-induced CNS disease were highly informative for deciphering the role of anti-MOG responses in autoimmune disease. These models were based on the transgenic expression in mice of MOG-specific T cell receptors, either alone (71, 78) or in combination with MOG-specific B cell receptors (74, 97) and gave striking results:

The overexpression of MOG-specific T cell receptors in transgenic C57/BL6 (71) or SJL (78) mice lead to spontaneous optic neuritis (ON) in more than 30% of all animals and rendered the animals hyper-susceptible to the induction of ON in response to sensitization with suboptimal amounts of MOG (71), or to a severe spontaneous relapsing-remitting EAE with episodes often altering between different CNS compartments in more than 60% of all male, and more than 80% of all females within 160 days after birth (78). In these animals, the transgenic T cells expanded MOG-specific B cells from the endogenous immune repertoire, which produced pathogenic autoantibodies binding to a conformational epitope on native MOG protein (78). Overexpression of MOG-specific T cell receptors in NOD mice led to MOG-specific $CD4^+$ and $CD8^+$ T cell responses at the same time (79). These animals revealed that $CD8^+$ MOG-specific T cells may be weakly encephalitogenic (79) and are able to regulate and attenuate $CD4^+$ driven immune responses by modulating APC functions and reducing $CD4^+$ T cell responses (80, 83).

Mice genetically engineered to express MOG-specific receptors on T and B cells (74, 97) showed a class switch of MOG Abs to an IgG1 subtype, and spontaneously developed inflammatory demyelinating CNS disease (74, 97). Most interestingly, spontaneous development of disease in these animals crucially depended on the presence of commensal microbiota in the gut (98).

Although many seminal observations on MOG-reactive T and B cell responses derive from murine EAE models, it is important to know that in these animals, large lesions with myelin loss are mainly caused by axonal degeneration with secondary demyelination, while primary demyelination is sparse (99, 100). Therefore, it is necessary to also study MOG autoreactivity in the marmoset

**TABLE 3 | The role of B cells in experimental autoimmune encephalomyelitis (EAE) animal models.**

| Reference | Year | Findings |
|---|---|---|
| Hjelmstrom et al. (92) | 1998 | B cell-independent demyelination in myelin oligodendrocyte glycoprotein (MOG)-induced EAE mice |
| Litzenburger et al. (89) | 1998 | MOG-specific B cells accelerate and exacerbate EAE, but are not able to induce spontaneous disease or demyelination without induced EAE |
| Stefferl et al. (87) | 1999 | Major histocompatibility complex (MHC) and MHC-linked effects can influence the antibody response and thereby disease severity in MOG-induced EAE |
| Lyons et al. (90) | 1999 | B-cell-deficient mice immunized with $MOG_{35–55}$ induced EAE but not mice immunized with recombinant full-length MOG |
| Forsthuber et al. (88) | 2001 | MOG peptide 97–108 is the immunodominant human leukocyte antigen (HLA)-DR4-restricted T cell epitope in transgenic mice and is presented by human B cells expressing HLA-DR4 (DRB1*0401) |
| Lyons et al. (91) | 2002 | MOG-specific B cells and serum reconstitute the ability for inducing inflammatory EAE effects in B cell-deficient mice |
| Fillatreau et al. (93) | 2002 | IL-10 production of B cells regulate type 1 immunity and play a key role in EAE recovery |
| Svensson et al. (96) | 2002 | B cell-deficient mice with different genetic backgrounds (C57BL/10 and DBA/1) immunized with $MOG_{1–125}$ showed decreased demyelination but inflammation was not affected |
| Bettelli et al. (97) | 2006 | TCRMOG × IgHMOG mice develop severe EAE, with inflammatory lesions in the spinal cord and optic nerves |
| Pollinger et al. (78) | 2009 | Transgenic mice expressing $MOG_{92–106}$ specific T cells expand endogenous MOG-specific B cells, producing conformational, (epitope independent) Abs, and enhancing demyelinating EAE in a relapsing-remitting EAE model |
| Molnarfi et al. (94) | 2013 | MOG-specific B cells play a critical role in the EAE pathogenesis due to its function as an antigen-presenting cells |
| Parker Harp et al. (95) | 2015 | B cells directly interact with dendritic cells and enhance CD4 driven EAE severity in mice |
| Flach et al. (58) | 2016 | MOG-specific B cells accelerate MOG T cell driven EAE inflammation and disease severity |

(*Callithrix jacchus*), in which MOG-induced EAE resembles human demyelinating diseases more closely (100–102). When these animals are immunized with the recombinant IgV domain of rat MOG, they developed lesions which were very similar to chronic multiple sclerosis (MS) plaques with mononuclear cell infiltrates, primary demyelination, and astrogliosis (103), even at the ultrastructural level (65). Moreover, some animals developed a progressive form of EAE, which was triggered by cytotoxic effector memory T cells and further promoted demyelination in the gray matter (76, 82). As seen before in mice and rats, the marmoset $CD4^+$ T cell response against MOG may cover several different epitopes, only one of which is highly encephalitogenic (104).

Cumulatively, these animal models revealed that

- autoimmune responses to MOG can be induced in many different species
- the susceptibility to MOG is determined by MHC- and non-MHC genes
- anti-MOG responses typically involve $CD4^+$ T cells and complement-fixing Abs of the IgG1 subtype
- the MOG-specific T cell repertoire contains T cells specific for several different T cell epitopes which vary between different species and substrains dependent on the MHC haplotype
- not all MOG-specific T cells are encephalitogenic
- MOG-specific B cells have Ab-dependent and Ab-independent effects on tissue damage
- different types of anti-MOG Abs exist, but only those recognizing conformational epitopes on the extracellular domain of MOG are pathogenic
- MOG-specific autoantibodies in the circulation specific for such conformational epitopes are harmless, unless these Abs gain access to the CNS *via* an opened blood-brain barrier in an inflammatory environment
- MOG-specific Abs can cross-react with other proteins like butyrophilin
- the extent of demyelination caused by anti-MOG Abs depends on MHC-dependent and MHC-independent factors.

## CLINICAL RELEVANCE OF MOG Abs IN DEMYELINATING DISEASES

As outlined above, MOG is one of the best-studied autoantigens for experimental autoimmune models for MS. Attempts to translate these findings into the human disease have yielded controversial results, especially with regard to MOG Abs as a prognostic biomarker in MS (105, 106) [reviewed in Berger et al. (107)]. These results were caused by the use of inappropriate methods (e.g., immunoblotting, ELISA) and antigens (recombinant human MOG produced in *Escherichia coli*, MOG peptides) to determine disease-specific MOG Abs. However, with improved detection methods using correctly folded and glycosylated MOG protein expressed in mammalian cells for radioimmunoassays, flow cytometry, and immunofluorescence, MOG Abs were found in a subset of predominantly pediatric patients with acute disseminated encephalomyelitis (ADEM), aquaporin-4 (AQP4) seronegative neuromyelitis optica spectrum disorders (NMOSD), monophasic or recurrent isolated ON, or transverse myelitis (TM), in atypical MS, brainstem encephalitis, and *N*-methyl-D-aspartate receptor-encephalitis with overlapping demyelinating syndromes, but rarely in classical MS (50, 55, 56, 108–176). Since low-titer MOG Abs are often found in MS patients and controls, most of these studies have used either a "high-titer" cut-off or an IgG1 secondary Ab to increase specificity. Like many other autoantibodies, e.g., to AQP4, MOG Abs are therefore only present in rare diseases indicating widely established immunological tolerance to most autoantigens.

These findings, however, raise the important question whether MOG Abs are associated with a specific clinical phenotype like AQP4 Abs are associated with NMOSD (177). We have therefore reviewed the literature and compared all studies, which have analyzed the presence of MOG Abs in inflammatory demyelinating disorders (MS, ADEM, and AQP4 Ab seronegative and seropositive NMOSD) in comparison with a control group of patients with other neurological disorders or healthy controls. Results from these studies are shown in **Table 4** and **Figure 1**. We have identified 26 studies which fulfilled these criteria (50, 55, 56, 109–116, 119, 121, 126, 130, 132, 134, 137, 141, 147, 148, 152, 156, 158, 165, 174). Only 13 of these studies included a control group with 50 or more individuals and only 5 studies included more than 100 controls (**Table 4**). Further, many patients and controls were repeatedly analyzed in some studies and therefore we decided not to include a statistical analysis of the reviewed publications. The specificity of these studies was calculated using the frequency of MOG Abs in other neurological disorders or healthy controls determined by the methods shown in **Table 4**. The overall specificity of these studies was 98.5% [95% confidence interval (CI) 97.8–99] and thus 1.5% (range 0–6%) of all controls were seropositive for MOG Abs (**Table 4**; **Figure 1**). The sensitivity of these studies was calculated using the frequency of MOG Abs in inflammatory demyelinating disorders determined by the methods shown in **Table 4**. The presence of MOG Abs in MS was analyzed in 23/26 studies and the overall sensitivity for MS was 5.1% (95% CI 4.2–6.1) and thus 5.1% (range 0–46.7%) of all MS patients were seropositive for MOG Abs. The highest frequency of MOG Abs within MS patients was found in pediatric MS patients and in one of the initial studies not using a high-titer cut-off. Therefore, it can be concluded that MOG Abs are rare in MS, particularly in adult MS, but are still found in a few patients in several studies. Since MOG Abs are associated with MS-like neuropathology (136, 149, 167, 172, 178, 179), they might play a role in pathophysiology in these patients and therefore the current practice to use MS as a negative control group for MOG Abs (141) should be regarded with caution. The presence of MOG Abs in ADEM was analyzed in 13/26 studies and the overall sensitivity for ADEM was 36.4% (95% CI 31.4–41.7; range 17.7–47.4%) and thus ADEM was the most frequent clinical presentation associated with MOG Abs. Again, the frequency of MOG Abs was highest in pediatric patients. Since the 26 studies used different clinical criteria for NMOSD, we reviewed the studies for the presence of MOG Abs in AQP4 seronegative patients with ON, TM, or NMOSD. The presence of MOG Abs in these conditions was analyzed in 15/26 studies and the overall sensitivity was 26.9% (95% CI 23.9–30.1; range 9.2–63.5%). Finally, the presence of MOG Abs in AQP4 seropositive NMOSD was analyzed in 13/26

**TABLE 4 | Studies reporting the presence of myelin oligodendrocyte glycoprotein (MOG) antibodies (Abs) in patients with inflammatory demyelinating disorders in comparison to a control group of patients with other neurological disorders and/or healthy controls.**

| Reference | Method | Patients | Multiple sclerosis | Acute disseminated encephalomyelitis | Aquaporin-4 (AQP4)− optic neuritis/transverse myelitis/neuromyelitis optica spectrum disorders (NMOSD) | AQP4+ NMOSD | Controls |
|---|---|---|---|---|---|---|---|
| Lalive et al. (109) | FACS | ad | 1/92 (1%) | n.a. | n.a. | n.a. | 1/37 (3%) |
| Zhou et al. (50) | FACS | ad | 25/210 (12%) | n.a. | n.a. | n.a. | 8/187 (4%) |
| O'Connor et al. (110) | RIA | ad, ped | 3/140 (2%) | 13/69 (19%) | n.a. | n.a. | 1/133 (1%) |
| Brilot et al. (112) | FACS | ad, ped | 0/54 (0%) | 8/19 (42%) | n.a. | n.a | 0/73 (0%) |
| McLaughlin et al. (111) | FACS | ad, ped | 39/385 (10%) | n.a. | n.a. | 0/13 (0%) | 6/214 (3%) |
| Selter et al. (113) | FACS | ped | n.a. | 9/19 (47%) | n.a. | n.a. | 0/58 (0%) |
| Di Pauli et al. (115) | IF-HT | ad, ped | 2/89 (2%) | 12/27 (44%) | n.a. | n.a. | 1/105 (1%) |
| Lalive et al. 2011 (114) | FACS | ped | 1/22 (5%) | 3/11 (27%) | n.a. | n.a. | 0/20 (0%) |
| Mader et al. (55) | IF-HT | ad, ped | 2/71 (3%) | 14/33 (42%) | 9/23 (39%) | 1/75 (1%) | 3/101 (3%) |
| Probstel et al. (116) | FACS | ad, ped | 14/127 (11%) | 19/54 (35%) | n.a. | n.a. | 0/63 (0%) |
| Kitley et al. (119) | IF | ad | 0/75 (0%) | n.a. | 4/27 (15%) | 0/44 (0%) | 0/23 (0%) |
| Rostasy et al. (121) | IF-HT | ped | 1/11 (9%) | 13/29 (45%) | 7/29 (24%) | 0/2 (0%) | 0/23 (0%) |
| Dale et al. (56) | FACS | ped | 7/15 (47%) | 11/24 (46%) | 13/24 (54%) | n.a. | 0/24 (0%) |
| Martinez-Hernandez et al. (134) | IF-HT | ad | 0/64 (0%) | n.a. | 14/52 (27%) | 2/45 (4%) | 0/30 (0%) |
| Ramanathan et al. (130) | FACS | ad | 1/76 (1%) | n.a. | 9/23 (39%) | n.a. | 0/52 (0%) |
| Elong Ngono et al. (132) | IF-HT | ad | 1/16 (6%) | n.a. | n.a. | n.a. | 1/24 (4%) |
| Ketelslegers et al. (147) | FACS | ped | n.a. | 10/24 (42%) | 4/29 (14%) | n.a. | 0/44 (0%) |
| Probstel et al. (137) | FACS | ad | 0/48 (0%) | n.a. | 4/17 (24%) | 0/31 (0%) | 0/39 (0%) |
| Waters et al. (141) | IF-IgG1 | ad | 0/76 (0%) | 7/16 (44%) | 40/63 (64%) | 0/130 (0%) | 0/13 (0%) |
| Fernandez-Carbonell et al. (152) | FACS | ped | 4/45 (9%) | 3/7 (43%) | 4/14 (29%) | 0/2 (0%) | 0/23 (0%) |
| Jarius et al. (174) | IF-HT | ad, ped | 0/139 (0%) | n.a. | 50/202 (25%) | 0/83 (0%) | 1/98 (1%) |
| Kim et al. (148) | IF-IgG1 | ad | 0/29 (0%) | 1/6 (17%) | 15/163 (9%) | 0/49 (0%) | 0/72 (0%) |
| Spadaro et al. (165) | FACS | ad | 5/181 (3%) | n.a. | n.a. | n.a. | 0/39 (0%) |
| van Pelt et al. (158) | FACS | ad | n.a. | n.a. | 20/61 (33%) | 0/41 (0%) | 0/8 (0%) |
| Overall | | | 106/196 (5%) | 123/338 (36%) | 193/727 (27%) | 3/515 (1%) | 22/1527 (1%) |

*The percentage of MOG Ab seropositivity was determined using the methods indicated in the table.*
*ad, adult; ped, pediatric; n.a., not analyzed; FACS, fluorescence-activated cell sorting; IF, immunofluorescence assay; IF-HT, immunofluorescence assay with high-titer cut-off; IF-IgG1, immunofluorescence assay for IgG1 Abs; RIA, radio immunoprecipitation assay.*

studies and the overall sensitivity was 2% (95% CI 1.2–3.4; range 1.2–3.4%). Thus, the presence of MOG Abs in AQP4 Ab-positive NMOSD is in the range of the control group.

In conclusion, these studies revealed that MOG Abs are associated with heterogeneous clinical presentations and a younger age of onset in human inflammatory demyelinating diseases but a clear common clinical phenotype is missing.

The histopathology associated with MOG Abs has been described in few patients including NMOSD, atypical demyelination, CIS, and ADEM (136, 149, 167, 172, 178, 179) (**Table 5**). All cases showed demyelinating lesions with features of MS pattern II, with well-demarcated confluent plaques with loss of myelin, relative preservation of axons, well-preserved astrocytes, and numerous macrophages containing myelin debris. The inflammatory infiltrates were predominantly composed of perivascular and parenchymal T-cells and some perivascular B-cells. Moreover, the deposition of terminal complement complex C9neo was reported indicating complement-dependent cytotoxicity (136, 167). All lesions were characterized by well-preserved oligodendrocytes that were partly MOG-negative, most likely compatible with preoligodendrocytes. Demyelination associated with MOG Abs differs from AQP4 seropositive NMOSD that characteristically shows loss of astrocytes with deposition of IgG and terminal complement complex C9neo, inflammatory infiltrates including the presence of neutrophilic and eosinophilic granulocytes, and elevated glial fibrillary acidic protein levels in CSF (180).

These similar immunopathological findings compatible with MS pattern II supports a humoral immune pathogenesis in patients with MOG Abs. Since the histopathological lesion type is independent from the clinical presentation the demyelinating lesions may be included under the term "MOG antibody syndrome."

## EPITOPE RECOGNITION AND SPECIES SPECIFICITY OF HUMAN MOG Abs

As MOG Ab binding has been shown to be dependent on the correct folding and glycosylation pattern of their antigen, studies were directed toward the binding motifs/epitopes of these Abs with the aim to identify specific binding patterns for diseases. Mayer and colleagues (122) performed epitope recognition studies of MOG Abs from several demyelinating diseases and seven distinct binding patterns were found. However, no clinical correlation between the binding patterns and different disease entities could be shown. Furthermore, these Abs were directed against only a single epitope or multiple epitopes and an association between glycosylation and an increased binding capacity

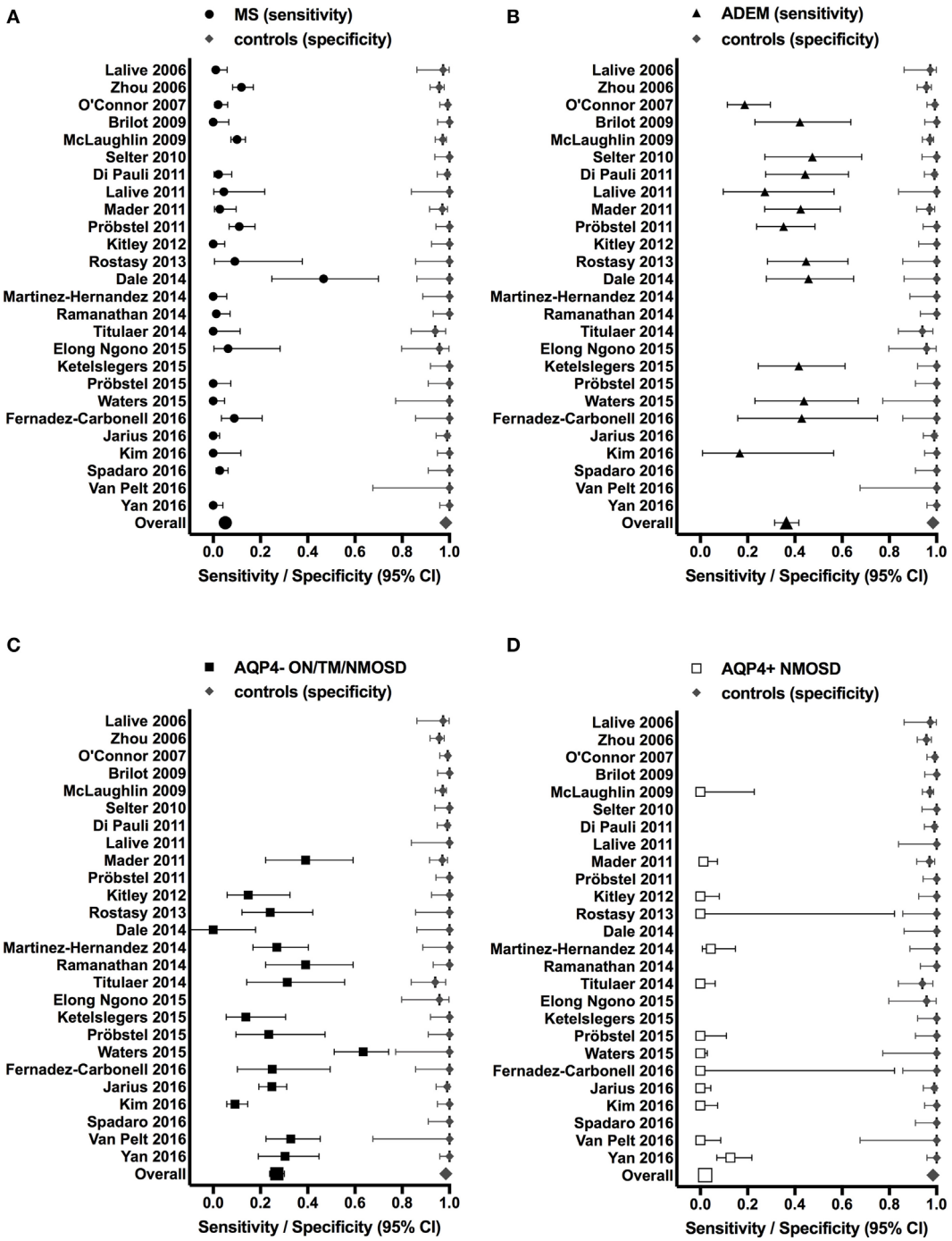



**FIGURE 1 | Studies reporting the presence of myelin oligodendrocyte glycoprotein (MOG) antibodies (Abs) in patients with inflammatory demyelinating disorders (A) MS; (B) ADEM; (C) AQP4− ON/TM/NMOSD; (D) AQP4+ NMOSD (sensitivity, left side of each graph) in comparison to a control group of patients with other neurological disorders and/or healthy controls (specificity, right side of each graph)**. Sensitivities and specificities are indicated by symbols with error bars (95% confidence intervals). Specificities were calculated using the frequency of MOG Abs in other neurological disorders or healthy controls determined by the methods shown in **Table 4**. Sensitivities were calculated using the frequency of MOG Abs in inflammatory demyelinating disorders determined by the methods shown in **Table 4**.

could not be detected. The most frequent epitopes were found in the CC′-loop and FG-loop of the extracellular IgV domain of correctly folded human MOG protein. Within the CC′-loop, AA P42 was essential for binding and therefore human MOG Abs did not bind either to rodent MOG, which has a serine at position 42, or to mutated human MOG P42S (122). These findings were confirmed and extended by Sepulveda et al. (166) who demonstrated that only a subset of human MOG Abs is also

**TABLE 5 | Neuropathological findings in patients with myelin oligodendrocyte glycoprotein (MOG) antibody (Ab)-associated demyelination.**

| Reference | Number of cases | Sex, age (years) | Clinical presentation | Findings |
|---|---|---|---|---|
| Konig et al. (178) | 1 | F, 49 | RRMS | Multiple sclerosis (MS) pattern II; oligodendrocytes in lesion preserved ($CNPase^+$; MOG not determined) |
| Spadaro et al. (136) | 1 | F, 66 | Recurrent myelitis + brainstem involvement | MS pattern II; oligodendrocytes preserved ($CNPase^+$; $MOG^-$) |
| Di Pauli et al. (149) | 1 | M, 71 | Acute disseminated encephalomyelitis (ADEM)/acute MS | MOG and aquaporin-4 Ab positive; MS pattern II; oligodendrocytes preserved ($CNPase^+$, $MOG^-$) |
| Jarius et al. (172) | 1 | F, 63 | CIS | MS pattern II; oligodendrocytes preserved ($CNPase^+$, $MOG^+$) |
| Wang et al. (167) | 1 | F, 67 | Neuromyelitis optica spectrum disorders | Pattern classification not done; well-demarcated demyelinating lesion with preserved axons and astrocytes |
| Körtvélyessy et al. (179) | 2 | M, 49<br>M, 34 | ADEM | Intrathecal MOG Ab synthesis; MS pattern II; one patient with overlapping features of pattern III (early MAG loss, apoptotic oligodendrocytes in addition to complement deposition) |

reactive to rodent MOG epitopes as analyzed by cell-based assays and tissue immunohistochemistry and this reactivity to rodent MOG did not correlate with a specific clinical phenotype. Finally, it has been already demonstrated that species differences of MOG lead to the activation of different pathogenic mechanisms in EAE induced with rodent or human $MOG_{35–55}$ or recombinant MOG (26, 92, 181).

## MOG Abs: EPIPHENOMENON OR INDICATIVE FOR DISEASE PHENOTYPE

The animal experiments described above clearly indicated that murine MOG Abs can be pathogenic. Furthermore, pathologic similarities to ADEM have been shown in transgenic MOG-IgG mice infected with several neurotrophic encephalitogenic viruses, exacerbating virus-induced CNS inflammation. These similarities were indicated by clinical defined extensive perivascular infiltrates (mixed inflammatory cell population, e.g., lymphocytes, neutrophils, NK cells, and blood born macrophages) and perivenous demyelination (182, 183).

By contrast, only four studies aimed to investigate the pathogenic role of human MOG Abs *in vivo*. Whereas several studies indicated that human MOG Abs can activate complement and cellular-dependent cytotoxicity (50, 55, 112) *in vitro*, these mechanisms were not observed after transfer of human MOG Abs to rodents *in vivo*: the injection of concentrated serum samples from MOG Ab-positive patients into LEW rats with EAE did not increase the clinical score of the disease, but led to a minor increase in demyelination and axonal loss (50). Intrathecal injection of purified human MOG IgG caused reversible brain edema and myelin loss with very little complement deposition at the lesion site (57).

A different pathogenic mechanism for MOG Abs was proposed in two recent studies (58, 59). In the first study (58), it was demonstrated that MOG-specific B cells and their products (MOG Abs) activate MOG-specific effector T cells *via* CNS resident APC. A similar effect was demonstrated for peripheral APC in the second study (59). Both studies emphasize an important role for Ab-mediated antigen opsonization and accumulation in Fc receptor expressing APCs and subsequent increased antigen presentation and activation of specific T cells.

## ARE MOG Abs A PRIMARY OR A SECONDARY IMMUNE RESPONSE?

The findings discussed in the previous chapter raise the important question whether human MOG Abs are pathogenic themselves or just a epiphenomenal bystander or a secondary immune reaction due to previous demyelination (184). An example for a secondary immune response was shown in a study using a transgenic myelin-specific T cell mice model, which developed spontaneous EAE (98). In this model, an interaction between MOG-specific T and B cells is necessary for inflammatory demyelination, resulting in the activation of native B cells by dendritic cells presenting MOG peptides in the cervical lymph nodes (78). In a gut germ free environment, autoreactive T cell activation failed, and therefore the signal cascade for producing autoantibodies producing B cells was significantly reduced, but increased after microbial re-colonization. One potential mechanism mediating the onset of spontaneous EAE is molecular mimicry, activating encephalitogenic T cells, with subsequent inflammation of the CNS and second, it leads to an activation of native MOG-specific B cells recruited to the CNS tissue *via* locally produced MOG material or drained into the CNS along peripheral lymph nodes.

But even if MOG Abs would only be a secondary immune reaction they still could be clinically relevant biomarkers such as seen in diabetes type I, an autoimmune disease affecting insulin producing β-cells in the pancreas. Four autoantibodies to insulin (185), glutamic acid decarboxylase (186), Islet antigen-2 (187), and zinc transporter 8 (188) have been identified as highly specific biomarkers to predict this disease. There is more than an 80% probability of developing diabetes in children and adolescents, if 2/4 autoantibodies are detected [reviewed in Bonifacio (189)]. However, these autoantibodies are not pathogenic itself, but rather indicate a disturbed immune activity or an underlying T cell-mediated autoimmune process (190). Similarly, it could be that human MOG Abs play only a minor role in the pathophysiology of inflammatory demyelination, but are highly specific markers for affected patients.

## CONCLUSION

In the past years, autoantibodies emerged as important biomarkers in neurological autoimmune diseases. One of the best examples for these biomarkers is AQP4 Abs as diagnostic marker for NMOSD. Numerous studies have now established a possible similar role for MOG Abs that are associated with a very heterogeneous age-dependent clinical presentation and MS-like neuropathology. The exact pathologic effect of human MOG Abs is still unclear and needs to be critically investigated in order to clarify the immunopathological role of these Abs.

## AUTHOR CONTRIBUTIONS

PP prepared the main body of the manuscript and tables. MB and TB participated in the preparation of the manuscript. RH participated in the preparation of the manuscript and prepared tables and figures. MR supervised the work and participated in the preparation of the manuscript and figures and tables. All authors approved the final version of the manuscript.

## ACKNOWLEDGMENTS

The authors are grateful to A. Navarro for proof-reading and comments on the manuscript.

## FUNDING

PP and MR are supported by a research grant from the Fonds zur Förderung der wissenschaftlichen Forschung, Austria (FWF graduate program W1206 SPIN).

## REFERENCES

1. Delarasse C, Della Gaspera B, Lu CW, Lachapelle F, Gelot A, Rodriguez D, et al. Complex alternative splicing of the myelin oligodendrocyte glycoprotein gene is unique to human and non-human primates. *J Neurochem* (2006) 98(6):1707–17. doi:10.1111/j.1471-4159.2006.04053.x
2. Pham-Dinh D, Mattei MG, Nussbaum JL, Roussel G, Pontarotti P, Roeckel N, et al. Myelin/oligodendrocyte glycoprotein is a member of a subset of the immunoglobulin superfamily encoded within the major histocompatibility complex. *Proc Natl Acad Sci U S A* (1993) 90(17):7990–4. doi:10.1073/pnas.90.17.7990
3. Brunner C, Lassmann H, Waehneldt TV, Matthieu JM, Linington C. Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase in the CNS of adult rats. *J Neurochem* (1989) 52(1):296–304. doi:10.1111/j.1471-4159.1989.tb10930.x
4. Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* (1988) 130(3):443–54.
5. Martini R, Schachner M. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. *J Cell Biol* (1986) 103(6 Pt 1):2439–48. doi:10.1083/jcb.103.6.2439
6. Boyle LH, Traherne JA, Plotnek G, Ward R, Trowsdale J. Splice variation in the cytoplasmic domains of myelin oligodendrocyte glycoprotein affects its cellular localisation and transport. *J Neurochem* (2007) 102(6):1853–62. doi:10.1111/j.1471-4159.2007.04687.x
7. Marta CB, Montano MB, Taylor CM, Taylor AL, Bansal R, Pfeiffer SE. Signaling cascades activated upon antibody cross-linking of myelin oligodendrocyte glycoprotein: potential implications for multiple sclerosis. *J Biol Chem* (2005) 280(10):8985–93. doi:10.1074/jbc.M413174200
8. Gardinier MV, Amiguet P, Linington C, Matthieu JM. Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily. *J Neurosci Res* (1992) 33(1):177–87. doi:10.1002/jnr.490330123
9. Guggenmos J, Schubart AS, Ogg S, Andersson M, Olsson T, Mather IH, et al. Antibody cross-reactivity between myelin oligodendrocyte glycoprotein and the milk protein butyrophilin in multiple sclerosis. *J Immunol* (2004) 172(1):661–8. doi:10.4049/jimmunol.172.1.661
10. Krishnamoorthy G, Saxena A, Mars LT, Domingues HS, Mentele R, Ben-Nun A, et al. Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of multiple sclerosis. *Nat Med* (2009) 15(6):626–32. doi:10.1038/nm.1975
11. Cong H, Jiang Y, Tien P. Identification of the myelin oligodendrocyte glycoprotein as a cellular receptor for rubella virus. *J Virol* (2011) 85(21):11038–47. doi:10.1128/JVI.05398-11
12. Garcia-Vallejo JJ, Ilarregui JM, Kalay H, Chamorro S, Koning N, Unger WW, et al. CNS myelin induces regulatory functions of DC-SIGN-expressing, antigen-presenting cells via cognate interaction with MOG. *J Exp Med* (2014) 211(7):1465–83. doi:10.1084/jem.20122192
13. von Budingen HC, Mei F, Greenfield A, Jahn S, Shen YA, Reid HH, et al. The myelin oligodendrocyte glycoprotein directly binds nerve growth factor to modulate central axon circuitry. *J Cell Biol* (2015) 210(6):891–8. doi:10.1083/jcb.201504106
14. Delarasse C, Daubas P, Mars LT, Vizler C, Litzenburger T, Iglesias A, et al. Myelin/oligodendrocyte glycoprotein-deficient (MOG-deficient) mice reveal lack of immune tolerance to MOG in wild-type mice. *J Clin Invest* (2003) 112(4):544–53. doi:10.1172/JCI15861
15. Linares D, Mana P, Goodyear M, Chow AM, Clavarino C, Huntington ND, et al. The magnitude and encephalogenic potential of autoimmune response to MOG is enhanced in MOG deficient mice. *J Autoimmun* (2003) 21(4):339–51. doi:10.1016/j.jaut.2003.09.001
16. Lebar R, Baudrimont M, Vincent C. Chronic experimental autoimmune encephalomyelitis in the Guinea pig. Presence of anti-M2 antibodies in central nervous system tissue and the possible role of M2 autoantigen in the induction of the disease. *J Autoimmun* (1989) 2(2):115–32. doi:10.1016/0896-8411(89)90149-2
17. Linington C, Lassmann H. Antibody responses in chronic relapsing experimental allergic encephalomyelitis: correlation of serum demyelinating activity with antibody titre to the myelin/oligodendrocyte glycoprotein (MOG). *J Neuroimmunol* (1987) 17(1):61–9. doi:10.1016/0165-5728(87)90031-2
18. Kabat EA, Wolf A, Bezer AE. The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J Exp Med* (1947) 85(1):117–30. doi:10.1084/jem.85.1.117
19. Seil FJ, Falk GA, Kies MW, Alvord EC Jr. The in vitro demyelinating activity of sera from Guinea pigs sensitized with whole CNS and with purified encephalitogen. *Exp Neurol* (1968) 22(4):545–55. doi:10.1016/0014-4886(68)90148-9
20. Lebar R, Boutry JM, Vincent C, Robineaux R, Voisin GA. Studies on autoimmune encephalomyelitis in the Guinea pig. II. An in vitro investigation on the nature, properties, and specificity of the serum-demyelinating factor. *J Immunol* (1976) 116(5):1439–46.
21. Lebar R, Vincent C, Fischer-le Boubennec E. Studies on autoimmune encephalomyelitis in the Guinea pig – III. A comparative study of two autoantigens of central nervous system myelin. *J Neurochem* (1979) 32(5):1451–60. doi:10.1111/j.1471-4159.1979.tb11084.x
22. Lassmann H, Brunner C, Bradl M, Linington C. Experimental allergic encephalomyelitis: the balance between encephalitogenic T lymphocytes and demyelinating antibodies determines size and structure of demyelinated lesions. *Acta Neuropathol* (1988) 75(6):566–76. doi:10.1007/BF00686201
23. Bradl M, Linington C. Animal models of demyelination. *Brain Pathol* (1996) 6(3):303–11. doi:10.1111/j.1750-3639.1996.tb00857.x

24. Adelmann M, Wood J, Benzel I, Fiori P, Lassmann H, Matthieu JM, et al. The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. *J Neuroimmunol* (1995) 63(1):17–27. doi:10.1016/0165-5728(95)00124-7
25. von Budingen HC, Hauser SL, Fuhrmann A, Nabavi CB, Lee JI, Genain CP. Molecular characterization of antibody specificities against myelin/oligodendrocyte glycoprotein in autoimmune demyelination. *Proc Natl Acad Sci U S A* (2002) 99(12):8207–12. doi:10.1073/pnas.122092499
26. Marta CB, Oliver AR, Sweet RA, Pfeiffer SE, Ruddle NH. Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology. *Proc Natl Acad Sci U S A* (2005) 102(39):13992–7. doi:10.1073/pnas.0504979102
27. de Graaf KL, Albert M, Weissert R. Autoantigen conformation influences both B- and T-cell responses and encephalitogenicity. *J Biol Chem* (2012) 287(21):17206–13. doi:10.1074/jbc.M111.304246
28. Bourquin C, Schubart A, Tobollik S, Mather I, Ogg S, Liblau R, et al. Selective unresponsiveness to conformational B cell epitopes of the myelin oligodendrocyte glycoprotein in H-2b mice. *J Immunol* (2003) 171(1):455–61. doi:10.4049/jimmunol.171.1.455
29. Berer K, Wekerle H, Krishnamoorthy G. B cells in spontaneous autoimmune diseases of the central nervous system. *Mol Immunol* (2011) 48(11):1332–7. doi:10.1016/j.molimm.2010.10.025
30. Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, et al. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol* (1994) 153(10):4349–56.
31. Kerlero de Rosbo N, Mendel I, Ben-Nun A. Chronic relapsing experimental autoimmune encephalomyelitis with a delayed onset and an atypical clinical course, induced in PL/J mice by myelin oligodendrocyte glycoprotein (MOG)-derived peptide: preliminary analysis of MOG T cell epitopes. *Eur J Immunol* (1995) 25(4):985–93. doi:10.1002/eji.1830250419
32. Mendel I, Kerlero de Rosbo N, Ben-Nun A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur J Immunol* (1995) 25(7):1951–9. doi:10.1002/eji.1830250723
33. Linington C, Berger T, Perry L, Weerth S, Hinze-Selch D, Zhang Y, et al. T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system. *Eur J Immunol* (1993) 23(6):1364–72. doi:10.1002/eji.1830230627
34. Delarasse C, Smith P, Baker D, Amor S. Novel pathogenic epitopes of myelin oligodendrocyte glycoprotein induce experimental autoimmune encephalomyelitis in C57BL/6 mice. *Immunology* (2013) 140(4):456–64. doi:10.1111/imm.12155
35. Shetty A, Gupta SG, Varrin-Doyer M, Weber MS, Prod'homme T, Molnarfi N, et al. Immunodominant T-cell epitopes of MOG reside in its transmembrane and cytoplasmic domains in EAE. *Neurol Neuroimmunol Neuroinflamm* (2014) 1(2):e22. doi:10.1212/NXI.0000000000000022
36. Trotter J, DeJong LJ, Smith ME. Opsonization with antimyelin antibody increases the uptake and intracellular metabolism of myelin in inflammatory macrophages. *J Neurochem* (1986) 47(3):779–89. doi:10.1111/j.1471-4159.1986.tb00679.x
37. Schluesener HJ, Sobel RA, Linington C, Weiner HL. A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J Immunol* (1987) 139(12):4016–21.
38. Kerlero de Rosbo N, Honegger P, Lassmann H, Matthieu JM. Demyelination induced in aggregating brain cell cultures by a monoclonal antibody against myelin/oligodendrocyte glycoprotein. *J Neurochem* (1990) 55(2):583–7. doi:10.1111/j.1471-4159.1990.tb04173.x
39. Scolding NJ, Compston DA. Oligodendrocyte-macrophage interactions in vitro triggered by specific antibodies. *Immunology* (1991) 72(1):127–32.
40. Vass K, Heininger K, Schafer B, Linington C, Lassmann H. Interferon-gamma potentiates antibody-mediated demyelination in vivo. *Ann Neurol* (1992) 32(2):198–206. doi:10.1002/ana.410320212
41. Linington C, Engelhardt B, Kapocs G, Lassman H. Induction of persistently demyelinated lesions in the rat following the repeated adoptive transfer of encephalitogenic T cells and demyelinating antibody. *J Neuroimmunol* (1992) 40(2–3):219–24. doi:10.1016/0165-5728(92)90136-9
42. Piddlesden SJ, Lassmann H, Zimprich F, Morgan BP, Linington C. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. *Am J Pathol* (1993) 143(2):555–64.
43. Genain CP, Nguyen MH, Letvin NL, Pearl R, Davis RL, Adelman M, et al. Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. *J Clin Invest* (1995) 96(6):2966–74. doi:10.1172/JCI118368
44. Johns TG, Kerlero de Rosbo N, Menon KK, Abo S, Gonzales MF, Bernard CC. Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J Immunol* (1995) 154(10):5536–41.
45. Ichikawa M, Johns TG, Adelmann M, Bernard CC. Antibody response in Lewis rats injected with myelin oligodendrocyte glycoprotein derived peptides. *Int Immunol* (1996) 8(11):1667–74. doi:10.1093/intimm/8.11.1667
46. Menon KK, Piddlesden SJ, Bernard CC. Demyelinating antibodies to myelin oligodendrocyte glycoprotein and galactocerebroside induce degradation of myelin basic protein in isolated human myelin. *J Neurochem* (1997) 69(1):214–22. doi:10.1046/j.1471-4159.1997.69010214.x
47. Van der Goes A, Kortekaas M, Hoekstra K, Dijkstra CD, Amor S. The role of anti-myelin (auto)-antibodies in the phagocytosis of myelin by macrophages. *J Neuroimmunol* (1999) 101(1):61–7. doi:10.1016/S0165-5728(99)00133-2
48. Marta CB, Taylor CM, Coetzee T, Kim T, Winkler S, Bansal R, et al. Antibody cross-linking of myelin oligodendrocyte glycoprotein leads to its rapid repartitioning into detergent-insoluble fractions, and altered protein phosphorylation and cell morphology. *J Neurosci* (2003) 23(13):5461–71.
49. von Budingen HC, Hauser SL, Ouallet JC, Tanuma N, Menge T, Genain CP. Frontline: epitope recognition on the myelin/oligodendrocyte glycoprotein differentially influences disease phenotype and antibody effector functions in autoimmune demyelination. *Eur J Immunol* (2004) 34(8):2072–83. doi:10.1002/eji.200425050
50. Zhou D, Srivastava R, Nessler S, Grummel V, Sommer N, Bruck W, et al. Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. *Proc Natl Acad Sci U S A* (2006) 103(50):19057–62. doi:10.1073/pnas.0607242103
51. Urich E, Gutcher I, Prinz M, Becher B. Autoantibody-mediated demyelination depends on complement activation but not activatory Fc-receptors. *Proc Natl Acad Sci U S A* (2006) 103(49):18697–702. doi:10.1073/pnas.0607283103
52. Jagessar SA, Smith PA, Blezer E, Delarasse C, Pham-Dinh D, Laman JD, 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(4):326–40. doi:10.1097/NEN.0b013e31816a6851
53. Harrer MD, von Budingen HC, Stoppini L, Alliod C, Pouly S, Fischer K, et al. Live imaging of remyelination after antibody-mediated demyelination in an ex-vivo model for immune mediated CNS damage. *Exp Neurol* (2009) 216(2):431–8. doi:10.1016/j.expneurol.2008.12.027
54. Ohtani S, Kohyama K, Matsumoto Y. Autoantibodies recognizing native MOG are closely associated with active demyelination but not with neuroinflammation in chronic EAE. *Neuropathology* (2011) 31(2):101–11. doi:10.1111/j.1440-1789.2010.01131.x
55. Mader S, Gredler V, Schanda K, Rostasy K, Dujmovic I, Pfaller K, et al. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. *J Neuroinflammation* (2011) 8:184. doi:10.1186/1742-2094-8-184
56. Dale RC, Tantsis EM, Merheb V, Kumaran RY, Sinmaz N, Pathmanandavel K, et al. Antibodies to MOG have a demyelination phenotype and affect oligodendrocyte cytoskeleton. *Neurol Neuroimmunol Neuroinflamm* (2014) 1(1):e12. doi:10.1212/NXI.0000000000000012
57. Saadoun S, Waters P, Owens GP, Bennett JL, Vincent A, Papadopoulos MC. Neuromyelitis optica MOG-IgG causes reversible lesions in mouse brain. *Acta Neuropathol Commun* (2014) 2:35. doi:10.1186/2051-5960-2-35
58. Flach AC, Litke T, Strauss J, Haberl M, Gomez CC, Reindl M, et al. Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease. *Proc Natl Acad Sci U S A* (2016) 113(12):3323–8. doi:10.1073/pnas.1519608113
59. Kinzel S, Lehmann-Horn K, Torke S, Hausler D, Winkler A, Stadelmann C, et al. Myelin-reactive antibodies initiate T cell-mediated CNS autoimmune disease by opsonization of endogenous antigen. *Acta Neuropathol* (2016) 132(1):43–58. doi:10.1007/s00401-016-1559-8
60. Devaux B, Enderlin F, Wallner B, Smilek DE. Induction of EAE in mice with recombinant human MOG, and treatment of EAE with a

MOG peptide. *J Neuroimmunol* (1997) 75(1–2):169–73. doi:10.1016/S0165-5728(97)00019-2
61. Slavin A, Ewing C, Liu J, Ichikawa M, Slavin J, Bernard CC. Induction of a multiple sclerosis-like disease in mice with an immunodominant epitope of myelin oligodendrocyte glycoprotein. *Autoimmunity* (1998) 28(2):109–20. doi:10.3109/08916939809003872
62. Weissert R, Wallstrom E, Storch MK, Stefferl A, Lorentzen J, Lassmann H, et al. MHC haplotype-dependent regulation of MOG-induced EAE in rats. *J Clin Invest* (1998) 102(6):1265–73. doi:10.1172/JCI3022
63. Storch MK, Stefferl A, Brehm U, Weissert R, Wallstrom E, Kerschensteiner M, et al. Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple sclerosis pathology. *Brain Pathol* (1998) 8(4):681–94. doi:10.1111/j.1750-3639.1998.tb00194.x
64. Encinas JA, Wicker LS, Peterson LB, Mukasa A, Teuscher C, Sobel R, et al. QTL influencing autoimmune diabetes and encephalomyelitis map to a 0.15-cM region containing Il2. *Nat Genet* (1999) 21(2):158–60. doi:10.1038/5941
65. Raine CS, Cannella B, Hauser SL, Genain CP. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation. *Ann Neurol* (1999) 46(2):144–60. doi:10.1002/1531-8249(199908)46:2<144::AID-ANA3>3.0.CO;2-K
66. Abdul-Majid KB, Jirholt J, Stadelmann C, Stefferl A, Kjellen P, Wallstrom E, et al. Screening of several H-2 congenic mouse strains identified H-2(q) mice as highly susceptible to MOG-induced EAE with minimal adjuvant requirement. *J Neuroimmunol* (2000) 111(1–2):23–33. doi:10.1016/S0165-5728(00)00360-X
67. Kerlero de Rosbo N, Brok HP, Bauer J, Kaye JF, 't Hart BA, Ben-Nun A. Rhesus monkeys are highly susceptible to experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein: characterisation of immunodominant T- and B-cell epitopes. *J Neuroimmunol* (2000) 110(1–2):83–96. doi:10.1016/S0165-5728(00)00306-4
68. Bourquin C, Iglesias A, Berger T, Wekerle H, Linington C. Myelin oligodendrocyte glycoprotein-DNA vaccination induces antibody-mediated autoaggression in experimental autoimmune encephalomyelitis. *Eur J Immunol* (2000) 30(12):3663–71. doi:10.1002/1521-4141(200012)30:12<3663::AID-IMMU3663>3.0.CO;2-7
69. Brok HP, Uccelli A, Kerlero De Rosbo N, Bontrop RE, Roccatagliata L, de Groot NG, 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(2):1093–101. doi:10.4049/jimmunol.165.2.1093
70. Weissert R, de Graaf KL, Storch MK, Barth S, Linington C, Lassmann H, et al. MHC class II-regulated central nervous system autoaggression and T cell responses in peripheral lymphoid tissues are dissociated in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. *J Immunol* (2001) 166(12):7588–99. doi:10.4049/jimmunol.166.12.7588
71. Bettelli E, Pagany M, Weiner HL, Linington C, Sobel RA, Kuchroo VK. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J Exp Med* (2003) 197(9):1073–81. doi:10.1084/jem.20021603
72. Sun D, Zhang Y, Wei B, Peiper SC, Shao H, Kaplan HJ. Encephalitogenic activity of truncated myelin oligodendrocyte glycoprotein (MOG) peptides and their recognition by CD8+ MOG-specific T cells on oligomeric MHC class I molecules. *Int Immunol* (2003) 15(2):261–8. doi:10.1093/intimm/dxg023
73. Smith PA, Heijmans N, Ouwerling B, Breij EC, Evans N, van Noort JM, et al. Native myelin oligodendrocyte glycoprotein promotes severe chronic neurological disease and demyelination in Biozzi ABH mice. *Eur J Immunol* (2005) 35(4):1311–9. doi:10.1002/eji.200425842
74. Krishnamoorthy G, Lassmann H, Wekerle H, Holz A. Spontaneous opticospinal encephalomyelitis in a double-transgenic mouse model of autoimmune T cell/B cell cooperation. *J Clin Invest* (2006) 116(9):2385–92. doi:10.1172/JCI28330
75. de Graaf KL, Barth S, Herrmann MM, Storch MK, Wiesmuller KH, Weissert R. Characterization of the encephalitogenic immune response in a model of multiple sclerosis. *Eur J Immunol* (2008) 38(1):299–308. doi:10.1002/eji.200737475
76. Kap YS, Smith P, Jagessar SA, Remarque E, Blezer E, Strijkers GJ, 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(3):1326–37. doi:10.4049/jimmunol.180.3.1326
77. Matsumoto Y, Park IK, Hiraki K, Ohtani S, Kohyama K. Role of pathogenic T cells and autoantibodies in relapse and progression of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis in LEW.1AV1 rats. *Immunology* (2009) 128(1 Suppl):e250–61. doi:10.1111/j.1365-2567.2008.02955.x
78. Pollinger B, Krishnamoorthy G, Berer K, Lassmann H, Bosl MR, Dunn R, et al. Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit endogenous MOG-specific B cells. *J Exp Med* (2009) 206(6):1303–16. doi:10.1084/jem.20090299
79. Bettini M, Rosenthal K, Evavold BD. Pathogenic MOG-reactive CD8+ T cells require MOG-reactive CD4+ T cells for sustained CNS inflammation during chronic EAE. *J Neuroimmunol* (2009) 213(1–2):60–8. doi:10.1016/j.jneuroim.2009.05.017
80. York NR, Mendoza JP, Ortega SB, Benagh A, Tyler AF, Firan M, et al. Immune regulatory CNS-reactive CD8+T cells in experimental autoimmune encephalomyelitis. *J Autoimmun* (2010) 35(1):33–44. doi:10.1016/j.jaut.2010.01.003
81. Anderson AC, Chandwaskar R, Lee DH, Sullivan JM, Solomon A, Rodriguez-Manzanet R, et al. A transgenic model of central nervous system autoimmunity mediated by CD4+ and CD8+ T and B cells. *J Immunol* (2012) 188(5):2084–92. doi:10.4049/jimmunol.1102186
82. Jagessar SA, Heijmans N, Blezer EL, Bauer J, Blokhuis JH, Wubben JA, et al. 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* (2012) 42(1):217–27. doi:10.1002/eji.201141863
83. Ortega SB, Kashi VP, Tyler AF, Cunnusamy K, Mendoza JP, Karandikar NJ. The disease-ameliorating function of autoregulatory CD8 T cells is mediated by targeting of encephalitogenic CD4 T cells in experimental autoimmune encephalomyelitis. *J Immunol* (2013) 191(1):117–26. doi:10.4049/jimmunol.1300452
84. Haanstra KG, Jagessar SA, Bauchet AL, Doussau M, Fovet CM, Heijmans N, et al. Induction of experimental autoimmune encephalomyelitis with recombinant human myelin oligodendrocyte glycoprotein in incomplete Freund's adjuvant in three non-human primate species. *J Neuroimmune Pharmacol* (2013) 8(5):1251–64. doi:10.1007/s11481-013-9487-z
85. Curtis AD II, Taslim N, Reece SP, Grebenciucova E, Ray RH, Rosenbaum MD, et al. The extracellular domain of myelin oligodendrocyte glycoprotein elicits atypical experimental autoimmune encephalomyelitis in rat and macaque species. *PLoS One* (2014) 9(10):e110048. doi:10.1371/journal.pone.0110048
86. Herrera SL, Palmer VL, Whittaker H, Smith BC, Kim A, Schellenberg AE, et al. Damage to the optic chiasm in myelin oligodendrocyte glycoprotein-experimental autoimmune encephalomyelitis mice. *Magn Reson Insights* (2014) 7:23–31. doi:10.4137/MRI.S19750
87. Stefferl A, Brehm U, Storch M, Lambracht-Washington D, Bourquin C, Wonigeit K, et al. Myelin oligodendrocyte glycoprotein induces experimental autoimmune encephalomyelitis in the "resistant" brown Norway rat: disease susceptibility is determined by MHC and MHC-linked effects on the B cell response. *J Immunol* (1999) 163(1):40–9.
88. Forsthuber TG, Shive CL, Wienhold W, de Graaf K, Spack EG, Sublett R, et al. T cell epitopes of human myelin oligodendrocyte glycoprotein identified in HLA-DR4 (DRB1*0401) transgenic mice are encephalitogenic and are presented by human B cells. *J Immunol* (2001) 167(12):7119–25. doi:10.4049/jimmunol.167.12.7119
89. Litzenburger T, Fassler R, Bauer J, Lassmann H, Linington C, Wekerle H, et al. B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. *J Exp Med* (1998) 188(1):169–80. doi:10.1084/jem.188.1.169
90. Lyons JA, San M, Happ MP, Cross AH. B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. *Eur J Immunol* (1999) 29(11):3432–9. doi:10.1002/(SICI)1521-4141(199911)29:11<3432::AID-IMMU3432>3.0.CO;2-2
91. Lyons JA, Ramsbottom MJ, Cross AH. Critical role of antigen-specific antibody in experimental autoimmune encephalomyelitis induced by recombinant myelin oligodendrocyte glycoprotein. *Eur J Immunol* (2002) 32(7):1905–13. doi:10.1002/1521-4141(200207)32:7<1905::AID-IMMU1905>3.0.CO;2-L

92. Hjelmstrom P, Juedes AE, Fjell J, Ruddle NH. B-cell-deficient mice develop experimental allergic encephalomyelitis with demyelination after myelin oligodendrocyte glycoprotein sensitization. *J Immunol* (1998) 161(9):4480–3.
93. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* (2002) 3(10):944–50. doi:10.1038/ni833
94. Molnarfi N, Schulze-Topphoff U, Weber MS, Patarroyo JC, Prod'homme T, Varrin-Doyer M, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* (2013) 210(13):2921–37. doi:10.1084/jem.20130699
95. Parker Harp CR, Archambault AS, Sim J, Ferris ST, Mikesell RJ, Koni PA, et al. B cell antigen presentation is sufficient to drive neuroinflammation in an animal model of multiple sclerosis. *J Immunol* (2015) 194(11):5077–84. doi:10.4049/jimmunol.1402236
96. Svensson L, Abdul-Majid KB, Bauer J, Lassmann H, Harris RA, Holmdahl R. A comparative analysis of B cell-mediated myelin oligodendrocyte glycoprotein-experimental autoimmune encephalomyelitis pathogenesis in B cell-deficient mice reveals an effect on demyelination. *Eur J Immunol* (2002) 32(7):1939–46. doi:10.1002/1521-4141(200207)32:7<1939::AID-IMMU1939>3.0.CO;2-S
97. Bettelli E, Baeten D, Jager A, Sobel RA, Kuchroo VK. Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. *J Clin Invest* (2006) 116(9):2393–402. doi:10.1172/JCI28334
98. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* (2011) 479(7374):538–41. doi:10.1038/nature10554
99. Lassmann H, Bradl M. Multiple sclerosis: experimental models and reality. *Acta Neuropathol* (2017) 133(2):223–44. doi:10.1007/s00401-016-1631-4
100. Hoftberger R, Leisser M, Bauer J, Lassmann H. Autoimmune encephalitis in humans: how closely does it reflect multiple sclerosis? *Acta Neuropathol Commun* (2015) 3(1):80. doi:10.1186/s40478-015-0260-9
101. 't Hart BA, Gran B, Weissert R. EAE: imperfect but useful models of multiple sclerosis. *Trends Mol Med* (2011) 17(3):119–25. doi:10.1016/j.molmed.2010.11.006
102. 't Hart BA, van Kooyk Y, Geurts JJ, Gran B. The primate autoimmune encephalomyelitis model; a bridge between mouse and man. *Ann Clin Transl Neurol* (2015) 2(5):581–93. doi:10.1002/acn3.194
103. Massacesi L, Genain CP, Lee-Parritz D, Letvin NL, Canfield D, Hauser SL. Active and passively induced experimental autoimmune encephalomyelitis in common marmosets: a new model for multiple sclerosis. *Ann Neurol* (1995) 37(4):519–30. doi:10.1002/ana.410370415
104. Genain CP, Hauser SL. Experimental allergic encephalomyelitis in the New World monkey *Callithrix jacchus*. *Immunol Rev* (2001) 183:159–72. doi:10.1034/j.1600-065x.2001.1830113.x
105. Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N Engl J Med* (2003) 349(2):139–45. doi:10.1056/NEJMoa022328
106. Kuhle J, Pohl C, Mehling M, Edan G, Freedman MS, Hartung HP, et al. Lack of association between antimyelin antibodies and progression to multiple sclerosis. *N Engl J Med* (2007) 356(4):371–8. doi:10.1056/NEJMoa063602
107. Berger T, Reindl M. Antibody biomarkers in CNS demyelinating diseases – a long and winding road. *Eur J Neurol* (2015) 22(8):1162–8. doi:10.1111/ene.12759
108. Pache F, Zimmermann H, Mikolajczak J, Schumacher S, Lacheta A, Oertel FC, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 4: afferent visual system damage after optic neuritis in MOG-IgG-seropositive versus AQP4-IgG-seropositive patients. *J Neuroinflammation* (2016) 13(1):282. doi:10.1186/s12974-016-0720-6
109. Lalive PH, Menge T, Delarasse C, Della Gaspera B, Pham-Dinh D, Villoslada P, et al. Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. *Proc Natl Acad Sci U S A* (2006) 103(7):2280–5. doi:10.1073/pnas.0510672103
110. O'Connor KC, McLaughlin KA, De Jager PL, Chitnis T, Bettelli E, Xu C, et al. Self-antigen tetramers discriminate between myelin autoantibodies to native or denatured protein. *Nat Med* (2007) 13(2):211–7. doi:10.1038/nm1488
111. McLaughlin KA, Chitnis T, Newcombe J, Franz B, Kennedy J, McArdel S, et al. Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis. *J Immunol* (2009) 183(6):4067–76. doi:10.4049/jimmunol.0801888
112. Brilot F, Dale RC, Selter RC, Grummel V, Kalluri SR, Aslam M, et al. Antibodies to native myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. *Ann Neurol* (2009) 66(6):833–42. doi:10.1002/ana.21916
113. Selter RC, Brilot F, Grummel V, Kraus V, Cepok S, Dale RC, et al. Antibody responses to EBV and native MOG in pediatric inflammatory demyelinating CNS diseases. *Neurology* (2010) 74(21):1711–5. doi:10.1212/WNL.0b013e3181e04096
114. Lalive PH, Hausler MG, Maurey H, Mikaeloff Y, Tardieu M, Wiendl H, et al. Highly reactive anti-myelin oligodendrocyte glycoprotein antibodies differentiate demyelinating diseases from viral encephalitis in children. *Mult Scler* (2011) 17(3):297–302. doi:10.1177/1352458510389220
115. Di Pauli F, Mader S, Rostasy K, Schanda K, Bajer-Kornek B, Ehling R, et al. Temporal dynamics of anti-MOG antibodies in CNS demyelinating diseases. *Clin Immunol* (2011) 138(3):247–54. doi:10.1016/j.clim.2010.11.013
116. Probstel AK, Dornmair K, Bittner R, Sperl P, Jenne D, Magalhaes S, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. *Neurology* (2011) 77(6):580–8. doi:10.1212/WNL.0b013e318228c0b1
117. Kezuka T, Usui Y, Yamakawa N, Matsunaga Y, Matsuda R, Masuda M, et al. Relationship between NMO-antibody and anti-MOG antibody in optic neuritis. *J Neuroophthalmol* (2012) 32(2):107–10. doi:10.1097/WNO.0b013e31823c9b6c
118. Rostasy K, Mader S, Schanda K, Huppke P, Gartner J, Kraus V, et al. Anti-myelin oligodendrocyte glycoprotein antibodies in pediatric patients with optic neuritis. *Arch Neurol* (2012) 69(6):752–6. doi:10.1001/archneurol.2011.2956
119. Kitley J, Woodhall M, Waters P, Leite MI, Devenney E, Craig J, et al. Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. *Neurology* (2012) 79(12):1273–7. doi:10.1212/WNL.0b013e31826aac4e
120. Huppke P, Rostasy K, Karenfort M, Huppke B, Seidl R, Leiz S, et al. Acute disseminated encephalomyelitis followed by recurrent or monophasic optic neuritis in pediatric patients. *Mult Scler* (2013) 19(7):941–6. doi:10.1177/1352458512466317
121. Rostasy K, Mader S, Hennes EM, Schanda K, Gredler V, Guenther A, et al. Persisting myelin oligodendrocyte glycoprotein antibodies in aquaporin-4 antibody negative pediatric neuromyelitis optica. *Mult Scler* (2013) 19(8):1052–9. doi:10.1177/1352458512470310
122. Mayer MC, Breithaupt C, Reindl M, Schanda K, Rostasy K, Berger T, et al. Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases. *J Immunol* (2013) 191(7):3594–604. doi:10.4049/jimmunol.1301296
123. Woodhall M, Coban A, Waters P, Ekizoglu E, Kurtuncu M, Shugaiv E, et al. Glycine receptor and myelin oligodendrocyte glycoprotein antibodies in Turkish patients with neuromyelitis optica. *J Neurol Sci* (2013) 335(1–2):221–3. doi:10.1016/j.jns.2013.08.034
124. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, Takahashi T, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* (2014) 82(6):474–81. doi:10.1212/WNL.0000000000000101
125. Kitley J, Waters P, Woodhall M, Leite MI, Murchison A, George J, et al. Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study. *JAMA Neurol* (2014) 71(3):276–83. doi:10.1001/jamaneurol.2013.5857
126. Titulaer MJ, Hoftberger R, Iizuka T, Leypoldt F, McCracken L, Cellucci T, et al. Overlapping demyelinating syndromes and anti-*N*-methyl-D-aspartate receptor encephalitis. *Ann Neurol* (2014) 75(3):411–28. doi:10.1002/ana.24117
127. Hacohen Y, Absoud M, Woodhall M, Cummins C, De Goede CG, Hemingway C, et al. Autoantibody biomarkers in childhood-acquired demyelinating syndromes: results from a national surveillance cohort. *J Neurol Neurosurg Psychiatry* (2014) 85(4):456–61. doi:10.1136/jnnp-2013-306411

128. Tanaka M, Tanaka K. Anti-MOG antibodies in adult patients with demyelinating disorders of the central nervous system. *J Neuroimmunol* (2014) 270(1–2):98–9. doi:10.1016/j.jneuroim.2014.03.001
129. Miyauchi A, Monden Y, Watanabe M, Sugie H, Morita M, Kezuka T, et al. Persistent presence of the anti-myelin oligodendrocyte glycoprotein autoantibody in a pediatric case of acute disseminated encephalomyelitis followed by optic neuritis. *Neuropediatrics* (2014) 45(3):196–9. doi:10.1055/s-0034-1371179
130. Ramanathan S, Reddel SW, Henderson A, Parratt JD, Barnett M, Gatt PN, et al. Antibodies to myelin oligodendrocyte glycoprotein in bilateral and recurrent optic neuritis. *Neurol Neuroimmunol Neuroinflamm* (2014) 1(4):e40. doi:10.1212/NXI.0000000000000040
131. Amano H, Miyamoto N, Shimura H, Sato DK, Fujihara K, Ueno S, et al. Influenza-associated MOG antibody-positive longitudinally extensive transverse myelitis: a case report. *BMC Neurol* (2014) 14:224. doi:10.1186/s12883-014-0224-x
132. Elong Ngono A, Lepetit M, Reindl M, Garcia A, Guillot F, Genty A, et al. Decreased frequency of circulating myelin oligodendrocyte glycoprotein B lymphocytes in patients with relapsing-remitting multiple sclerosis. *J Immunol Res* (2015) 2015:673503. doi:10.1155/2015/673503
133. Tsuburaya RS, Miki N, Tanaka K, Kageyama T, Irahara K, Mukaida S, et al. Anti-myelin oligodendrocyte glycoprotein (MOG) antibodies in a Japanese boy with recurrent optic neuritis. *Brain Dev* (2015) 37(1):145–8. doi:10.1016/j.braindev.2014.02.002
134. Martinez-Hernandez E, Sepulveda M, Rostasy K, Hoftberger R, Graus F, Harvey RJ, et al. Antibodies to aquaporin 4, myelin-oligodendrocyte glycoprotein, and the glycine receptor alpha1 subunit in patients with isolated optic neuritis. *JAMA Neurol* (2015) 72(2):187–93. doi:10.1001/jamaneurol.2014.3602
135. Baumann M, Sahin K, Lechner C, Hennes EM, Schanda K, Mader S, et al. Clinical and neuroradiological differences of paediatric acute disseminating encephalomyelitis with and without antibodies to the myelin oligodendrocyte glycoprotein. *J Neurol Neurosurg Psychiatry* (2015) 86(3):265–72. doi:10.1136/jnnp-2014-308346
136. Spadaro M, Gerdes LA, Mayer MC, Ertl-Wagner B, Laurent S, Krumbholz M, et al. Histopathology and clinical course of MOG-antibody-associated encephalomyelitis. *Ann Clin Transl Neurol* (2015) 2(3):295–301. doi:10.1002/acn3.164
137. Probstel AK, Rudolf G, Dornmair K, Collongues N, Chanson JB, Sanderson NS, et al. Anti-MOG antibodies are present in a subgroup of patients with a neuromyelitis optica phenotype. *J Neuroinflammation* (2015) 12:46. doi:10.1186/s12974-015-0256-1
138. Hacohen Y, Absoud M, Deiva K, Hemingway C, Nytrova P, Woodhall M, et al. Myelin oligodendrocyte glycoprotein antibodies are associated with a non-MS course in children. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(2):e81. doi:10.1212/NXI.0000000000000081
139. Symmonds M, Waters PJ, Kuker W, Leite MI, Schulz UG. Anti-MOG antibodies with longitudinally extensive transverse myelitis preceded by CLIPPERS. *Neurology* (2015) 84(11):1177–9. doi:10.1212/WNL.0000000000001370
140. Morris KA, Waters P, Woodhall MR, Kuker W, Vincent A, Leite MI, et al. A 41-year-old woman with acute weakness and encephalopathy associated with MOG antibodies. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(3):e88. doi:10.1212/NXI.0000000000000088
141. Waters P, Woodhall M, O'Connor KC, Reindl M, Lang B, Sato DK, et al. MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(3):e89. doi:10.1212/NXI.0000000000000089
142. Ikeda K, Kiyota N, Kuroda H, Sato DK, Nishiyama S, Takahashi T, et al. Severe demyelination but no astrocytopathy in clinically definite neuromyelitis optica with anti-myelin-oligodendrocyte glycoprotein antibody. *Mult Scler* (2015) 21(5):656–9. doi:10.1177/1352458514551455
143. Nakajima H, Motomura M, Tanaka K, Fujikawa A, Nakata R, Maeda Y, et al. Antibodies to myelin oligodendrocyte glycoprotein in idiopathic optic neuritis. *BMJ Open* (2015) 5(4):e007766. doi:10.1136/bmjopen-2015-007766
144. Hyun JW, Kim SH, Huh SY, Kim W, Yun J, Joung A, et al. Idiopathic aquaporin-4 antibody negative longitudinally extensive transverse myelitis. *Mult Scler* (2015) 21(6):710–7. doi:10.1177/1352458514551454
145. Hoftberger R, Sepulveda M, Armangue T, Blanco Y, Rostasy K, Cobo Calvo A, et al. Antibodies to MOG and AQP4 in adults with neuromyelitis optica and suspected limited forms of the disease. *Mult Scler* (2015) 21(7):866–74. doi:10.1177/1352458514555785
146. Chalmoukou K, Alexopoulos H, Akrivou S, Stathopoulos P, Reindl M, Dalakas MC. Anti-MOG antibodies are frequently associated with steroid-sensitive recurrent optic neuritis. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(4):e131. doi:10.1212/NXI.0000000000000131
147. Ketelslegers IA, Van Pelt DE, Bryde S, Neuteboom RF, Catsman-Berrevoets CE, Hamann D, et al. Anti-MOG antibodies plead against MS diagnosis in an acquired demyelinating syndromes cohort. *Mult Scler* (2015) 21(12):1513–20. doi:10.1177/1352458514566666
148. Kim SM, Woodhall MR, Kim JS, Kim SJ, Park KS, Vincent A, et al. Antibodies to MOG in adults with inflammatory demyelinating disease of the CNS. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(6):e163. doi:10.1212/NXI.0000000000000163
149. Di Pauli F, Hoftberger R, Reindl M, Beer R, Rhomberg P, Schanda K, et al. Fulminant demyelinating encephalomyelitis: insights from antibody studies and neuropathology. *Neurol Neuroimmunol Neuroinflamm* (2015) 2(6):e175. doi:10.1212/NXI.0000000000000175
150. Numa S, Kasai T, Kondo T, Kushimura Y, Kimura A, Takahashi H, et al. An adult case of anti-myelin oligodendrocyte glycoprotein (MOG) antibody-associated multiphasic acute disseminated encephalomyelitis at 33-year intervals. *Intern Med* (2016) 55(6):699–702. doi:10.2169/internalmedicine.55.5727
151. Adachi T, Yasui K, Takahashi T, Fujihara K, Watanabe Y, Nakashima K. Anti-myelin oligodendrocyte glycoprotein antibodies in a patient with recurrent optic neuritis involving the cerebral white matter and brainstem. *Intern Med* (2016) 55(10):1351–4. doi:10.2169/internalmedicine.55.6122
152. Fernandez-Carbonell C, Vargas-Lowy D, Musallam A, Healy B, McLaughlin K, Wucherpfennig KW, et al. Clinical and MRI phenotype of children with MOG antibodies. *Mult Scler* (2016) 22(2):174–84. doi:10.1177/1352458515587751
153. Piccolo L, Woodhall M, Tackley G, Jurynczyk M, Kong Y, Domingos J, et al. Isolated new onset 'atypical' optic neuritis in the NMO clinic: serum antibodies, prognoses and diagnoses at follow-up. *J Neurol* (2016) 263(2):370–9. doi:10.1007/s00415-015-7983-1
154. Baumann M, Hennes EM, Schanda K, Karenfort M, Kornek B, Seidl R, et al. Children with multiphasic disseminated encephalomyelitis and antibodies to the myelin oligodendrocyte glycoprotein (MOG): extending the spectrum of MOG antibody positive diseases. *Mult Scler* (2016) 22(14):1821–9. doi:10.1177/1352458516631038
155. Kakalacheva K, Regenass S, Wiesmayr S, Azzi T, Berger C, Dale RC, et al. Infectious mononucleosis triggers generation of IgG auto-antibodies against native myelin oligodendrocyte glycoprotein. *Viruses* (2016) 8(2):E51. doi:10.3390/v8020051
156. Yan Y, Li Y, Fu Y, Yang L, Su L, Shi K, et al. Autoantibody to MOG suggests two distinct clinical subtypes of NMOSD. *Sci China Life Sci* (2016) 59(12):1270–81. doi:10.1007/s11427-015-4997-y
157. Cobo-Calvo A, Sepulveda M, Bernard-Valnet R, Ruiz A, Brassat D, Martinez-Yelamos S, et al. Antibodies to myelin oligodendrocyte glycoprotein in aquaporin 4 antibody seronegative longitudinally extensive transverse myelitis: clinical and prognostic implications. *Mult Scler* (2016) 22(3):312–9. doi:10.1177/1352458515591071
158. van Pelt ED, Wong YY, Ketelslegers IA, Hamann D, Hintzen RQ. Neuromyelitis optica spectrum disorders: comparison of clinical and magnetic resonance imaging characteristics of AQP4-IgG versus MOG-IgG seropositive cases in the Netherlands. *Eur J Neurol* (2016) 23(3):580–7. doi:10.1111/ene.12898
159. Ramanathan S, Prelog K, Barnes EH, Tantsis EM, Reddel SW, Henderson AP, et al. Radiological differentiation of optic neuritis with myelin oligodendrocyte glycoprotein antibodies, aquaporin-4 antibodies, and multiple sclerosis. *Mult Scler* (2016) 22(4):470–82. doi:10.1177/1352458515593406
160. Akaishi T, Sato DK, Nakashima I, Takeshita T, Takahashi T, Doi H, et al. MRI and retinal abnormalities in isolated optic neuritis with myelin oligodendrocyte glycoprotein and aquaporin-4 antibodies: a comparative study. *J Neurol Neurosurg Psychiatry* (2016) 87(4):446–8. doi:10.1136/jnnp-2014-310206
161. Sepulveda M, Armangue T, Sola-Valls N, Arrambide G, Meca-Lallana JE, Oreja-Guevara C, et al. Neuromyelitis optica spectrum disorders:

comparison according to the phenotype and serostatus. *Neurol Neuroimmunol Neuroinflamm* (2016) 3(3):e225. doi:10.1212/NXI.0000000000000225
162. Akaishi T, Nakashima I, Takeshita T, Mugikura S, Sato DK, Takahashi T, et al. Lesion length of optic neuritis impacts visual prognosis in neuromyelitis optica. *J Neuroimmunol* (2016) 293:28–33. doi:10.1016/j.jneuroim.2016.02.004
163. Polat I, Yis U, Karaoglu P, Ayanoglu M, Ozturk T, Guleryuz H, et al. Myelin oligodendrocyte glycoprotein antibody persistency in a steroid-dependent ADEM case. *Pediatrics* (2016) 137(5):e20151958. doi:10.1542/peds.2015-1958
164. Siritho S, Sato DK, Kaneko K, Fujihara K, Prayoonwiwat N. The clinical spectrum associated with myelin oligodendrocyte glycoprotein antibodies (anti-MOG-Ab) in Thai patients. *Mult Scler* (2016) 22(7):964–8. doi:10.1177/1352458515614093
165. Spadaro M, Gerdes LA, Krumbholz M, Ertl-Wagner B, Thaler FS, Schuh E, et al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* (2016) 3(5):e257. doi:10.1212/NXI.0000000000000257
166. Sepulveda M, Armangue T, Martinez-Hernandez E, Arrambide G, Sola-Valls N, Sabater L, et al. Clinical spectrum associated with MOG autoimmunity in adults: significance of sharing rodent MOG epitopes. *J Neurol* (2016) 263(7):1349–60. doi:10.1007/s00415-016-8147-7
167. Wang JJ, Jaunmuktane Z, Mummery C, Brandner S, Leary S, Trip SA. Inflammatory demyelination without astrocyte loss in MOG antibody-positive NMOSD. *Neurology* (2016) 87(2):229–31. doi:10.1212/WNL.0000000000002844
168. Thulasirajah S, Pohl D, Davila-Acosta J, Venkateswaran S. Myelin oligodendrocyte glycoprotein-associated pediatric central nervous system demyelination: clinical course, neuroimaging findings, and response to therapy. *Neuropediatrics* (2016) 47(4):245–52. doi:10.1055/s-0036-1583184
169. Lechner C, Baumann M, Hennes EM, Schanda K, Marquard K, Karenfort M, et al. Antibodies to MOG and AQP4 in children with neuromyelitis optica and limited forms of the disease. *J Neurol Neurosurg Psychiatry* (2016) 87:897–905. doi:10.1136/jnnp-2015-311743
170. Hacohen Y, Nishimoto Y, Fukami Y, Lang B, Waters P, Lim MJ, et al. Paediatric brainstem encephalitis associated with glial and neuronal autoantibodies. *Dev Med Child Neurol* (2016) 58(8):836–41. doi:10.1111/dmcn.13090
171. Azumagawa K, Nomura S, Shigeri Y, Jones LS, Sato DK, Nakashima I, et al. Post-vaccination MDEM associated with MOG antibody in a subclinical *Chlamydia* infected boy. *Brain Dev* (2016) 38(7):690–3. doi:10.1016/j.braindev.2016.02.004
172. Jarius S, Metz I, Konig FB, Ruprecht K, Reindl M, Paul F, et al. Screening for MOG-IgG and 27 other anti-glial and anti-neuronal autoantibodies in 'pattern II multiple sclerosis' and brain biopsy findings in a MOG-IgG-positive case. *Mult Scler* (2016) 22(12):1541–9. doi:10.1177/1352458515622986
173. Kaneko K, Sato DK, Nakashima I, Nishiyama S, Tanaka S, Marignier R, et al. Myelin injury without astrocytopathy in neuroinflammatory disorders with MOG antibodies. *J Neurol Neurosurg Psychiatry* (2016) 87(11):1257–9. doi:10.1136/jnnp-2015-312676
174. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. *J Neuroinflammation* (2016) 13(1):279. doi:10.1186/s12974-016-0719-z
175. Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. *J Neuroinflammation* (2016) 13(1):280. doi:10.1186/s12974-016-0718-0
176. Jarius S, Kleiter I, Ruprecht K, Asgari N, Pitarokoili K, Borisow N, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 3: brainstem involvement – frequency, presentation and outcome. *J Neuroinflammation* (2016) 13(1):281. doi:10.1186/s12974-016-0717-1
177. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
178. Konig FB, Wildemann B, Nessler S, Zhou D, Hemmer B, Metz I, et al. Persistence of immunopathological and radiological traits in multiple sclerosis. *Arch Neurol* (2008) 65(11):1527–32. doi:10.1001/archneur.65.11.1527
179. Körtvélyessy P, Breu M, Pawlitzki M, Metz I, Heinze H-J, Matzke M, et al. ADEM-like presentation, anti-MOG antibodies, and MS pathology: TWO case reports. *Neurol Neuroimmunol Neuroinflamm* (2017) 4(3):e335. doi:10.1212/NXI.0000000000000335
180. Misu T, Hoftberger R, Fujihara K, Wimmer I, Takai Y, Nishiyama S, et al. Presence of six different lesion types suggests diverse mechanisms of tissue injury in neuromyelitis optica. *Acta Neuropathol* (2013) 125(6):815–27. doi:10.1007/s00401-013-1116-7
181. Oliver AR, Lyon GM, Ruddle NH. Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice. *J Immunol* (2003) 171(1):462–8. doi:10.4049/jimmunol.171.9.4934
182. Burrer R, Buchmeier MJ, Wolfe T, Ting JP, Feuer R, Iglesias A, et al. Exacerbated pathology of viral encephalitis in mice with central nervous system-specific autoantibodies. *Am J Pathol* (2007) 170(2):557–66. doi:10.2353/ajpath.2007.060893
183. Young NP, Weinshenker BG, Parisi JE, Scheithauer B, Giannini C, Roemer SF, et al. Perivenous demyelination: association with clinically defined acute disseminated encephalomyelitis and comparison with pathologically confirmed multiple sclerosis. *Brain* (2010) 133(Pt 2):333–48. doi:10.1093/brain/awp321
184. Reindl M, Di Pauli F, Rostasy K, Berger T. The spectrum of MOG autoantibody-associated demyelinating diseases. *Nat Rev Neurol* (2013) 9(8):455–61. doi:10.1038/nrneurol.2013.118
185. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* (1983) 222(4630):1337–9. doi:10.1126/science.6362005
186. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* (1990) 347(6289):151–6. doi:10.1038/347151a0
187. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, et al. Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* (1994) 152(6):3183–8.
188. Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci U S A* (2007) 104(43):17040–5. doi:10.1073/pnas.0705894104
189. Bonifacio E. Predicting type 1 diabetes using biomarkers. *Diabetes Care* (2015) 38(6):989–96. doi:10.2337/dc15-0101
190. Pihoker C, Gilliam LK, Hampe CS, Lernmark A. Autoantibodies in diabetes. *Diabetes* (2005) 54(Suppl 2):S52–61. doi:10.2337/diabetes.54.suppl_2.S52

**Conflict of Interest Statement:** The Neurological Research Laboratory (Medical University of Innsbruck and Tirol Kliniken) receives payments for antibody assays (AQP4- and anti-neuronal antibodies) and for MOG and AQP4 antibody validation experiments organized by Euroimmun (Germany).

frontiers in Immunology

ORIGINAL RESEARCH
published: 08 May 2017
doi: 10.3389/fimmu.2017.00514

# Reactivity to Novel Autoantigens in Patients with Coexisting Central Nervous System Demyelinating Disease and Autoimmune Thyroid Disease

*Judith M. Greer[1]*, Simon Broadley[2,3] and Michael P. Pender[4,5]*

*[1] Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia, [2] School of Medicine, Griffith University, Southport, QLD, Australia, [3] Department of Neurology, Gold Coast University Hospital, Southport, QLD, Australia, [4] Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia, [5] Department of Neurology, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia*

OPEN ACCESS

**Edited by:**
*Robert Weissert, University of Regensburg, Germany*

**Reviewed by:**
*Herman Waldmann, University of Oxford, UK*
*Salvatore Benvenga, University of Messina, Italy*

***Correspondence:**
*Judith M. Greer*
*j.greer@uq.edu.au*

**Specialty section:**
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

**Received:** *23 February 2017*
**Accepted:** *18 April 2017*
**Published:** *08 May 2017*

**Citation:**
*Greer JM, Broadley S and Pender MP (2017) Reactivity to Novel Autoantigens in Patients with Coexisting Central Nervous System Demyelinating Disease and Autoimmune Thyroid Disease. Front. Immunol. 8:514. doi: 10.3389/fimmu.2017.00514*

Several lines of evidence suggest a definite and unique link between CNS demyelinating diseases and autoimmune thyroid disease (AITD). The aim of the current study was to systematically compare the clinical and laboratory features of patients with coexistent AITD and CNS demyelinating disease with those of patients with just CNS demyelinating disease. Forty-four patients with coexisting CNS demyelinating disease and AITD were identified and their clinical and radiological features were recorded. Blood and DNA were collected and tested for HLA type and for the response of T cells and antibodies to a variety of antigens. Patients with multiple sclerosis (MS) without AITD and healthy individuals were included as controls. Patients with coexisting AITD and CNS demyelinating disease were almost exclusively female (43/44) and had prominent spinal cord involvement as the main neurological finding. The HLA molecules carried by individuals with CNS demyelinating disease and AITD differed from both other MS patients and healthy individuals. Furthermore, patients with both CNS disease and AITD showed less T cell reactivity than patients with MS alone to myelin proteolipid protein, but, compared to other groups, showed elevated levels of T cell reactivity to the calcitonin gene-related peptide, which is present in both the CNS and the thyroid, and elevated levels of T cell and antibody to the leucine-rich repeat-containing G-protein coupled receptor 4 (LGR4), a molecule that is expressed in the brainstem and spinal cord, and which is a homolog of the thyroid-stimulating hormone receptor. We suggest that reactivity of autoreactive immune cells in these patients against antigens present in both the thyroid and the spinal cord is a potential mechanism underlying the pattern of lesion development in the CNS in patients with coexisting AITD and MS and might indicate a novel mechanism of disease pathogenesis in these patients.

**Keywords: multiple sclerosis, neuromyelitis optica spectrum disorder, autoimmune thyroid disease, calcitonin gene-related peptide, LGR4, T cells, autoantibodies, autoantigens**

## INTRODUCTION

We have had a long-term interest in the question of why some patients with autoimmune CNS demyelinating disease tend to develop lesions in relatively restricted parts of the CNS. In some patients, lesion distribution appears to correlate closely to the HLA type of the patients and the dominant myelin antigen-specific T cell reactivity restricted by those HLA types (1, 2). In other cases, we have noted that patients who have another coexisting autoimmune disease often tend to have a similar lesion distribution, HLA restriction, and T cell myelin antigen reactivity pattern when compared to other patients who have the same combination of autoimmune diseases. For example, we have shown that patients with a combination of multiple sclerosis (MS) and psoriasis tend to have prominent involvement of the brainstem and cerebellum lesions and to carry HLA-DRB1*07 (3) and have elevated levels of T cell reactivity against the 184–210 region of myelin proteolipid protein (PLP) (unpublished data).

Neuromyelitis optica (NMO) is another example of restricted lesion distribution in CNS demyelinating diseases, being originally characterized primarily by the presence of optic nerve and longitudinally extensive spinal cord lesions and the presence of aquaporin 4 (AQP4) autoantibodies. More recently, NMO spectrum disorders (NMOSD) has become the favored terminology for this syndrome, as it is now recognized that some patients have variant lesion distribution, with the presence of either autoantibodies against AQP4 or myelin oligodendrocyte glycoprotein (MOG). Interestingly, it has been reported that there is an increased incidence of non-organ-specific autoimmunity in NMOSD (4), and the coexistence of systemic lupus erythematosus (SLE) or Sjögren's syndrome in AQP4 autoantibody positive patients with NMOSD actually strengthens confidence in the NMOSD diagnosis (5).

In both MS and NMOSD, there have been reports of increased prevalence of thyroid dysfunction and anti-thyroid antibodies (6–10). We have observed that MS patients who have coexisting autoimmune thyroid disease (AITD) tend to have more extensive spinal cord involvement than other MS patients, irrespective of whether the MS or the thyroid disease develops first (11). Links between disease affecting the corticospinal tract and thyroid disease have been recognized for many years. In 1888, Charcot described paraplegia-like symptoms in a patient with severe hyperthyroidism (12). Hyperthyroidism has been reported as a contributing factor in corticospinal tract malfunction (13) and posterolateral myelopathy (14), and encephalopathy associated with hypothyroidism (Hashimoto encephalopathy) has been recognized for many years (15). In addition, increased IgG, myelin basic protein and activated helper T cells have been found in the cerebrospinal fluid of acute necrotizing myelopathy associated with thyroid cancer, suggesting that immune-mediated demyelination may be occurring in this condition (16).

There could be several explanations for the links between autoimmune CNS demyelinating disease and AITD. First, the HLA type of patients might predispose them to development of both AITD and CNS demyelinating disease, by allowing presentation of pathogenic epitopes specific for each of these disorders. The two main types of AITD, Graves' disease (hyperthyroidism) and Hashimoto's thyroiditis (hypothyroidism), have both been linked primarily to carriage of the HLA-DRB1*03-DQB1*02-DQA1*0501 (DR3) genotype in Caucasians (17). MS is generally thought of as involving linkage to HLA-DRB1*15:01; however, around 40% of MS patients are negative for DRB1*15:01 (1), and HLA-DRB1*03 has also been reported to confer an increased risk for development of MS (18). Interestingly, studies on NMOSD patients from Caucasian, Afro-Caribbean, and Indian populations have all found an association with HLA-DRB1*03 (19–21). Second, there may be cross-reactivity between antigens present in the CNS and in the thyroid, either through expression of the identical antigens in both organs or through expression of related proteins with conserved epitope domains in the different organs. Other possibilities include altered thyroid function inducing changes in the CNS, including the induction of neoantigens; however, our observation that patients with coexisting CNS and thyroid disease have similar patterns of lesion distribution, irrespective of whether they initially had CNS or thyroid disease, suggests that this last mechanism is less likely. Finally, molecular mimicry between various microbes and self-antigens has been postulated to underpin development of autoimmunity, and it is of interest to note that MS, NMOSD, and AITD have all been linked to infection with microbes, such as hepatitis C virus and *Helicobacter pylori*, suggesting the possibility that such infections could induce autoimmune diseases targeting more than one organ (22–26).

The aim of the current study was to investigate HLA molecules and T cell and autoantibody reactivity to CNS and thyroid antigens in patients with coexisting CNS demyelinating disease and AITD. We show that such patients differ in these respects from healthy individuals and MS patients without AITD.

## MATERIALS AND METHODS

### Patients and Controls

This study was approved by the Royal Brisbane and Women's Hospital Human Research Ethics Committee, The University of Queensland Medical Research Ethics Committee, and Griffith University Human Research Ethics Committee. Up to 20 mL of blood was obtained from patients with coexisting autoimmune CNS demyelinating disease and AITD, patients with MS alone, and healthy individuals who had no history of AITD. Written informed consent was obtained prior to blood collection. Where sufficient blood was available, 5 mL was used as a source of serum, 2 mL was used to extract genomic DNA, and the remainder was used for T cell assays. Only 3–5 mL of blood was available from four patients with autoimmune CNS demyelinating disease and AITD, in which case plasma was used rather than serum, and T cell assays were tested only for reactivity to one of the antigens of interest, calcitonin gene-related peptide (CGRP—see below for more detail on antigens used). Blood was not available from 11 of the patients with coexisting AITD and MS. Patient and control demographics for individuals from whom blood was collected are shown in **Table 1**.

### HLA Typing

Genomic DNA was prepared using NucleoSpin Blood DNA extraction kits (Macherey-Nagel, Düren, Germany) as previously

**TABLE 1 | Demographics of patients and controls whose blood was used in this study.**

| Group | *N* | Age at time of blood collection (mean ± SE) | M:F | CNS disease type | | | | Disease duration (mean ± SE) | EDSS (mean ± SE) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | RR-MS | SP-MS | PP-MS | RR-NMO | | |
| CNS demyelinating disease + autoimmune thyroid disease | 33 | 50.9 ± 1.8 | 1:32 | 12 | 11 | 6 | 4 | 13.1 ± 1.8 | 5.1 ± 0.4 |
| Hypo | 19 | 53.7 ± 2.2 | 1:18 | 5 | 6 | 5 | 3 | 15.5 ± 2.3 | 5.4 ± 0.4 |
| Hyper | 14 | 47.1 ± 3.0 | 0:14 | 7 | 5 | 1 | 1 | 10.1 ± 2.3 | 4.6 ± 0.8 |
| Other multiple sclerosis (MS) (T cell assays) | 20 | 46.0 ± 3.0 | 4:16 | 12 | 5 | 3 | 0 | 10.6 ± 2.4 | 4.3 ± 0.7 |
| Other MS (LGR4 and thyroid-stimulating hormone receptor serum assays) | 13 | 41.1 ± 3.0 | 3:10 | 10 | 1 | 2 | 0 | 10.1 ± 1.9 | 3.3 ± 0.5 |
| Other MS (calcitonin gene-related peptide serum assays) | 14 | 39.0 ± 2.5 | 2:12 | 9 | 2 | 3 | 0 | 7.2 ± 2.5 | 4.0 ± 0.7 |
| Healthy controls (HC) (T cell assays) | 20 | 41.4 ± 2.5 | 4:16 | NA | NA | NA | NA | NA | NA |
| HC (serum assays) | 11 | 36.2 ± 3.1 | 1:10 | NA | NA | NA | NA | NA | NA |

*M:F, number of males:number of females; RR-MS, relapsing-remitting multiple sclerosis; SP-MS, secondary progressive MS; PP-MS, primary progressive MS; RR-NMO, relapsing-remitting NMO; EDSS, Expanded Disability Status Scale score; NA, not applicable.*

described (2). Dynal low and high resolution SSP kits (Dynal Biotech, Thermo Fisher, Australia) were used to type for HLA-DR, -DQA, -DQB, and -DP alleles, following the manufacturer's recommended protocols. Results for the patients with coexisting CNS demyelinating disease and AITD are reported to the four digit level, when it was able to be determined. For comparison with larger groups of MS patients and healthy controls (HC), alleles were grouped at the two digit level.

## Peptides and Antigens

For T cell assays, peptides from PLP, the thyroid-stimulating hormone receptor (TSHR), LGR4 (a homolog of the TSHR), and the α and β types of CGRP were used to stimulate T cells. The PLP peptides used ($PLP_{184–199}$ + $PLP_{190–209}$) were overlapping peptides against which we have previously reported elevated levels of T cell reactivity in approximately 40% of MS patients (2). TSHR and LGR4 are members of the same LGR family and have some regions of sequence homology (27). The three peptides chosen from each of these molecules are from the extracellular portion of the protein in each instance. CGRP is a neuropeptide that has been reported to be expressed in both the thyroid and the spinal cord (28). The sequences of peptides used are given in **Table 2**. α-CGRP and β-CGRP were purchased from Bachem (Bubendorf, Switzerland). The other peptides were synthesized by Mimotopes (Melbourne, VIC, Australia). Because the PLP peptides are moderately hydrophobic they were dissolved in 0.2 M acetic acid as 5 mg/mL stock solutions. Stock solutions of the other antigens were made in water. The peptides were diluted in tissue culture medium immediately prior to use. For T cell assays, peptides were used in four pools: CGRP (α-CGRP + β-CGRP), PLP ($PLP_{184–199}$ + $PLP_{190–209}$), LGR4 (pool of the three LGR4 peptides), and TSHR (pool of the three TSHR peptides). Concanavalin A (Con A) was used as a positive control in all T cell assays.

## Assessment of T Cell Proliferation

Blood was centrifuged through Ficoll and the peripheral blood mononuclear cells (PBMC) were collected at the interface, washed, and used fresh in T cell assays. Assays testing for reactivity to CGRP were done using a $^3$H-thymidine uptake assay, as previously described (2). In brief, $1.5 \times 10^5$ fresh PBMC/well were cultured in U-bottom 96-well plates (Nunc) in the presence or absence of CGRPα + CGRPβ (at concentrations ranging from 1 to 50 μg/mL) or Con A (2 μg/mL) for 6 days, with [$^3$H]thymidine being added during the final 18 h. Cells were then harvested onto glass-fiber mats, and the cpm determined in a Betaplate counter (Beckman Coulter). The cell division index (CDI) was determined by the formula: CDI = (Mean cpm of peptide-containing wells)/(Mean cpm of control wells without peptide). The mean CDI reported is the maximum value over the different antigen dilutions. For the other antigens, reactivity was tested using CFSE assays, as we have previously described (2). Briefly, PBMC were labeled with 2 nM CFSE (Invitrogen), as previously described (29) and incubated with peptide pools (tested at 10 and 25 μg/mL concentrations of each peptide) for 10 days in phenol red-free X-vivo 15 serum-free medium (Lonza). Cells were then washed, stained with PerCP-labeled anti-CD4 or anti-CD8 antibodies (BD Biosciences), and analyzed by flow cytometry, with gating on the lymphocyte population. The number of cells with reduced CFSE staining in the $CD4^+$ or $CD8^+$ group divided by the total number of $CD4^+$ or $CD8^+$ cells was used to determine the percentage of cells dividing in response to antigen. The CDI was calculated as the percentage of cells dividing in response to antigen/percentage of cells dividing without antigen. The highest level of proliferation is reported in the results, regardless of the concentration of

**TABLE 2 | Sequences of peptides used in this study.**

| Peptide designation | Sequence |
|---|---|
| $PLP_{184–199}$ | QSIAFPSKTSASIGSL |
| $PLP_{190–209}$ | SKTSASIGSLCADARMYGVL |
| CGRPα | ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF |
| CGRPβ | ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF |
| $LGR4_{38–51}$[a] | DRRVDCSGKGLTAV |
| $LGR4_{529–542}$[a] | FKPCEYLLGSWMIR |
| $LGR4_{609–623}$[a] | GIWWETGSGCKVAGS |
| $TSHR_{36–49}$[b] | DFRVTCKDIQRIPS |
| $TSHR_{405–418}$[b] | FNPCEDIMGYKFLR |
| $TSHR_{485–499}$[b] | AIDWQTGPGCNTAGF |

[a]*Accession number for LGR4 sequence is Q9BXB1.*

[b]*Accession number for thyroid-stimulating hormone receptor sequence is P16473.*

antigen or whether the responding cells were $CD4^+$ or $CD8^+$ (the $CD4^+$ response was the highest is all expect one assay), in order to keep the results compatible with the assays for CGRP, where the type of responding cell is not determined.

## Detection of CGRP Antibodies

ELISA was used to test for the presence of antibodies against CGRP. Plates (Nunc Maxisorb) were coated with a mixture of α-CGRP and β-CGRP (each at 0.5 μg/well) + bovine serum albumin (BSA) (2.5μg/well) or with BSA (2.5μg/well) alone (control wells) in 0.2 M bicarbonate buffer. Plates were blocked with 2% skimmed milk in PBS-Tween 20, 100 μL of each diluted serum or plasma sample (1/40 dilution) was added in triplicate to control wells and wells containing CGRP, and the plates incubated overnight at room temperature in a humidified chamber. After four washes, 100 μL of alkaline phosphatase-labeled anti-human polyvalent Igs (G + A + M) (Sigma-Aldrich) was added to each well and plates were incubated for 2 h at room temperature in a humidified chamber. Plates were washed again and 200 μL of *p*-nitrophenyl phosphate substrate (Sigma-Aldrich) was added to each well. The reaction was stopped after 15 min by addition of 25 μL 3N NaOH, and the absorbance read at 405 in a plate reader (Paradigm). The CGRP-specific response was determined by subtracting the mean absorbance value for the wells coated with BSA alone from that of wells coated with BSA + CGRP.

## Cell-Based Assays to Test for Antibodies against AQP4, MOG, TSHR, and LGR4

Anti-AQP4 and anti-MOG antibodies were detected by fixed cell-based assay using a commercially available kit as per the manufacturer's instructions (EUROIMMUN, Germany). The presence of antibodies specific for TSHR and for LGR4 was assessed using Cos7 cells transduced with lentiviral particles expressing full length human TSHR or LGR4 (under the control of a cytomegalovirus promoter) together with a GFP marker (to identify transduced cells) and puromycin resistance gene (for selection of transduced cells) were obtained from Applied Biological Materials Inc. (Richmond, BC, Canada). COS-7 cells were grown to 75% confluency and then transduced using polybrene-assisted uptake of particles, using the manufacturer's recommended protocol. Successfully transduced cells were selected in media containing 20 μg/mL puromycin. The transduced cells or non-transduced Cos7 cells (as controls) were plated in 16-well chamber slides. Once cells were confluent, the slides were washed, fixed in freshly prepared 4% paraformaldehyde, and then stored in PBS with 0.01% azide at 4°C until required. Sera/plasma was added to wells at 1:20 dilution (1 h at 4°C). Each sample was tested on at least two wells of each cell line. Each slide contained control wells that had either no primary antibody or no primary and no secondary antibody added. After 3× 5 min washes in PBS-T, HRP-labeled anti-human Ig (G + A + M) was added to wells for 1 h at 4°C. Slides were washed again, and then nickel-enhanced DAB substrate (Sigma) was added for 5 min. Cellular DAB staining was assessed in a blinded fashion for each well.

## Statistical Analyses

GraphPad Prism 7.0 was used for analysis. For comparisons of frequencies, contingency tables were analyzed using a two-way Fisher's exact test. Where correction for multiple comparisons was applied, that is noted in the text. Where a comparison among three or more groups was to be made, data were first assessed to determine if they were normally distributed. If they were, ANOVA with Bonferroni correction for multiple comparisons was used to compare groups. If the data were not normally distributed, the Kruskal–Wallis test with Dunn's multiple comparisons test was used. $p < 0.05$ was considered to be significant throughout.

# RESULTS

## Features of Patients with Coexisting CNS Demyelinating Disease and AITD

We identified 44 patients with CNS demyelinating disease who had also been diagnosed with coexisting AITD. Of these 44 patients, all were of Caucasian background and only 1 (2.3%) was male. Four patients (10.8%, all female) met diagnostic criteria for NMOSD (5) and were positive for anti-AQP4 antibodies using a cell-based assay. All other patients (except case #227) met the 2005 and/or 2010 Revised McDonald criteria for MS (30, 31) and were negative for anti-AQP4 and anti-MOG autoantibodies using cell-based assays. Of the 40 MS patients with coexisting AITD, the distribution of disease course (50% relapsing-remitting MS, 33% secondary progressive MS, and 17.5% primary progressive MS) is typical for MS. Also in this group, symptoms of AITD started several months after commencement of treatment with IFN-β in four female patients, as we have previously reported in two of these patients (32); these patients were excluded from some of the analyses, as outlined in subsequent sections. None of the patients in the study were treated with alemtuzumab (which has also been linked to subsequent development of AITD).

The clinical characteristics of patients with CNS demyelinating disease and AITD are presented in **Table 3**. The majority of patients [25/40 (62.5%) in the MS group and 3/4 (75%) in the NMOSD group] had hypothyroidism. Of the 28 hypothyroid patients, 20 had overt hypothyroidism, 4 had subclinical disease, and this information was not recorded for the other four patients. Of the patients with hyperthyroidism, all but one had been treated with radioactive iodine or by thyroidectomy, and were taking thyroxine at the time of blood collection. The hyperthyroid patient who had not had such treatment was in remission at the time of blood collection. None of the hyperthyroid patients had ophthalmopathy. Excluding the patients who developed AITD following commencement of IFN-β treatment, the onset of AITD preceded that of CNS demyelinating disease in 17 patients, developed at the same time in 3 cases (including two of the patients with NMOSD), followed the onset of CNS demyelinating disease in 17 cases, and was unknown for 3 cases. Interestingly, however, even in this small number of patients there was a significant difference ($p < 0.05$) in the proportions of hypothyroid vs hyperthyroid patients who developed AITD before CNS demyelinating disease (**Table 4**). Hyperthyroidism

**TABLE 3 | Clinical characteristics of patients with multiple sclerosis (MS) and autoimmune thyroid disease (AITD).**

| ID No | Diagnosis | Cerebrospinal fluid Oligo IgG | Age MS onset | Age AITD onset | Thyroid type | Other autoimmune disease | Family history of autoimmunity | Major site of clinical involvement at attack | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 133 | RR-MS | NT | 43 | 42 | Hyper | None | Yes | SC | SC | SC | BS Cb | SC | | |
| 200 | RR-MS | NT | 32 | 16 | Hypo | None | Yes | SC | ON | SC | SC | SC ON | | |
| 269 | RR-MS | NT | 32 | 28 | Hyper | None | Yes | SC | SC | SC | | | | |
| 271 | RR-MS | Yes | 29 | 26 | Hyper | None | Yes | SC | SC Cb | BS | SC | | | |
| 274 | RR-MS | NT | 35 | <22 | Hyper | ANA 1:40 | Adopted | ON SC | SC | SC | SC | | | |
| 291 | RR-MS | NT | 23 | 51 | Hypo | None | No | ON | ON | SC | | | | |
| 299 | RR-MS | Yes | 57 | 51 | Hyper | None | Yes | BS SC | BS | SC | Cerebral | SC | | |
| 316 | RR-MS | Yes | 41 | 38 | Hypo | None | Yes | SC | SC | SC | SC | SC | | |
| 332 | RR-MS | No | 43 | 50 | Hypo | None | Yes | SC | BS SC | | | | | |
| 393 | RR-MS | NT | 55 | 35 | Hyper | None | Yes | SC | SC | | | | | |
| 493 | RR-MS | NT | 33 | 42 (after IFNβ) | Hyper | Crohn's | No | SC | SC | SC | BS SC | | | |
| 582 | RR-MS | NT | 30 | 38 (after IFNβ) | Hypo | None | Yes | Cerebrum | SC | SC | SC | | | |
| 609 | RR-MS | Yes | 35 | 30 | Hyper | None | Yes | ON | SC | | | | | |
| 610 | RR-MS | Yes | 17 | 44 (after IFNβ) | Hypo | None | Not known | ON | BS | SC | SC | SC | | |
| 611 | RR-MS | NT | 43 | ~50 | Hyper | None | Yes | ON Cb | BS | SC | BS | | | |
| 612 | RR-MS | NT | 25 | 37 | Hypo | None | No | ON | BS | SC | SC | | | |
| 613 | RR-MS | Yes | 40 | Not known | Hypo | None | Yes | BS | BS | BS | BS | BS | BS | BS |
| 615 | RR-MS | NT | 24 | 44 | Hypo | None | Yes | SC | BS | | | | | |
| 616 | RR-MS | Yes | 43 | Not known | Hypo | None | Not known | SC | SC | SC | SC | ON | | |
| 617 | RR-MS | NT | 35 | 36 | Hypo | None | Yes | BS | SC | SC | | | | |
| 31 | SP-MS | No | 34 | 55 | Hypo | Positive lupus anticoagulant | Yes | SC | BS | SC | SC | BS | BS ON | SC |
| 43 | SP-MS | Yes | 26 | 48 | Hypo | None | Yes | SC | Progressive SC | | | | | |
| 145 | SP-MS | NT | 40 | 65 | Hyper | Autoimmune hepatitis | Yes | SC | Cerebrum, SC and ON in secondary progression | | | | | |

(*Continued*)

**TABLE 3 | Continued**

| ID No | Diagnosis | Cerebrospinal fluid Oligo IgG | Age MS onset | Age AITD onset | Thyroid type | Other autoimmune disease | Family history of autoimmunity | Major site of clinical involvement at attack | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 171 | SP-MS | NT | 26 | 45 | Hypo | None | Yes | SC | SC BS | SC ON | Further attacks of unclear localization | | | |
| 182 | SP-MS | Yes | 23 | 29 | Hyper | None | Yes | BS | ON | | Progressive SC | | | |
| 261 | SP-MS | Yes | 39 | 16 | Hypo | Possible uveitis | Yes | SC | SC | SC | SC | SC ON | SC | SC |
| 317 | SP-MS | Yes | 37 | 49 | Hypo | Psoriasis | Yes | BS | SC | | Progressive SC | | | |
| 370 | SP-MS | NT | 40 | 19 | Hyper | None | Yes | ON | SC | | Progressive SC | | | |
| 374 | SP-MS | No | 36 | Not known | Hypo | None | Yes | BS | SC | SC | Progressive SC | | | |
| 492 | SP-MS | Yes | 30 | 34 | Hypo | Psoriasis | No | BS | SC | SC | ON | | Progressive SC | |
| 494 | SP-MS | NT | 39 | 47 | Hyper | None | No | ON | | | Progressive SC | | | |
| 593 | SP-MS | Yes | 35 | 49 | Hyper | None | Not known | BS | SC | SC | | | | |
| 608 | SP-MS | Yes | 23 | 37 (after IFNβ) | Hypo | None | Yes | ON SC | BS | BS SC | BS | BS | | |
| 50 | PP-MS | Yes | 46 | 59 | Hypo | None | Yes | SC was initial and remained only site of clinical involvement | | | | | | |
| 193 | PP-MS | Yes | 38 | 28 | Hypo | IDDM age 35 | Yes | Cerebrum | | | SC | | | |
| 227 | PP-MS | NT | 49 | Before MS | Hypo | Dermatitis herpetiformis | Yes | | | Progressive SC | | | | |
| ♂313 | PP-MS | Yes | 57 | 57 | Hypo | Crohn's, alopecia areata | No | | | Progressive SC | | | | |
| 337 | PP-MS | Yes | 50 | 48 | Hypo | None | Yes | | | Progressive SC | | | | |
| 384 | PP-MS | No | 34 | 25 | Hyper | None | Yes | | | Progressive SC | | | | |
| 614 | PP-MS | Yes | 48 | 14 | Hypo | None | Not known | | | Progressive Cb and BS | | | | |
| 207 | NMO spectrum disorders (NMOSD) | No | 70 | 70 | Hypo | Myasthenia gravis | Yes | SC | SC | Died | | | | |
| 219 | NMOSD | Yes | 26 | 9 | Hyper | Mild psoriasis | Yes | SC | SC | SC | SC | SC | SC | SC |
| 268 | NMOSD | No | 29 | 29 | Hypo | ITP; APC Abs | No | ON | SC | SC | SC | SC | | |
| 328 | NMOSD | NT | 25 | 40 | Hypo | None | Yes | ON | ON | ON | ON | SC | | |

*RR-MS, relapsing-remitting MS; SP-MS, secondary progressive MS; PP-MS, primary progressive MS; RR-NMO, relapsing-remitting NMO; SC, spinal cord; BS, brainstem; ON, optic nerve; Cb, cerebellum; IDDM, insulin-dependent (type 1) diabetes mellitus; ITP, immune thrombocytopenic purpura; APC Abs, anti-phosphatidyl choline antibodies; ANA, anti-nuclear antibodies; ♂, male patient.*

preceded the onset of CNS demyelinating disease in the majority of cases (66.7%), whereas hypothyroidism occurred prior to development of CNS demyelinating disease in only 31.8% of cases.

Eleven of the 44 patients (25%) had an additional autoimmune disease, most commonly psoriasis or Crohn's disease. Of the 37 patients where a family history was known, 30 (81.1%) had other family members with an autoimmune disease, most commonly AITD, but also MS, psoriasis, Crohn's disease, rheumatoid arthritis, and SLE. The personal or family incidence of other autoimmune diseases did not appear to be related to whether the AITD type was hypothyroidism or hyperthyroidism.

In most of the patients, the spinal cord was the most common site of clinical involvement (**Table 3**), with 68% of the 152 attacks recorded in these patients primarily affecting the spinal cord. Eighteen patients also had attacks involving the optic nerve, and 17 patients had attacks involving the brainstem. In most of the MS cases, there was also clinical and/or magnetic resonance imaging evidence of cerebral lesions, but symptoms from these lesions were generally mild.

**TABLE 4 | Time of onset of CNS demyelinating disease compared to onset of autoimmune thyroid disease (AITD) differs in patients with hypothyroidism vs hyperthyroidism.**

| | CNS disease occurred first | AITD occurred first | CNS disease and AITD commenced concurrently |
|---|---|---|---|
| Hypo ($n = 22$) | 12 | 7 | 3 |
| Hyper ($n = 15$) | 5 | 10 | 0 |

## HLA Typing

The HLA types carried by the patients with coexisting CNS demyelinating disease and AITD are shown in **Table 5**, subgrouped according to the type of thyroid disease (hypo vs hyper) and whether AITD developed prior to or after the CNS disease. Blood was not available for HLA typing from 11 patients (9 with hypothyroidism and 2 with hyperthyroidism). The most commonly

**TABLE 5 | HLA types carried by people with CNS demyelinating disease and autoimmune thyroid disease (AITD).**

| | AITD type | ID No. | DRB1[a] | DQA1[a] | DQB1[a] | DPB1[a] |
|---|---|---|---|---|---|---|
| **CNS disease occurred prior to AITD** | | | | | | |
| | Hypo | 31 | 03:01, 15:01 | 05:01, 01:02 | 02:01, 06:02 | 03:01, 04:01 |
| | Hypo | 43 | 04:04, 15:01 | 03:01, 01:02 | 03, 06:02 | 02:01, 04:01 |
| | Hypo | 50 | 07:01, 13:02 | 02:01, 01:02 | 03:03, 06:04 | 04:01, 04:01 |
| | Hypo | 171 | 01:01/2, 03:01 | 01:01, 05:01 | 05, 02:01/2 | 04:01, 04:02 |
| | Hypo | 291 | 08, 15:01 | 04:01, 01:02 | 04:01/2, 06:02 | 04:01, 04:01 |
| | Hypo | 317 | 03, 10:01 | 05:01, 01:05 | 02, 05 | 02:01, 04:01 |
| | Hypo | 328 | 15:01, 15:01 | 01:02, 01:02 | 06:02, 06:02 | ND |
| | Hypo | 332 | 01:01/2, 03:01 | 01:01, 05:01 | 05, 02:01/2 | 03:01, 04:01 |
| [a] | Hypo | 582 | 01:03, 15 | ND | ND | ND |
| | Hyper | 145 | 04:07, 15:01 | 03:03, 01:02 | 030:2, 06:02 | 03:01, 04:01 |
| | Hyper | 182 | 03, 15:01 | 05:01, 01:02 | 02:01/2, 06:02 | 03:01, 61:01N |
| [a] | Hyper | 493 | 01:03, 15:01 | 05:05, 01:02 | 03:01, 06:02 | 04:01, 04:02 |
| | Hyper | 494 | 04:02, 15:01 | 03:01, 01:02 | 03, 06:02 | 02:01, 04:01 |
| | Hyper | 593 | 03, 04:02 | ND | ND | ND |
| **CNS disease and AITD commenced at the same time** | | | | | | |
| | Hypo | 207 | 03, 03 | 05:01, 05:01 | 0201/2, 0201/2 | 0201, 0202 |
| | Hypo | 268 | 04:01, 15:01 | 03:02, 01:02 | 0301, 0602 | 0401, 0401 |
| | Hypo | 313 | 03:01, 13:03 | 05:01, 05:05 | 0201/2, 0301 | 0101, 0602 |
| **AITD occurred prior to CNS disease** | | | | | | |
| | Hypo | 193 | 03:05, 04:02 | 05:01, 03:01 | 02:01/2, 03:02 | 79:01, 79:01 |
| | Hypo | 200 | 07:01, 15:01 | 02:01, 01:02 | 02:01/2, 06:02 | 03:01, 19:01 |
| | Hypo | 227 | 07:01, 07:01 | 02:01, 02:01 | 02:01, 03:03/6 | 04:01, 09:01 |
| | Hypo | 261 | 13:01, 15:01 | 01:03, 01:02 | 06:03, 06:02 | 04:01, 05:01 |
| | Hypo | 316 | 15:01, 15:01 | 01:02, 01:02 | 06:02, 06:02 | ND |
| | Hypo | 337 | 15:01, 16:01 | 01:02, 01:02 | 06:02, 05 | 04:01, 10:01 |
| | Hyper | 133 | 01:01/2, 04:01 | 01:01, 03:01 | 05, 03:02 | 03:01, 04:01 |
| | Hyper | 219 | 11, 13:14 | 01:05, 05:05 | 05, 03:01 | 04:01, 04:01 |
| | Hyper | 269 | 01:01/2, 03 | 01:01, 05:01 | 05, 02 | 01:01, 20:01 |
| | Hyper | 271 | 03:01, 07:01 | 02:01, 05:01 | 02:01/2, 02:01/2 | 01:01, 04:01 |
| | Hyper | 274 | 03:01, 08:01 | 04:01, 05:01 | 02:01/2, 04:01/2 | 04:01, 04:01 |
| | Hyper | 299 | 01:03, 04 | ND | ND | ND |
| | Hyper | 370 | 10:01, 13:02 | 01:05, 01:02 | 05, 06:04 | |
| | Hyper | 384 | 04:01, 15:01 | 03:03, 01:02 | 03:02, 06:02 | 04:01, 04:02 |
| | Hyper | 393 | 07:01, 15:02 | 02:01, 01:02 | 03:01, 06:01 | ND |
| **Commencement of AITD in relation to CNS disease not known** | | | | | | |
| | Hypo | 374 | 03, 04:04 | 05:01, 03:01 | 02:01/2, 03:02 | 02:02, 14:01 |

[a]*Disease commenced after β-IFN treatment; ND = not done.*

found genotype was DRB1*03–DQA1*05–DQB1*02: this genotype has previously been reported to occur commonly in patients with AITD. Interestingly, four of the five patients (80%) who developed MS prior to hyperthyroidism carried the MS-related DRB1*15:01 allele, whereas only one (11.1%) of the nine patients (eight MS and one NMOSD) who developed hyperthyroidism prior to CNS demyelinating disease carried this allele. The HLA-DR genotypic and allelic frequencies for these groups are compared with those of 159 healthy individuals and 277 patients with MS alone (all at the two digit serotyping level) in **Table 6**. In comparison with the MS group, patients with CNS demyelinating disease and hypothyroidism had an elevated frequency of carriage of HLA-DR3 (significantly different without correction for multiple comparisons). The frequency of carriage of DRB1*15:01 allele among patients with coexisting CNS demyelinating disease and AITD was intermediate between the healthy control group and the MS group, but did not reach statistical significance in comparison with either.

## T Cell Reactivity

Given the pronounced involvement of the spinal cord in patients with coexisting CNS demyelinating disease and AITD, T cell responses against two antigens that have been reported to be present at high levels in the spinal cord compared to other parts of the nervous system, CGRP, and LGR4, were tested. CGRP levels have been reported to be upregulated not only in the spinal cord but also in the diseased thyroid gland. LGR4, which is expressed in the CNS, particularly in the spinal cord, and also in other sites throughout the body, notably the skin and gastrointestinal tract, is a homolog of TSHR, the target of autoantibodies in Graves' disease. We also assessed T cell reactivity against the immunodominant region of PLP ($PLP_{184–209}$), since we have previously found that T cell responses to PLP are elevated in a significant proportion of patients with MS (2, 33, 34). Sufficient blood was available for T cell assays from 15 CNS disease + hypothyroid patients and 10 CNS disease + hyperthyroidism patients. In addition, 20 patients with MS alone and 20 healthy individuals without known thyroid disease were also tested.

Compared to patients with MS alone and to HC, patients with coexisting CNS demyelinating disease and hypothyroidism showed a significant increase in T cell reactivity to CGRP ($p \leq 0.03$) (**Figure 1**). T cell reactivity to CGRP was slightly increased in the patients with coexisting CNS demyelinating disease and hyperthyroidism but was not significantly different from that in the group with MS alone or in the HC. In contrast, patients with coexisting CNS demyelinating disease and hyperthyroidism showed significantly increased T cell responses to a panel of three LGR4 peptides, compared to all other groups ($p \leq 0.005$) (**Figure 1**). A smaller subgroup (5–8 per group) of those tested for reactivity to LGR4 was also tested for T cell reactivity to the homologous peptides from TSHR. Once again, elevated reactivity to the TSHR peptides was also seen only in the patients with coexisting CNS demyelinating disease and hyperthyroidism (**Figure 1**); however, owing to the smaller numbers of individuals tested and the variation in the responses of the patients, this did not reach statistical significance.

**TABLE 6 | Frequency of HLA-DRB1 alleles in patients with coexisting CNS demyelinating disease and autoimmune thyroid disease (AITD) in comparison to healthy individuals and multiple sclerosis (MS) patients without AITD.**

| DRB1 allele | Healthy controls (HC) ($n = 159$) | | MS alone ($n = 277$) | | CNS disease + AITD ($n = 33$) | | CNS disease + hyperthyroidism ($n = 14$) | | CNS disease + hypothyroidism ($n = 19$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Genotypic (%) | Allelic (%) | Genotypic (%) | Allelic (%) | Genotypic (%) | Allelic (%) | Genotypic (%) | Allelic (%) | Genotypic (%) | Allelic (%) |
| 01 | 28 (17.6) | 28 (8.8) | 39 (14.1) | 39 (7.0) | 7 (21.2) | 7 (10.6) | 4 (28.6) | 4 (14.3) | 3 (15.8) | 3 (7.9) |
| 03 | 35 (22.0) | 36 (11.3) | 60 (21.7) | 69 (12.5) | 13 (39.4)* | 14 (21.2)* | 5 (35.7) | 5 (17.8) | 8 (42.1)* | 9 (23.7)* |
| 04 | 46 (28.9) | 49 (15.4) | 70 (25.3) | 75 (13.5) | 10 (30.3) | 10 (15.2) | 6 (42.9) | 6 (21.4) | 4 (21.0) | 4 (10.5) |
| 07 | 37 (23.3) | 41 (12.9) | 44 (15.9) | 48 (8.7) | 5 (15.2) | 6 (9.1) | 2 (14.3) | 2 (7.1) | 3 (15.8) | 4 (10.5) |
| 08 | 12 (7.5) | 12 (3.8) | 17 (6.1) | 17 (3.1) | 2 (6.1) | 2 (3.0) | 1 (7.0) | 1 (3.6) | 1 (5.3) | 1 (2.6) |
| 09 | 7 (4.4) | 7 (2.2) | 3 (1.1) | 3 (0.5) | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 2 (1.3) | 2 (0.6) | 1 (0.4) | 1 (0.2) | 2 (6.1) | 2 (3.0) | 1 (7.0) | 1 (3.6) | 1 (5.3) | 1 (2.6) |
| 11 | 25 (15.7) | 26 (8.2) | 24 (8.7) | 24 (4.3) | 1 (3.0) | 1 (1.5) | 1 (7.0) | 1 (3.6) | 0 | 0 |
| 12 | 11 (6.9) | 11 (3.5) | 9 (3.2) | 9 (1.6) | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 31 (19.5) | 31 (9.7) | 47 (17.0) | 50 (9.0) | 5 (15.2) | 5 (7.5) | 2 (14.3) | 2 (7.1) | 3 (15.8) | 3 (7.9) |
| 14 | 16 (10.1) | 17 (5.3) | 11 (4.0) | 12 (2.2) | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 50 (31.4) | 56 (17.6) | 171 (61.7)*** | 200 (36.1)*** | 16 (48.5) | 18 (27.3) | 6 (42.9) | 6 (21.4) | 10 (52.6) | 12 (31.6) |
| 16 | 2 (1.3) | 2 (0.6) | 6 (2.2) | 7 (1.3) | 1 (3.0) | 1 (1.5) | 0 | 0 | 1 (5.3) | 1 (2.6) |

*$p \leq 0.05$ vs HC or MS alone, before correction for multiple comparisons.
***$p < 0.0001$ vs HC, after correction for multiple comparisons.

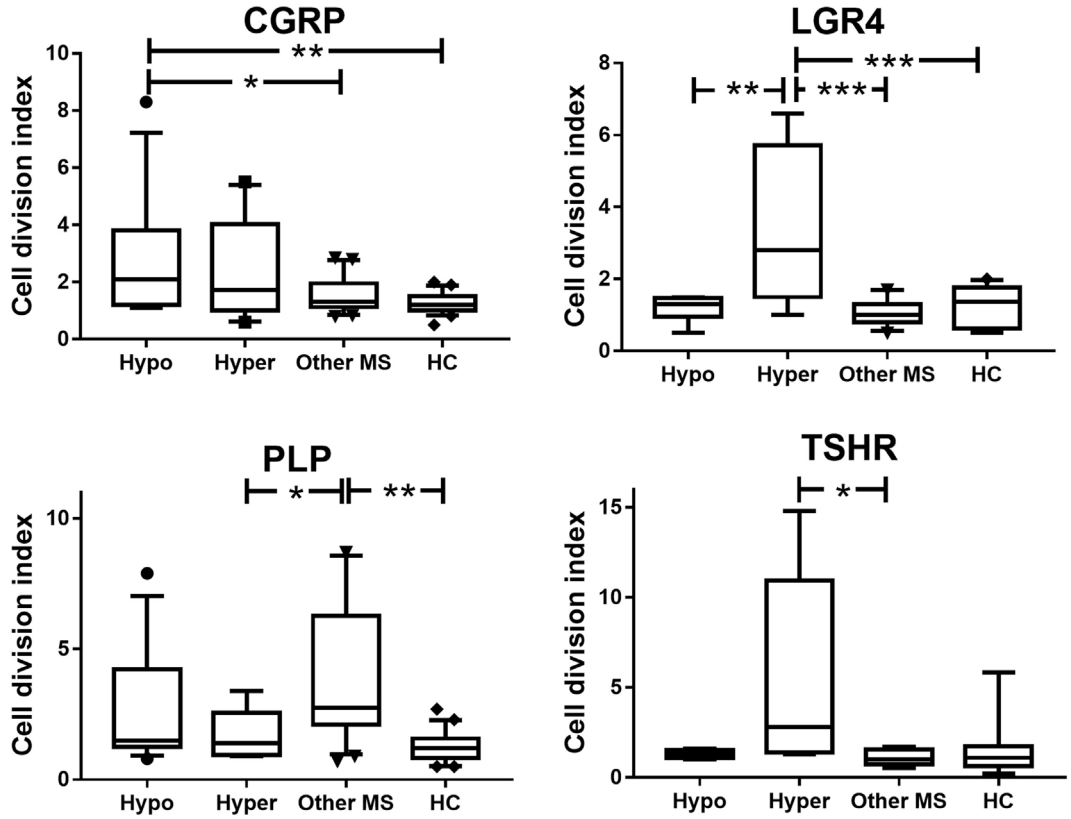



**FIGURE 1 | Proliferative responses to calcitonin gene-related peptide (CGRP), LGR4, thyroid-stimulating hormone receptor (TSHR), and proteolipid protein (PLP) in patients with coexisting CNS demyelinating disease and autoimmune hypothyroidism (Hypo) or hyperthyroidism (Hyper), multiple sclerosis (MS) alone, or healthy controls (HC)**. Because the data are not normally distributed, they were analyzed used the Kruskal–Wallis test (non-parametric equivalent of ANOVA), with Dunn's multiple comparisons test to allow for multiple comparisons being made in the figure. The box of the box and whisker plots extends from the 25th to the 75th percentiles of the results. The line in the middle of the box is plotted at the median. The whiskers are drawn down to the 10th percentile and up to the 90th. Points below and above the whiskers are drawn as individual points. *$p < 0.05$ and **$p < 0.001$, compared to the indicated groups.

Compared to the HC, patients with MS without AITD showed significantly elevated T cell reactivity to the $PLP_{184–209}$ region (**Figure 1**). In patients with coexisting CNS demyelinating disease and AITD, T cell reactivity to $PLP_{184–209}$ was intermediate between that in patients with MS without AITD and that in HC but did not differ significantly from that in either of these groups.

Taken together, these finding show that patients with coexisting CNS demyelinating disease and AITD have increased T cell reactivity directed against antigens present in both the CNS and thyroid or against molecules for which CNS and thyroid homologs exist.

## Antibody Reactivity

Levels of antibodies specific for CGRP were measured in an ELISA assay, using the whole CGRP molecule immobilized on the ELISA plate. Apart from one patient with coexisting MS and hypothyroidism (patient 43), who showed highly elevated serum levels of antibodies against CGRP (>50-fold higher than the level for any other individuals in any group), none of the other patients with coexisting CNS demyelinating disease and AITD showed antibody levels higher than the levels for healthy individuals or other MS patients (data not shown). Interestingly, multiple samples had been collected from patient 43 over 8 years, and testing of all of these samples showed a sustained response to CGRP across the entire 8 years (not shown).

**TABLE 7 | Frequency of antibodies against aquaporin 4 (AQP4), myelin oligodendrocyte glycoprotein (MOG), LGR4 and thyroid-stimulating hormone receptor (TSHR).**

| Patient group | Positive staining of cells transfected with | | | |
|---|---|---|---|---|
| | AQP4 (%) | MOG (%) | LGR4 (%) | TSHR (%) |
| NMO spectrum disorders and autoimmune thyroid disease (AITD) ($n = 4$) | 4 (100) | 0 (0) | 1 (25) | 2 (50%) |
| Multiple sclerosis (MS) and AITD ($n = 30$) | 0 (0) | 0 (0) | 10 (43.3) | 11 (36.7) |
| Hypo ($n = 18$) | | | 2 (11.1) | 3 (16.7) |
| Hyper ($n = 12$) | | | 8 (66.7) | 8 (66.7) |
| MS only ($n = 14$) | ND | ND | 0 (0) | 2 (14.3) |

*ND, not determined.*

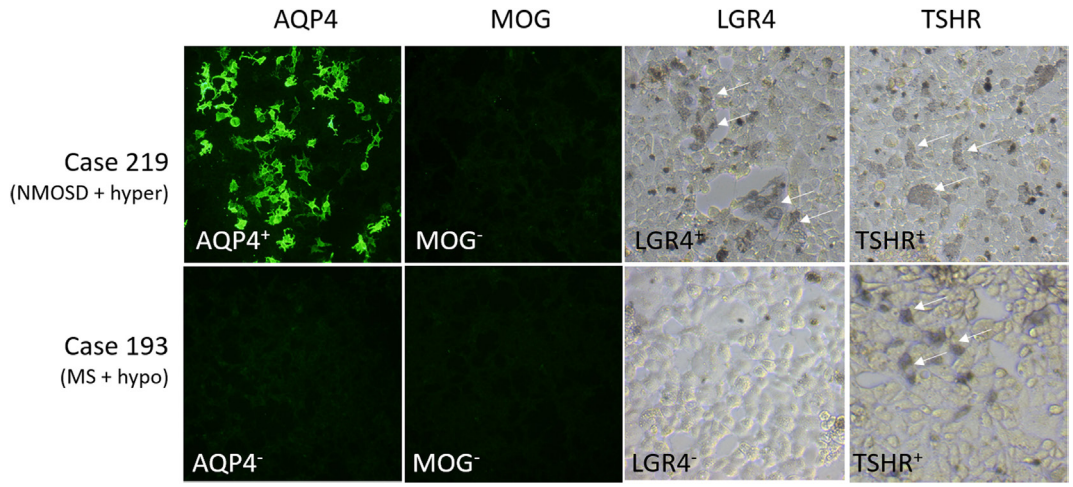



**FIGURE 2 | Representative images of staining of cells expressing aquaporin 4 (AQP4), myelin oligodendrocyte glycoprotein (MOG), LGR4, or thyroid-stimulating hormone receptor (TSHR)**. Results for two cases, #219 [NMO spectrum disorders (NMOSD) patient with hyperthyroidism who is positive for antibodies against AQP4, LGR4, and TSHR] and #193 [multiple sclerosis (MS) patient with hypothyroidism who is positive for antibodies against TSHR, but not the other proteins], are shown. Human antibody labeling of AQP4- and MOG-transfected cells was detected with FITC-labeled anti-human IgG, so that positive cells fluoresced green. Human antibody labeling of LGR4- or TSHR-transduced cells was detected by HRP-labeled anti-human IgG + A + M, followed by nickel-enhanced DAB staining, so that positive cells are labeled by a blue/gray precipitate. Arrows indicate positively stained cells.

Other antibodies were measured using cell-based assays. Results are shown in **Table 7**, with representative images in **Figure 2**. Only patients with NMOSD had anti-AQP4 antibodies. None of the patients tested in this study had detectable anti-MOG antibodies using this commercial assay. Labeling of LGR4-transduced cells occurred in 1 of the 4 NMOSD + AITD patients (patient with hyperthyroidism), and in 10 of 30 patients with MS + AITD. In the latter group, 8 of the 10 patients with anti-LGR4 antibodies had hyperthyroidism, consistent with our finding of increased T cell reactivity to LGR4s in patients with concomitant hyperthyroidism and CNS demyelinating disease. Similarly, antibodies specific for TSHR were found mainly in patients with hyperthyroidism (9 of a total of 13 positives). Not all patients who were positive for TSHR antibody also reacted against LGR4 and *vice versa*, suggesting specific targeting of each molecule in different patients.

## DISCUSSION

In this paper, we report that patients with coexisting CNS demyelinating disease and AITD show novel immune reactivity to CGRP and LGR4 which is not found in MS patients who do not have AITD and HC. The patterns of immune reactivity to these molecules differed between patients with autoimmune hypothyroidism and patients with autoimmune hyperthyroidism, suggesting that immune reactivity targeting these molecules might have a role in the development of the different types of AITD. Also of interest was the clinical presentation of patients, with predominant spinal cord involvement in most patients during one or more attacks.

Patients with increased reactivity to CGRP were distributed among both the hypothyroid and hyperthyroid subgroups, but responses were slightly higher in the hypothyroid group. In contrast to the reactivity to LGR4 in the hyperthyroid group, there were no obvious differences in reactivity to CGRP between patients who developed AITD prior to vs after the CNS disease. Within the CNS, CGRP has been reported to be selectively distributed throughout sensory, motor, and autonomic areas of the spinal cord (35), which may partly explain the predominance of spinal cord disease seen in the patients with coexisting AITD and CNS disease. Reactivity against other thyroid antigens, such as thyroglobulin and thyroperoxidase, were not specifically assessed in the current study, as we focused on antigens that were expressed in the spinal cord; however, antibodies against these molecules were measured as part of the clinical workup of these patients, but there were no significant correlations between the antibody levels and reactivity to CGRP or LGR4 (data not shown).

The most interesting group of patients was those with autoimmune hyperthyroidism preceding the onset of CNS demyelinating disease. They carried HLA molecules different from those generally associated with MS or NMOSD and had significantly elevated T cell and antibody reactivity to LGR4. TSHR and LGR4 both belong to the LGR family, but whereas TSHR belong to the Group A LGR family, which have 7–9 leucine-rich repeats, LGR4 belongs to the Group B family, which have 17 leucine-rich repeats (27). Overall, there is only about 20% identity and 45% similarity between the two molecules, but there are several parts of the molecules, particular in their shared seven transmembrane domain, where the regions of identity and similarity are higher. Studies in patients with Graves' disease have shown a variety of T cell epitopes in TSHR, with none appearing to be dominant (36). The three peptides from TSHR, and the three corresponding peptides of LGR4 used in this study, which were chosen based

on their predicted extracellular location and on their predicted ability to bind to HLA molecules commonly found in MS, only represent a small selection of possible T cell epitopes. It would be useful in the future to undertake a larger T cell study utilizing overlapping peptides across the whole of the similar parts of these molecules. However, even with the limited number of peptides used, we could still see significantly elevated responses to LGR4 in the subgroup of patients with coexisting AITD and CNS demyelinating disease. We postulate that, in these patients, CNS disease might be a direct consequence of immunological cross-reactivity between LGR4 and TSHR, with disease spreading from the thyroid to the CNS.

There are many case reports in the literature of CNS disorders, including myelopathy, myelitis, and ataxia occurring together with thyroid disease (13, 37–41). In many cases, it has been reported that the CNS symptoms can be reversed by normalizing the levels of thyroid hormones, suggesting that the CNS symptoms were caused directly by the altered thyroid hormone levels. However, there are some reports where such therapy has not reversed the CNS disease, but rather the CNS syndrome has remained and progressed (37, 42, 43). In one such study, where persistent cerebellar ataxia was associated with elevated levels of autoantibodies against thyroglobulin and thyroperoxidase, the authors concluded that the most likely cause of the cerebellar degeneration in the patients was autoimmune attack (43). The results of the current study lend further support to the idea that autoreactivity against antigens present in the thyroid could spread to the CNS.

The idea of intra-CNS epitope spreading in animal models of MS and in MS itself is well established (44, 45), although we are not aware of reports where disease spreads from the CNS to another organ. However, another example of where cross-reactivity between related antigens might play a role in spreading of autoimmune disease from a peripheral organ to the CNS (or *vice versa*) is in patients with coexisting type 1 diabetes (T1D) and stiff person syndrome (SPS). In both T1D and SPS, a major target of autoimmune attack is glutamic acid decarboxylase (GAD). GAD exists in two isoforms of differing molecular weight and encoded by separate genes, GAD65 and GAD67, and while both isoforms are present in the CNS, only GAD65 is present in the pancreas. Autoantibodies and T cells from patients with T1D appear to target different epitopes of GAD65 when compared to patients with SPS, and generally the response to antigens in SPS appears to be broader than in T1D, with a larger number of epitopes of both GAD65 and GAD67 being recognized (46, 47).

It is of interest to note that development of AITD has been reported to follow treatment with several of the drugs commonly used in MS, including IFN-β and anti-CD52 antibody (alemtuzumab/Campath/Lemtrada). IFN-β has been reported to enhance the production of the B cell activating factor (BAFF), which could potentially enhance antibody-mediated autoimmune disease. In contrast, anti-CD52 antibody has been suggested to lead to development of other autoimmune disease through the depletion of regulatory T cells. The reason(s) why these treatments results predominantly in AITD is as yet uncertain. Our results suggest that there could be cross-reactivity between antigens in the CNS and the thyroid, and since AITD is likely to be predominantly an autoantibody-mediated disease, enhancement of the autoantibody response or removal of regulatory cells that normally keep the action of these autoantibodies under control could result in the emergence of AITD.

Some studies (48–50) but not others (10, 51) have shown an increased occurrence of other autoimmune diseases in patients with MS and their first-degree relatives, and NMOSD has also been linked to coexistence of other autoimmune diseases in addition to AITD (52). Irrespective of whether or not the overall incidence of other autoimmune diseases is higher or the same as the general population, it is of interest why some patients appear to be so susceptible to multiple autoimmune diseases. It is generally considered that patients who develop multiple autoimmune diseases carry HLA types that predispose them to development of certain combinations of autoimmune diseases, and this would almost certainly be important in determining such potential. However, at present, our knowledge of the specific antigens involved and their HLA restriction is insufficient to predict combinations of autoimmune diseases with any certainty. It is notable that out of all the potential autoimmune diseases that could occur, a fairly restricted number of diseases were reported in both the personal and family histories of patients with coexisting CNS demyelinating disease and AITD, primarily thyroid disease, MS, diseases affecting the skin (including psoriasis, alopecia areata, and dematitis herpetiformis), and disease affecting the gastrointestinal tract (Crohn's disease, celiac disease). This specific combination of diseases is of interest, as both CGRP and LGR4 are strongly expressed not only in the thyroid and the spinal cord but also in the gastrointestinal tract and in the skin. The number of patients in the current study is too small to determine whether elevated reactivity to CGRP and/or LGR4 definitely correlates with the development of other autoimmune diseases affecting the gastrointestinal tract or skin, but this would be an interesting study for the future. It is also of interest to note that one of the NMOSD patients in our study also had myasthenia gravis, since acetylcholinesterase, one of the targets of autoantibodies in myasthenia gravis, has an extensive degree of homology with thyroglobulin (53). Since myasthenia gravis co-occurs with NMOSD at a relatively high frequency, it would be of interest to determine if patients with both of these diseases were more likely to develop AITD than other NMOSD patients.

The reason for the coexistence of CNS demyelinating disease and AITD almost exclusively in females (43 of the 44 patients in this study were female) is unclear. Most studies report a higher incidence in females than males for MS (typically ~3:1 female: male, although in the south-east part of Queensland where this study was conducted the ratio appears to be higher at ~6:1) (54, 55), NMOSD (2–5.5:1) (20) and AITD (~5:1) (56), and it may just be that the risk associated with getting CNS disease and AITD together represents a fairly straightforward multiplicative relationship between the risk of getting each disease alone. Alternatively, there could be differences in regulation/expression of these antigens in female vs male CNS tissue, dimorphism in the imprinting of the genes encoding these molecules (57), or other mechanisms that remain to be elucidated.

The further study of patients with coexisting AITD and CNS demyelinating disease may help to identify new antigenic targets within the CNS and to explain previous observations of linkages between altered thyroid metabolism and subsequent CNS disease.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the NHMRC National Statement on Ethical Conduct in Human Research. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Royal Brisbane and Women's Hospital Human Research Ethics Committee, The University of Queensland Medical Research Ethics Committee and the Griffith University Human Research Ethics Committee.

## AUTHOR CONTRIBUTIONS

JG and MP designed the study. MP and SB recruited patients, assessed patients included in the study, and edited the manuscript. JG undertook the laboratory-based research and wrote the manuscript.

## ACKNOWLEDGMENTS

The authors would like to thank Shannon Beasley, Peter Csurhes, Aakanksha Dixit, and Taahli Sharman-Selvidge for technical assistance, Stefan Blum and Meng Tan for checking patient charts, and Kaye Hooper, Bernie Gazzard, and Rosalie Scott for collecting blood samples. Funding for this project was provided through the National Health and Medical Research Council of Australia (GNT0351404) and Multiple Sclerosis Research Australia.

## REFERENCES

1. Greer JM. The role of HLA in MS susceptibility and phenotype. *Curr Top Behav Neurosci* (2015) 26:1–27. doi:10.1007/7854_2014_357
2. Greer JM, Csurhes PA, Muller DM, Pender MP. Correlation of blood T cell and antibody reactivity to myelin proteins with HLA type and lesion localization in multiple sclerosis. *J Immunol* (2008) 180(9):6402–10. doi:10.4049/jimmunol.180.9.6402
3. Ganesvaran G, Greer JM, Pender MP. Prominent brainstem and cerebellar involvement in multiple sclerosis with psoriasis. *Mult Scler* (2009) 15(6):763–6. doi:10.1177/1352458509103612
4. Pittock SJ, Lennon VA, de Seze J, Vermersch P, Homburger HA, Wingerchuk DM, et al. Neuromyelitis optica and non organ-specific autoimmunity. *Arch Neurol* (2008) 65(1):78–83. doi:10.1001/archneurol.2007.17
5. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* (2015) 85(2):177–89. doi:10.1212/WNL.0000000000001729
6. Karni A, Abramsky O. Association of MS with thyroid disorders. *Neurology* (1999) 53(4):883–5. doi:10.1212/WNL.53.4.883
7. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* (2004) 364(9451):2106–12. doi:10.1016/S0140-6736(04)17551-X
8. Li H, Dai Y, Wu AM, Sun X, Lin Y, Lv K, et al. Anti-thyroid antibodies and cerebrospinal fluid findings in neuromyelitis optica spectrum disorders. *J Neuroimmunol* (2015) 281:38–43. doi:10.1016/j.jneuroim.2015.02.014
9. Munteis E, Cano JF, Flores JA, Martinez-Rodriguez JE, Miret M, Roquer J. Prevalence of autoimmune thyroid disorders in a Spanish multiple sclerosis cohort. *Eur J Neurol* (2007) 14(9):1048–52. doi:10.1111/j.1468-1331.2007.01882.x
10. Ramagopalan SV, Dyment DA, Valdar W, Herrera BM, Criscuoli M, Yee IM, et al. Autoimmune disease in families with multiple sclerosis: a population-based study. *Lancet Neurol* (2007) 6(7):604–10. doi:10.1016/S1474-4422(07)70132-1
11. Greer JM, Pender MP. Consistent patterns of clinical and laboratory findings in patients with co-existent autoimmune thyroid disease and autoimmune demyelinating disease in the central nervous system. *J Neuroimmunol* (2012) 253(1–2):141. doi:10.1016/j.jneuroim.2012.10.001
12. Charcot J. Leçons du mardi à la Salpêtrière: polyclinique, 1887–1889. *Bibliothèque nationale*. Paris: Hachette (1975) p. 239–43.
13. Garcia CA, Fleming RH. Reversible corticospinal tract disease due to hyperthyroidism. *Arch Neurol* (1977) 34(10):647–8. doi:10.1001/archneur.1977.00500220081018
14. Melamed E, Berman M, Lavy S. Posterolateral myelopathy associated with thyrotoxicosis. *N Engl J Med* (1975) 293(15):778–9. doi:10.1056/NEJM197510092931519
15. Olmez I, Moses H, Sriram S, Kirshner H, Lagrange AH, Pawate S. Diagnostic and therapeutic aspects of Hashimoto's encephalopathy. *J Neurol Sci* (2013) 331(1–2):67–71. doi:10.1016/j.jns.2013.05.009
16. Kuroda Y, Miyahara M, Sakemi T, Matsui M, Ryu T, Yamaguchi M, et al. Autopsy report of acute necrotizing opticomyelopathy associated with thyroid cancer. *J Neurol Sci* (1993) 120(1):29–32. doi:10.1016/0022-510X(93)90020-Y
17. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: associations and mechanisms of action. *Curr Genomics* (2007) 8(7):453–65. doi:10.2174/138920207783591690
18. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* (2011) 476(7359):214–9. doi:10.1038/nature10251
19. Deschamps R, Paturel L, Jeannin S, Chausson N, Olindo S, Bera O, et al. Different HLA class II (DRB1 and DQB1) alleles determine either susceptibility or resistance to NMO and multiple sclerosis among the French Afro-Caribbean population. *Mult Scler* (2011) 17(1):24–31. doi:10.1177/1352458510382810
20. Pandit L, Asgari N, Apiwattanakul M, Palace J, Paul F, Leite MI, et al. Demographic and clinical features of neuromyelitis optica: a review. *Mult Scler* (2015) 21(7):845–53. doi:10.1177/1352458515572406
21. Zephir H, Fajardy I, Outteryck O, Blanc F, Roger N, Fleury M, et al. Is neuromyelitis optica associated with human leukocyte antigen? *Mult Scler* (2009) 15(5):571–9. doi:10.1177/1352458508102085
22. Gavalas E, Kountouras J, Boziki M, Zavos C, Polyzos SA, Vlachaki E, et al. Relationship between *Helicobacter pylori* infection and multiple sclerosis. *Ann Gastroenterol* (2015) 28(3):353–6.
23. Long Y, Gao C, Qiu W, Hu X, Shu Y, Peng F, et al. *Helicobacter pylori* infection in neuromyelitis optica and multiple sclerosis. *Neuroimmunomodulation* (2013) 20(2):107–12. doi:10.1159/000345838
24. Mader EC Jr, Richeh W, Ochoa JM, Sullivan LL, Gutierrez AN, Lovera JF. Tumefactive multiple sclerosis and hepatitis C virus 2a/2C infection: dual benefit of long-term interferon beta-1a therapy? *J Neurol Sci* (2015) 349(1–2):239–42. doi:10.1016/j.jns.2014.12.034
25. Mariotto S, Ferrari S, Monaco S. HCV-related central and peripheral nervous system demyelinating disorders. *Inflamm Allergy Drug Targets* (2014) 13(5):299–304. doi:10.2174/1871528113666140908113841
26. Guarneri F, Benvenga S. Environmental factors and genetic background that interact to cause autoimmune thyroid disease. *Curr Opin Endocrinol Diabetes Obes* (2007) 14(5):398–409. doi:10.1097/MED.0b013e3282ef1c48
27. Xu JG, Huang C, Yang Z, Jin M, Fu P, Zhang N, et al. Crystal structure of LGR4-Rspo1 complex: insights into the divergent mechanisms of ligand recognition by leucine-rich repeat G-protein-coupled receptors (LGRs). *J Biol Chem* (2015) 290(4):2455–65. doi:10.1074/jbc.M114.599134
28. Petermann JB, Born W, Chang JY, Fischer JA. Identification in the human central nervous system, pituitary, and thyroid of a novel calcitonin gene-related

peptide, and partial amino acid sequence in the spinal cord. *J Biol Chem* (1987) 262(2):542–5.
29. Mannering SI, Morris JS, Jensen KP, Purcell AW, Honeyman MC, van Endert PM, et al. A sensitive method for detecting proliferation of rare autoantigen-specific human T cells. *J Immunol Methods* (2003) 283(1–2): 173–83. doi:10.1016/j.jim.2003.09.004
30. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* (2011) 69(2):292–302. doi:10.1002/ana.22366
31. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* (2005) 58(6):840–6. doi:10.1002/ana.20703
32. McDonald ND, Pender MP. Autoimmune hypothyroidism associated with interferon beta-1b treatment in two patients with multiple sclerosis. *Aust N Z J Med* (2000) 30(2):278–9. doi:10.1111/j.1445-5994.2000.tb00823.x
33. Greer JM, Csurhes PA, Cameron KD, McCombe PA, Good MF, Pender MP. Increased immunoreactivity to two overlapping peptides of myelin proteolipid protein in multiple sclerosis. *Brain* (1997) 120(Pt 8):1447–60. doi:10.1093/brain/120.8.1447
34. Pender MP, Csurhes PA, Greer JM, Mowat PD, Henderson RD, Cameron KD, et al. Surges of increased T cell reactivity to an encephalitogenic region of myelin proteolipid protein occur more often in patients with multiple sclerosis than in healthy subjects. *J Immunol* (2000) 165(9):5322–31. doi:10.4049/jimmunol.165.9.5322
35. Gibson SJ, Polak JM, Bloom SR, Sabate IM, Mulderry PM, Ghatei MA, et al. Calcitonin gene-related peptide immunoreactivity in the spinal cord of man and of eight other species. *J Neurosci* (1984) 4(12):3101–11.
36. Morshed SA, Latif R, Davies TF. Delineating the autoimmune mechanisms in Graves' disease. *Immunol Res* (2012) 54:191–203. doi:10.1007/s12026-012-8312-8
37. Cremer GM, Goldstein NP, Paris J. Myxedema and ataxia. *Neurology* (1969) 19(1):37–46. doi:10.1212/WNL.19.1.37
38. Jellinek EH, Kelly RE. Cerebellar syndrome in myxoedema. *Lancet* (1960) 276(7144):225–7. doi:10.1016/S0140-6736(60)91424-0
39. Soderbergh G. Encore un cas de myxoedeme avec symptomes cerebelleux. *Rev Neurol (Paris)* (1911) 22:86–9.
40. Bassi S, Albizzati MG, Beltramelli A, Frattola L. Encephalomyelitis with thyrotoxicosis. *J Neurol* (1978) 218(4):293–5. doi:10.1007/BF00312885
41. Frisbie JH, Fisch B. Graves' disease complicating myelopathy. *Paraplegia* (1992) 30(8):593–7. doi:10.1038/sc.1992.121
42. Bonuccelli U, Nuti A, Monzani F, De Negri F, Muratorio A. Familial occurrence of hypothyroidism and cerebellar ataxia. *Funct Neurol* (1991) 6(2):171–5.
43. Selim M, Drachman DA. Ataxia associated with Hashimoto's disease: progressive non-familial adult onset cerebellar degeneration with autoimmune thyroiditis. *J Neurol Neurosurg Psychiatry* (2001) 71(1):81–7. doi:10.1136/jnnp.71.1.81
44. Tuohy VK, Yu M, Yin L, Kawczak JA, Johnson JM, Mathisen PM, et al. The epitope spreading cascade during progression of experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunol Rev* (1998) 164(1):93–100. doi:10.1111/j.1600-065X.1998.tb01211.x
45. Vanderlugt CL, Begolka WS, Neville KL, Katz-Levy Y, Howard LM, Eagar TN, et al. The functional significance of epitope spreading and its regulation by co-stimulatory molecules. *Immunol Rev* (1998) 164:63–72. doi:10.1111/j.1600-065X.1998.tb01208.x
46. Lohmann T, Hawa M, Leslie RD, Lane R, Picard J, Londei M. Immune reactivity to glutamic acid decarboxylase 65 in stiffman syndrome and type 1 diabetes mellitus. *Lancet* (2000) 356(9223):31–5. doi:10.1016/S0140-6736(00)02431-4
47. O'Sullivan EP, Behan LA, King TF, Hardiman O, Smith D. A case of stiff-person syndrome, type 1 diabetes, celiac disease and dermatitis herpetiformis. *Clin Neurol Neurosurg* (2009) 111(4):384–6. doi:10.1016/j.clineuro.2008.11.006
48. Barcellos LF, Kamdar BB, Ramsay PP, DeLoa C, Lincoln RR, Caillier S, et al. Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study. *Lancet Neurol* (2006) 5(11):924–31. doi:10.1016/S1474-4422(06)70552-X
49. Dobson R, Giovannoni G. Autoimmune disease in people with multiple sclerosis and their relatives: a systematic review and meta-analysis. *J Neurol* (2013) 260(5):1272–85. doi:10.1007/s00415-012-6790-1
50. Henderson RD, Bain CJ, Pender MP. The occurrence of autoimmune diseases in patients with multiple sclerosis and their families. *J Clin Neurosci* (2000) 7(5):434–7. doi:10.1054/jocn.2000.0693
51. Roshanisefat H, Bahmanyar S, Hillert J, Olsson T, Montgomery S. Shared genetic factors may not explain the raised risk of comorbid inflammatory diseases in multiple sclerosis. *Mult Scler* (2012) 18(10):1430–6. doi:10.1177/1352458512438240
52. Zhang B, Zhong Y, Wang Y, Dai Y, Qiu W, Zhang L, et al. Neuromyelitis optica spectrum disorders without and with autoimmune diseases. *BMC Neurol* (2014) 14:162. doi:10.1186/s12883-014-0162-7
53. Mappouras DG, Philippou G, Haralambous S, Tzartos SJ, Balafas A, Souvatzoglou A, et al. Antibodies to acetylcholinesterase cross-reacting with thyroglobulin in myasthenia gravis and Graves's disease. *Clin Exp Immunol* (1995) 100(2):336–43. doi:10.1111/j.1365-2249.1995.tb03674.x
54. Harbo HF, Gold R, Tintore M. Sex and gender issues in multiple sclerosis. *Ther Adv Neurol Disord* (2013) 6(4):237–48. doi:10.1177/1756285613488434
55. Taylor BV, Lucas RM, Dear K, Kilpatrick TJ, Pender MP, van der Mei IA, et al. Latitudinal variation in incidence and type of first central nervous system demyelinating events. *Mult Scler* (2010) 16(4):398–405. doi:10.1177/1352458509359724
56. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol* (2014) 35(3):347–69. doi:10.1016/j.yfrne.2014.04.004
57. Gregg C, Zhang J, Butler JE, Haig D, Dulac C. Sex-specific parent-of-origin allelic expression in the mouse brain. *Science* (2010) 329(5992):682–5. doi:10.1126/science.1190831

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 27 February 2017
doi: 10.3389/fimmu.2017.00165

# Autophagy and Autophagy-Related Proteins in CNS Autoimmunity

*Christian W. Keller[1] and Jan D. Lünemann[1,2]**

[1] Institute of Experimental Immunology, Laboratory of Neuroinflammation, University of Zürich, Zürich, Switzerland,
[2] Department of Neurology, University Hospital Zürich, Zürich, Switzerland

Autophagy comprises a heterogeneous group of cellular pathways that enables eukaryotic cells to deliver cytoplasmic constituents for lysosomal degradation, to recycle nutrients, and to survive during starvation. In addition to these primordial functions, autophagy has emerged as a key mechanism in orchestrating innate and adaptive immune responses and to shape $CD4^+$ T cell immunity through delivery of peptides to major histocompatibility complex (MHC) class II-containing compartments (MIICs). Individual autophagy proteins additionally modulate expression of MHC class I molecules for $CD8^+$ T cell activation. The emergence and expansion of autoreactive $CD4^+$ and $CD8^+$ T cells are considered to play a key role in the pathogenesis of multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis. Expression of the essential autophagy-related protein 5 (Atg5), which supports T lymphocyte survival and proliferation, is increased in T cells isolated from blood or brain tissues from patients with relapsing-remitting MS. Whether Atgs contribute to the activation of autoreactive T cells through autophagy-mediated antigen presentation is incompletely understood. Here, we discuss the complex functions of autophagy proteins and pathways in regulating T cell immunity and its potential role in the development and progression of MS.

**Keywords: autophagy pathways, non-canonical autophagy, multiple sclerosis, EAE/MS, oligodendrocyte death, antigen presentation**

OPEN ACCESS

***Edited by:***
*Robert Weissert,*
*University of Regensburg, Germany*

***Reviewed by:***
*Laura Santambrogio,*
*Albert Einstein College of Medicine, USA*
*Craig Michael Walsh,*
*University of California Irvine, USA*

***Correspondence:**
*Jan D. Lünemann*
*jan.luenemann@uzh.ch*

***Specialty section:***
*This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Immunology*

***Received:*** *23 December 2016*
***Accepted:*** *02 February 2017*
***Published:*** *27 February 2017*

***Citation:***
*Keller CW and Lünemann JD (2017) Autophagy and Autophagy-Related Proteins in CNS Autoimmunity. Front. Immunol. 8:165. doi: 10.3389/fimmu.2017.00165*

## INTRODUCTION

Organisms with subcellular compartmentalization and membrane-bound organelles face constant challenges in order to maintain metabolic integrity and homeostasis. Autophagy comprises a set of evolutionary conserved catabolic pathways that converge in the guided direction of assigned cargo to the endolysosomal system (1–3). At first, autophagy was predominantly recognized for its contribution in keeping energy homeostasis, and degradation of aberrant protein aggregates (4, 5) as well as mediator of development-associated forms of cell death (6). In recent years, however, autophagy's function has exceeded the originally allotted role as a mere protein degradation system alongside the proteasomal machinery. In addition to regulating cellular proteostasis and cell death, autophagy pathways are increasingly being recognized for actively participating in physiological and pathological immune responses. In doing so, autophagy pathways limit intracellular proliferation of pathogens (7), restrict secretion of proinflammatory mediators (8), and tweak T cell responses by orchestrating both loading and preservation of antigens on and surface-expression of antigen-presenting molecules (9–12).

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), which is driven by a complex interplay of genetic, environmental, metabolic, and immunological

factors. The strong genetic association between MS and the major histocompatibility complex (MHC) allele DRB1*1501 suggests that $CD4^+$ T cell-mediated antigen responses play a key role in its pathogenesis. This notion is supported by the presence of clonally expanded T and B cells in MS lesions and cerebrospinal fluid samples derived from MS patients (13–15). In this review, we will first illustrate the biology of distinct autophagy pathways and their function in regulating adaptive immunity in order to discuss how the autophagy machinery potentially interferes with pathogenic adaptive immune responses in MS.

## AUTOPHAGY

Canonical autophagy implicates at least three distinct pathways: macroautophagy (MA), microautophagy (MI), and chaperone-mediated autophagy (CMA) (16). Although all of the above mentioned pathways coalesce in the lysosome, they considerably differ (albeit showing some overlap), in their means of cargo transportation, triggering events, and regulatory factors.

## MACROAUTOPHAGY

Macroautophagy, the canonical autophagy pathway *sensu strictu*, is evolutionary conserved from yeast to mammalian cells and characterized by highly regulated membrane reorganization processes with the subsequent *de novo* formation of a 0.5–1.5 μm wide double-membraned vesicle termed autophagosome (17). Upon sequestering neighboring parts of the cytoplasm, the autophagosome subsequently fuses with lysosomes resulting in enzymatic cargo break down (18, 19). The process is partitioned in five sequential steps (1. induction/nucleation, 2. elongation, 3. closing/maturation, 4. fusion, 5. degradation) that are orchestrated by hierarchies of autophagy-related genes/proteins (Atgs) and other essential components, in a tightly regulated enzymatic cascade (16, 20, 21) (**Figure 1**). Albeit originally identified in yeast, mammalian counterparts for many Atgs have been characterized and some Atgs are so far exclusively reported in mammalian cells and lack yeast orthologs (21). Among all autophagy pathways, MA is to date the most extensively investigated one and, depending on the target constituent encompasses subentities such as macromitophagy, -pexophagy, -xenophagy, and -lipophagy (21–25).

### Atg Machinery and Autophagosome Formation

*De novo* synthesis and maturation of the autophagosome as well as the trafficking of such vesicles to and fusion with lysosomes are distinctive features of MA in opposition to other autophagy pathways. Formation of this typifying vesicle requires approximately 5–10 min and is under the control of an ever-growing number of Atgs (19, 21, 26, 27). The finalized autophagosome is usually swiftly turned over but may reach a half-life of 10–25 min (28, 29). The key proteins that initiate and govern the formation of the autophagosome can be assembled in functionally designated groups: unc 51-like kinase (ULK) complex (1), the class III phosphatidylinositide 3-kinase (PI3K) complex (2), the Atg2/WD repeat domain phosphoinositide-interacting protein (WIPI) complex and the Atg9 cycling system (3), the Atg12-conjugation system (4), and the microtubule-associated protein 1 light chain 3 (LC3)-conjugation system (5) (19, 21, 30).

The autophagosome emanates from the double-membraned phagophore (also called isolation membrane), which sequesters and closes around designated parts of the cytoplasm to form the completed autophagosome. The emergence of said phagophore constitutes the induction/nucleation phase. However, the exact assembly platform and membrane source for the generation of these initial structures are still debated. Similar to the pre-autophagosomal structure that is observed adjacent to the vacuole in yeast, an autophagosome formation site, represented by dot-like accumulations of Atgs, has been identified in mammalian cells (31). Primary suspect organelles to provide membranes include specialized PI3P-enriched endoplasmic reticulum (ER) domains coined omegasomes (32). 3D electron tomography studies corroborated these results by showing that the double-membraned phagophore originates in between two protruding ER flaps. The phagophore then entwines one of the two extensions and finally buds off the ER containing the previously enfolded ER flap. This model is further supported by the fact that >70% of autophagosomes contain ER-derived cargo (33, 34). Nevertheless, other membrane sources for autophagosome generation have been suggested. Among them is the outer mitochondrial membrane (35). These two opposing results might be brought together by a recent study that identified ER-mitochondria contact sites as the originating platform for the autophagosome initiation (36). The VAMP3-dependent heterotypic fusion between early endosome-derived $Atg9^+$ vesicles and recycling endosome-derived $Atg16L1^+$ vesicles has also been suggested to contribute to autophagosome precursor generation (37). Additionally, ER exit sites (38, 39), the ER-Golgi intermediate compartment together with coat protein complex II (40, 41), the plasma membrane (42), and a novel compartment comprised of $Atg9^+$ vesicles and tubules (43) were implicated in providing membrane material. It is conceivable that different subentities of MA preferentially harness distinct membrane sources. Possibly, there is a hierarchy of membrane reservoirs that sequentially may serve as alternative when other sources have been exploited. Furthermore, various tissues with specific composition of subcellular compartments may differ in their means to utilize membranes for autophagosome generation.

### The Atg Core Machinery

The ULK complex is an upstream Atg-unit and, by differential phosphorylation of ULK1 (the main mammalian ortholog to yeast Atg1), a direct target of MA regulation *via* target of rapamycin complex 1 (TORC1) and AMP-activated protein kinase (AMPK), respectively (44, 45). ULK1/2 builds a stable complex with FIP200, Atg13, and Atg101 (46–48). Not composition of the complex itself but rather differential phosphorylation of its members procures promotion or inhibition of MA. In a state of MA inactivation, TORC1 restrains the process by coordinate phosphorylation of ULK1/2 and Atg13. During activation, MA-promoting phosphorylation of ULK1/2 *via* AMPK, ULK1/2 autophosphorylation, and ULK1/2-mediated phosphorylation of Atg13 and FIP200 occur followed by translocation of the entire

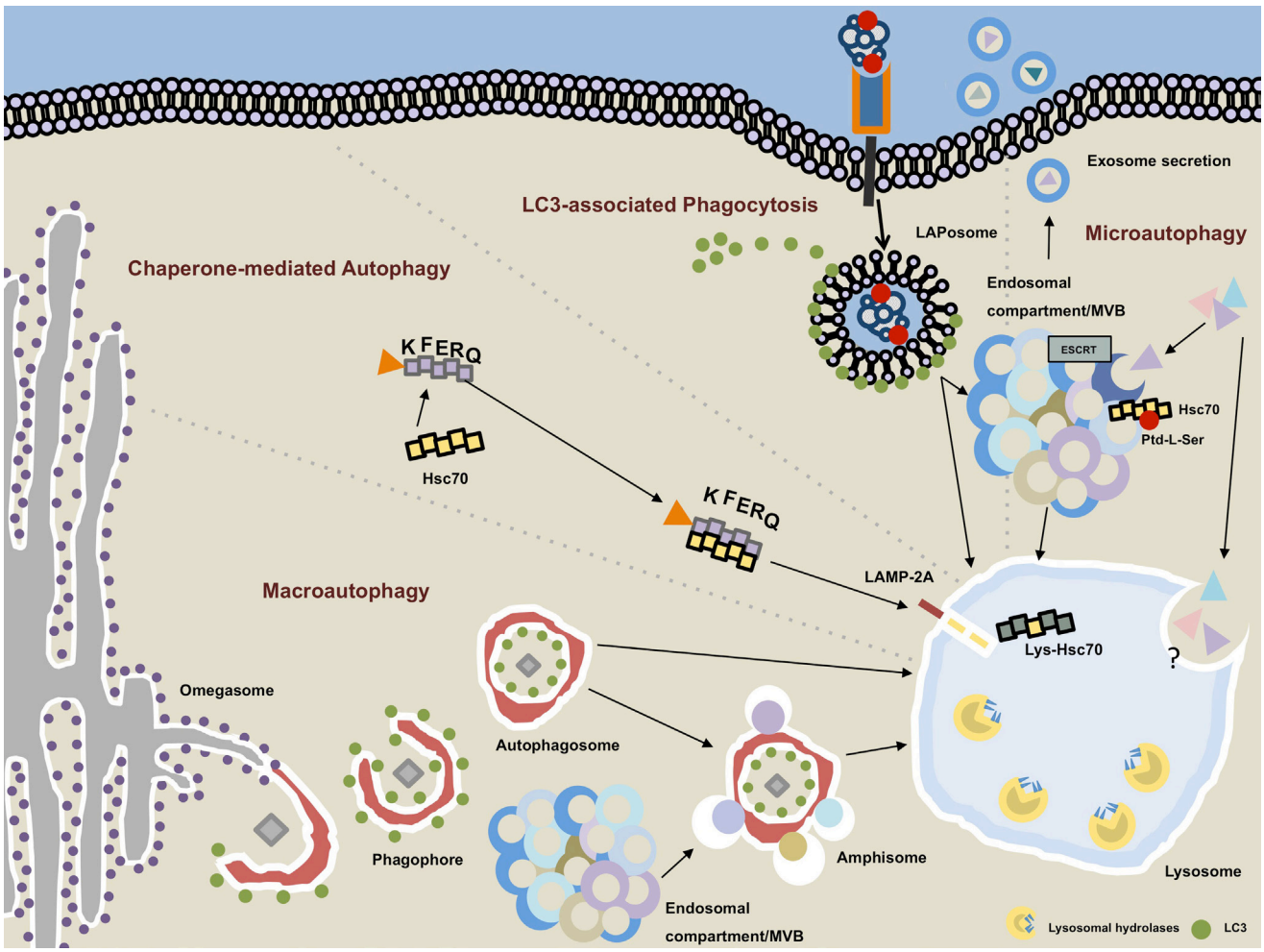



**FIGURE 1 | Autophagy pathways converge in the lysosomal compartment**. Macroautophagy (MA): the phagophore emanates most likely from endoplasmic reticulum-derived membrane sources at a PI3P-rich (not depicted) structure called the omegasome. Upon recruitment of microtubule-associated protein 1 light chain 3 (LC3) on the outer and inner leaflet of the forming autophagosome, cytoplasmic cargo is engulfed and LC3 removed from the outer membrane as the autophagosome is closed. The completed vesicles may now either be further matured *via* step-wise fusion with the endocytic compartment resulting in the generation of amphisomes or immediately fuse with hydrolytic enzymes-containing lysosomes. Chaperone-mediated autophagy: target proteins that contain an exposed KFERQ sequence motif are guided in an hsc70-dependent manner toward lysosome-associated membrane protein type 2A (LAMP-2A), which resides in the lysosomal membrane. Upon unfolding of the target protein, LAMP-2A multimers together with lysosomal hsc70 facilitate the transport into the lysosomal lumen. LC3-associated phagocytosis: ligation of an appropriate receptor (e.g., TLR2, Dectin-1, TIM4, etc.) leads to receptor-mediated phagocytosis recruitment and binding of LC3 to the outer membrane of the LAPosome. By analogy with the MA pathway, completed LAPosomes may either fuse with other endocytic vesicles or directly merge with lysosomes. Microautophagy: cytoplasmic cargo can be directly targeted to endosomal compartments/multivesicular bodies (MVB) in an hsc70-, phosphatidylserine (Ptd-L-Ser)-, and endosomal sorting complexes required for transport (ESCRT)-dependent manner. Exosomes containing cytoplasmic material may emanate from the MVB and be secreted into the extracellular space. The molecular events and regulatory processes orchestrating the direct invagination of cytoplasmic constituents into lysosomes remain largely unbeknownst.

complex to autophagosomal initiation sites possibly on tubulovesicular areas comprised of ER and Atg9$^+$ vesicles (46, 49–52). Atg101 may facilitate phosphorylation of Atg13 and minimize its proteasomal degradation (53, 54).

## The Class III PI3K Complex

The tetrameric core of this complex consists of vacuolar protein sorting protein (Vps)34 (a phosphoinositide 3 kinase), Vps15 (a regulatory subunit of Vps34 also called p150), Beclin 1 (ortholog of Atg6), and Atg14L (aka Atg14 and Barkor) (27, 55, 56). NRBF2 (ortholog of Atg38) is a recently identified additional subunit of the complex that augments the enzymatic activity of the lipid kinase Vps34 (57–59). The lipid kinase complex is recruited to autophagosomal initiation sites in an ULK1-complex/Atg9-dependent manner (31, 60).

The key function of the complex is to produce PI3P *via* Vps34-mediated phosphorylation of phosphatidylinositol. Presence and accumulation of PI3P are essential for MA as PI3P-enriched membrane areas then function as platforms to which downstream PI3P-binding partners can be recruited. Regulation of MA occurs

also on the level of the PI3P complex in that death-associated protein kinase promotes autophagosome formation by unleashing Beclin 1 from the Bcl-2/Bcl-XL complex (61).

## Atg2/WIPI Complex and the Atg9 Cycling System

WIPI1/2 (mammalian ortholog to yeast Atg18) and its binding partner Atg2 are among the PI3P-binding effector molecules that are recruited to PI3P-enriched sites upon PI3PK complex activation (31, 62–64). Since PI3P is present on various subcellular membranes, up until recently it remained a conundrum as of how organelle-specific WIPI-binding is achieved. Studies in yeast have now suggested that WIPI-recruitment to the phagophore requires a dual determinant: PI3P as well as presence of Atg2 (65). The Atg2/WIPI complex is thought to be downstream of the two previous complexes but lateral to the following two conjugation systems (21). Its presence is essential for the formation of autophagosomes probably in part due to recruitment of lipidated LC3 (mammalian ortholog of yeast Atg8) to autophagosomal initiation sites and shielding of lipidated LC3 from Atg4-mediated deconjugation (66).

The transmembrane protein Atg9 can be found in the trans-Golgi network and on late endosomes under nutrient-rich (hence MA-inactive) conditions (67). Upon starvation (and induced MA activity) however, $Atg9^+$ vesicles translocate from the trans-Golgi network and colocalize with LC3 (67). Possibly, Atg2 and WIPI1/2 mediate Atg9-shuttling between autophagosomal initiation sites and peripheral membrane sources (68). However, its interaction and association with the autophagosome appear to be transient since completed autophagosomes are devoid of Atg9 (69). It was also suggested that local fusion of $Atg9^+$ vesicles operates as an initiation step in the generation of autophagosomes (43). Possibly, Atg9 functions in provision of membrane material for generation of autophagosomes or even removal of early autophagosomal molecules from the site of autophagosome generation (21). The exact interplay between the individual molecules of this unit and their overall function remains subject to further investigation.

## The Atg12-Conjugation System

The first of two ubiquitin-like conjugation systems that orchestrate autophagosome formation consists of the MA-essential molecules Atg12, Atg7, Atg10, Atg5, and Atg16L1 (21). Constitutively and independent of cellular nutrient status, Atg12 is activated *via* E1-like enzyme Atg7, followed by transfer to E2-like enzyme Atg10 which catalyzes the covalent conjugation of Atg12 to Atg5. *Via* binding to Atg5, the conjugate then forms a complex with Atg16L1 (70). WIPI2 now attracts the Atg5–Atg12–Atg16L1 complex by means of a recently identified binding site in Atg16L1 to the site of autophagosome generation (71). Additionally, it has been suggested that Atg16L1 is also recruited to the phagophore *via* binding to ULK-complex member FIP200 (72, 73). On site, the complex then functions as an E3-like enzyme for the second conjugation system. The complex is regularly found on the outer membrane of the phagophore but dissociates from there upon completion of the autophagosome (26).

## The LC3-Conjugation System

The second and final conjugation system is comprised of the ubiquitin-like LC3, the hydrolase Atg4, Atg7, and Atg3, which function as E1- and E2-like enzymes, respectively. The pro-form of LC3 is cleaved by Atg4 leading to exposure of a C-terminal glycine residue (74, 75). The resulting LC3-I is then lipidated with phosphatidylethanolamine (PE) at said glycine residue by means of Atg3 and Atg7 (76–78). This PE-lipidation of LC3 is assisted by the Atg5–Atg12-conjugates *via* E3-like activity (70, 79). Additionally, the Atg5–Atg12–Atg16L1-complex guides and determines LC3 to its subcellular destination (79). This lipidated form of LC3, also called LC3-II is initially found symmetrically distributed on the inner and outer membrane of the phagophore (75, 76, 80). There it appears to aid in elongation of the phagophore as well as coordinate tethering and hemifusion of its membranes and finally the closing of the phagophore to an autophagosome (81–84). Upon vesicle closure, LC3 on the outer membrane is being cleaved from PE *via* Atg4 while intraluminal LC3 stays associated with the organelle which makes LC3-II to this day a valuable marker for autophagosomes (80, 85). In addition to its contribution during membrane reorganization, LC3 mediates MA-cargo selectivity by functioning as an adaptor molecule (86).

# REGULATION OF MA

Most mammalian cells carry out MA on a constitutive level at varying degrees. However, depending on the cell type, macroautophagic activity can be induced and modulated in numerous ways. Primarily nutrient deprivation is a potent stimulus of autophagy and starvation-induced autophagy is a common means to investigate MA under experimental conditions (87, 88). The process receives regulatory input on both a systemic and a cellular level (88). Among the most upstream regulatory units of the MA machinery is the antagonistic interplay of coordinate phosphorylation by the two serine/threonine protein kinases AMPK and TORC1 (44, 45) (**Figure 2**). For insights into transcriptional and epigenetic regulation of MA, we kindly refer the reader to excellent recent review articles (89–91).

## mTOR/TORC1

Target of rapamycin complex 1 is one of two functional units of mTOR. Notably, as opposed to TORC2, which has been implicated as an inhibitory entity of CMA activity (92), TORC1 is actually a binding partner for the eponymous rapamycin and is the relevant subunit that participates in negatively regulating MA (93). Various signals that reflect a cell's metabolic status (free amino acids, growth factors, fatty acids, oxygen, etc.) are integrated at the level of TORC1 which, during its activated state, restrains MA *via* phosphorylation of the upstream MA-machinery members ULK1/2 at serine 757 and ATG13 at serine 258 and is therefore considered a potent negative regulator of MA (46, 47, 94). In keeping with its cardinal function as a coordinator of metabolic homeostasis, two major inputs for TORC1 downstream signaling are free amino acids and growth factors (88). Growth factors signal *via* the PI3K–Akt–tuberous sclerosis complex (TSC)1/TSC2 pathway, eventually leading to disinhibition of the small

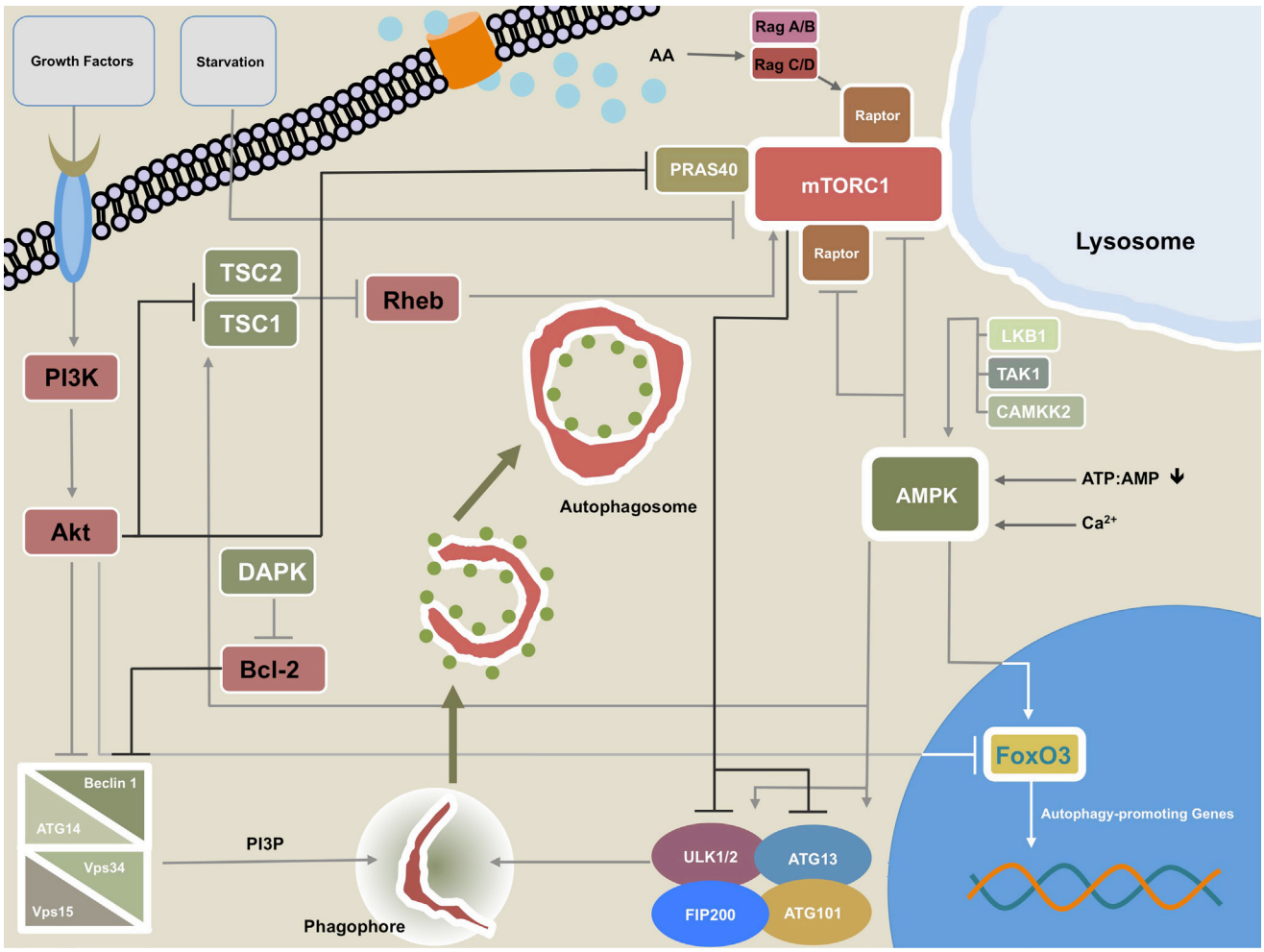



**FIGURE 2 | Regulatory network of macroautophagy (MA)**. This figure illustrates the most important regulatory interplay of molecules that govern MA activity. Growth factors can signal through phosphatidylinositide 3-kinase (PI3K) to Akt, which in turn inhibits the Beclin 1-containing class III PI3K complex. Akt may also repress transcription of autophagy-promoting genes by direct inhibition of transcription factor forkhead box protein O3 (FoxO3). Additionally, Akt reduces macroautophagic activity by inhibiting tuberous sclerosis complex (TSC)1/TSC2. Akt interaction with PRAS40 promotes mTORC1 activity thereby further damping MA. The calcium/calmodulin ($Ca^{2+}$/CaM) serine/threonine kinase death-associated protein kinase (DAPK) fosters MA through phosphorylation of Beclin 1 at T119 which leads to dissociation of Beclin 1 from Bcl-2. The TSC1/TSC2 complex can promote MA by inhibiting the mTORC1 activator and GTPase Ras homolog enriched in brain (Rheb). AMPK and mTORC1 are the two cardinal regulatory units that control MA. mTORC1 may repress the process by phosphorylation of ULK1/2 and/or Atg13. mTORC1 activity is increased by amino acid sensing Rag GTPases *via* interaction with Raptor. The Rag GTPases also facilitate translocation of mTORC1 to the lysosomal membrane, which serves as a prerequisite for the aforementioned mTORC1-promoting activity of Rheb. AMPK promotes MA by several means: phosphorylation of ULK1/2 and/or Atg13 at different residues than mTORC1, direct interaction with FoxO3, and by directly disinhibiting the inhibitory units, mTORC1 and Raptor. AMPK itself can be triggered by increasing levels of AMP relative to ATP, free $Ca^{2+}$, and activity of liver kinase B1 (LKB1), transforming growth factor-β-activating kinase 1 (TAK1), and CAMKK2.

GTPase Ras homolog enriched in brain (Rheb) which then directly activates TORC1 (95–97). Alternatively, the mTOR binding partner proline-rich Akt/PKB substrate 40 kDa (PRAS40) is directly phosphorylated by Akt which in turn leads to revoking of PRAS40-mediated inhibition of mTOR (98). Recently, Akt has been shown to also hamper MA in a direct manner *via* phosphorylation of Beclin 1 (99). Indirectly, Akt may repress MA *via* inhibiting forkhead box protein O3 (FoxO3) members-dependent transcription of autophagy-promoting genes (100–102).

Target of rapamycin complex 1 does not directly detect free amino acids and the actual amino acid sensing entity remains yet to be identified (88). Detection of free amino acids by innominate cytoplasmic sensors facilitates the Rag-GTPases-dependent translocation of TORC1 to the lysosomal membrane, which is a spatial requirement for Rheb-mediated activation of TORC1 (88, 103, 104).

## AMPK

AMPK is primarily an energy-sensing kinase that in response to metabolic stress and depleted cellular energy resources such as a low ATP:AMP ratio initiates catabolism (105). In doing so, AMPK is also a positive regulator of MA-mediated proteolysis

(106). However, even under nutrient-rich conditions AMPK may act toward MA induction in a $Ca^{2+}$-dependent manner (107, 108). Although essential for starvation-induced MA, AMPK might not suffice as a stand-alone signal but is likely to require additional cues in order to fully initiate MA (107, 108). Liver kinase B1, calcium/calmodulin-dependent protein kinase kinase-β (CAMKKβ), and transforming growth factor-β-activating kinase 1 are upstream regulators which through phosphorylating its catalytic α-subunit activate AMPK (109–113).

AMPK promotes MA by different pathways such as initiation of Rheb inhibition through TSC1/TSC2 (114), phosphorylation of the mTOR binding partner raptor, thereby inhibiting TORC1 (115), or direct phosphorylation of ULK1/2 (at serine 317 and serine 777) or Atg13 (at serine 224) which activates these family members of the upstream MA machinery (44, 45, 94). Furthermore, AMPK positively regulates MA through activation of the transcriptional factor FoxO3 which in turn transactivates MA-essential genes (116, 117).

## MICROAUTOPHAGY

During MI, a vaguely characterized autophagosome-independent process, superfluous cytoplasmic constituents including whole organelles are directly engulfed by surface invaginations of lyso- or endosomal surfaces leading to the budding of cargo-containing microvesicles into the endolysosomal lumina where degradation takes place (118–121) (**Figure 1**). MI initially has been termed unselective; however, numerous reports in yeast and mammalian cells challenged this concept and described more discerning mechanisms and functions underlying this pathway (118, 120, 122). For instance, the MI-assisted transport of endosomes to lysosomes appears to be a crucial event during murine embryogenesis in a Rab7-dependent manner (123). Endosomal sorting complexes required for transport (ESCRT)-machinery-dependent microlipophagy is essential during cellular adaptation to cell stress caused by disruption of lipidostasis in yeast (124). Schuck and colleagues characterized the specific uptake of compromised ER into the yeast vacuole and coined the term ERphagy (125). Other cargo selective MI entities in yeast include micromitophagy (126, 127), micropexophagy (128, 129), and piecemeal microautophagy of the nucleus (130–132). To which extent these processes take place in mammalian cells remains largely obscure (133). Little is known about MI and its regulation on a molecular level in mammalian cells and most findings rely on electron microscope-based morphological alterations such as lysosomal wrapping and flap-like lysosomal membrane extensions (133). The picture becomes even more hazy when acknowledging that some forms of MI require the core machinery needed for MA (see above) but others do not (120, 128, 132, 134). However, distinct cargo selective MI pathways have also been identified in mammalian cells. For instance, heat shock cognate protein of 70 kDa (hsc70)-dependent targeting of cytosolic KFERQ sequence motif-containing proteins into late endosomes and multivesicular bodies (MVB), termed endosomal MI, has recently been identified in murine antigen-presenting cells (APCs) (120). Although this newly characterized process resembles the selective pathway of CMA (see below) into lysosomes, this endosome/MVB-destined MI does not require unfolding of target proteins and is lysosome-associated membrane protein type 2A (LAMP-2A) independent (120). Also different from CMA, endosomal MI is not only dependent on hsc70 but also heavily relies on ESCRT I and III and involves electrostatic binding of hsc70 to negatively charged endosomal phosphatidylserine (Ptd-L-Ser) (120, 135). Fission yeast Nbr1, a partial homolog of mammalian MA receptor NBR1, has been recently identified as a receptor for ESCRT-dependent endosomal MI (136, 137). Two recent reports further elaborated on this novel pathway. Mukherjee and colleagues describe an endosomal MI-like process in the fat body of *Drosophila melanogaster*. In accordance with what had been reported previously, this process is KFERQ- and hsc70-dependent and relies on ESCRT I-, II-, and III-mediated endosomal MVB formation. Although this pathway is independent of MA-machinery members—*atg5*, *atg7*, and *atg12*—it can be induced by prolonged starvation and by mTOR signaling under nutrient-rich conditions (138). A second report shows that by means of its oligomerization capabilities, hsc70 promotes membrane deformations, which supports presynaptic endosomal MI and increases the local turnover of target proteins. Importantly, the degree of presynaptic endosomal MI fosters the release of neurotransmitters into the synapse (139).

Intraluminal vesicles generated within MVBs give rise to exosomes which in a controlled and coordinated manner are released to the extracellular space by most cells (140, 141). Aside from their physiological function in cell-to-cell communication and coordinating immune responses, these vesicles of endocytic origin have also been implicated in propagating neurodegenerative processes (141–143). Conclusive evidence points toward a substantial role of endosomal MI in providing cargo for this unique secretory pathway (120, 141). It will be of great importance to elucidate the mechanisms and triggers that decide whether MVB content is targeted toward intracellular degradation by the lysosome or instead is fed into the secretory pathway *via* exosomes.

## CHAPERONE-MEDIATED AUTOPHAGY

Chaperone-mediated autophagy is a selective degradation process devoid of autophagosome formation during which substrate proteins are directly targeted to the lysosome (144) (**Figure 1**). There is no known equivalent in yeast and evolutionary speaking CMA has emerged only recently, thus far exclusively being identified in mammals (145). Most cells constitutively carry out CMA but the process is upregulated in response to oxidative stress (146), hypoxia (147), DNA damage (148), or protracted nutrient deprivation (149). Unlike MI and MA, which may also handle surplus organelles and non-protein macromolecules, CMA exclusively processes proteins that contain a pentapeptide motif in their amino acid sequence (KFERQ) (150). CMA encompasses at least five distinct steps (1. recognition, 2. binding, 3. unfolding, 4. translocation, 5. disassembly) (144): target proteins bind with their KFERQ-recognition motif to the cytosolic chaperone hsc70 and the substrate:chaperone complex translocates to the lysosomal membrane (recognition) (151). There, LAMP-2A, a

CMA-receptor, binds the substrate:chaperone complex *via* its cytosolic tail and subsequently the designated protein undergoes unfolding in a hsc70-dependent manner probably assisted by co-chaperons on the cytosolic side of the lysosome (binding and unfolding) (152, 153). Binding of the substrate:chaperone complex to monomeric LAMP-2A not only initiates protein unfolding but also recruitment of further LAMP-2A molecules and subsequent assembly of high molecular weight multimers that then, by help of lysosomal hsc70 (lys-hsc70) enable passage of target proteins to the luminal side of the lysosome (translocation) (154). Upon release of the target protein into the lysosomal lumen, the LAMP-2A multimers are hsp70-dependently broken down into monomers which are then available for another cycle of ligand binding and uptake (disassembly) (154). Recently, more light has been shed on the molecular regulatory pathways controlling CMA. Glial fibrillary acidic protein (GFAP) together with elongation factor 1α tunes assembly and disassembly of the LAMP-2A translocation complexes at the lysosomal membrane (155). Phosphorylation of GFAP by lysosomal Akt1 leads to destabilization of such translocation complexes and consequently reduced CMA activity. The antagonistic interplay between PH domain leucine-rich repeat-containing protein phosphatase 1 (PHLPP1) as a negative and mTORC2 as a positive regulator upstream of lysosomal Akt1 signaling therefore appears to play a central role in modulating CMA activity (92). Different from MI and MA, CMA processes one molecule at the time and does not require morphological changes of lysosomal membranes such as invaginations, wrapping, flap-like extensions, or fusion with other membranes (156, 157). The main rate limiting factor in CMA is abluminal LAMP-2A availability which is regulated by the degree of LAMP-2A degradation by a metalloprotease and cathepsin A at the site of designated lipid microdomains in the lysosomal membrane (158). As previously mentioned, a prerequisite for CMA processing of a target protein is the presence of an amino acid sequence motif biochemically related to KFERQ (150). In contrast to the biochemical earmarking of a molecule by means of ubiquitination, which may designate the target for proteasomal degradation or recognition by ubiquitin-recognizing adaptor molecules, the KFERQ consensus motif is pre-existing in CMA-target proteins (145). It is therefore likely that the pentapeptide motif is not ubiquitously accessible for binding to hsc70 in order to prevent premature protein translocation to the lysosomal lumen *via* CMA. Possible mechanisms to condition a target for this destination include but are not limited to, partial unfolding, disassembly of multimers, cleavage of proteins, and posttranslational modifications such as phosphorylation or acetylation of amino acid residues (145, 159, 160). Cytosolic accumulation of aberrant proteins is a hallmark of age-related proteinopathies of the brain and other tissues. A central function of CMA is the removal of misfolded or unwanted proteins and dysfunction in CMA in senescent cells has been implicated in promoting neurodegeneration (161–163). Not considering posttranslational alterations, roughly 30% of cytosolic proteins bear a sequence motif biochemically related to KFERQ and are therefore potentially subject to CMA-mediated lysosomal degradation (145, 150, 151). Due to the heterogenous nature of this protein pool it does not come as a surprise that CMA can be regarded as a pivotal gatekeeper in energy homeostasis that also fine-tunes vital cellular processes by limiting key metabolic enzymes (144).

## AUTOPHAGOSOME MATURATION AND FUSION WITH THE ENDOLYSOSOMAL SYSTEM

A hallmark of all autophagic pathways is their convergence into the lysosomal system. During MA, this event requires the coordinated membrane fusion of the autophagosome and endolysosomal vesicles (**Figure 1**). Prior to terminal fusion with lysosomes, autophagosomes may (or may not) fuse with vesicles of the endocytic compartments like early or late endosomes. The resulting amphisomes subsequently fuse with lysosomes to form autolysosomes. These partly sequential, partly parallel fusion events underscore the dynamic nature of MA. Hence, observed accumulation of autophagosomes needs to be carefully interpreted, for it can mean both *bona fide* induction of the process and blocked lysosomal fusion.

Autophagosomes are widely distributed throughout the cytoplasm. For these vesicles to fuse with lysosomes and late endosomes (which are predominantly located juxtanuclear), autophagosomes need to be efficiently guided toward this area. Members of the cytoskeleton have been shown to orchestrate the regulated trafficking of autophagosomes from the periphery to sites at which membrane fusion occurs. In fact, microtubules might even aid in autophagosome formation and subsequent fusion with endosomal compartments (164, 165). In an antagonistic interplay, the members of the motor protein family dynein/dynactin complex and kinesin have shown to be involved in guiding autophagosomes alongside microtubules toward lysosome-rich areas, a process during which the trafficked vesicles become increasingly acidified as they approach the juxtanuclear area (166, 167). Consequently, disruption of the dynein machinery exacerbates aberrant protein aggregation in experimental models of neurodegeneration due to dysfunctional MA (168). Interestingly, dynamic distribution of lysosomes within the cytoplasm may actually constitute a mechanism by which autophagic flux is regulated (169).

In accordance with general membrane reorganization, also the fusion of autophagosomes with endolysosomal vesicles is in large parts orchestrated by members of small GTPases called Rabs, membrane-tethering complexes, and SNAREs. The molecular specifics have been reviewed in detail elsewhere (170, 171). Studies in yeast suggest that Atg4-mediated cleavage of the LC3 ortholog Atg8 from the outer membrane constitutes one prerequisite that renders the autophagosome ready for fusion (172, 173). Possibly, absence of molecules that are involved in the early phase of autophagosome biogenesis functions as yet another signal (170).

Our understanding of the precise molecular events during autophagosome biogenesis and fusion has significantly improved during the last decade and enticing models have been proposed as on how the phagophore is initiated. However, one needs to be cautious as of how the aforementioned molecular interplay of

Atgs can be generalized since most results were obtained studying starvation-induced MA. There is evidence that autophagosomal biogenesis, and subsequent fusion partners, -sites, and -mechanisms are highly dependent on induction stimulus and cargo (170). Furthermore, the degree as to which these processes are actually carried out in sequence remains enigmatic. It is likely that numerous steps occur in parallel and distinct Atgs might not only carry out a single function but take on several tasks within the cascade. The molecular interactions between Atgs and the kinetics of the process might also significantly differ within the mammalian species and even on a tissue level, there might be specifics to MA that need to be taken into account.

Finally, at least for some Atgs that are essential for MA, an even more promiscuous role has begun to unfold in that these proteins also facilitate MA-independent functions in cellular reprograming, dynamic membrane re-distribution, pathogen clearance, and antigen presentation (11, 174–178).

## NON-CANONICAL AUTOPHAGY PATHWAYS

Non-canonical autophagy comprises a set of recently characterized pathways that either result in autophagosome formation but omit the usage of distinct parts of the classical MA-machinery or autophagosome-independent pathways that utilize key components of the MA network (16, 179). One can at least differentiate five distinct entities: *LC3-associated phagocytosis (LAP)*, *Beclin 1-independent autophagy*, *autophagosome formation from multiple phagophores and pathogen-specific autophagy modification*, *autophagy-associated unconventional protein secretion*, and *defective ribosomal products-containing autophagosome-rich blebs*. Here, we focus on LAP, since this pathway has been associated with antigen processing and presentation, and kindly refer the reader for specifics on the other non-canonical autophagy pathways to excellent reviews elsewhere (16, 179, 180).

### LC3-Associated Phagocytosis

During phagocytosis, a specialized way of endocytosis, cells internalize solid extracellular constituents in a receptor-mediated fashion. The resulting phagosome is being step-wise matured and subsequently fuses with the lysosomal compartment in order to break down the incorporated material (181). Recently, a novel organelle, the single-membraned $LC3^+$ phagosome or LAPosome, has been identified and the process of its generation and fate was coined LAP (**Figure 3**) (182, 183). LAP, that links both phagocytosis of extracellular cargo and members of the autophagy molecular core machinery, is initiated by ligation of a variety of extracellular receptors (12, 182, 184–186). Although some components of the MA machinery are essential for LAP (e.g., Atg5, Atg7, LC3, Beclin 1), the process is independent of others (e.g., the ULK complex including ULK1/2, FIP200, Atg13, Atg101, WIPI1).

LC3-associated phagocytosis-triggering receptors include toll-like receptors (TLRs), Fc-receptors, C-type lectins, and Ptd-L-Ser-binding receptors (12, 182, 184–186). Upon activation of a LAP-triggering surface receptor, a PI3PK complex that differs in its composition from the one involved in MA, is recruited to the cytosolic membrane of the phagosome followed by the recruitment of the NADPH oxidase NOX2. These events are not preceded by involvement of the canonical ULK complex. The LAP-associated PI3PK complex is made up by Beclin 1, Vps34, and Vps15 (analogous to MA) but is devoid of Atg14 (187). Instead, it includes UVRAG and Rubicon (not members of the canonical PI3PK complex). Similar to MA, the PI3PK complex is set out to generate PI3P on the LAPosome. The association of the modified PI3PK complex on the LAPosome is potentially liaised *via* Rubicon which, together with PI3P also appears to be key in recruiting, stabilizing, and activating the NADPH oxidase NOX2 to the organelle (187). Rubicon mediates NOX2 stabilization by interaction of its serine-rich domain (AA 567–625) with the NOX2 subunit $p22^{phox}$ whereas PI3P stabilizes the NOX2 subunit $p40^{phox}$ (187, 188). LAPosomal PI3P in concert with NOX2-dependent reactive oxygen species (ROS) production then initiates the two canonical MA conjugation systems (see above), which results in efficient deposition of lipidated LC3 on the outer LAPosomal membrane (187). Consequently, LAP is highly dependent on the MA proteins Atg5, Atg12, Atg16L1, Atg7, Atg3, Atg4, and lipidated LC3. Molecules that have been described to be dispensable for canonical MA but are thought to be essential for LAP include NOX2 and Rubicon (187).

Taken together and in contrast to the canonical MA pathway, during which LC3 conjugation to the phagophore allows for recruitment of cytoplasmic substrates into forming autophagosomes by means of LC3-binding anchor proteins such as p62/sequestosome 1, LAP handles formerly extracellular particles that access the cell through phagocytosis and occurs without the formation of a double-membraned vesicle and in the absence of p62/sequestosome 1 on $LC3^+$ single-membraned phagosomes (179, 183, 187).

So far LAP has been implicated in efficient clearance of *Saccharomyces cerevisiae* and *Aspergillus fumigatus* (182, 187). However, in the case of *Listeria monocytogenes* infection, LAPosomes have been described as *Listeria*-containing compartments that promote persistent infection (189). In plasmacytoid dendritic cells (DCs), LAP, induced upon binding of immune complexes through FcγR, is required to assemble the interferon regulatory factor 7 (IRF7)-signaling compartment which is essential for IRF7 activation and subsequent IFNα secretion downstream of TLR9 ligation (186). LysM-$Cre^+$ conditional knockout mice for LAP-essential proteins mainly target $CD11b^+$/$F4/80^+$ macrophages and $CD11b^+Ly6G^+$ neutrophils. Aged mice that are deficient of LAP activity in these myeloid subsets spontaneously acquire a lupus-like phenotype (190).

Several questions remain unanswered. The functional benefit of coupling lipidated LC3 to a phagosome is still not completely understood. Some have argued that the coating of the phagosome with LC3-II allows the vesicle to travel faster along microtubules which results in faster fusion with the lysosomal compartment (182, 185, 191). However, studies that report more rapid cargo degradation were predominantly obtained using murine macrophages. Human studies on the other hand show that in prototypical APCs such as DCs, LAP seems to retain antigenic

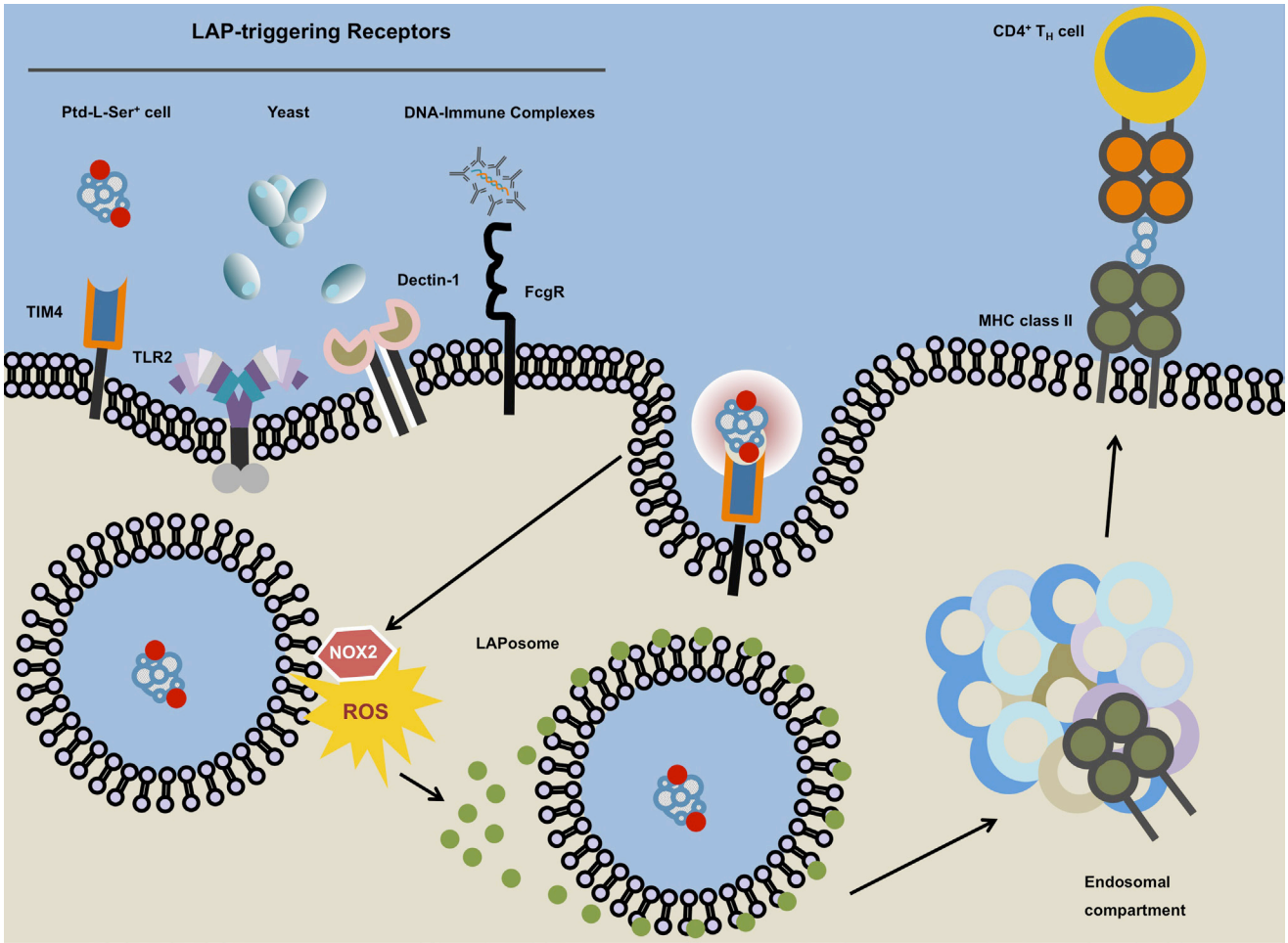



**FIGURE 3 | Microtubule-associated protein 1 light chain 3 (LC3)-associated phagocytosis**. The ligation of LC3-associated phagocytosis (LAP)-triggering receptors such as phosphatidylserine (Ptd-L-Ser)-recognizing TIM4, TLR2, Dectin-1, or Fcγ receptors will ensue receptor-mediated phagocytosis and subsequent phosphatidylinositide 3-kinase-dependent association of the NADPH oxidase NOX2 with the phagosome. The NOX2-derived reactive oxygen species (ROS) and laposomal PI3P (not depicted) mediate the recruitment and binding of LC3 to the outer membrane of the LAPosome. Instead of terminal fusion with the lysosomal compartment, the completed LAPosome may also fuse with endosomal vesicles including major histocompatibility complex (MHC) class II-containing compartments (MIICs) in which after enzymatic digestion, LAPosomal content can be loaded upon MHC class II molecules followed by the presentation of the resulting peptides to $T_H$ cells.

cargo for sustained MHC class II antigen presentation rather than promoting its degradation (12). Antigenic containment and stabilization in combination with low levels of lysosomal proteases have been speculated to be a crucial mechanism by which DCs maintain efficient and prolonged antigen presentation (12, 183, 192). Lee and colleagues reported that in murine DCs, TLR-dependent phagocytosed HSV-2 can be found in LC3$^+$ single-membraned vesicles reminiscent of LAPosomes. In absence of LAP-essential protein Atg5, subsequent HSV-2-specific CD4$^+$ T cell responses were markedly reduced, arguing for inefficient MHC class II-dependent presentation of LAP-deficient DCs toward cognate T cells (193). Interestingly, pharmacological induction of canonical MA by TORC1 inhibitor rapamycin did not lead to enhanced MHC class II presentation of viral antigens arguing for a non-MA pathway linking phagocytosis and MHC class II presentation (193).

## AUTOPHAGY PATHWAYS IN CNS AUTOIMMUNITY

Multiple sclerosis is a chronic neuroinflammatory condition during which autoaggressive leukocytes invade across a compromised blood–brain barrier into the CNS. On site, the infiltrated immune cells, in concert with CNS-resident cells mediate progressive axonal demyelination and engender spatio-temporally disseminated lesions within the CNS which entails diffuse cytodegeneration in gray and white matter areas of the CNS (194) (**Figure 4**).

Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model of MS in which disease is induced by immunization of animals with myelin-derived antigenic peptides together with adjuvant or by adoptively transferring pre-activated myelin-specific CD4$^+$ T cells in naïve recipients (195–197). EAE is a predominantly T cell-driven model and a pathomechanistic

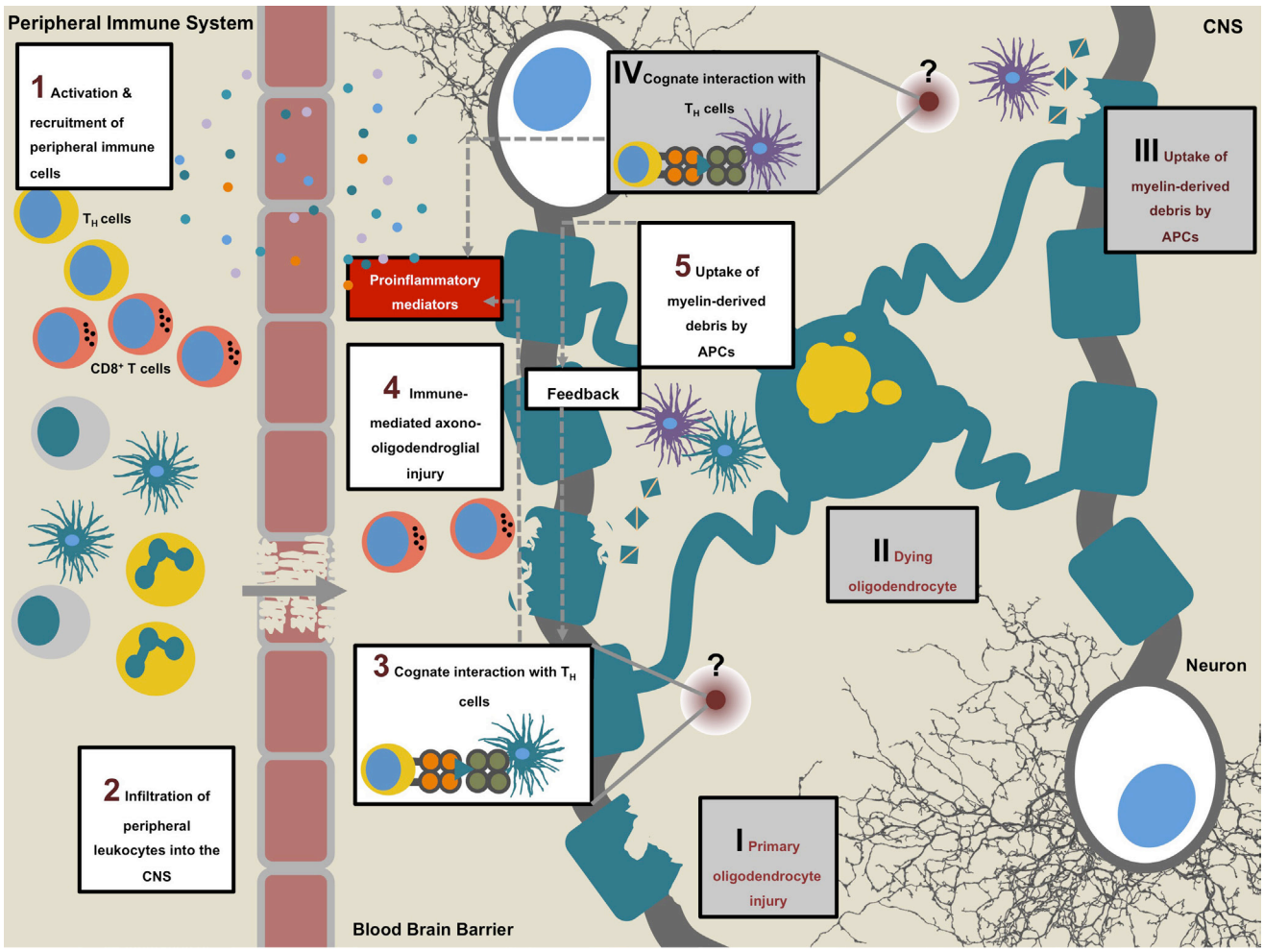



**FIGURE 4 | Multiple sclerosis (MS) pathogenesis**. Sequence of events that initiates the onset of disease (1–5). MS is suspected to be an autoimmune condition during which peripheral autoaggressive T cells cross into the central nervous system (CNS) where, in concert with other leukocytes, they mediate axono-oligodendroglial damage and unleash a circle of self-perpetuating neuroinflammation (1–5). An alternative to this "outside-in" sequence is the "inside-out" model, which proposes a primary disease triggering event within the CNS (I–IV): primary oligodendrocyte damage of unknown cause may lead to dying and disintegrating oligodendrocytes followed by the uptake of myelin-derived material by CNS-resident antigen-presenting cells (APCs). Upon further intracellular processing of the phagocytosed material, APCs present antigenic myelin-derived peptides to $T_H$ cells (at an as of yet unknown site) which in turn initiate influx of peripheral immune cells by secretion of proinflammatory cytokines and chemokines. On site, infiltrating immune cells, including cytotoxic $CD8^+$ T cells and myeloid cells perpetrate secondary oligodendrocyte damage.

prerequisite for CNS damage is the local reactivation of $CD4^+$ T cells specific for myelin-derived antigens (198–202). Reactivated $CD4^+$ T cells together with activated myeloid cells initiate a cascade of proinflammatory events, which is believed to perpetuate CNS tissue damage leading to the development of clinical EAE symptoms (203–205).

How and by which APC subset pathogenic $CD4^+$ T cells are being locally reactivated and cause tissue damage during the course of MS and EAE is, however, incompletely understood. This is in part due to the fact that the detailed antigen-processing route of how myelin self-antigens are processed and presented toward $CD4^+$ T cells has not been fully elucidated.

Given their diverse, yet often essential functions in immune surveillance, cell survival, proteostasis, antigen presentation, and regulating cellular energy levels, a role for autophagy pathways in the complex pathobiology of MS can been proposed in many aspects of the condition including but not limited to the innate and adaptive branches of the immune system. In the following sections, we will discuss how autophagy pathways potentially interfere with pathogenic adaptive immune responses in MS.

## AUTOPHAGY IN T CELLS

Autophagy has a central but complex role in cell survival in numerous cell types, functioning either as a pro-survival or as a cell death mechanism depending on the cell type, the nature of the death stimulus, and subsequent compensatory changes. Atg5-deficient $CD4^+$ and $CD8^+$ T cells develop normally in the thymus, but fail to repopulate the periphery because of massive cell death and fail to undergo efficient proliferation after T cell receptor

stimulation (206). Using a T cell-specific conditional knockout of Beclin 1 (CD4-Cre Beclin $1^{fl/fl}$), Kovacs et al. later elaborated on the underlying mechanism and confirmed that in absence of Beclin 1 in the $CD4^+$ $CD8^+$ T cell compartment, homeostasis was disturbed. In line with previous reports, thymic development of the T cell compartment was normal; however, numbers of $CD8^+$ T cells in secondary lymphoid organs were drastically diminished (>3-fold), whereas $CD4^+$ T cell were less affected (reduction of $CD4^+$ T cell numbers amounted to <2-fold). The steady state fractions of naïve and memory T cells were unchanged between conditional KO mice and controls. Upon activation with anti-CD3/anti-CD28, however, T cells readily underwent apoptosis. Increased susceptibility to cell death was linked to MA-dependent degradation of pro-apoptotic proteins and seems to be differentially relevant in $CD4^+$ T cell subsets (207).

Additionally, Atg16L1-specific deletion in T cells results in spontaneous intestinal inflammation and inbalances of different $T_H$ cell subsets in the gut. While intestinal T regulatory cells (TREGs) are drastically reduced in number, IL-13-producing $T_H2$ cells expand (208). TREG-specific deletion of Atg5 and Atg7 entailed reduced frequencies and survival of this subset and leads to defective self-tolerance (209).

These data indicate a critical role for the autophagy machinery in lymphocyte development and function and suggest that MA may be essential for both T lymphocyte survival and proliferation in the steady state and after immune activation.

In line with these assumptions, CD4-Cre Beclin $1^{fl/fl}$ mice are completely protected from active induction of EAE (207). Interestingly, the defect in the $CD4^+$ T cell compartment during EAE is much more pronounced in the $T_H1$ fraction but only mildly present in $T_H17$. Subsequent *in vitro* experiments further confirmed that $T_H1$ cells are significantly more prone to undergo apoptosis in absence of autophagy (207).

In addition to the observed protection of EAE upon T cell-specific ablation of autophagy, Atg5 transcript and protein levels in circulating T cells positively correlated with the disease score in mice that had been actively immunized with $MOG_{35–55}$ peptide. Furthermore, peripheral T cell-derived RNA analyses suggested a significant increase in Atg5 expression in patients with active relapsing-remitting MS as compared to healthy controls. Atg5 RNA levels in postmortem brain tissue from patients with secondary progressive MS (SPMS) were markedly elevated and strong Atg5 immunoreactivity was observed in confocal microscopy analyses (210).

In contrast to what Kovacs and colleagues had reported, another study that used Beclin 1-deficient $Rag1^{-/-}$ chimeras, found that although Beclin 1 is essential for the maintenance of early lymphocyte progenitor subsets, it is widely expendable for the homeostasis and function of peripheral T and B cells (207, 211). Differential efficiencies of genetic targeting and overall differences in the genetic ablation models may partly account for these discrepancies.

Recently, an additional role for autophagy proteins in the maintenance of $CD8^+$ memory T cells has emerged. Mice that lack Atg7 in T cells showed reduced $CD8^+$ T cell-dependent recall responses upon infection with influenza and MCMV (212). Impairment of $CD8^+$ T cell memory in elderly individuals is commonly observed and in part responsible for the reduced efficacy of vaccination with aging (213). Interestingly, Puleston et al. also found reduced levels of autophagic activity in $CD8^+$ cells of aged mice. Accordingly, upregulation of autophagy with the polyamine spermidine could restore memory $CD8^+$ T cell function to vaccination in aged mice (212). In contrast to what Pua and colleagues had reported previously (206), another study described how $CD8^+$ cells downregulate autophagic activity upon activation and during proliferation. This was followed by increased activity before the contraction phase. Absence of the autophagy proteins Atg5 and Atg7 lead to impaired formation of a $CD8^+$ T cell memory pool. Moreover, metabolomics analysis disclosed mitochondrial fatty acid oxidation to be dysfunctional in Atg7-deficient T cells at the transition to the memory phase (214).

Aside from the role of autophagy in T cell survival and homeostasis, also CMA has been implicated in regulating $CD4^+$ T cell responses. The negative regulators of TCR signaling, RCAN1, and Itch have been identified as substrates for CMA. Thereby, CMA-controlled degradation of these molecules fine-tunes T cell responses which is exemplified by the significant reduction in activation-induced proliferation of $CD4^+$ T cells that lack LAMP-2A expression (215). Although so far, dysfunctional CMA in T cell has not been formally reported to occur in MS or its model systems it is known that mice lacking Itch are prone to develop a hyperactive T cell-driven systemic progressive autoimmune condition (216).

Similar to T cell development, B cells require autophagy both during development and maintenance in the periphery. Atg5-deficient pro-B cells do not efficiently develop into pre-B cells, but instead, seem to die at increased frequencies of apoptosis (217). Altogether, these data suggest that highly proliferative lymphocyte compartments during development or in response to immune activation, as seen in autoimmune CNS conditions, require the autophagic machinery to efficiently mobilize nutrients and maintain cellular fitness. However, further studies are needed to clarify differential roles of autophagy pathways in lymphocyte subsets particularly in the context of T cell-driven autoimmunity.

## AUTOPHAGY AND ANTIGEN PRESENTATION

Similar to other autoimmune diseases, HLA-DR and -DQ alleles within the HLA class II region on chromosome 6p21 are by far the strongest risk-conferring genes and are thought to account for 10–60% of the genetic risk of MS (218). The size of the autoreactive T cell pool is limited by mechanisms of tolerance; however, it is clear that potentially dangerous cells persist in the immune system of healthy individuals without causing damage. Although it is generally accepted that HLA class II molecules influence autoimmune disease risk by regulating the emergence, activation, and expansion of autoreactive $CD4^+$ T lymphocytes, our knowledge of how HLA class II-mediated antigen presentation confers risk for autoimmune diseases and regulates $CD4^+$ T cell autoreactivity at the molecular level is incompletely understood (219, 220).

The cellular architecture of MS lesions is dynamic in its neuropathological features including leukocyte composition (221).

Commonly, T cell infiltration occurs in two distinct waves (222, 223). Initially, MS lesions are characterized by oligodendrocyte damage and commencing demyelination accompanied by microglia that show an activated phenotype. However, in these initial lesions lymphocytes are scarce. Thereupon progressive demyelination occurs, and myelin constituents are phagocytosed by microglia and other myeloid cells which ensues a dramatic T cell influx (224).

Onset of EAE is fully dependent on the presence of myelin-specific $T_H$ cells and local reactivation of these pathogenic culprits is essential in developing CNS autoimmunity (198–202). To this effect, on-site presence and activity of $CD11c^+$ APCs is an instrumental prerequisite for disease induction during the effector phase of EAE (199). Moreover, intravital 2-photon microscopy analyses revealed that during EAE, $CD11c^+$ APCs show a strong proinflammatory phenotype, express a chemokine profile complementary to the chemokine receptor profile expressed on encephalitogenic T cells in the CNS and depict close spatial interactions with these lymphocytes. Hence, depletion of $CD11c^+$ APCs abrogated enrichment of pathogenic T cells in the CNS and significantly alleviated disease course upon adoptive transfer EAE (225). Active $MOG_{35–55}$ immunization of mice that lack expression of Atg7 in the $CD11c^+$ compartment results in ameliorated EAE disease course (226). Therefore, further elucidation of the underlying molecular events that drive these cognate interactions between the triad of (CNS resident) APC, myelin antigen, and encephalitogenic T cell, might unveil new potential therapeutic objectives.

The classical MHC class II pathway, the MA pathway, and LAP are potential degradation pathways resulting in MHC class II presentation of self-antigens. The first compelling evidence for the existence of an endogenous MHC class II pathway came from a study on describing how the endogenous measles virus matrix and nucleocapsid protein-derived antigens could be presented to $CD4^+$ T cells *via* MHC class II (227). Only 1 year later, Nuchtern et al. confirmed these findings for yet another viral antigen by reporting the efficient loading of influenza A matrix protein-derived peptides onto MHC class II (228). Further proof for the existence of endogenous MHC class II loading pathways was obtained through the direct analysis of peptides bound to MHC class II molecules. In fact, around 30% of natural ligands eluted from MHC class II molecules from a multitude of cell types were found to be derived from endogenous protein sources (229). In a next step, MA was newly identified as a pathway that delivers endogenous antigenic constituents into MIICs for subsequent recognition by $CD4^+$ T cells (9, 10, 230). Interestingly, the ability to deliver endogenous antigens to MHC class II seems to be differentially relevant depending on the APC (231). MA is constitutively active in a variety of MHC class II positive APCs such as DCs and B cells (9, 230, 232) and can be induced following immune stimulation of these cells through germline-encoded pattern-recognition receptors such as TLRs or inflammatory cytokines (233–235). Co-localization studies indicate that in professional APCs, autophagosomes frequently fuse with MIICs, and that experimental delivery of antigens to autophagosomes by targeting the autophagosomal membrane through fusion with LC3 results in robust recognition by antigen-specific $CD4^+$ T cells (10, 232). Additionally, not only hematopoietic APCs but also thymic epithelial cells expend MA to generate endogenously derived MHC class II-bound peptides for positive selection of $CD4^+$ T cells (236).

Nevertheless, myelin-derived candidate antigens are not known to be expressed in professional APCs therefore an intracellular loading of these peptides onto MHC class II molecules in the context of CNS autoimmunity appears counterintuitive. LAP couples phagocytosis of extracellular solid cargo to key members of the autophagy machinery (179, 187). A prerequisite for LAP is the engagement of germline-encoded pattern-recognition receptors or Ptd-L-Ser receptors through phagocytosed material. The role of TLR signaling in the context of EAE has partly been investigated. TLR9 and, to some extend, TLR2 signaling mediates the pathogenicity of EAE, whereas TLR1- and 6-deficiency in mice did not have an impact on the development of active EAE (237, 238). Importantly, also adoptive transfer EAE-induction was dependent on TLR2 signaling, discarding the assumption that microbial-derived constituents in the adjuvant preparation during active EAE serve as ligands (238). Additionally, both studies showed that deficiency of the downstream TLR-signaling molecule myeloid differentiation primary response gene 88 (MyD88) abrogates disease (237, 238).

Ample data demonstrate that oligodendrocyte injury and death represented by primary demyelination in absence of inflammatory infiltrates constitute early events in the disease course and are considered specific pathogenic features of MS (239). Sequential MRI analyses revealed that subtle changes in white matter areas can be detected prior to apparent lesions (240). Reduction of macromolecular material and a focal increase of free water could be detected as early as several months before the active lesion occurred. The study's authors concluded that the reduction of magnetization exchange rate was likely to result from primary myelin injury (241). Furthermore, genome-wide epigenetic differences in the DNA methylation status have been reported between normal appearing white matter derived from MS patients and non-diseased control brains (242). The observed hypermethylation and subsequent diminished expression of loci in MS affected brains included genes that control oligodendrocyte survival (*BCL2L2* and *NDRG1*), suggesting that augmented susceptibility to injury precedes inflammatory infiltration. Importantly, a recent study showed that oligodendrocyte death is sufficient to induce encephalitogenic T cell responses and subsequent neuroinflammation *in vivo* (243). It is therefore conceivable that primary death of oligodendrocytes of yet unknown cause leads to subsequent uptake of myelin-derived antigenic debris by CNS-resident APCs and the following mounting of a myelin-specific adaptive response (244). The non-canonical autophagy pathway LAP may facilitate uptake and ensuing presentation of myelin protein-derived antigenic constituents, thus promoting local reactivation of encephalitogenic $T_H$ cells.

Particularly early MS lesions depict enhanced gene expression related to ROS production. The most pronounced changes were found for transcripts encoding subunits of the NADPH oxidase complex 2 (NOX2), i.e., CYBA, CYBB, and NCF1, supporting the concept of oxidative stress as a pivotal pathogenic factor particularly early during the disease. NOX2 complex expression is

detectable in up to 20% of myeloid cells, predominantly localized in areas of initial tissue damage at the edge of actively demyelinating lesions and is associated with the presence of CNS-infiltrating T cells suggesting that NOX2 expression by myeloid cells may contribute to CNS inflammation *via* recruitment or expansion of T cells within MS lesions (245). NOX2 is expressed in macrophages, DCs, microglia, neutrophils, and eosinophils (246). In resting conditions, the enzyme complex is highly glycosylated and resides in intracellular secondary granules and, at lower amounts, in the plasma membrane. After a triggering event, its subunits gp91 and p22 are translocated to the plasma membrane at the site of the forming phagosome, and the cytosolic subunits (p40, p47, p67) are recruited to assemble the active NOX2 complex (247). NOX2 function, generation of ROS, has initially been linked to killing of intraphagosomal pathogens by providing the adequate environment for protease function. In professional APCs, whose principal function is to take up, process and present antigens in order to initiate and shape adaptive immune responses rather than pathogen killing or clearance of cell debris, NOX2 expression was shown to regulate antigen presentation due to its ability to control the level of antigen degradation by altering phagosomal pH levels (248). Indeed, DCs lacking NOX2 show enhanced phagosomal acidification and increased antigen degradation, resulting in impaired cross presentation (248). In addition to cell type-dependent tuning of phagosomal acidification, NOX2 has recently also been implicated in regulating serine and cysteine proteases (cathepsins B, L, and S) within phagosomes (249) and it has been suggested that increased hydrolysis of critical regions within the encephalitogenic $MOG_{35–55}$ antigen by cysteine cathepsins in the early phagosome contribute to the reduced incidence and delayed onset of EAE reported in NOX2-deficient mice (250). It is conceivable that NOX2 as essential component of the non-canonical autophagy pathway LAP not only affects levels of phagosomal proteolysis as previously shown, but facilitates the induction of an alternative pathway for antigen loading onto MHC class II molecules, thereby contributing to $CD4^+$ T cell-mediated augmentation of autoimmune CNS inflammation.

Altogether, the aforementioned studies suggest that the autophagic machinery could potentially contribute to the initiation and maintenance of $CD4^+$ T cell-driven CNS tissue injury through delivery of $CD4^+$ T cell antigens into MIICs.

## OUTLOOK: AUTOPHAGY PATHWAYS IN CNS AUTOIMMUNITY BEYOND ANTIGEN PRESENTATION AND LYMPHOCYTE SURVIVAL

There is a profound body of evidence showing that mitochondrial dysfunction is a commonly observed feature in active MS plaques (194, 251–253). In lesions of patients suffering from acute MS, immunohistochemical analyses revealed mitochondrial defects selectively in respiratory chain complex IV subunit COX-I. Mitochondrial damage was observed in oligodendrocytes and axons, but was not present in macrophages or microglia (251). Mitochondria, while being a primary source thereof, are at the same time particularly susceptible to free radical-mediated damage and respiratory chain complex IV subunit COX-I expression is in part regulated by posttranslational modification of mRNA by nitric oxide (NO) (254–256). It has been suggested that myeloid cell-derived secretion of active mediators including NO in close proximity to oligodendrocytes mediates selective COX-I degradation with subsequent mitochondria impairment (221, 257–260). NMDA-receptor signaling has been shown to induce NADPH oxidase (NOX) activity in oligodendrocyte precursor cells and the subsequent production of ROS can drive both further oligodendrocyte differentiation and myelination (261, 262).

Malregulated oligodendrocyte-intrinsic events may also contribute to ROS-mediated mitochondrial damage in oligodendrocytes during MS. Mitochondrial homeostasis (mitostasis) and quality control is ensured by consecutive cycles of fusion and fission tightly coupled to mitophagy-mediated removal of aberrant organelles (263, 264) and, complex IV deficiency as well as concomitant cellular energy deficit can in part be compensated for by mitochondrial hyperfusion (265). It is therefore conceivable that malfunctioning of the autophagic axis within this regulatory network leads to disturbance of compensatory mechanisms during free radical induced COX-I deficiency (194, 263). Interestingly, as opposed to the observed functional and morphological impairment of mitochondria in highly active lesions, older and inactive lesions depict enhanced mitochondrial count and activity (266, 267).

In comparison to other cell types, neurons depict an unique geometry and organelles such as mitochondria are also needed in distal axonal regions afar from the neuronal soma where mitophagy-mediated lysosomal degradation takes place (268, 269). Duly distribution of mitochondria within axons is critical for neuronal physiology and the constant turnover of dysfunctional specimens in these post-mitotic cells requires well-regulated motor-protein-driven trafficking and anchoring of mitochondria alongside microtubules (269). Temporary increase in mitochondria number, size, and activity as well as subcellular re-location is believed to constitute an axonoprotective mechanism in the light of an elevated energy consumption during chronic axonal injury (270). This compensatory mitochondrial response relies on anchoring molecule syntaphilin, which mediates docking of mitochondria through interaction with microtubules since knockout of syntaphilin results in exacerbated degeneration of demyelinated neurons (270). Syntaphilin-anchored mitochondria have been reported to also recruit Parkin for subsequent mitophagy (268).

In addition to COX-I deficiency observed in acute MS lesions of the white matter, progressive disease also features mitochondrial alterations in deeper cortical areas reminiscent of prototypic neurodegenerative disorders with autophagy-associated pathoetiology such as Alzheimer's disease (AD) and Parkinson's disease (271, 272). Patients suffering from SPMS show significantly higher levels of clonally expanded mtDNA deletions in neurons of layer VI as compared to age-matched controls (272). Increased clonally expanded mtDNA deletions were also observed in layer VI adjacent subcortical white matter which suggests that in both instances soluble factors such as inflammation-associated free radicals in active lesions mediate mitochondrial degeneration. Although this needs to be clearly addressed experimentally, the

possibility has been raised that accumulations of mtDNA deletions during MS might also yield mutations that could compromise autophagy-mediated quality control of mitochondria (194).

In the process of ATP generation by mitochondrial oxidative phosphorylation, ROS arise that in order to maintain cellular integrity and to prevent oxidative stress, need to be eliminated by means of several antioxidants such as NADPH, cytochrome *c*, and most importantly glutathione (273). However, aside from their detrimental role when produced in large quantities, if not exceedingly present, ROS, occupy also important signaling functions within the cell, interact with redox-sensitive proteins, and work toward maintaining cellular homeostasis in response to cell stress (274). Also Atgs have been described to be sensitive to ROS-mediated posttranslational modification. For example, Atg4 is oxidized and thereby inactivated by $H_2O_2$, which subsequently facilitates conjugation of LC3 to membranes (275). Furthermore, redox-sensitive nuclear transcription factor and histone deacetylase sirtuin 1 (SIRT1), which is highly expressed in neurons, promotes autophagic activity directly by complexing and deacetylation of essential autophagy-machinery proteins and indirectly by deacetylation of FoxO members which in turn leads to promotion of autophagy (274, 276).

Portending oxidative damage, strong immunoreactivity for oxidized phospholipids in neurons of active cortical MS lesions has been described to be specific to MS and was not observed to the same degree in tuberculous meningitis, AD, and age-matched controls (239). In accordance with these observations, antioxidant enzymes such as superoxide dismutase 1 and 2, heme oxigenase 1, and catalase have been shown to be increased in active MS lesions. Interestingly, this upregulation of antioxidant defense molecules was particularly present in astrocytes and myelin-containing macrophages but not in oligodendrocytes (277). It has been suggested before that the extreme susceptibility of oligodendrocytes to ROS-mediated damage is in part due to insufficient compensatory expression of protective enzymes (277, 278).

Increased mitochondrial-derived ROS accumulation and concomitant oxidative damage is believed to constitute a key feature of cellular aging and this process appears to be accelerated and augmented during MS particularly in oligodendrocytes and neurons (239, 279, 280). In line with this, treatment of aged mice with mTORC1 inhibitor and MA inducer rapamycin increased longevity in mice (281). It is likely that at least in part this effect was mediated by the compound's capability to bridle injurious mitochondrial ROS production and mtDNA accumulation per induction of mitophagy (282).

Whole organelles such as mitochondria are not targeted for lysosomal degradation *via* LAMP-2A-dependent CMA. However, during cell stress, CMA does participate indirectly in mitochondrial quality control in neurons by removing non-functional PARK7, a redox-sensitive glycase with antioxidative properties (283).

In order to target autophagy pathways for therapeutic purposes in MS it will be crucial to fully elucidate the degree to which individual pathways (e.g., MI, MA, CMA, and LAP) contribute to pathology. This will be challenging since impairment of one pathway may lead to both, compensatory responses and concomitant attenuation in the remaining pathways (145). To this end, robust methods need to be established in order reliably distinguish and monitor discrete autophagy pathways. Taken together, these data suggest that regenerative and protective processes in oligodendrocytes and demyelinated axons are highly reliant on meticulously functioning mitochondrial quality control, during which autophagic pathways play a pivotal role (194, 269). Future research will show whether the autophagic machinery, in addition to its function in regulating innate and adaptive immune responses, interferes with neurobiological features of MS such as axonal degeneration.

## AUTHOR CONTRIBUTIONS

Both authors have made substantial, intellectual, and equally valuable contribution to the work and approved it for publication.

## ACKNOWLEDGMENTS

The authors thank Christian Münz (University of Zürich, Switzerland) for critically reviewing this manuscript.

## FUNDING

CWK was supported by a Forschungskredit doctoral fellowship (FK-14-021) provided by the University of Zürich. JDL was supported by the Swiss National Foundation (31003A-169664), the Novartis Foundation for medical-biological research, the Sassella Foundation, the Hartmann Müller Foundation, and the Swiss Multiple Sclerosis Society. The authors declare no conflict of interests.

## REFERENCES

1. Deter RL, Baudhuin P, De Duve C. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol* (1967) 35:C11–6.
2. Deter RL, De Duve C. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J Cell Biol* (1967) 33:437–49.
3. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* (2011) 147:728–41. doi:10.1016/j.cell.2011.10.026
4. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* (2006) 441:880–4. doi:10.1038/nature04723
5. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* (2006) 441:885–9. doi:10.1038/nature04724
6. Denton D, Nicolson S, Kumar S. Cell death by autophagy: facts and apparent artefacts. *Cell Death Differ* (2012) 19:87–95. doi:10.1038/cdd.2011.146
7. Thurston TLM, Wandel MP, von Muhlinen N, Foeglein Á, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* (2012) 482:414–8. doi:10.1038/nature10744
8. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. *Nature* (2008) 456:264–8. doi:10.1038/nature07383
9. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, et al. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* (2005) 307:593–6. doi:10.1126/science.1104904

10. Schmid D, Pypaert M, Münz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* (2007) 26:79–92. doi:10.1016/j.immuni.2006.10.018
11. Loi M, Müller A, Steinbach K, Niven J, Barreira da Silva R, Paul P, et al. Macroautophagy proteins control MHC class I levels on dendritic cells and shape anti-viral CD8(+) T cell responses. *Cell Rep* (2016) 15:1076–87. doi:10.1016/j.celrep.2016.04.002
12. Romao S, Gasser N, Becker AC, Guhl B, Bajagic M, Vanoaica D, et al. Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. *J Cell Biol* (2013) 203:757–66. doi:10.1083/jcb.201308173
13. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* (2000) 192:393–404. doi:10.1084/jem.192.3.393
14. Jacobsen M, Cepok S, Quak E, Happel M, Gaber R, Ziegler A, et al. Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients. *Brain* (2002) 125:538–50. doi:10.1093/brain/awf059
15. von Büdingen HC, Kuo TC, Sirota M, van Belle CJ, Apeltsin L, Glanville J, et al. B cell exchange across the blood-brain barrier in multiple sclerosis. *J Clin Invest* (2012) 122:4533–43. doi:10.1172/JCI63842
16. Codogno P, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat Rev Mol Cell Biol* (2011) 13:7–12. doi:10.1038/nrm3249
17. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. *Cell Struct Funct* (2002) 27:421–9. doi:10.1247/csf.27.421
18. Deretic V. Autophagy in leukocytes and other cells: mechanisms, subsystem organization, selectivity, and links to innate immunity. *J Leukoc Biol* (2016) 100:969–78. doi:10.1189/jlb.4MR0216-079R
19. Feng Y, He D, Yao Z, Klionsky DJ. The machinery of macroautophagy. *Cell Res* (2014) 24:24–41. doi:10.1038/cr.2013.168
20. Tsukada M, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* (1993) 333:169–74.
21. Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* (2011) 27:107–32. doi:10.1146/annurev-cellbio-092910-154005
22. Randow F, Youle RJ. Self and nonself: how autophagy targets mitochondria and bacteria. *Cell Host Microbe* (2014) 15:403–11. doi:10.1016/j.chom.2014.03.012
23. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* (2011) 469:323–35. doi:10.1038/nature09782
24. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. *Nature* (2009) 458:1131–5. doi:10.1038/nature07976
25. Klionsky DJ, Cuervo AM, Dunn WA Jr, Levine B, van der Klei I, Seglen PO. How shall I eat thee? *Autophagy* (2007) 3:413–6. doi:10.4161/auto.4377
26. Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, et al. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J Cell Biol* (2001) 152:657–68. doi:10.1083/jcb.152.4.657
27. Suzuki H, Osawa T, Fujioka Y, Noda NN. Structural biology of the core autophagy machinery. *Curr Opin Struct Biol* (2016) 43:10–7. doi:10.1016/j.sbi.2016.09.010
28. Schworer CM, Shiffer KA, Mortimore GE. Quantitative relationship between autophagy and proteolysis during graded amino acid deprivation in perfused rat liver. *J Biol Chem* (1981) 256:7652–8.
29. Pfeifer U. Inhibition by insulin of the formation of autophagic vacuoles in rat liver. A morphometric approach to the kinetics of intracellular degradation by autophagy. *J Cell Biol* (1978) 78:152–67.
30. Shibutani ST, Yoshimori T. A current perspective of autophagosome biogenesis. *Cell Res* (2014) 24:58–68. doi:10.1038/cr.2013.159
31. Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* (2010) 6:764–76. doi:10.4161/auto.6.6.12709
32. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* (2008) 182:685–701. doi:10.1083/jcb.200803137
33. Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen E-L. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* (2009) 5:1180–5. doi:10.4161/auto.5.8.10274
34. Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* (2009) 11:1433–7. doi:10.1038/ncb1991
35. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, et al. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* (2010) 141:656–67. doi:10.1016/j.cell.2010.04.009
36. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, et al. Autophagosomes form at ER-mitochondria contact sites. *Nature* (2013) 495:389–93. doi:10.1038/nature11910
37. Puri C, Renna M, Bento CF, Moreau K, Rubinsztein DC. Diverse autophagosome membrane sources coalesce in recycling endosomes. *Cell* (2013) 154:1285–99. doi:10.1016/j.cell.2013.08.044
38. Guo Y, Chang C, Huang R, Liu B, Bao L, Liu W. AP1 is essential for generation of autophagosomes from the trans-Golgi network. *J Cell Sci* (2012) 125:1706–15. doi:10.1242/jcs.093203
39. Zoppino FCM, Militello RD, Slavin I, Alvarez C, Colombo MI. Autophagosome formation depends on the small GTPase Rab1 and functional ER exit sites. *Traffic* (2010) 11:1246–61. doi:10.1111/j.1600-0854.2010.01086.x
40. Ge L, Melville D, Zhang M, Schekman R. The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. *Elife* (2013) 2:e00947. doi:10.7554/eLife.00947
41. Ge L, Zhang M, Schekman R. Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER-Golgi intermediate compartment. *Elife* (2014) 3:e04135. doi:10.7554/eLife.04135
42. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* (2010) 12:747–57. doi:10.1038/ncb2078
43. Mari M, Griffith J, Rieter E, Krishnappa L, Klionsky DJ, Reggiori F. An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. *J Cell Biol* (2010) 190:1005–22. doi:10.1083/jcb.200912089
44. Kim J, Kundu M, Viollet B, Guan K-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* (2011) 13:132–41. doi:10.1038/ncb2152
45. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* (2011) 331:456–61. doi:10.1126/science.1196371
46. Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* (2009) 284:12297–305. doi:10.1074/jbc.M900573200
47. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* (2009) 20:1981–91. doi:10.1091/mbc.E08-12-1248
48. Hara T, Mizushima N. Role of ULK-FIP200 complex in mammalian autophagy: FIP200, a counterpart of yeast Atg17? *Autophagy* (2009) 5:85–7. doi:10.4161/auto.5.1.7180
49. Karanasios E, Walker SA, Okkenhaug H, Manifava M, Hummel E, Zimmermann H, et al. Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. *Nat Commun* (2016) 7:12420. doi:10.1038/ncomms12420
50. Chang Y-Y, Neufeld TP. An Atg1/Atg13 complex with multiple roles in TOR-mediated autophagy regulation. *Mol Biol Cell* (2009) 20:2004–14. doi:10.1091/mbc.E08-12-1250
51. Chan EYW, Kir S, Tooze SA. siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J Biol Chem* (2007) 282:25464–74. doi:10.1074/jbc.M703663200
52. Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, et al. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J Cell Biol* (2008) 181:497–510. doi:10.1083/jcb.200712064
53. Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* (2009) 5:973–9. doi:10.4161/auto.5.7.9296
54. Mercer CA, Kaliappan A, Dennis PB. A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy* (2009) 5:649–62. doi:10.4161/auto.5.5.8249

55. Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* (2008) 19:5360–72. doi:10.1091/mbc.E08-01-0080
56. Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* (2008) 105:19211–6. doi:10.1073/pnas.0810452105
57. Araki Y, Ku WC, Akioka M, May AI, Hayashi Y, Arisaka F, et al. Atg38 is required for autophagy-specific phosphatidylinositol 3-kinase complex integrity. *J Cell Biol* (2013) 203:299–313. doi:10.1083/jcb.201304123
58. Young LN, Cho K, Lawrence R, Zoncu R, Hurley JH. Dynamics and architecture of the NRBF2-containing phosphatidylinositol 3-kinase complex I of autophagy. *Proc Natl Acad Sci U S A* (2016) 113:8224–9. doi:10.1073/pnas.1603650113
59. Ohashi Y, Soler N, García Ortegón M, Zhang L, Kirsten ML, Perisic O, et al. Characterization of Atg38 and NRBF2, a fifth subunit of the autophagic Vps34/PIK3C3 complex. *Autophagy* (2016) 12:2129–44. doi:10.1080/15548627.2016.1226736
60. Matsunaga K, Morita E, Saitoh T, Akira S, Ktistakis NT, Izumi T, et al. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J Cell Biol* (2010) 190:511–21. doi:10.1083/jcb.200911141
61. Zalckvar E, Berissi H, Eisenstein M, Kimchi A. Phosphorylation of Beclin 1 by DAP-kinase promotes autophagy by weakening its interactions with Bcl-2 and Bcl-XL. *Autophagy* (2009) 5:720–2. doi:10.4161/auto.5.5.8625
62. Obara K, Sekito T, Niimi K, Ohsumi Y. The Atg18-Atg2 complex is recruited to autophagic membranes via phosphatidylinositol 3-phosphate and exerts an essential function. *J Biol Chem* (2008) 283:23972–80. doi:10.1074/jbc.M803180200
63. Polson HE, de Lartigue J, Rigden DJ, Reedijk M, Urbé S, Clague MJ, et al. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* (2010) 6:506–22. doi:10.4161/auto.6.4.11863
64. Velikkakath AKG, Nishimura T, Oita E, Ishihara N, Mizushima N. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Mol Biol Cell* (2012) 23:896–909. doi:10.1091/mbc.E11-09-0785
65. Rieter E, Vinke F, Bakula D, Cebollero E, Ungermann C, Proikas-Cezanne T, et al. Atg18 function in autophagy is regulated by specific sites within its β-propeller. *J Cell Sci* (2013) 126:593–604. doi:10.1242/jcs.115725
66. Nair U, Cao Y, Xie Z, Klionsky DJ. Roles of the lipid-binding motifs of Atg18 and Atg21 in the cytoplasm to vacuole targeting pathway and autophagy. *J Biol Chem* (2010) 285:11476–88. doi:10.1074/jbc.M109.080374
67. Young AR, Chan EY, Hu XW, Köchl R, Crawshaw SG, High S, et al. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J Cell Sci* (2006) 119:3888–900. doi:10.1242/jcs.03172
68. Reggiori F, Tucker KA, Stromhaug PE, Klionsky DJ. The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. *Dev Cell* (2004) 6:79–90. doi:10.1016/S1534-5807(03)00402-7
69. Orsi A, Razi M, Dooley HC, Robinson D, Weston AE, Collinson LM, et al. Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, are required for autophagy. *Mol Biol Cell* (2012) 23:1860–73. doi:10.1091/mbc.E11-09-0746
70. Geng J, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series. *EMBO Rep* (2008) 9:859–64. doi:10.1038/embor.2008.163
71. Dooley HC, Razi M, Polson HE, Girardin SE, Wilson MI, Tooze SA. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. *Mol Cell* (2014) 55:238–52. doi:10.1016/j.molcel.2014.05.021
72. Gammoh N, Florey O, Overholtzer M, Jiang X. Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex-dependent and -independent autophagy. *Nat Struct Mol Biol* (2013) 20:144–9. doi:10.1038/nsmb.2475
73. Nishimura T, Kaizuka T, Cadwell K, Sahani MH, Saitoh T, Akira S, et al. FIP200 regulates targeting of Atg16L1 to the isolation membrane. *EMBO Rep* (2013) 14:284–91. doi:10.1038/embor.2013.6
74. Hemelaar J, Lelyveld VS, Kessler BM, Ploegh HL. A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE-16, MAP1-LC3, GABARAP, and Apg8L. *J Biol Chem* (2003) 278:51841–50. doi:10.1074/jbc.M308762200
75. Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci* (2004) 117:2805–12. doi:10.1242/jcs.01131
76. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* (2000) 19:5720–8. doi:10.1093/emboj/19.21.5720
77. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, et al. A ubiquitin-like system mediates protein lipidation. *Nature* (2000) 408:488–92. doi:10.1038/35044114
78. Tanida I, Sou YS, Ezaki J, Minematsu-Ikeguchi N, Ueno T, Kominami E. HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and GABAA receptor-associated protein-phospholipid conjugates. *J Biol Chem* (2004) 279:36268–76. doi:10.1074/jbc.M401461200
79. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell* (2008) 19:2092–100. doi:10.1091/mbc.E07-12-1257
80. Kimura S, Noda T, Yoshimori T. Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy* (2007) 3:452–60. doi:10.4161/auto.4451
81. Nakatogawa H, Ichimura Y, Ohsumi Y. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* (2007) 130:165–78. doi:10.1016/j.cell.2007.05.021
82. Fujita N, Hayashi-Nishino M, Fukumoto H, Omori H, Yamamoto A, Noda T, et al. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol Biol Cell* (2008) 19:4651–9. doi:10.1091/mbc.E08-03-0312
83. Weidberg H, Shvets E, Shpilka T, Shimron F, Shinder V, Elazar Z. LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. *EMBO J* (2010) 29:1792–802. doi:10.1038/emboj.2010.74
84. Sou YS, Waguri S, Iwata J, Ueno T, Fujimura T, Hara T, et al. The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. *Mol Biol Cell* (2008) 19:4762–75. doi:10.1091/mbc.E08-03-0309
85. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* (2016) 12:1–222. doi:10.1080/15548627.2015.1100356
86. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. *Autophagy* (2011) 7:279–96. doi:10.4161/auto.7.3.14487
87. Mizushima N, Klionsky DJ. Protein turnover via autophagy: implications for metabolism. *Annu Rev Nutr* (2007) 27:19–40. doi:10.1146/annurev.nutr.27.061406.093749
88. Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. *Nature* (2015) 517:302–10. doi:10.1038/nature14190
89. Füllgrabe J, Klionsky DJ, Joseph B. The return of the nucleus: transcriptional and epigenetic control of autophagy. *Nat Rev Mol Cell Biol* (2014) 15:65–74. doi:10.1038/nrm3716
90. Lapierre LR, Kumsta C, Sandri M, Ballabio A, Hansen M. Transcriptional and epigenetic regulation of autophagy in aging. *Autophagy* (2015) 11:867–80. doi:10.1080/15548627.2015.1034410
91. Füllgrabe J, Ghislat G, Cho D-H, Rubinsztein DC. Transcriptional regulation of mammalian autophagy at a glance. *J Cell Sci* (2016) 129:3059–66. doi:10.1242/jcs.188920
92. Arias E, Koga H, Diaz A, Mocholi E, Patel B, Cuervo AM. Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. *Mol Cell* (2015) 59:270–84. doi:10.1016/j.molcel.2015.05.030
93. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* (2006) 124:471–84. doi:10.1016/j.cell.2006.01.016
94. Puente C, Hendrickson RC, Jiang X. Nutrient-regulated phosphorylation of ATG13 inhibits starvation-induced autophagy. *J Biol Chem* (2016) 291:6026–35. doi:10.1074/jbc.M115.689646
95. Long X, Ortiz-Vega S, Lin Y, Avruch J. Rheb binding to mammalian target of rapamycin (mTOR) is regulated by amino acid sufficiency. *J Biol Chem* (2005) 280:23433–6. doi:10.1074/jbc.C500169200

96. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb binds and regulates the mTOR kinase. *Curr Biol* (2005) 15:702–13. doi:10.1016/j.cub.2005.02.053
97. Avruch J, Lin Y, Long X, Murthy S, Ortiz-Vega S. Recent advances in the regulation of the TOR pathway by insulin and nutrients. *Curr Opin Clin Nutr Metab Care* (2005) 8:67–72. doi:10.1097/00075197-200501000-00010
98. Vander Haar E, Lee S-I, Bandhakavi S, Griffin TJ, Kim D-H. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* (2007) 9:316–23. doi:10.1038/ncb1547
99. Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, et al. Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. *Science* (2012) 338:956–9. doi:10.1126/science.1225967
100. Guo S, Sonenshein GE. Forkhead box transcription factor FOXO3a regulates estrogen receptor alpha expression and is repressed by the Her-2/neu/phosphatidylinositol 3-kinase/Akt signaling pathway. *Mol Cell Biol* (2004) 24:8681–90. doi:10.1128/MCB.24.19.8681-8690.2004
101. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell* (1999) 96:857–68.
102. Das TP, Suman S, Alatassi H, Ankem MK, Damodaran C. Inhibition of AKT promotes FOXO3a-dependent apoptosis in prostate cancer. *Cell Death Dis* (2016) 7:e2111. doi:10.1038/cddis.2015.403
103. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* (2010) 141:290–303. doi:10.1016/j.cell.2010.02.024
104. Betz C, Hall MN. Where is mTOR and what is it doing there? *J Cell Biol* (2013) 203:563–74. doi:10.1083/jcb.201306041
105. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* (2007) 8:774–85. doi:10.1038/nrm2249
106. Meley D, Bauvy C, Houben-Weerts JH, Dubbelhuis PF, Helmond MT, Codogno P, et al. AMP-activated protein kinase and the regulation of autophagic proteolysis. *J Biol Chem* (2006) 281:34870–9. doi:10.1074/jbc.M605488200
107. Høyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Mol Cell* (2007) 25:193–205. doi:10.1016/j.molcel.2006.12.009
108. Høyer-Hansen M, Jäättelä M. AMP-activated protein kinase: a universal regulator of autophagy? *Autophagy* (2007) 3:381–3.
109. Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A* (2004) 101:3329–35. doi:10.1073/pnas.0308061100
110. Hong S-P, Leiper FC, Woods A, Carling D, Carlson M. Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc Natl Acad Sci U S A* (2003) 100:8839–43. doi:10.1073/pnas.1533136100
111. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, et al. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* (2003) 13:2004–8. doi:10.1016/j.cub.2003.10.031
112. Herrero-Martín G, Høyer-Hansen M, García-García C, Fumarola C, Farkas T, López-Rivas A, et al. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. *EMBO J* (2009) 28:677–85. doi:10.1038/emboj.2009.8
113. Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, et al. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab* (2005) 2:9–19. doi:10.1016/j.cmet.2005.05.009
114. Inoki K, Zhu T, Guan K-L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* (2003) 115:577–90. doi:10.1016/S0092-8674(03)00929-2
115. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* (2008) 30:214–26. doi:10.1016/j.molcel.2008.03.003
116. Sanchez AM, Csibi A, Raibon A, Cornille K, Gay S, Bernardi H, et al. AMPK promotes skeletal muscle autophagy through activation of forkhead FoxO3a and interaction with Ulk1. *J Cell Biochem* (2012) 113:695–710. doi:10.1002/jcb.23399
117. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* (2007) 6:458–71. doi:10.1016/j.cmet.2007.11.001
118. Li W-W, Li J, Bao J-K. Microautophagy: lesser-known self-eating. *Cell Mol Life Sci* (2012) 69:1125–36. doi:10.1007/s00018-011-0865-5
119. Farré J-C, Krick R, Subramani S, Thumm M. Turnover of organelles by autophagy in yeast. *Curr Opin Cell Biol* (2009) 21:522–30. doi:10.1016/j.ceb.2009.04.015
120. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, et al. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell* (2011) 20:131–9. doi:10.1016/j.devcel.2010.12.003
121. Marzella L, Ahlberg J, Glaumann H. Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. *Virchows Arch B Cell Pathol Incl Mol Pathol* (1981) 36:219–34.
122. Santambrogio L, Cuervo AM. Chasing the elusive mammalian microautophagy. *Autophagy* (2011) 7:652–4. doi:10.4161/auto.7.6.15287
123. Kawamura N, Sun-Wada GH, Aoyama M, Harada A, Takasuga S, Sasaki T, et al. Delivery of endosomes to lysosomes via microautophagy in the visceral endoderm of mouse embryos. *Nat Commun* (2012) 3:1071. doi:10.1038/ncomms2069
124. Vevea JD, Garcia EJ, Chan RB, Zhou B, Schultz M, Di Paolo G, et al. Role for lipid droplet biogenesis and microlipophagy in adaptation to lipid imbalance in yeast. *Dev Cell* (2015) 35:584–99. doi:10.1016/j.devcel.2015.11.010
125. Schuck S, Gallagher CM, Walter P. ER-phagy mediates selective degradation of endoplasmic reticulum independently of the core autophagy machinery. *J Cell Sci* (2014) 127:4078–88. doi:10.1242/jcs.154716
126. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, Martinou JC. Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death Differ* (2005) 12:1613–21. doi:10.1038/sj.cdd.4401697
127. Bhatia-Kiššová I, Camougrand N. Mitophagy in yeast: actors and physiological roles. *FEMS Yeast Res* (2010) 10:1023–34. doi:10.1111/j.1567-1364.2010.00659.x
128. Manjithaya R, Nazarko TY, Farré J-C, Subramani S. Molecular mechanism and physiological role of pexophagy. *FEBS Lett* (2010) 584:1367–73. doi:10.1016/j.febslet.2010.01.019
129. Dunn WA Jr, Cregg JM, Kiel JA, van der Klei IJ, Oku M, Sakai Y, et al. Pexophagy: the selective autophagy of peroxisomes. *Autophagy* (2005) 1:75–83. doi:10.4161/auto.1.2.1737
130. Roberts P, Moshitch-Moshkovitz S, Kvam E, O'Toole E, Winey M, Goldfarb DS. Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae*. *Mol Biol Cell* (2003) 14:129–41. doi:10.1091/mbc.E02-08-0483
131. Krick R, Mühe Y, Prick T, Bredschneider M, Bremer S, Wenzel D, et al. Piecemeal microautophagy of the nucleus: genetic and morphological traits. *Autophagy* (2009) 5:270–2. doi:10.4161/auto.5.2.7639
132. Krick R, Muehe Y, Prick T, Bremer S, Schlotterhose P, Eskelinen EL, et al. Piecemeal microautophagy of the nucleus requires the core macroautophagy genes. *Mol Biol Cell* (2008) 19:4492–505. doi:10.1091/mbc.E08-04-0363
133. Mijaljica D, Prescott M, Devenish RJ. Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy* (2011) 7:673–82. doi:10.4161/auto.7.7.14733
134. Shpilka T, Elazar Z. Shedding light on mammalian microautophagy. *Dev Cell* (2011) 20:1–2. doi:10.1016/j.devcel.2010.12.010
135. Morozova K, Clement CC, Kaushik S, Stiller B, Arias E, Ahmad A, et al. Structural and biological interaction of hsc-70 protein with phosphatidylserine in endosomal microautophagy. *J Biol Chem* (2016) 291:18096–106. doi:10.1074/jbc.M116.736744
136. Liu XM, Sun LL, Hu W, Ding YH, Dong MQ, Du LL. ESCRTs cooperate with a selective autophagy receptor to mediate vacuolar targeting of soluble cargos. *Mol Cell* (2015) 59:1035–42. doi:10.1016/j.molcel.2015.07.034
137. Mizushima N. Nbr1, a receptor for ESCRT-dependent endosomal microautophagy in fission yeast. *Mol Cell* (2015) 59:887–9. doi:10.1016/j.molcel.2015.09.004
138. Mukherjee A, Patel B, Koga H, Cuervo AM, Jenny A. Selective endosomal microautophagy is starvation-inducible in *Drosophila*. *Autophagy* (2016) 12:1984–99. doi:10.1080/15548627.2016.1208887
139. Uytterhoeven V, Lauwers E, Maes I, Miskiewicz K, Melo MN, Swerts J, et al. Hsc70-4 deforms membranes to promote synaptic protein turnover

by endosomal microautophagy. *Neuron* (2015) 88:735–48. doi:10.1016/j.neuron.2015.10.012
140. Howitt J, Hill AF. Exosomes in the pathology of neurodegenerative diseases. *J Biol Chem* (2016) 291:26589–97. doi:10.1074/jbc.R116.757955
141. Baixauli F, López-Otín C, Mittelbrunn M. Exosomes and autophagy: coordinated mechanisms for the maintenance of cellular fitness. *Front Immunol* (2014) 5:403. doi:10.3389/fimmu.2014.00403
142. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* (2014) 14:195–208. doi:10.1038/nri3622
143. Brettschneider J, Del Tredici K, Lee VM-Y, Trojanowski JQ. Spreading of pathology in neurodegenerative diseases: a focus on human studies. *Nat Rev Neurosci* (2015) 16:109–20. doi:10.1038/nrn3887
144. Tasset I, Cuervo AM. Role of chaperone-mediated autophagy in metabolism. *FEBS J* (2016) 283:2403–13. doi:10.1111/febs.13677
145. Kaushik S, Cuervo AM. Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol* (2012) 22:407–17. doi:10.1016/j.tcb.2012.05.006
146. Kiffin R, Christian C, Knecht E, Cuervo AM. Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell* (2004) 15:4829–40. doi:10.1091/mbc.E04-06-0477
147. Hubbi ME, Hu H, Kshitiz, Ahmed I, Levchenko A, Semenza GL. Chaperone-mediated autophagy targets hypoxia-inducible factor-1α (HIF-1α) for lysosomal degradation. *J Biol Chem* (2013) 288:10703–14. doi:10.1074/jbc.M112.414771
148. Park C, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. *Nat Commun* (2015) 6:6823. doi:10.1038/ncomms7823
149. Cuervo AM, Knecht E, Terlecky SR, Dice JF. Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. *Am J Physiol* (1995) 269:C1200–8.
150. Dice JF. Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends Biochem Sci* (1990) 15:305–9.
151. Chiang HL, Terlecky SR, Plant CP, Dice JF. A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* (1989) 246:382–5.
152. Cuervo AM, Dice JF. A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* (1996) 273:501–3.
153. Salvador N, Aguado C, Horst M, Knecht E. Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. *J Biol Chem* (2000) 275:27447–56. doi:10.1074/jbc.M001394200
154. Bandyopadhyay U, Kaushik S, Varticovski L, Cuervo AM. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. *Mol Cell Biol* (2008) 28:5747–63. doi:10.1128/MCB.02070-07
155. Bandyopadhyay U, Sridhar S, Kaushik S, Kiffin R, Cuervo AM. Identification of regulators of chaperone-mediated autophagy. *Mol Cell* (2010) 39:535–47. doi:10.1016/j.molcel.2010.08.004
156. Bandyopadhyay U, Cuervo AM. Chaperone-mediated autophagy in aging and neurodegeneration: lessons from alpha-synuclein. *Exp Gerontol* (2007) 42:120–8. doi:10.1016/j.exger.2006.05.019
157. Massey A, Kiffin R, Cuervo AM. Pathophysiology of chaperone-mediated autophagy. *Int J Biochem Cell Biol* (2004) 36:2420–34. doi:10.1016/j.biocel.2004.04.010
158. Kaushik S, Massey AC, Cuervo AM. Lysosome membrane lipid microdomains: novel regulators of chaperone-mediated autophagy. *EMBO J* (2006) 25:3921–33. doi:10.1038/sj.emboj.7601283
159. Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates huntingtin and targets it for degradation by the proteasome and lysosome. *J Cell Biol* (2009) 187:1083–99. doi:10.1083/jcb.200909067
160. Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. *Mol Cell* (2011) 42:719–30. doi:10.1016/j.molcel.2011.04.025
161. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* (2004) 305:1292–5. doi:10.1126/science.1101738
162. Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J Clin Invest* (2008) 118:777–88. doi:10.1172/JCI32806
163. Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, et al. Interplay of LRRK2 with chaperone-mediated autophagy. *Nat Neurosci* (2013) 16:394–406. doi:10.1038/nn.3350
164. Fass E, Shvets E, Degani I, Hirschberg K, Elazar Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. *J Biol Chem* (2006) 281:36303–16. doi:10.1074/jbc.M607031200
165. Köchl R, Hu XW, Chan EYW, Tooze SA. Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. *Traffic* (2006) 7:129–45. doi:10.1111/j.1600-0854.2005.00368.x
166. Kimura S, Noda T, Yoshimori T. Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct Funct* (2008) 33:109–22. doi:10.1247/csf.08005
167. Maday S, Wallace KE, Holzbaur ELF. Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. *J Cell Biol* (2012) 196:407–17. doi:10.1083/jcb.201106120
168. Ravikumar B, Acevedo-Arozena A, Imarisio S, Berger Z, Vacher C, O'Kane CJ, et al. Dynein mutations impair autophagic clearance of aggregate-prone proteins. *Nat Genet* (2005) 37:771–6. doi:10.1038/ng1591
169. Korolchuk VI, Saiki S, Lichtenberg M, Siddiqi FH, Roberts EA, Imarisio S, et al. Lysosomal positioning coordinates cellular nutrient responses. *Nat Cell Biol* (2011) 13:453–60. doi:10.1038/ncb2204
170. Ganley IG. Autophagosome maturation and lysosomal fusion. *Essays Biochem* (2013) 55:65–78. doi:10.1042/bse0550065
171. Hyttinen JMT, Niittykoski M, Salminen A, Kaarniranta K. Maturation of autophagosomes and endosomes: a key role for Rab7. *Biochim Biophys Acta* (2013) 1833:503–10. doi:10.1016/j.bbamcr.2012.11.018
172. Nair U, Yen WL, Mari M, Cao Y, Xie Z, Baba M, et al. A role for Atg8-PE deconjugation in autophagosome biogenesis. *Autophagy* (2012) 8:780–93. doi:10.4161/auto.19385
173. Yu ZQ, Ni T, Hong B, Wang HY, Jiang FJ, Zou S, et al. Dual roles of Atg8-PE deconjugation by Atg4 in autophagy. *Autophagy* (2012) 8:883–92. doi:10.4161/auto.19652
174. Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, Miller BC, et al. Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. *Cell Host Microbe* (2008) 4:458–69. doi:10.1016/j.chom.2008.10.003
175. Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* (2008) 9:1004–10. doi:10.1038/nrm2529
176. Radoshevich L, Murrow L, Chen N, Fernandez E, Roy S, Fung C, et al. ATG12 conjugation to ATG3 regulates mitochondrial homeostasis and cell death. *Cell* (2010) 142:590–600. doi:10.1016/j.cell.2010.07.018
177. Subramani S, Malhotra V. Non-autophagic roles of autophagy-related proteins. *EMBO Rep* (2013) 14:143–51. doi:10.1038/embor.2012.220
178. Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, et al. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature* (2015) 528:565–9. doi:10.1038/nature16451
179. Münz C. Of LAP, CUPS, and DRibbles – unconventional use of autophagy proteins for MHC restricted antigen presentation. *Front Immunol* (2015) 6:200. doi:10.3389/fimmu.2015.00200
180. Cadwell K. Crosstalk between autophagy and inflammatory signalling pathways: balancing defence and homeostasis. *Nat Rev Immunol* (2016) 16:661–75. doi:10.1038/nri.2016.100
181. Gray M, Botelho RJ. Phagocytosis: hungry, hungry cells. *Methods Mol Biol* (2017) 1519:1–16. doi:10.1007/978-1-4939-6581-6_1
182. Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* (2007) 450:1253–7. doi:10.1038/nature06421
183. Romao S, Münz C. LC3-associated phagocytosis. *Autophagy* (2014) 10:526–8. doi:10.4161/auto.27606
184. Ma J, Becker C, Lowell CA, Underhill DM. Dectin-1-triggered recruitment of light chain 3 protein to phagosomes facilitates major histocompatibility complex class II presentation of fungal-derived antigens. *J Biol Chem* (2012) 287:34149–56. doi:10.1074/jbc.M112.382812
185. Martinez J, Almendinger J, Oberst A, Ness R, Dillon CP, Fitzgerald P, et al. Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated

phagocytosis is required for the efficient clearance of dead cells. *Proc Natl Acad Sci U S A* (2011) 108:17396–401. doi:10.1073/pnas.1113421108
186. Henault J, Martinez J, Riggs JM, Tian J, Mehta P, Clarke L, et al. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. *Immunity* (2012) 37:986–97. doi:10.1016/j.immuni.2012.09.014
187. Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat Cell Biol* (2015) 17:893–906. doi:10.1038/ncb3192
188. Ueyama T, Nakakita J, Nakamura T, Kobayashi T, Son J, Sakuma M, et al. Cooperation of p40(phox) with p47(phox) for Nox2-based NADPH oxidase activation during Fcγ receptor (FcγR)-mediated phagocytosis: mechanism for acquisition of p40(phox) phosphatidylinositol 3-phosphate (PI(3)P) binding. *J Biol Chem* (2011) 286:40693–705. doi:10.1074/jbc.M111.237289
189. Lam GY, Cemma M, Muise AM, Higgins DE, Brumell JH. Host and bacterial factors that regulate LC3 recruitment to *Listeria monocytogenes* during the early stages of macrophage infection. *Autophagy* (2013) 9:985–95. doi:10.4161/auto.24406
190. Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, et al. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature* (2016) 533:115–9. doi:10.1038/nature17950
191. Ma J, Becker C, Reyes C, Underhill DM. Cutting edge: FYCO1 recruitment to dectin-1 phagosomes is accelerated by light chain 3 protein and regulates phagosome maturation and reactive oxygen production. *J Immunol* (2014) 192:1356–60. doi:10.4049/jimmunol.1302835
192. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science* (2005) 307:1630–4. doi:10.1126/science.1108003
193. Lee HK, Mattei LM, Steinberg BE, Alberts P, Lee YH, Chervonsky A, et al. In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* (2010) 32:227–39. doi:10.1016/j.immuni.2009.12.006
194. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol* (2015) 14:183–93. doi:10.1016/S1474-4422(14)70256-X
195. Ben-Nun A, Wekerle H, Cohen IR. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol* (1981) 11:195–9.
196. Ben-Nun A, Wekerle H, Cohen IR. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. *Nature* (1981) 292:60–1.
197. Stromnes IM, Goverman JM. Passive induction of experimental allergic encephalomyelitis. *Nat Protoc* (2006) 1:1952–60. doi:10.1038/nprot.2006.284
198. Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ TH-17 cells in relapsing EAE. *Nat Immunol* (2007) 8:172–80. doi:10.1038/ni1430
199. Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, et al. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med* (2005) 11:328–34. doi:10.1038/nm1197
200. Tompkins SM, Padilla J, Dal Canto MC, Ting JP, Van Kaer L, Miller SD. De novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. *J Immunol* (2002) 168:4173–83. doi:10.4049/jimmunol.168.8.4173
201. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* (1988) 239:290–2.
202. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat Med* (2005) 11:335–9. doi:10.1038/nm1202
203. Huitinga I, van Rooijen N, de Groot CJ, Uitdehaag BM, Dijkstra CD. Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages. *J Exp Med* (1990) 172:1025–33.
204. Becher B, Prat A, Antel JP. Brain-immune connection: immuno-regulatory properties of CNS-resident cells. *Glia* (2000) 29:293–304. doi:10.1002/(SICI)1098-1136(20000215)29:4<293::AID-GLIA1>3.0.CO;2-A
205. Schreiner B, Heppner FL, Becher B. Modeling multiple sclerosis in laboratory animals. *Semin Immunopathol* (2009) 31:479–95. doi:10.1007/s00281-009-0181-4
206. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He Y-W. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J Exp Med* (2007) 204:25–31. doi:10.1084/jem.20061303
207. Kovacs JR, Li C, Yang Q, Li G, Garcia IG, Ju S, et al. Autophagy promotes T-cell survival through degradation of proteins of the cell death machinery. *Cell Death Differ* (2012) 19:144–52. doi:10.1038/cdd.2011.78
208. Kabat AM, Harrison OJ, Riffelmacher T, Moghaddam AE, Pearson CF, Laing A, et al. The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. *Elife* (2016) 5:e12444. doi:10.7554/eLife.12444
209. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat Immunol* (2016) 17:277–85. doi:10.1038/ni.3365
210. Alirezaei M, Fox HS, Flynn CT, Moore CS, Hebb AL, Frausto RF, et al. Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. *Autophagy* (2009) 5:152–8. doi:10.4161/auto.5.2.7348
211. Arsov I, Adebayo A, Kucerova-Levisohn M, Haye J, MacNeil M, Papavasiliou FN, et al. A role for autophagic protein beclin 1 early in lymphocyte development. *J Immunol* (2011) 186:2201–9. doi:10.4049/jimmunol.1002223
212. Puleston DJ, Zhang H, Powell TJ, Lipina E, Sims S, Panse I, et al. Autophagy is a critical regulator of memory CD8(+) T cell formation. *Elife* (2014) 3:2516. doi:10.7554/eLife.03706
213. Haq K, McElhaney JE. Immunosenescence: influenza vaccination and the elderly. *Curr Opin Immunol* (2014) 29:38–42. doi:10.1016/j.coi.2014.03.008
214. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, et al. Autophagy is essential for effector CD8(+) T cell survival and memory formation. *Nat Immunol* (2014) 15:1152–61. doi:10.1038/ni.3025
215. Valdor R, Mocholi E, Botbol Y, Guerrero-Ros I, Chandra D, Koga H, et al. Chaperone-mediated autophagy regulates T cell responses through targeted degradation of negative regulators of T cell activation. *Nat Immunol* (2014) 15:1046–54. doi:10.1038/ni.3003
216. Fang D, Elly C, Gao B, Fang N, Altman Y, Joazeiro C, et al. Dysregulation of T lymphocyte function in itchy mice: a role for Itch in TH2 differentiation. *Nat Immunol* (2002) 3:281–7. doi:10.1038/ni763
217. Miller BC, Zhao Z, Stephenson LM, Cadwell K, Pua HH, Lee HK, et al. The autophagy gene ATG5 plays an essential role in B lymphocyte development. *Autophagy* (2008) 4:309–14. doi:10.4161/auto.5474
218. Hillert J, Olerup O. HLA and MS. *Neurology* (1993) 43:2426–7.
219. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* (2005) 23:683–747. doi:10.1146/annurev.immunol.23.021704.115707
220. Nylander A, Hafler DA. Multiple sclerosis. *J Clin Invest* (2012) 122:1180–8. doi:10.1172/JCI58649
221. Lassmann H, van Horssen J. The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Lett* (2011) 585:3715–23. doi:10.1016/j.febslet.2011.08.004
222. Henderson APD, Barnett MH, Parratt JDE, Prineas JW. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann Neurol* (2009) 66:739–53. doi:10.1002/ana.21800
223. Marik C, Felts PA, Bauer J, Lassmann H, Smith KJ. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? *Brain* (2007) 130:2800–15. doi:10.1093/brain/awm236
224. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol* (2004) 55:458–68. doi:10.1002/ana.20016
225. Paterka M, Siffrin V, Voss JO, Werr J, Hoppmann N, Gollan R, et al. Gatekeeper role of brain antigen-presenting CD11c+ cells in neuroinflammation. *EMBO J* (2016) 35:89–101. doi:10.15252/embj.201591488
226. Bhattacharya A, Parillon X, Zeng S, Han S, Eissa NT. Deficiency of autophagy in dendritic cells protects against experimental autoimmune encephalomyelitis. *J Biol Chem* (2014) 289:26525–32. doi:10.1074/jbc.M114.575860
227. Jacobson S, Sekaly RP, Jacobson CL, McFarland HF, Long EO. HLA class II-restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. *J Virol* (1989) 63:1756–62.
228. Nuchtern JG, Biddison WE, Klausner RD. Class II MHC molecules can use the endogenous pathway of antigen presentation. *Nature* (1990) 343:74–6.
229. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanović S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* (1999) 50:213–9.

230. Nimmerjahn F, Milosevic S, Behrends U, Jaffee EM, Pardoll DM, Bornkamm GW, et al. Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. *Eur J Immunol* (2003) 33:1250–9. doi:10.1002/eji.200323730
231. Brazil MI, Weiss S, Stockinger B. Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. *Eur J Immunol* (1997) 27:1506–14.
232. Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Müller M, et al. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc Natl Acad Sci U S A* (2005) 102:7922–7. doi:10.1073/pnas.0501190102
233. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* (2007) 315:1398–401. doi:10.1126/science.1136880
234. Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *EMBO J* (2008) 27:1110–21. doi:10.1038/emboj.2008.31
235. Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* (2007) 27:135–44. doi:10.1016/j.immuni.2007.05.022
236. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* (2008) 455:396–400. doi:10.1038/nature07208
237. Prinz M, Garbe F, Schmidt H, Mildner A, Gutcher I, Wolter K, et al. Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. *J Clin Invest* (2006) 116:456–64. doi:10.1172/JCI26078
238. Miranda-Hernandez S, Gerlach N, Fletcher JM, Biros E, Mack M, Körner H, et al. Role for MyD88, TLR2 and TLR9 but not TLR1, TLR4 or TLR6 in experimental autoimmune encephalomyelitis. *J Immunol* (2011) 187:791–804. doi:10.4049/jimmunol.1001992
239. Fischer MT, Wimmer I, Höftberger R, Gerlach S, Haider L, Zrzavy T, et al. Disease-specific molecular events in cortical multiple sclerosis lesions. *Brain* (2013) 136:1799–815. doi:10.1093/brain/awt110
240. Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann Neurol* (1998) 43:809–14.
241. Fazekas F, Ropele S, Enzinger C, Seifert T, Strasser-Fuchs S. Quantitative magnetization transfer imaging of pre-lesional white-matter changes in multiple sclerosis. *Mult Scler* (2002) 8:479–84. doi:10.1191/1352458502ms860oa
242. Huynh JL, Garg P, Thin TH, Yoo S, Dutta R, Trapp BD, et al. Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. *Nat Neurosci* (2014) 17:121–30. doi:10.1038/nn.3588
243. Traka M, Podojil JR, McCarthy DP, Miller SD, Popko B. Oligodendrocyte death results in immune-mediated CNS demyelination. *Nat Neurosci* (2016) 19:65–74. doi:10.1038/nn.4193
244. Stys PK, Zamponi GW, van Minnen J, Geurts JJG. Will the real multiple sclerosis please stand up? *Nat Rev Neurosci* (2012) 13:507–14. doi:10.1038/nrn3275
245. Fischer MT, Sharma R, Lim JL, Haider L, Frischer JM, Drexhage J, et al. NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. *Brain* (2012) 135:886–99. doi:10.1093/brain/aws012
246. Lam GY, Huang J, Brumell JH. The many roles of NOX2 NADPH oxidase-derived ROS in immunity. *Semin Immunopathol* (2010) 32:415–30. doi:10.1007/s00281-010-0221-0
247. Petry A, Weitnauer M, Görlach A. Receptor activation of NADPH oxidases. *Antioxid Redox Signal* (2010) 13:467–87. doi:10.1089/ars.2009.3026
248. Savina A, Jancic C, Hugues S, Guermonprez P, Vargas P, Moura IC, et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. *Cell* (2006) 126:205–18. doi:10.1016/j.cell.2006.05.035
249. Rybicka JM, Balce DR, Chaudhuri S, Allan ERO, Yates RM. Phagosomal proteolysis in dendritic cells is modulated by NADPH oxidase in a pH-independent manner. *EMBO J* (2012) 31:932–44. doi:10.1038/emboj.2011.440
250. Allan ER, Tailor P, Balce DR, Pirzadeh P, McKenna NT, Renaux B, et al. NADPH oxidase modifies patterns of MHC class II-restricted epitopic repertoires through redox control of antigen processing. *J Immunol* (2014) 192:4989–5001. doi:10.4049/jimmunol.1302896
251. Mahad D, Ziabreva I, Lassmann H, Turnbull D. Mitochondrial defects in acute multiple sclerosis lesions. *Brain* (2008) 131:1722–35. doi:10.1093/brain/awn105
252. Campbell GR, Ohno N, Turnbull DM, Mahad DJ. Mitochondrial changes within axons in multiple sclerosis: an update. *Curr Opin Neurol* (2012) 25:221–30. doi:10.1097/WCO.0b013e3283533a25
253. Trapp BD, Stys PK. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurol* (2009) 8:280–91. doi:10.1016/S1474-4422(09)70043-2
254. Guo H, Wei J, Kuo PC. Nitric oxide inhibits expression of cytochrome B in endotoxin-stimulated murine macrophages. *Biochem Biophys Res Commun* (2001) 289:993–7. doi:10.1006/bbrc.2001.6107
255. Wei J, Guo H, Kuo PC. Endotoxin-stimulated nitric oxide production inhibits expression of cytochrome c oxidase in ANA-1 murine macrophages. *J Immunol* (2002) 168:4721–7. doi:10.4049/jimmunol.168.9.4721
256. Larsson N-G. Somatic mitochondrial DNA mutations in mammalian aging. *Annu Rev Biochem* (2010) 79:683–706. doi:10.1146/annurev-biochem-060408-093701
257. Bolaños JP, Almeida A, Stewart V, Peuchen S, Land JM, Clark JB, et al. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem* (1997) 68:2227–40. doi:10.1046/j.1471-4159.1997.68062227.x
258. Cross AH, Manning PT, Keeling RM, Schmidt RE, Misko TP. Peroxynitrite formation within the central nervous system in active multiple sclerosis. *J Neuroimmunol* (1998) 88:45–56. doi:10.1016/S0165-5728(98)00078-2
259. Liu JS, Zhao ML, Brosnan CF, Lee SC. Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. *Am J Pathol* (2001) 158:2057–66. doi:10.1016/S0002-9440(10)64677-9
260. Zeis T, Probst A, Steck AJ, Stadelmann C, Brück W, Schaeren-Wiemers N. Molecular changes in white matter adjacent to an active demyelinating lesion in early multiple sclerosis. *Brain Pathol* (2009) 19:459–66. doi:10.1111/j.1750-3639.2008.00231.x
261. Cavaliere F, Urra O, Alberdi E, Matute C. Oligodendrocyte differentiation from adult multipotent stem cells is modulated by glutamate. *Cell Death Dis* (2012) 3:e268. doi:10.1038/cddis.2011.144
262. Accetta R, Damiano S, Morano A, Mondola P, Paternò R, Avvedimento EV, et al. Reactive oxygen species derived from NOX3 and NOX5 drive differentiation of human oligodendrocytes. *Front Cell Neurosci* (2016) 10:146. doi:10.3389/fncel.2016.00146
263. Twig G, Hyde B, Shirihai OS. Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. *Biochim Biophys Acta* (2008) 1777:1092–7. doi:10.1016/j.bbabio.2008.05.001
264. Harris H, Rubinsztein DC. Control of autophagy as a therapy for neurodegenerative disease. *Nat Rev Neurol* (2011) 8:108–17. doi:10.1038/nrneurol.2011.200
265. Rolland SG, Motori E, Memar N, Hench J, Frank S, Winklhofer KF, et al. Impaired complex IV activity in response to loss of LRPPRC function can be compensated by mitochondrial hyperfusion. *Proc Natl Acad Sci U S A* (2013) 110:E2967–76. doi:10.1073/pnas.1303872110
266. Witte ME, Bø L, Rodenburg RJ, Belien JA, Musters R, Hazes T, et al. Enhanced number and activity of mitochondria in multiple sclerosis lesions. *J Pathol* (2009) 219:193–204. doi:10.1002/path.2582
267. Mahad DJ, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, et al. Mitochondrial changes within axons in multiple sclerosis. *Brain* (2009) 132:1161–74. doi:10.1093/brain/awp046
268. Cai Q, Zakaria HM, Simone A, Sheng Z-H. Spatial parkin translocation and degradation of damaged mitochondria via mitophagy in live cortical neurons. *Curr Biol* (2012) 22:545–52. doi:10.1016/j.cub.2012.02.005
269. Sheng Z-H. Mitochondrial trafficking and anchoring in neurons: new insight and implications. *J Cell Biol* (2014) 204:1087–98. doi:10.1083/jcb.201312123
270. Ohno N, Chiang H, Mahad DJ, Kidd GJ, Liu L, Ransohoff RM, et al. Mitochondrial immobilization mediated by syntaphilin facilitates survival of demyelinated axons. *Proc Natl Acad Sci U S A* (2014) 111:9953–8. doi:10.1073/pnas.1401155111

271. Yamamoto A, Yue Z. Autophagy and its normal and pathogenic states in the brain. *Annu Rev Neurosci* (2014) 37:55–78. doi:10.1146/annurev-neuro-071013-014149
272. Campbell GR, Ziabreva I, Reeve AK, Krishnan KJ, Reynolds R, Howell O, et al. Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. *Ann Neurol* (2011) 69:481–92. doi:10.1002/ana.22109
273. Gao L, Laude K, Cai H. Mitochondrial pathophysiology, reactive oxygen species, and cardiovascular diseases. *Vet Clin North Am Small Anim Pract* (2008) 38:137–55, vi. doi:10.1016/j.cvsm.2007.10.004
274. Lizama-Manibusan B, McLaughlin B. Redox modification of proteins as essential mediators of CNS autophagy and mitophagy. *FEBS Lett* (2013) 587:2291–8. doi:10.1016/j.febslet.2013.06.007
275. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* (2007) 26:1749–60. doi:10.1038/sj.emboj.7601623
276. Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, et al. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci U S A* (2008) 105:3374–9. doi:10.1073/pnas.0712145105
277. van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra CD, van der Valk P, et al. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. *Free Radic Biol Med* (2008) 45:1729–37. doi:10.1016/j.freeradbiomed.2008.09.023
278. Juurlink BH, Thorburne SK, Hertz L. Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. *Glia* (1998) 22:371–8.
279. Barja G. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. *Antioxid Redox Signal* (2013) 19:1420–45. doi:10.1089/ars.2012.5148
280. Haider L, Fischer MT, Frischer JM, Bauer J, Höftberger R, Botond G, et al. Oxidative damage in multiple sclerosis lesions. *Brain* (2011) 134:1914–24. doi:10.1093/brain/awr128
281. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* (2009) 460:392–5. doi:10.1038/nature08221
282. Martínez-Cisuelo V, Gómez J, García-Junceda I, Naudí A, Cabré R, Mota-Martorell N, et al. Rapamycin reverses age-related increases in mitochondrial ROS production at complex I, oxidative stress, accumulation of mtDNA fragments inside nuclear DNA, and lipofuscin level, and increases autophagy, in the liver of middle-aged mice. *Exp Gerontol* (2016) 83:130–8. doi:10.1016/j.exger.2016.08.002
283. Wang B, Cai Z, Tao K, Zeng W, Lu F, Yang R, et al. Essential control of mitochondrial morphology and function by chaperone-mediated autophagy through degradation of PARK7. *Autophagy* (2016) 12:1215–28. doi:10.1080/15548627.2016.1179401

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Advantages of publishing in Frontiers

**OPEN ACCESS**
Articles are free to read, for greatest visibility

**COLLABORATIVE PEER-REVIEW**
Designed to be rigorous – yet also collaborative, fair and constructive

**FAST PUBLICATION**
Average 85 days from submission to publication (across all journals)

**COPYRIGHT TO AUTHORS**
No limit to article distribution and re-use

**TRANSPARENT**
Editors and reviewers acknowledged by name on published articles

**SUPPORT**
By our Swiss-based editorial team

**IMPACT METRICS**
Advanced metrics track your article's impact

**GLOBAL SPREAD**
5'100'000+ monthly article views and downloads

**LOOP RESEARCH NETWORK**
Our network increases readership for your article

**Frontiers**
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
**www.frontiersin.org**

**Find us on**