SMGroup

Immunotherapy for Multiple Sclerosis Patients

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ABSTRACT

Therapeutic options for multiple sclerosis (MS) patients significantly increased over the last ten years mainly due to FDA approval of treatments originally developed for other diseases (e.g. anti-cancer drugs) that have immunomodulatory or immunosuppressive effects useful for MS. The immune system in general and T lymphocytes in particular are considered to play a central role in initiating and perpetuating the pathological immune response in MS. Current therapeutic approaches mediate non-T-cell-selective immune suppression or immune modulation (e.g., interferons), selective depletion of immune cell populations involving T cells (e.g., anti-CD52, alemtuzumab) or inhibition of specific molecular pathways to sequester leucocytes (e.g., natalizumab-mediated leukocyte sequestration approach). However, current treatment options in MS do not stop disease progression or reverse existing disabilities in MS patients. So far, levels of long-term disabilities under available treatments are identical to those seen in placebo groups. The authors believe a paradigm shift is necessary from immunomodulatory or immunosuppressive therapies which aim to prevent disease exacerbation, towards active

regenerative strategies, which intend to repair demyelinated brain and spinal cord lesions. This book chapter intends to highlight antibody-based therapies for the treatment of MS with focus on conventional and reparative antibodies including natural autoantibodies (NAbs) and (some) future options currently being tested in clinical trials.

INTRODUCTION

Multiple Sclerosis, an Inflammatory CNS Disease

MS is the most common chronic inflammatory, demyelinating disease of the CNS and typically results in neurological disability. MS is most often relapsing, and progresses in the white (and grey) matter of the CNS with unclear pathogenesis. So far, there is no treatment available to stop disease progression or reverse existing disabilities in MS patients. March 2015 statistics from the MS Foundation estimated more than 400,000 people in the United States and about 2.5 million people worldwide with MS (http://j.mp/MS_Statistics). The most common MS subtype, relapsing–remitting MS (RRMS) is present in 80-85 percent of patients and typically begins in the second or third decade of life with a female predominance of 2:1 and most recently 3:1. Patients typically improve spontaneously or respond to corticosteroids administered intravenously; a treatment paradigm using 1 gram methylprednisolone intravenously for each of 3 consecutive days without oral corticosteroid taper was initiated by *Moses Rodriguez* at the Mayo Clinic in 1983 (known at the Mayo Clinic as the "Rodriguez protocol"). This treatment has become the standard approach for treating acute exacerbations of MS throughout the world. Unfortunately, the patient's responsiveness to corticosteroids typically fades over time. A certain level of CNS dysfunction may persist between relapses or progresses over time (secondary progressive MS). Some of these deficits that persist after methylprednisolone therapy may respond to plasma exchange [1]. This was proven to be the case in a double blind placebo controlled trial where 40% of patients improved with true exchange [2].

Approximately 15-20 percent of MS patients are diagnosed with primary progressive MS, which progresses gradually in the absence of obvious relapses and remissions. Primary progressive MS has a similar incidence among men and women [3].

Pathological Hallmarks in MS

CNS demyelination is a primary inflammatory process and the pathological hallmark in MS leading to brain lesion formation and neurological deficits [3,4]. Demyelination disables saltatory nerve conduction and increases axonal vulnerability to environmental stressors, a key aspect in MS pathogenesis, which results in loss of neural function and ultimately neuronal death. Despite extensive investigations during the last decades the initial trigger(s) causing the disease have not been identified. Given the complexity of the disease with potentially different demyelinating disorders covered under the umbrella of MS, different mechanisms may contribute to tissue injury and MS lesion formation. Infiltrating T cells and peripheral macrophages attacking/engulfing myelinating oligodendrocytes are believed to initiate acute MS lesions [5]. This hypothesis is

strongly supported by studies using an inflammatory rodent model of MS, termed experimental autoimmune encephalomyelitis (EAE). Myelin proteins in combination with complete Freunds adjuvants (CFA) (typically mycobacteria tuberculosis plus mineral oil) induce EAE. Based on EAE studies in rodents, it was hypothesized that invading T-cells reactive to myelin components are the major disease initiators in MS [6,7]. Similar to EAE, demyelination of the spinal cord in the Theiler's murine encephalomyelitis (TMEV) - model of MS was shown to be T-cell mediated. Different from EAE, immunization with spinal cord homogenates containing substantial myelin components in TMEV-infected chronically demyelinated mice induce substantial remyelination of demyelinated spinal cord areas. IgM antibodies targeting myelin components are responsible for the beneficial outcome in this model, which questions a deleterious effect of anti-myelin antibodies in EAE and MS.

An alternative to the immune-mediated hypothesis is that oligodendrocyte death represents the first and earliest lesion stage, which in turn results in primary demyelination and secondary autoimmune inflammation. This hypothesis was first proposed by Rodriguez et al.[1,9,10] and later confirmed by Barnett and Prineas [11]. Levels of subsequent lesion extension and oligodendrocyte loss may follow the extent of secondary inflammation. Recent studies also emphasize mitochondrial damage with subsequent energy failure as a major contributor to MS pathogenesis [13-17], which may in part explain demyelination and oligodendrocyte apoptosis [18], death of small diameter axons [19,20], differentiation arrest of oligodendrocyte progenitor cells (OPCs) [21] and astrocytic dysfunction [22].

Immunotherapies using Conventional and Natural Antibodies for the Treatment of Multiple Sclerosis (Ms)

Immunosuppressive and immunomodulatory therapeutic approaches for MS

Current therapies using glatiramer acetate, β-interferons, immune suppressive agents, or adhesion molecule inhibitors ease the severity of acute attacks and lower their frequency but result in the same degree of long-term disability as seen in placebo treated patients. In addition, strong immune suppressive drugs facilitate fatal infections. We believe a paradigm shift is necessary from immunosuppressive/immunomodulatory strategies in MS, which aim to prevent disease exacerbation, towards active regenerative strategies, which intend to repair demyelinated brain and spinal cord lesions.

Here we discuss different immune therapy options with focus on monoclonal antibodies as potential therapeutic strategies for MS patients.

US Food and Drug Administration (FDA)-approved monoclonal antibodies for the treatment of MS

So far, Natalizumab and Alemtuzumab are the only US Food and Drug Administration (FDA)-approved monoclonal antibodies for the treatment of MS.

Natalizumab (Tysabri): Natalizumab is a humanized monoclonal antibody that targets the focal adhesion molecule integrin α-4 present on T lymphocytes and other immune cells. Integrin α-4 is essential for T lymphocytes to cross the BBB and enter the CNS lesion site. Natalizumab is effective in an immune-mediated animal model of MS where it prevents T lymphocytes from entering the CNS lesions and thereby lessening the disease [23]. When administered every four weeks in a phase III trial, natalizumab reduced the exacerbation rate by approximately 68% [24]. Natalizumab combined with beta-interferon resulted in an additional decrease in disease activity [25]. However, Natalizumab poses an increased risk of CNS virus infections leading to progressive multifocal leukoencephaolpathy (PML). As of 31st of August 2015 there were 588 cases of natalizumab-associated PML among 142,000 patients (http://bit.ly/natpml0815) [26].

Alemtuzumab (Campath-1H): The monoclonal antibody Alemtuzumab (Campath) targets an antigen (CD52) present on monocytes and lymphocytes and causes systemic suppression of the immune system that can last for up to a year. The primary indication for Alemtuzumab is the treatment of chronic lymphocytic leukemia. A phase III trial in patients with relapsing-remitting MS demonstrated lower relapse rates compared to controls but without having an effect on the degree of disability [27]. An earlier phase II trial was terminated due to development of idiopathic thrombocytopenia in three patients [27]. Approximately 23 % of patients under Alemtuzumab develop autoimmune thyroid disease and may be at risk to develop PML or other potentially fatal opportunistic infections.

The main safety concerns include autoimmune thyroid problems and idiopathic thrombocytopenic purpura (ITP) for which patients will require monitoring.

Non FDA-approved monoclonal antibodies for the treatment of MS

Rituximab (Rituxan): The chimeric monoclonal antibody Rituximab binds and destroys B cells through binding to a phosphorylated glycoprotein (CD20). Rituximab was primarily used for diseases characterized by overactive B cells. Based on antibody-mediated injury seen in an immune-mediated animal model of MS (EAE), and its assumed predictive value for relapsing and progressive MS, Rituximab was considered as an alternative treatment for MS and studied in more detail. Rituximab lowers the rate of relapses in MS patients by 50 % as determined 48 weeks after administration [28]. However, the clinical outcome in patients with primary progressive disease was not significantly different [29]. In addition, Rituximab may also cause PML and is associated with reactivation of dormant prior infections like hepatitis B [30].

Daclizumab (Zenepax): The humanized monoclonal antibody Daclizumab binds to the interleukin-2 receptor (CD25) on T cells, B cells and thymocytes [31]. Daclizumab was primarily used to prevent rejection of transplanted organs. With respect to disease related processes CD25 is also expressed in neoplastic B cells, neuroblastomas and tumor infiltrating lymphocytes. Daclizumab improved the MRI outcome and clinical scores in patients with MS in combination with β-interferon or in monotherapy [31]. So far, there are no reports of opportunistic infections

or other life-threatening adverse effects with daclizumab. This is in contrast to side-effects seen with natalizumab and rituximab and may be based on its rather immune modulatory as opposed to immune suppressive effects. It was assumed that daclizumab eradicates certain CD4 and CD8 T lymphocyte populations through increasing levels of $CD56^+$ natural killer cells [29]. A phase III trial using daclizumab versus β-interferon demonstrated a 45 % reduction in annual relapses compared to β-interferon in patients with relapsing multiple sclerosis.

Ocrelizumab: The humanized monoclonal antibody Ocrelizumab targets the phosphorylated glycoprotein CD20 (see Rituximab) on circulating B lymphocytes but not plasma cells. Similar to Rituximab, Ocrelizumab causes depletion of B lymphocytes through complement- and antibody-dependent cytotoxicity plus stimulation of apoptosis. An important reason for its development was the detection of anti-chimeric neutralizing antibodies against the chimeric antibody Rituximab in 24% of patients. This disturbing outcome is likely to be reduced in patients treated with the humanized antibody Ocrelizumab. The favorable outcome in a phase II trial comparing Ocrelizumab against IFNβ-1a and placebo led to a phase III study in primary progressive MS and relapsing-remitting MS [32]. There are, however, safety concerns using Ocrelizumab based on the phase II MS trial noted above with a 41-year old patient dying from brain edema 14 weeks into the trial. A phase III trial of Ocrelizumab in systemic lupus erythematosus was discontinued due to opportunistic infections after methotrexate exposure [33] .

Ofatumumab (Arzerra): Ofatumumab is a fully human monoclonal antibody targeting CD20. Ofatumumab was previously FDA approved in combination with chlorambucil, for the treatment of previously untreated patients with chronic lymphocytic leukemia (CLL). Results from phase I/II trials using Ofatumumab vs. placebo for relapsing-remitting MS reported no major safety concerns. Ofatumumab-treatment resulted in a ≥90% reduction in new T1 gadolinium-enhancing lesions for all doses of Ofatumumab ≥30 mg (**P** < .001) [34].

Off-Label use of polyclonal intravenous immunoglobulins (IVIgG) for the treatment of relapsing-remitting Multiple sclerosis (RRMS) patients

Intravenous Immunoglobulins (IVIgG): IVIgG is a therapeutic compound prepared from pools of plasma obtained from several thousand healthy blood donors. IVIgG contains small amounts of NAbs and typically lacks immunoglobulin isotypes other than IgGs, which is, however, dependent on the manufacturing process used. The advantage of large donor pools is increased reactivity of individual antibodies to certain antigens. Theoretically, IVIgG comprises the entire range of reactivity exhibited by IgG of normal human sera. The reactivity can target foreign antigens, including bacterial and viral antigens, as well as self-antigens such as membrane-associated self-molecules, intracellular and extracellular antigens. It was found that the self-reactivity of normal serum IgG with solubilized extracts of normal homologous tissues targeted a conserved and limited set of dominant antigens [35-37]. The constant interaction between antibodies, antibody molecules and variable regions of antigen receptors on B cells is an essential component of selection of immune repertoires and establishment of tolerance to self [38,39].

Components of IVIgG preparations, production process and product safety

Intact IgG molecules form the primary component of IVIgG, which is produced by commercial manufacturers as well as not-for-profit organizations. Due to diverse manufacturers in the market, several quality markers of IVIgG preparations including purity, pH, osmolarity, sodium and sugar content may vary between IVIgG preparations [40]. IVIgG preparations undergo viral inactivation and may contain trace amounts of IgA, anti-A and anti-B (IgG antibodies directed against human blood group antigens), soluble CD4, CD8, HLA molecules and certain cytokines [41-43]. Most of the methodology used to isolate and purify IVIgG dates back to the late 1950s. The more recently tweaked methodology that includes caprylate precipitation followed by anion exchange chromatography is currently used [44]. Characteristics of commercially prepared IVIgG are a pH range of 4-6, with ≥240 mosmol/kg, a total protein quantity of ≥30 g/l with ≤3% of immune aggregates. Finally, the product should test negative for the surface antigen of the hepatitis B virus (HBsAg), HIV p24 antigen, anti-HIV-1 antibodies, anti-HIV-2 antibodies and anti-hepatitis C virus (HCV) antibodies [41].

Clinical use of IVIgG preparations: After demonstrating an attenuated clearance of platelets in a child with immune thrombocytopenic purpura (ITP) [45] followed by a similar effect in adults with ITP [46], IVIgG was licensed for use in ITP. IVIgG was also tested and used in the clinic as a replacement low-dose therapy for primary and secondary immunodeficiencies. Diseases in this category include PID [47], X-linked agammaglobulinaemia (XLA), acquired hypogammaglobulinemia, common variable immunodeficiency, X-linked hyper-immunoglobulin (Ig)M, severe combined immunodeficiency, HIV infection, Wiskott–Aldrich syndrome and selective IgG class deficiency [48,49]. Other currently licensed indications for IVIgG include Kawasaki disease, Guillain-Barrè syndrome, and chronic inflammatory demyelinating polyneuropathy (CIDP). However, its initial success in treatment of ITP led to its use in treating many systemic inflammatory and autoimmune disorders affecting the joints, skin, hematopoietic system and central nervous system [48]. Licensed uses account for less than half of the annual worldwide sales of IVIgG preparations; most sales are for “off-label” IVIgG applications [50]. In addition to antibody-mediated diseases, IVIgG is also effective in several disorders caused by derailment of cellular immunity, such as dermatomyositis, multiple sclerosis (MS), graft-versus-host disease (GvHD) in recipients of allogeneic bone marrow transplants and treatment of cellular rejection after organ transplantation [51-53].

Mechanisms of action of IVIgG: Infused IVIgG has a half-life of 21 days in immuno-competent individuals; however, the beneficial effect of IVIgG extends beyond its half-life. This suggests that IVIgG therapy not only neutralizes pathogenic antibodies but also induces lasting changes in the cellular compartment of the immune system. The precise mechanism by which IVIgG suppresses inflammation is multi-factorial with many working hypotheses postulated: inhibition of the activation cascade of macrophages, dendritic cells, and pathogenic T-cells (Th1 cells, Th17 cells); expansion of regulatory T-cells; modulation of B-cell responses and inhibition of pathogenic

autoantibody production; suppression of inflammatory cytokine production; neutralization of pathogenic autoantibodies via anti-idiotypes and inhibition of complement [54].

Potential use of IVIgG for the treatment of relapsing-remitting MS patients: A proven track record of minimal side effects of IVIgG in treated patients and promising data from experimental animal models of MS prompted the initiation of clinical trials for the treatment of relapsing-remitting MS (RRMS).

Initial European clinical studies showed beneficial effects of IVIgG for RRMS. An Austrian study was multicenter, randomized, double-blind and placebo-controlled with patients receiving monthly doses of 0.15–0.2 g/kg for 2 years [55] . IVIgG treated patients showed reduced clinical disability levels as measured by the absolute change in Kurtzke's expanded disability status scale (EDSS) score. The beneficial outcome for RRMS patients was confirmed in 1998 by showing reduced numbers of gadolinium-enhancing lesions on monthly serial MRI scans in the IVIgG arm of the study. In addition, the number of exacerbation-free patients was significantly higher during IVIgG treatment [56]. A double-blind, placebo-controlled study in Poland reported that an IVIgG dose of 0.2 g/kg administered once per month for 12 months is equally effective as 0.4 g/kg in reducing MS activity [57]. Subsequent data from a retrospective, multicenter observational study over 5 years reported that IVIgG treatment (0.24 ± 0.15 g/kg/month) resulted in a 69% reduction in the mean annual relapse rate [58] . Collectively, these studies suggested that IVIgG is effective in reducing the relapse rate in RRMS [3,59].

In contrast, a randomized, double-blinded, placebo-controlled trial of IVIgG immunoglobulin performed in MS patients with persistent muscle weakness indicated no difference in the degree of change in strength between treatment groups [59]. There was no apparent benefit in relapse behavior or impairment measures during the 6-month observation period in 67 enrolled patients. No benefit was observed in either patients who remained clinically stable or in those with evidence of disease activity. Patients with active MS during the trial worsened in all groups. It was concluded that IVIgG does not reverse muscle weakness in MS. The isometric muscle strength measurement was a reliable indicator of strength [59].

The negative outcome of IVIgG for the treatment of RRMS (and possibly secondary progressive MS) was confirmed in the randomized, double-blinded, placebo-controlled PRIVIG study using two doses of IVIgG or placebo. After 1 year, the differences in relapse-free patients did not differ statistically between treatment groups and no difference was observed in numbers of newly active MRI lesions. It was concluded that IVIgG treatment had no beneficial effect in RRMS patients in doses ranging from 0.2 to 0.4 g/kg [60].

In addition, a randomized, double-blind Phase II trial of intravenous IVIgG in inflammatory demyelinating optic neuritis did not reverse persistent visual loss from optic neuritis to a degree that merits general use [61]. 55 patients with persistent acuity loss after optic neuritis were randomized to receive either IVIg 0.4 g/kg daily for 5 days followed by three single infusions

monthly for 3 months, or placebo. The trial was terminated by the National Eye Institute because of negative results after 55 of the planned 60 patients had been enrolled. Analysis of this data indicated that a difference between treatment groups was not observed for the primary outcome measure, improvement in logMAR visual scores at 6 months ($p = 0.766$) [61].

The negative outcome in three independent studies seriously questioned the utility of IVIgG for the treatment of RRMS or in MS patients with optic neuritis.

While many auto-inflammatory diseases have proved responsive to IVIgG therapy the outcome of these MS trials is still puzzling. Several factors may have impacted the different outcome. It is of note that patients enlisted in the Mayo Clinic trial had fixed deficits and therefore potentially secondary progressive disease, while the European trials focused on RRMS only. This would suggest that IVIgG may be effective early during RRMS but not during later disease stages (secondary progressive MS). In addition, patient cohorts enlisted in mentioned MS trials were relatively small and not uniform, which makes comparisons between different trials particularly difficult [62].

IVIgG is currently not considered practical for the treatment of MS. IVIgG may prevent relapses after a first demyelinating event, but a beneficial effect in patients with RRMS is unclear. Use of IVIgG may be considered in patients with severe relapses that are non-responsive to corticosteroids [63].

Intravenous IgM, an alternative to Intravenous IgG: Immunoglobulin M (IgM) is an important component discarded during IVIgG preparations. Given the plasma concentration of IgM, ~18 tons of pooled IgM may be discarded every year. Studies performed in the past have documented the beneficial effect of polyclonal human IgM molecules.

Treatment of chronically TMEV-infected mice with polyclonal human IgM resulted in enhanced remyelination when compared with IVIgG. Moreover, polyclonal human IgM promoted remyelination to a degree comparable to the monoclonal human IgM termed rHIgM22 [64]. IVIgM also prevents complement activation *in vitro* and *in vivo* in a rat model of acute inflammation [65] and inhibits classical pathway complement activation, but not bactericidal activity, of human serum [66] . Stehr et al. [67], reported on the use of IgM-enriched solution on polymorphonuclear neutrophil function, bacterial clearance and lung histology in endotoxemia, a condition in which both pro-inflammatory and anti-inflammatory cascade systems are simultaneously initiated similar to sepsis. Their results documented a striking pulmonary protective effect of IVIgM, enhanced reticuloendothelial system bacterial clearance, increased *in vivo* phagocytosis efficiency, and an especially beneficial effect on LPS-induced pulmonary histological changes [67]. The *in vivo* therapeutic efficacy of IVIgM was also confirmed in experimental models of uveitis, myasthenia gravis, and multiple sclerosis [64,68,69]. The mechanisms of action of IVIgM include the induction of apoptosis of lymphoid cell lines and human peripheral blood mononuclear cells [70], the suppression of T cell functions *in vitro* and a delay in the activation of T lymphocytes

in SCID mice [71]. IVIgM may overcome, at least in part, the shortage of IVIgG; however, further work is warranted to appreciate the true beneficial potential of polyclonal IgM antibodies.

Strategies to repair CNS lesions in multiple sclerosis patients using monoclonal antibodies

Reparative monoclonal antibodies are a potential novel class of therapeutics for MS patients with the ability to actively repair CNS lesions in animal models of MS. At the time of this writing, the human monoclonal antibody Li81 (BIIB033) targeting LINGO-1 is being tested in phase II clinical trials in patients with relapsing remitting multiple sclerosis (RRMS) or with secondary progressive multiple sclerosis (SPMS). The trial started in April 2013 and is due for completion in early 2016.

The human remyelination promoting antibody rHIgM22 successfully completed phase I trial for patients with all clinical presentations of MS [72]. In this study, rHIgM22 showed excellent safety and tolerability at all doses tested. Furthermore, HIgM22 was detected in the cerebral spinal fluid (CSF) two days after intravenous injection (i.e., ≥0.05 ng/ml) in all rHIgM22-treated patients at two dose levels. Even 29 days after treatment, rHIgM22 was measurable in the CSF of 5 out of 12 patients [73]. This human data demonstrates that IgM antibodies are able to cross the BBB and persist in the CSF (>40 % of patients) for almost a month after treatment.

The remainder of this section emphasizes characteristics of reparative human antibodies, identified antigens and proposed mechanism(s) of action for these antibody classes.

Anti-LINGO-1 antibody (Li81) (BIIB033): The anti-LINGO-1 antibody Li81 (BIIB033) is a potential treatment for multiple sclerosis.

LINGO-1 and myelination: LINGO-1 ("Leucine rich repeat and Immunoglobin-like domain-containing protein 1") is a functional component (co-receptor) of the Nogo receptor signaling complex and interacts with the ligand-binding Nogo-66 receptor (NogoR). Lingo-1 is almost exclusively expressed in CNS neurons and oligodendrocytes during both embryonic and postnatal stages [72-79]. LINGO-1 is a negative regulator of myelination and involved in inhibition of axon regeneration through a complex together with NgR1, Nogo-66 and p75 (trimolecular receptor complex) [75,77]. Three major inhibitory factors for myelination Nogo, oligodendrocyte myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG) share this receptor complex, and accomplish inhibition of myelination through RhoA-GTP upregulation. In addition, LINGO-1 also inhibits oligodendrocyte progenitor cell (OPC) differentiation and myelination though a different mechanism that requires RhoA activation but does not involve p75 or NgR1 [76].

LINGO-1 is also involved in the regulation of neural apoptosis by inhibiting WNK3 kinase activity. It has been shown that blocking the extracellular domain of LINGO-1 disrupts the interaction between receptor kinases and LINGO-1 which directly attenuates inhibition of neuronal survival. However among the four WNK family members, only WNK3 has been shown to regulate and increase cell survival in a capase-3-dependant pathway [80,81].

Anti-LINGO-1 (Li81) immunotherapy (BIIB033)

Animal models: Results from animal models demonstrated promotion of spinal cord remyelination in an experimental autoimmune encephalomyelitis model [73,75]. The murine monoclonal antibody mAb3B5 recognizes mouse LINGO-1 and is a surrogate antibody for the human monoclonal antibody Li81 (BIIB033). mAb3B5, improves axon function in mice after experimental spinal cord demyelination [82]. In cell culture models, anti-LINGO-1 stimulated OPC differentiation and myelination [78].

Results from human studies: The anti-LINGO-1 antibody Li81 targets the leucine-rich repeat domain of LINGO-1, which blocks contact points in LINGO-1 required for protein oligomerization. Binding of the antibodies Fab region (responsible for antigen binding) to LINGO-1 results in the formation of a stable complex comprised of 2 copies of LINGO-1 and 2 copies of the Fab region [79]. The resulting complex blocks epitopes in the LINGO-1 IgG domain that are involved in oligodendrocyte differentiation [79]. Anti-LINGO-1 immunotherapy is intended to stimulate regrowth of the myelin sheath, which is damaged in MS brain lesions. Anti-LINGO-1 is thought to promote the development of oligodendrocytes, the cells which maintain myelin coating around neuronal axons. It has been found that LINGO-1 antagonists such as Li81 significantly improve and regulate survival after neural injury caused by the protein. Anti-LINGO-1 antibodies block the action of LINGO-1, allowing young cells to mature into oligodendrocytes. This may restore or repair damaged myelin, offering the potential for preventing or possibly reversing disability.

Serum pharmacokinetics of BIIB033 have been determined in control groups and MS cohorts with similar outcomes in both groups. The antibodies serum half-life ranges from 15 to 24 days after intravenous administration [80]. Intravenous doses >3 mg per kg in humans lead to serum concentrations that are predicted to have pharmacological activity, based on remyelination studies in rats. Cerebrospinal fluid (CSF) pharmacokinetics resulted in variable outcomes amongst tested individuals [80]. Ratio of brain and spinal cord to plasma concentrations is likely to be about 0.1% in humans compared to 0.1 to 0.4% in rats [81].

RENEW CLINICAL TRIAL

In April 2015 results from the Phase II clinical trial RENEW (Biogen) were published, which tested safety and efficacy of anti-LINGO-1 in optic neuritis, a disease that involves inflammation of the optic nerve which may lead to complete blindness in affected patients. The most common cause of optic neuritis is MS. 82 participants with their first episode of optic neuritis were given 6 doses of anti-LINGO-1 once every 4 weeks for 24 weeks total or a placebo. The study concluded that anti-LINGO-1 produced better signal quality across the optic nerves impacted by optical neuritis suggesting that some level of myelin repair had taken place. The patients taking part in the study showed a 34% improvement in nerve signals as compared to controls at week 24 but not with a change in the thickness of retinal layers or with improved visual function; at week 32,

12 weeks after the last dose, additional latency recovery was measured (a 41% improvement) (www.clinicaltrials.gov/ct2/show/NCT01721161; a)

SYNERGY Clinical Trial

The clinical trial SYNERGY (Biogen) tested efficacy and safety of anti-LINGO-1 in patients with relapsing remitting multiple sclerosis (RRMS) when used concurrently with β-interferon 1a (Avonex). The trial is due for completion in June 2016. 416 participants receive β-interferon 1a once weekly plus placebo or 3 mg, 10 mg, 30 mg or 100 mg of anti-LINGO-1 per kilogram of weight. Anti-LINGO-1 is given once monthly for 84 weeks. The primary outcome measure is the percentage of participants who display improved neurophysical or cognitive function (www.clinicaltrials.gov/ct2/show/NCT01864148).

Natural Antibodies (rHIgM22)

Even though the existence of NAbs (also sometimes termed "naturally occurring antibodies") was met with initial skepticism, pioneering work by Avrameas [82-84] and Notkins [85,86] , established convincing evidence that NAbs are part of the human innate immunoglobulin repertoire [83,87] (Table 1).

Table 1: Properties of polyreactive versus monospecific monoclonal antibodies.

	Polyreactive monoclonal antibody	Monospecific monoclonal antibody
Antigen	Many structurally diverse and unrelated antigens	Single antigen
Affinity	Low (K_d: 10^{-4} to 10^{-7})	High (K_d: 10^{-7} to 10^{-11})
Sequence	Germline or near germline with few somatic mutations, no affinity maturation	Somatically mutated, affinity maturated
Number of potentially allowed conformations inside the antibody's antigen binding pocket	more than one conformation allowed	only one conformation allowed (lock and key fit mechanism)
Immunoglobulin subtype	IgM > IgA and IgG	IgG > IgM, IgA
Half-life time	IgM:~8 h; IgG: ~10 h; IgA: ~8 h	IgM:~35 h; IgG: ~280 h; IgA: ~26 h

NAbs utilize germline-encoded genes directed against foreign antigens, self- and altered self-structures [82] and are present in newborns without stimulation by foreign antigens [88]. In contrast, conventional antibodies require external stimuli for their production. NAbs are polyreactive by definition with few or no somatic mutations in the antibody's variable light and heavy chain, which are required for high affinity binding of a single antigen. NAbs of the IgM isotype are found in invertebrates and vertebrates. High levels of IgG NAbs and to a lower extent IgM and IgA isotypes are detected in vertebrates [89]. In general, NAbs bind their antigen with low affinity but high avidity [90], which describes the combined synergistic strength of multiple bond interactions rather than the sum of bonds between antigen and antibody. In contrast, conventional antibodies, typically of the IgG isotype, undergo affinity maturation and contain somatic mutations to ensure high-affinity antigen binding, which is commonly linked to the antibody's monospecificity.

Accumulating evidence categorize NAbs as natural systemic surveillance molecules that tag damaged cells and foreign pathogens for elimination by the immune system through opsonization or antibody-dependent cellular cytotoxicity. Some NAbs can actively signal in cancer and brain cells. The ability of identified NAbs to detect and sometimes induce apoptosis in tumor cells may play an important function in tumor surveillance [91-94] . In mice and humans, another class of NAbs, termed remyelination promoting antibodies, actively promotes repair in demyelinated spinal cord areas [61,65,95-99]

Remyelination-Promoting Antibodies Are A Subclass of Nabs

All identified monoclonal remyelination promoting antibodies are of germline origin or near germline with few somatic mutations, thus having the cardinal features of physiologic natural antibodies. So far, all identified remyelination promoting antibodies with NAb features are IgMs (O1, O4, A2B5, HNK-1, rHIgM22, except high-affinity anti-Lingo IgG antibodies, which stimulate remyelination in rodents but do not have NAb features).

Characterization of Remyelination-Promoting Antibodies

All remyelination promoting antibodies with known antigens are polyreactive, which is the result of their rather flexible antigen binding site. Thus, antibodies with identified antigens bind to at least one or multiple sphingolipids, which are glycosylated lipids with ceramide or sphingosine backbone and essential lipid raft components. Only the hydrophilic carbohydrate moiety of the sphingolipids is exposed to the cell surface and, therefore, detectable by antibodies. This emphasizes the carbohydrate moiety and excludes the lipid backbone as the essential part of the antigen.

The dissociation constants (K_d) of the monoclonal (mouse) remyelination promoting IgMs O4 and O1 are unusually high for polyreactive natural antibodies ($\sim 0.9 \times 10^{-9}$ M for O4 and O1 compared to K_d's of typically 10^{-4} to 10^{-7} M for natural antibodies) [100](Table 1). A study by Paz Soldan et al. [101] indicated that all tested remyelination promoting IgMs induce a Ca^{2+}-influx in astrocytes (GFAP+), OPCs and immature OLs [101]. IgM-mediated effects in astrocytes and oligodendrocytes were, however, independent from each other, based on different signaling mechanisms and based on different Ca^{2+}pools (ER-stored Ca^{2+} for astrocytes and extracellular Ca^{2+} for oligodendrocytes) [101] . The AMPA glutamate receptor was shown to be responsible for IgM-mediated calcium-influx into OPCs and immature OLs whereas IgMs-stimulated calcium-influx into astrocytes was mediated through phospholipase C-mediated generation of IP3 and subsequent gating of IP3-sensitive channels [101]. In summary, all well-characterized remyelination promoting antibodies are of germline origin, belong to the IgM isotype and induce calcium influx into astrocytes, OPCs and immature OLs.

Common Antigens And Mechanistic Theories for Remyelination Promoting Antibodies

Common antigens

The ganglioside-binding antibody A2B5 targets several sialylated glycosphingolipids

(= gangliosides) due to their similar carbohydrate epitope [102]. HNK-1 recognizes the glycosphingolipid 3-sulfoglucuronyl paragloboside (SGPG) [103] as well as the carbohydrate epitope of the glycoproteins MAG and P0 [103]. The mouse IgM O1 binds to galactocerebroside and similar glycosphingolipids [104] , whereas O4 targets sulfated galactocerebroside (sulfatide), seminolipid, the unknown pro-oligodendroblast antigen (POA) and cholesterol (O4) [105].

Figure 1 shows by double immunofluorescence primary oligodendrocyte-lineage cells targeted by the human remyelination promoting antibody rHIgM22 (red in all overlays) combined with mouse remyelination promoting antibodies A2B5, O4, and O1 (all IgMs, all green in overlays) or commercially available anti-MBP, anti-Olig-2 and anti-MOG antibodies (all IgGs, all green in overlays) (30 minutes live staining on ice to prevent endocytosis of antibodies before fixation and possibly permeabilization) (Figure 1).

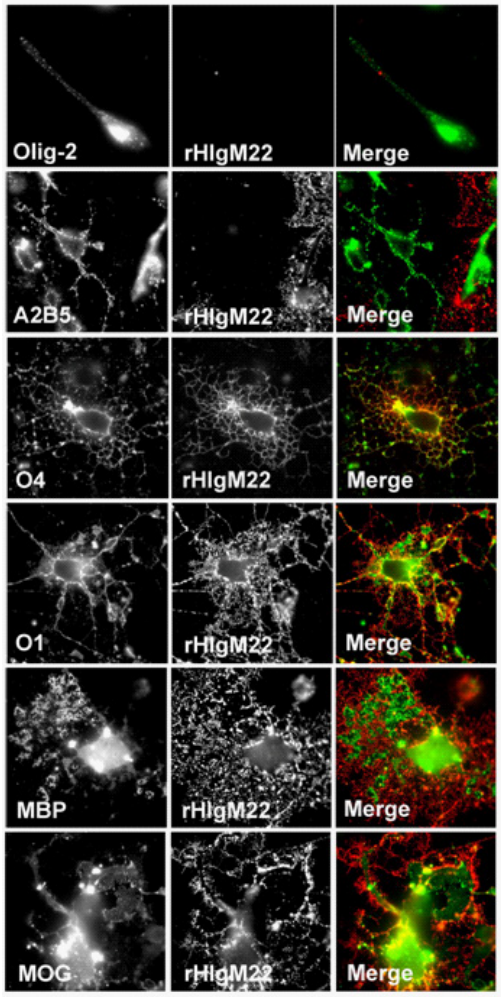

Figure 1: 30 minutes live staining on ice to prevent endocytosis of antibodies before fixation and possibly permeabilization.

A more recent study shifted our focus from glycosphingolipids to glycoproteins as an alternative class of antigens for remyelination promoting antibodies. As mentioned earlier, the carbohydrate, but not the lipid moiety of sphingolipids, is accessible to antibodies and may be sufficient for antibody binding when linked to either a lipid or protein backbone. The relatively simple carbohydrate structure on sphingolipids compared to the complex glycosylation found on many glycoproteins suggests that carbohydrate building blocks responsible for antibody binding can be found on glycoproteins as well. Inoko et al. [106] demonstrated binding of the remyelination promoting IgM A2B5 to a novel set of brain-derived glycoproteins as shown by Western blots [106]. It is well accepted that A2B5 binds to c-series gangliosides GT3 and GQ1c. However, c-series gangliosides and their O-acetyl derivatives are majorly expressed during early developmental stages in the CNS but seldom during adulthood in vertebrates [107]. Cerebellar stellate neurons are the only known exception in the adult human CNS that express c-series gangliosides [108]. This raises the question whether CNS remyelination promotion in adult mice by A2B5 is mediated through binding to glycosphingolipids or glycoproteins [109].

Mechanistic Strategies

Glycosphingolipids and cholesterol are essential components of lipid rafts, which act as signaling platforms at the level of the plasma membrane in cells. Lipid rafts may enable or disable interactions between many different cell-surface receptors (proteins) to transduce extracellular stimuli over the plasma membrane into the cytoplasmic space (general lipid raft concept). Pentameric IgM molecules can bind and cluster up to ten antigens at a time on different cells. We hypothesize that IgMs targeting glycosphingolipids stabilize existing rafts or stimulate the formation of new lipid rafts at the plasma membrane, thereby enhancing the effects of extracellular stimuli via existing cellular signaling pathways (**lipid raft hypothesis**).

Alternatively, remyelination-promoting IgMs may be involved in the opsonization of cellular debris and dead or apoptotic cells in a lesion site. Remyelination promoting antibodies 94.03 and 79.08 [109], O1 and the human sulfatide binding IgM DS1F8 (Kirschning et al. 1999) prominently stain filaments in astrocytes or HeLa cells (when stained with antibodies after fixation and permeabilization), which are identified as microtubule-like structures [110]. Figure 2 emphasizes binding of remyelination promoting antibodies 94.03 and 79.03 to filamentous structures present in astrocytes (arrows) (Figure 2). Binding to intracellular filamentous structures is also common with antibodies targeting galactosylceramide and sulfatide [111]. This may indicate epitope similarities between oligodendrocyte specific glycosphingolipids present at the cell surface and in internal pools relative to intracellular cytoskeletal proteins detected by polyreactive antibodies. The ability of certain remyelination promoting antibodies to target both membrane lipids and attached cytoskeletal proteins may significantly facilitate lesion clearance by the immune system and help to repopulate demyelinated areas with oligodendrocyte progenitor cells.

In summary, all remyelination promoting IgM mAbs bind to glycosphingolipids and potentially to glycoproteins at the cell surface of OPCs/OLs. It remains elusive whether effects seen *in vivo*

and *in vitro* are mediated through binding to cell-surface glycosphingolipids, glycoproteins and/or internal cytoskeletal structures. Given the fact that antibody repair is mediated by a single peripheral injection, it is possible that IgM-induced repair mechanism may also be mediated by peripheral factors that seep into the CNS.

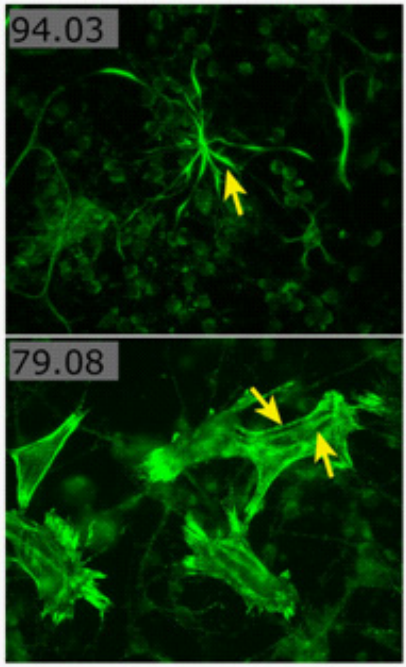

Figure 2: Binding of remyelination promoting antibodies 94.03 and 79.03 to filamentous structures present in astrocytes.

Low amounts of rHIgM22 are effective to stimulate remyelination in TMEV-infected animals

The effective dose of rHIgM22 to stimulate spinal cord remyelination in TMEV-infected mice is as low as 500 ng per mouse when administered i.p. in a single bolus injection. Remyelination was completed five weeks after the IgM injection. Higher antibody doses (up to 1000 fold) were not more effective in enhancing higher levels of remyelination compared to lower antibody doses [99]. It has been assumed that this is the limitation of the model during the chronic axonal phase of the disease. In addition, multiple antibody doses of rHIgM22 in TMEV-infected mice were not more effective compared to a single antibody dose.

In summary, rHIgM22 is a highly potent therapeutic in mice, mediates its effects within weeks and causes long-lasting tissue repair. It also suggests a molecular and cellular memory effect long after destruction of the antibody.

The integrity of the IgM molecule is required to stimulate remyelination

Chemical or enzymatical cleavage of rHIgM22 into pseudo-IgG monomers resulted in lack of efficacy for the antibody to stimulate remyelination. Similarly, switching the antibody class from IgM to IgG for the mouse monoclonal IgM 94.03 or the human antibody rHIgM22 led to antibodies that had effects similar to PBS and entirely different compared to the intact pentameric IgM molecule with respect to stimulation of remyelination. This may either suggest that antigen clustering on a single living cell or clustering of multiple cells by the IgM molecule is essential for its cellular effects.

NATURAL ANTIBODIES AND THEIR POTENTIAL BENEFICIAL OR DELETERIOUS EFFECTS IN THE PERIPHERAL NERVOUS SYSTEM

Elevated levels of certain immunoglobulins, termed autoantibodies targeting glycosphingolipids and the myelin protein MAG, are sometimes found in peripheral neuropathies [111]. The constant association of anti-glycosphingolipid antibodies with peripheral nervous system (PNS) dysimmune neuropathies supports a pathogenic link between anti-glycosphingolipid antibodies and neuropathy (e.g., Miller Fisher syndrome (MFS); Guillain-Barre syndrome (GBS); neuropathy associated with IgM monoclonal gammopathy (PN+IgM); chronic inflammatory demyelinating polyneuropathy (CIDP)). This includes immunoglobulins of the isotypes IgG, IgA and IgM. Anti-MAG antibodies and >20 different glycosphingolipids have been associated with chronic and acute peripheral neuropathic syndromes of including GM1b, GD1a, GM1, GalNAc-GD1a (acute motor axonal neuropathy), GQ1b, GD3, GD1b, GT1a (sensory variants of GBS) [112] and sulfatide (GBS, CIDP) [113].

In contrast to their suggested involvement in peripheral neuropathies, gangliosides promote neurite outgrowth and regeneration both *in vivo* and *in vitro* [114]. Clinical trials using purified gangliosides in neuromuscular disorders did not show sufficient efficacy but also did not give rise to an immune response followed by increased pathology [115]. Intramuscular doses of purified bovine ganglioside mixtures were used therapeutically for years throughout Europe, and it is widely believed that these protein-free sphingolipids are not antigenic and do not elicit immune-mediated side-effects. Experimentally, several studies failed to show that gangliosides enhance autoimmune demyelination in the PNS [116] and did not induce neurological signs of neuropathies or neuropathological changes [117]. In addition, passive transfer of anti-GM1 antibodies failed to transfer the disease [118]. All of this argues against the hypothesis that antibodies targeting glycosphingolipids are involved in the pathogenesis of peripheral neuropathies.

Interestingly, antibodies associated with peripheral neuropathies can bind to similar or identical antigens as remyelination-promoting antibodies and can be of IgM isotype similar to remyelination-promoting antibodies. At least some antibodies associated with peripheral neuropathies may act through complement fixation [119], which results in pore formation and cellular destruction after plasma-membrane binding. In contrast to these antibodies, the

remyelination-promoting antibody rHIgM22 does not fix complement and does not target Schwann cells or peripheral nerves (unpublished data). Toxicology studies in primates and rodents using 1000-fold higher amounts of rHIgM22 than the therapeutic dose demonstrated no pathological effects in the PNS. Lack of antibody binding to peripheral nerves, however, suggests that rHIgM22 does not stimulate remyelination in the peripheral nervous system.

Unlike rHIgM22, remyelination-promoting antibodies O4 (sulfatide), O1 (galactosylceramide) and HNK1 (anti-SGPG, MAG) do bind to cell surface antigens on Schwann cells. It has not been determined whether these antibodies stimulate remyelination in the PNS. Surprisingly, antibodies associated with peripheral neuropathies target the same antigens as the mentioned promoters of remyelination (see above). The different outcome of various antibodies targeting identical antigens on myelinating oligodendrocytes versus Schwann cells raises the question whether antibodies associated with peripheral neuropathies actively exacerbate the disease course or merely represent a bystander effect. Generation of anti-sphingolipid antibodies may occur after the axonal and myelin destruction due to increasing amounts of cellular debris.

Given the number of different diseases covered under the umbrella of "peripheral neuropathies" and their individual complexity, it is extremely difficult to extrapolate which antibodies actively participate in the pathogenesis of different neuropathies based on clinical studies using immunosuppressive and immunomodulatory drugs. However, the efficacy of current immune therapies such as rituximab, prednisolone and cyclophosphamide in neuropathies with anti-MAG IgM antibodies remains unproven [120]. Plasma exchange (PE) seems to be effective in patients with paraproteinemic neuropathies associated with high IgG or IgA antibodies but not IgM antibodies [121]. Similarly, corticosteroids, when administered in monotheraphy, were not effective in IgM-associated neuropathies [122]. In support of suggested differences between different antibody isotypes in paraproteinemic neuropathies, IgM-associated distal demyelinating symmetric neuropathies respond rather poorly to immunosuppressive therapy [123].

We conclude that IgMs targeting sphingolipids are unlikely pathogenic. However, it remains elusive whether remyelination promoting IgM antibodies O4, O1 and HNK-1 are beneficial for patients with peripheral neuropathies.

CONCLUSIONS

There is an immediate need to identify new treatment strategies for basically all neurologic diseases including MS and neurodegenerative diseases. In demyelinating diseases like MS the therapeutic focus should be on strategies that actively induce brain lesion repair and stimulation of remyelination. Targeting the immune system did not prevent or reverse long term disabilities. Future prospects for remyelination therapies are encouraging. Potential combinatorial therapeutic approaches could include agents that target the immune system to eliminate deleterious immune system-mediated injury and perhaps anti-LINGO antibodies or rHIgM22 that stimulate remyelination. Indeed, human monoclonal IgMs that target the CNS may enhance the permissive

environment for regenerating lost myelin. The very low amount of the human antibody rHIgM22 necessary for stimulation of remyelination in mice and an open BBB during acute phases of the human disease are encouraging and give hope for MS patients.

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