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The Immunological Basis for Treatment of Multiple Sclerosis

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Abstract

During the last few years, the concept of multiple sclerosis (MS) as a pure inflammatory disease mediated by myelin reactive T cells has been challenged. Neither the specificity nor the mechanisms triggering or perpetuating the immune response are understood. Genetic studies have so far not identified therapeutic targets outside the HLA complex, but epidemiological and immunological studies have suggested putative pathogenetic factors which may be important in therapy or prevention, including the Epstein–Barr virus and vitamin D. Advances in the treatment of MS have been reached by manipulating the immune response where the pathogenesis of MS intersects experimental autoimmune encephalomyelitis, most recently by blocking T-cell migration through the blood–brain barrier. Antigen-specific approaches are effective in experimental models driven by a focused immune response against defined autoantigens, but MS may not fit into this concept. Novel candidate autoantigens which are not constitutively expressed in the brain, such as protein α-B crystallin or IgG V-region idiotopes, as well as evidence of pathogenetic heterogeneity and complexity, suggest that treating MS by tolerizing the immune system against an universal MS antigen may be a *fata morgana.* Further characterization of MS subtypes may lead to individualized treatment. However, shared immunological features, such as intrathecal production of oligoclonal IgG, suggest that potential therapeutic targets may be shared by most MS patients.

Introduction

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS). The disease affects approximately 2.5 million persons worldwide, and is second only to trauma as the cause of acquired neurological disability in young adults. MS displays remarkable clinical heterogeneity. More than 80% present with relapsing remitting MS, with full or partial recovery of neurological deficits between the relapses. Most of these patients develop secondary progressive MS, with progressive clinical deterioration. Approximately 10–15% of the patients have primary progressive MS without evident relapses from the onset. Fifteen per cent of the patients with relapsing remitting MS have benign disease course with minimal disability after 15 years, but a majority of them develops a substantial neurological handicap. Patients with primary progressive MS have no effect of available immunomodulatory therapies, and face the most severe prognosis.

The basic pathology characterized by perivascular leucocyte infiltration and axonal transsection was recognized in the middle of the 18th century, and the intrathecal synthesis of IgG was described during World War II [1]. The animal model experimental autoimmune encephalomyelitis (EAE) was developed more than 75 years ago to study acute disseminated encephalomyelitis complicating vaccination with rabies virus grown in brain tissue [2]. Several therapeutic targets in MS have been identified in the EAE model, and therapeutic progress has mainly been achieved where the pathogenesis of MS and EAE intersects, such as the transmigration of lymphocytes across the blood–brain barrier. During the last 15 years, five immunomodulatory drugs have been approved for the treatment of relapsing remitting MS, and several others have entered clinical trials (Table 1).

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Table 1 Some current and emerging therapeutic agents in MS, extended from [100] by information from National Institute of Health website http://www.clinicaltrials.gov/ and National MS society website http://www.nationalmssociety.org/.

Disease component	MS type	Assumed mechanism	Target	Agent	Current status
Inflammation	Relapsing-remitting	Downregulating MHC and costimulation	Type 1 IFNR	IFN-β1a, IFN-β1b	Approved/phase IV
		Blocking lymphocyte trafficking	$A_4\beta_1$-integrin	Natalizumab	Approved
			S1PR	Fingolimod	Phase III
		Th1–Th2 shift, Treg, trophic factors	HLA, TCR	Glatiramer acetate	Approved/phase IV
		Reduce T-cell activation	PPAR-γ	Pioglitazone	Phase 1
			HMG-CoA reductase	Statins	Phase II/III
			IL-2R	Dacluzimab	Phase II
			Vitamin D receptor	Vitamin D	Phase II completed
		B-cell depletion	CD20	Rituximab	Phase III
		Leucocyte depletion	CD52	Alemtuzumab	Phase III (suspended)
			Adenosine deaminase	Cladribine	Phase III
			DNA	Cyclofosfamide	Phase II
				Mitxantrone	Approved/phase IV
Neurodegeneration	Progressive	Blocking sodium influx	Sodium channels	Lamotrigine	Phase II
		Brain-derived nerve growth factor	TrkB	Glatiramer acetate	Approved/phase IV
		Blocking glutamate neurotransmission	NMDA receptors	Memantine	Phase II
		Growth factors	Demyelinated neurons	Mesenchymal stem cells	Phase 1/IIA

IFNR, interferon receptor; PPAR, peroxisome proliferator-activated receptor; S1PR, sphingosine-1-phosphate receptor; TrkB, tyrosine kinase receptor B.

Current and emerging MS therapy is only partially effective, particularly because the long-term effect on progression of disability is poor. Interferons and glatiramer acetate are immunomodulators which modulate T-cell activation and reduce inflammatory mediators (Table 1), and reduce the relapse rate in relapsing remitting MS with one-third. The anti-neoplastic drug mitoxantrone suppresses the proliferation of lymphocytes and macrophages, impairs antigen presentation and inhibit B-cell function and antibody production, and is used for severe forms of relapsing remitting and secondary progressive MS. Natalizumab targets $\alpha 4\beta 1$-integrin mediated cell migration across the blood–brain barrier, and is probably more effective than glatiramer acetate and interferons [3], but is associated with an 1:1500 annual risk of progressive multifocal leukoencephalopathy (PML). PML is a lethal or devastating opportunistic infection caused by JC virus, and is probably a consequence of reduced immunosurveillance of the brain. Fingolimod, also called FTY720, reduced relapse rate by almost 50% in a proof-of-concept study [4]. Fingolimod inactivates sphingosine-1-phosphate receptors which are necessary for thymocytes and lymphocytes to egress from the thymus and secondary lymphoid organs, where the cells become sequestered [5]. Opportunistic infections may, therefore, be a concern also for fingolimod.

In our view, the main factor limiting the improvement of MS therapy is not limited translation from basic research to pharmaceutical agents, but rather the restricted knowledge of the aetiology and pathogenesis of MS. The mechanisms breaking immune tolerance, the specificity and pathogenetic significance of the immune response, the mechanisms perpetuating it and the relationship between inflammation and neuronal degeneration are not fully understood. In this paper, we explore some aspects of MS which are poorly mirrored by current animal models, and point out possible implications for therapy.

Aetiology: genes

A genetic basis for MS is evident from the concordance rate of 13–30% in monozygotic twins and 3% in dizygotic twins [6]. It is commonly believed that this leaves at least 70% of MS aetiology to environmental factors. However, it is often forgotten that monozygotic twins cease to be genetically identical as the immune system develops, because V(D)J recombination in the T-cell receptor (TCR) and immunoglobulin (Ig) genes and the somatic hypermutation in immunoglobulin V-genes will lead to a different set of TCR and Ig. Thus, the stochastic factors involved in MS development may be the random generation of B-cell receptors and TCR [7]. Association with HLA-A3 was noticed in 1972 [8], and was soon found to be secondary to a primary association with HLA-DR2. Since then, several association studies and genome wide linkage screens have been performed, and the only association that has been shown consistently in Northern Europeans is to the HLA-DR15 haplotype

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(DRB1*1501, DQB1*0602). This association is quite strong (relative risk approximately 4), but carrying the HLA- DR15 haplotype has little or no influence on the disease course or the severity [9]. There is, however, a gene-dose effect, as homozygosity for HLA-DR15 is associated with severe MS [10]. A dose effect of HLA class II genes is also observed in celiac disease [11], probably caused by enhanced presentation of gluten peptides [12]. A similar mechanism could be operating in MS, but cannot be established as long as the target antigen of the immune response has not been defined.

Association with HLA class II supports a prominent role for $CD4^+$ T cells in MS, and the identification of the susceptibility alleles is important in attempts to develop specific immunotherapy. However, strong linkage disequilibrium makes it difficult to establish whether DRB1*1501 or DQB1*0602 confer the disease risk. Some Norwegian MS patients carry DQB1*0602 and not DRB1*1501, while none have DRB1*1501 in the absence of DQB1*0602, suggesting that DQB1*0602 is the primary susceptibility allele [13]. In line with this, association to HLA-DQB1*0602 and not to HLA-DRB1 alleles was found in Afro-Brazilian MS patients [14]. However, later studies on Afro-Americans and Sardinians suggest that MS is most closely associated with HLA DRB1*1501 rather than DQB1*0602 [15].

Although association of genome wide significance to loci outside the HLA region has not been found in MS, approximately 50 such loci have been identified in rodent EAE models [16]. MS is extremely heterogenous, and polymorphisms in non-HLA genes might be important in subgroups of patients, as suggested by their association with cytotoxic T-lymphocyte antigen (CTLA)-4 in MS patients from families with accumulation of other autoimmune diseases [17]. Subgrouping of MS patients is being hampered by the lack of available biomarkers. However, antibodies against aquaporin (AQP)-4 were recently found in serum from NMO patients, a demyelinating disease of the optic nerves and spinal cord closely related to MS, and is now used as a biomarker to distinguish NMO from common MS [18] This observation supports that subgroups of common MS may also be immunologically distinguishable, and respond differently to immunological treatment.

Aetiology: environmental triggers of disease

Multiple sclerosis is most frequent in industrialized countries with temperate climate. People migrating from low- to high-risk areas before the age of 15 acquire an increased MS risk, suggesting that environmental factors in early life trigger MS. This is supported by the emergence of MS among the black population of the Caribbean islands, where MS has been rare [19]. Increase in MS incidence is most prominent in Martinique, which has received a substantial 'return migration' from metropolitan France, where MS is more common. Those who had lived in metropolitan France until 15 years of age had the highest MS risk. Studies of adoptees and step-siblings suggest that familial clustering of MS is caused by shared genes and not by shared environment [20]. Environmental triggers of MS are, therefore, likely to be widely distributed in areas where MS is common, and not rare microbes or toxins selectively striking those who subsequently develop MS.

Epstein–Barr virus (EBV) infection and vitamin D deficiency are examples illustrating the value of combining epidemiology and immunology. EBV infects a majority of the population. Delayed primary infection is common in developed countries and is associated with infectious mononucleosis, which increases MS risk with a factor of approximately 2.5 [21]. EBV infection is closely associated with MS, because virtually all MS patients are EBV seropositive [22], including children who are otherwise often EBV seronegative [23]. Moreover, MS risk is strongly correlated with the titre of EBV nuclear antigen (EBNA)-antibodies prior to disease [24].

The mechanism linking MS and EBV is not established, but could involve the activation of myelin basic protein (MBP)-specific T cells by cross-recognition of EBV. This is supported by the finding that a T-cell clone from an MS patient cross-recognized an MBP peptide presented by DRα1*0101, DRβ1*1501 and an EBV peptide presented by DRα1*0101, DRβ1*0101 [25]. To test the relevance of cross-reactive T cells in MS, we generated DR-restricted CSF T-cell clones specific to the EBV peptide from an MS patient with the relevant DR alleles [26]. Eight of the 14 EBV-specific T-cell clones cross-recognized the MBP peptide, suggesting that cross-reactive T cells are prevalent in the CSF. However, it must be emphasized that EBV-specific T cells were only detected in CSF from one of the two patients studied, and that this MS patient displayed brisk proliferative T-cell responses to MBP in blood, which is quite uncommon, and the results may, therefore, not be fully representative for MS.

The association between MS and vitamin D was first suggested from observations of covariation between the MS incidence and fish consumption in Norway [27], and is supported by the north-south gradient of MS prevalence in Australia; the MS risk being more than seven times higher in Tasmania than in tropical Queensland [28]. MS incidence correlates inversely with past exposure to UV radiation [29], as well as vitamin D levels in the blood prior to onset of MS [30].1,25-dihydroxyvitamin D3 receptors are expressed on activated lymphocytes [31], and picomolar concentrations of 1,25-dihydroxyvitamin D3 suppress IL-2 induced T-cell proliferation [32]. 1,25-dihydroxyvitamin D3 has been shown to prevent and suppress progression of EAE [33]. Suppression of EAE is

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associated with the modulation of the JAK/STAT pathway in the IL12/IFN-γ axis, leading to Th2 differentiation [34]. 1,25-dihydroxyvitamin D3 fails to inhibit EAE in IL-10 deficient mice, and may enhance an IL-10 dependent anti-inflammatory loop [35]. Vitamin D supplementation to MS patients increased serum TGF-β levels [36], but the clinical effect of vitamin D supplementation is not settled.

Inflammation versus neurodegeneration

Active white matter MS lesions are characterized by activated microglia and macrophages containing myelin debris, reactive astrocytes, T-cell infiltration, a few B cell and plasma cells, demyelinated axons and variable axonal destruction [37]. Thus, MS involves both inflammation and neurodegeneration, but the temporal and causal relationship between these components of MS is controversial, and could differ between the various regions of the brain. MS has been regarded as a disease of the white matter but during the last few years, widespread grey matter involvement has been re-discovered. Whereas demyelination is a shared feature between white and grey matter MS lesions, inflammation is much less prominent in grey matter compared with white matter lesions. The number of T cells and macrophages in cortical MS lesions is comparable to that of cortex from non-neurological control patients [38]. The extent of grey matter involvement has so far been hard to study *in vivo,* but seems to be most prominent in the late stages of the disease.

T-cell infiltrates are present in the spinal cord also from patients with neurodegenerative diseases like amyotrophic lateral sclerosis [39]. An extreme view would be that MS is a primary degenerative disease, with a secondary immune response which could be either reparative, detrimental or both. This view is supported by observations in a study of biopsies and autopsies from acute MS cases, showing that activation of microglia and oligodendrocyte apoptosis preceded T-cell infiltration [40]. Interestingly, experimental data show that MBP-specific T cells may contribute to protection against the CNS damage after trauma. After partial crush injuries of the optic nerve or spinal cord, rats injected with MBP-specific T cells recovered better than control rats injected with OVA-specific T cells [41]. MBP-specific T cells accumulated in the lesions, suggesting that protection is mediated by infiltrating T cells. Activated MBP-specific T cells produce brain-derived nerve growth factor upon activation, and neuroprotective effects of T cells may involve secretion of trophic factors [42].

During the last few years, the heterogeneity of MS has extended to comprise the pathology of active demyelinating lesions, which may reflect different pathogenetic pathways in MS. Based on 51 biopsies and 32 autopsies, Lucchinetti et al. [43] identified four patterns of white matter demyelination. T cells were present in all patterns, but pattern I was compatible with demyelination induced by macrophages and their toxic products, pattern II by antibodies and complement, and pattern III and IV with virus or toxins rather than immune-mediated cytotoxicity. Only one pattern was present in each patient. The relevance of this subtyping was recently supported by a therapeutic trial of plasma exchange: All the 10 patients with pattern II, but none of the nine patients with patterns either I or III responded favourably [44]. However, it must be emphasized that the subtyping is based on a highly selected material which probably has an over-representation of atypical MS cases, as biopsy is not performed as a diagnostic procedure in MS unless other diseases like tumour, infection or vasculitis are suspected.

T cells in MS and EAE

$CD4^+$ T-cell responses against MBP, myelin oligodendrocyte protein (MOG), myelin associated protein (MAG) and proteolipid protein (PLP) have been extensively studied in EAE and MS, reviewed in [45]. To some extent, human and murine immunodominant epitopes overlap. In MS, HLA DRB1*1501 restricted $CD4^+$ T-cell responses have been found particularly against MBP 85–99 [46, 47], but T cells from both MS patients and controls seem to recognize several epitopes spread throughout the MBP molecule [48].

In EAE, immunization with adjuvant and myelin proteins or adoptive transfer of activated myelin specific $CD4^+$ T cells elicits a Th1-cell response that orchestrates an attack on CNS myelin. Furthermore, EAE develops spontaneously in transgenic mice expressing human TCR specific for MBP, HLA-DRα1*0101, DRβ1*1501 and human CD4 [49]. The prominent role for myelin-specific $CD4^+$ T cells in MS is less obvious. MBP-specific $CD4^+$ T cells are part of the normal naïve T-cell repertoire and have been repeatedly detected in comparable frequencies in the blood of MS patients and healthy controls in proliferation assays [46, 50–53]. Thus, it is not evident that tolerance to myelin proteins is broken in MS.

Evidence supporting a role for myelin-specific $CD4^+$ T cells in MS includes increased frequencies of MBP-, PLP- and MAG-specific $CD4^+$ T cells in blood and CSF detected in ELISPOT assays compared with controls [54, 55], and the elevated precursor frequency in the blood of $CD4^+$ T cells specific for MBP 84–102 during clinical exacerbations [56]. Furthermore, MBP-specific $CD4^+$ T cells in blood are clonally expanded [47]. It has also been reported that MBP reactive T cells from MS patients display increased number of mutations in the hypoxanthine guanine phosphoribosyltransferase gene, which is a marker of the cell division [57].

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The encephalitogenic potential of myelin-specific $CD4^+$ T cells in humans was demonstrated in a clinical study of an altered peptide ligand corresponding to MBP 83–99. Subcutaneous injection of this altered peptide ligand was followed by clinical relapses and emergence of Th1 cells cross-recognizing the altered peptide ligand and MBP 83–99 [58]. However, this observation could just as well be the effect of non-physiological activation of MBP-specific T cells being present in the naïve repertoire, rather than being mediated by T cells of general pathogenic significance in MS.

Studies on $CD8^+$ T cells have played a minor role in MS and EAE research, although $CD8^+$ T cells outnumber $CD4^+$ T cells in the centre of MS lesions [59]. Clonal expansion of CSF and infiltrating $CD8^+$ T cells is more prominent than for $CD4^+$ T cells [60]. In one patient, 35% of infiltrating T cells belonged to a single $CD8^+$ T-cell clone, and $CD8^+$ T-cell clones were detected in the CSF several years after their initial discovery in CNS plaques [61]. Recently, it was shown that EAE could be induced by adoptive transfer of MBP- and MOG-specific $CD8^+$ T cells [62, 63]. Little is known about the specificity of $CD8^+$ T cells in MS. Increased precursor frequencies of $CD8^+$ T cells specific for transaldolase, as well as MBP 111–119 and MBP 87–95 have been reported in blood of HLA-A2 positive MS patients [64, 65].

Antigen-specific therapies

Given the limited effect and possible serious adverse effects of currently used MS treatment, antigen-specific therapy is an attractive approach. Prevention of EAE by injection of CNS homogenates was demonstrated 50 years ago [66], suggesting the therapeutic potential in human demyelinating disease [67]. Several approaches, including oral and intravenous administration of myelin proteins and peptides, antibodies against TCR of myelin-specific T cells and vaccination with myelin-specific T cells ameliorate or prevent EAE, reviewed in [68, 69]. Some promising results have also been achieved in MS. In a phase II clinical trial, intravenous infusions of an immunodominant MBP 82–98 peptide suppressed anti-MBP antibodies in CSF and delayed disease progression in all the 20 patients carrying the HLA haplotypes DR2 or DR4 [70]. A phase II study of infusion of 5, 20 or 50 mg of a peptide corresponding to MBP 82–98 with altered contact sites for the TCR, but preserved binding sites to HLA, was suspended due to anaphylactic reactions [71]. A Th2 response against the peptide aroused within 1 week and waned after 1 month, and was followed by a Th2 response against native MBP after 6–10 weeks. However, the only detectable clinical effect was a reduction in inflammatory activity measured radiologically in the subgroup which received 5 mg of altered peptide.

The concept of T-cell vaccination in MS emerged from the observation that the injection of attenuated MBP-specific T cells prevented EAE [72]. T-cell vaccination in autoimmune diseases is based on the assumption that TCR from autoaggressive T cells carry idiotopes within their hypervariable regions, which could be targeted by an idiotype-specific regulatory network [73]. Rapid depletion of myelin-specific T cells is mediated by $CD8^+$ anti-idiotypic $CD8^+$ T cells [74]. Furthermore, immunization with activated T cells induces immune responses against cellular activation markers, such as the IL-2 receptor α-chain and heat-shock protein 60 [75, 76]. In a recent vaccination study of MS patients with autologous T cells, regulatory T cells expanded by the vaccine specifically recognized peptides from the IL-2 receptor receptor α-chain. Similar results, accompanied by substantial clinical improvement, have been obtained in rheumatoid arthritis [77]. The clinical effect of T-cell vaccination in MS has not yet been established, but clinical trials are ongoing.

So far, clinical results of antigen-specific treatment of MS based on myelin proteins and myelin-specific T cells have generally been disappointing. An example was the negative results of oral administration of 8 mg MBP and 15 mg PLP in a phase III study including 515 MS patients [78], which followed the promising results obtained in a pilot study of oral tolerization with bovine myelin [79]. In addition to technical questions related to strategy of tolerance induction, antigen-specific therapies in MS face a fundamental problem as the target antigens have not been firmly identified. A prerequisite for antigen-specific treatment is the existence and identification of a dominant immunogen in each patient. T-cell responses against myelin antigens in MS are polyclonal and target diversified T-cell epitopes, and the pathogenetic significance is unknown and could be heterogeneous. Thus, antigens involved in EAE could be less relevant in MS, or epitope spreading could have broadened the specificity of the immune response beyond the initial trigger.

Another problem is the timing of treatment. As other experimental treatments, antigen-specific therapies are often offered to patients in an advanced stage of the disease. At this stage, degenerative processes may have become independent of inflammation. In line with this, treatment of secondary progressive MS patients with alemtuzumab, a monoclonal antibody targeting CD52 on all T cells and B cells, almost blocked intrathecal inflammation, but did not hinder the progression of brain atrophy and clinical disability [80].

Novel candidate T-cell target antigens in MS

During the last several years, two novel candidate T-cell autoantigens, which are not constitutively expressed in

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the brain or characterized in EAE, have been suggested in MS.

α-B crystallin

The small heat-shock protein α-B crystalline was identified by the stimulation of human T cells with fractions of myelin proteins from brain specimens [81]. The stimulating fraction was only detectable in MS brains, and was identified as α-B crystallin. Expression of α-B crystallin is upregulated in oligodendrocytes from active MS lesions, where the protein is detectable within phagosomes of perivascular macrophages [82]. EBV-transformed human B cells express α-B crystalline, and α-B crystalline-specific T cells recognize autologous EBV-transformed B cells [83]. The 'mistaken self' hypothesis suggests that α-B crystallin-specific T cells become activated during EBV infection, and that these T cells recognize α-B crystallin expressed in the inflamed brain [84].

Mice constitutively express α-B crystallin in lymphoid tissue, and seem to be resistant to EAE induced by this antigen [85]. α-B crystallin-specific T cells can be generated in α-B crystallin-knockout mice, but adoptive transfer of such T cells did not induce EAE in wild type mice [86]. However, transfer of α-B crystalline-specific T cells induced EAE in mice infected with avirulent *Semliki forest virus*, supporting that inflammation of the target organ is essential for disease induction [87].

Immunoglobulin idiotopes as T-cell antigens

B cells may present endogenous V-region sequences on MHC class II molecules to idiotope-specific T cells [88, 89]. T cells are generally tolerant to germline-encoded IgG, and somatic mutations seem to be critical for T-cell recognition of V-region epitopes [90]. The relevance of T-cell responses to IgG idiotopes in autoimmune diseases has been demonstrated in systemic experimental lupus [91]. Furthermore, idiotope-driven T–B-cell collaboration elicits autoimmune disease in transgenic mice [92].

Perpetuating intrathecal synthesis of oligoclonal IgG of unknown specificity [93], as well as intrathecal synthesis of IgG against several viruses [94, 95], are immunological hallmarks of MS. Clonally expanded B cells from MS brains and CSF have undergone somatic hypermutation [96, 97]. Thus, these sequences are non-self to immune system, and could, therefore, be recognized by idiotope-specific T cells. To test this hypothesis, we analysed T-cell responses in blood from MS patients and controls against IgG purified from autologous CSF [53]. T cells from 14 of the 21 MS and four of the 17 control patients recognized autologous CSF IgG. The amount of IgG which could be purified from each patient did not allow mapping of T-cell epitopes. To overcome this problem, we established EBV-transformed B-cell lines from CSF of two MS patients, which produced monoclonal IgG [98]. T-cell clones from blood and CSF from both patients recognized autologous, but not heterologous, monoclonal CSF IgG in the context of HLA DRB1*1501- or DRB1*1302-encoded molecules, and a T-cell epitope was mapped to a mutated framework region. These results suggest that idiotope-driven T–B-cell collaboration could offer an explanation to the perpetuating intrathecal synthesis of IgG in the absence of an overt T-cell antigen in MS.

So far, neither the association between immunopathological heterogeneity of white matter lesions nor the extent of cortical involvement and intrathecal production of IgG has been established. However, a vast majority of the patients display intrathecal synthesis of oligoclonal IgG, and idiotope-driven T–B-cell collaboration could, therefore, be a shared phenomenon in between different pathogenetic subtypes of MS. This process is most likely to occur within inflamed white matter rich in lymphocytes, but could also take place in ectopic meningeal germinal centres which are found predominantly in the later stages of the disease.

Modulation of idiotope-driven T–B-cell collaboration may be a potential mechanism for B-cell directed therapy in MS, including plasma exchange, mitoxantrone and rituximab, because these treatments may modulate idiotope-driven T–B-cell collaboration by removing IgG and B cells carrying immunogenic idiotopes. It should be kept in mind that the knowledge of the precise mechanism of action of several drugs used in MS is limited, and that treatments designed to target T cells may also have a substantial effect on B cells. In line with this, it was recently reported that $\alpha 4\beta 1$-integrin was more abundantly expressed on $CD19^+$ B cells than $CD3^+$ T cells [99]. B cells carrying immunogenic idiotopes, might therefore be a target also for natalizumab.

Concluding remarks

The complexity of MS includes combinations of genetic predisposition, environmental triggers, clinical presentations and possibly pathogenetic mechanisms. The search for novel treatments could either focus on identification of therapeutic targets in particular subgroups of patients, or identification of common targets where the pathogenetic pathways merge. The perpetuating intrathecal production of oligoclonal IgG might be such a common pathway, because it occurs early and is shared by a vast majority of relapsing remitting MS patients. However, as for several other features of the disease, we face uncertainties concerning the exact pathogenetic role of this phenomenon; it could mediate both protective and detrimental effects. This question will hardly be answered *in vitro*, and calls for even closer collaboration

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between researchers working with animal models and humans.

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