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Immune surveillance of the central nervous system in multiple sclerosis– Relevance for therapy and experimental models

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Abstract

Treatment of central nervous system (CNS) autoimmune disorders frequently involves the reduction, or depletion of immune-competent cells. Alternatively, immune cells are being sequestered away from the target organ by interfering with their movement from secondary lymphoid organs, or their migration into tissues. These therapeutic strategies have been successful in multiple sclerosis (MS), the most prevalent autoimmune inflammatory disorder of the CNS. However, many of the agents that are currently approved or in clinical development also have severe potential adverse effects that stem from the very mechanisms that mediate their beneficial effects by interfering with CNS immune surveillance.

This review will outline the main cellular components of the innate and adaptive immune system that participate in host defense and maintain immune surveillance of the CNS. Their pathogenic role in MS and its animal model experimental autoimmune encephalomyelitis (EAE) is also discussed. Furthermore, an experimental model is introduced that may assist in evaluating the effect of therapeutic interventions on leukocyte homeostasis and function within the CNS. This model or similar models may become a useful tool in the repertoire of pre-clinical tests of pharmacological agents to better explore their potential for adverse events.

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Introduction

The presentation of foreign antigen constitutes a key event in immune surveillance and host defense. Antigen presenting cells (APC) of the innate immune system recognize, capture, and present antigen to T lymphocytes, which subsequently initiate the cellular adaptive immune response.

Within the central nervous system (CNS), three compartments, the parenchyma, the cerebral perivascular spaces (CPVS), and the subarachnoid spaces play a critical role in antigen presentation. In the parenchyma, astrocytes are the most abundant CNS glial cell population, but their role as APCs remains controversial [1–5]. The most potent intrinsic APCs within the CNS parenchyma are microglial cells, and recent findings appear to suggest an important role for microglia cells in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of the human inflammatory disorder multiple sclerosis (MS) [6]. Thus, microglia cells are likely to participate in CNS immune surveillance.

The second CNS compartment that plays a crucial role in CNS antigen presentation are CPVS, or “Virchow-Robin spaces”. There is now abundant evidence that hematopoetically-derived APCs, including monocyte-derived macrophages [7,8], and dendritic cells (DCs) [9] reside and present antigen in CPVS, and that cells in this compartment play an essential part in the initiation and perpetuation of CNS autoimmune disease. B cells, which together with T cells provide an antigen-specific adaptive immune response, are also competent APCs that are abundantly present in the CPVS during inflammation [10].

A third compartment where antigen presentation occurs, and which therefore be critical for CNS immune surveillance is the subarachnoid space. Kivisakk et al demonstrated in the EAE model that $CD4^+$ T cells that were polarized to produce T helper (Th) cell 1 or Th 17 cytokines accumulate within the subarachnoid space early in the disease course [11]. Specifically, $CD4^+$ T cells could be detected in the subarachnoid space before they entered the spinal cord parenchyma. Within the subarachnoid space, $CD4^+$ T cells proliferated, and time-lapse microscopy indicated that these cells actively scanned the tissue and interacted with local major histocompatibility (MHC) class II^+ APC.

Disruption of the innate or adaptive immune response within the CNS is likely beneficial in CNS autoimmunity. Not surprisingly, most pharmacological agents that are currently approved for the therapy of MS were specifically designed to either diminish the absolute number of immune-competent leukocytes and their function in the periphery and subsequently in the CNS, or to reduce the ability of leukocytes to enter the brain and the spinal cord. These strategies have resulted in a meaningful decrease in clinical and paraclinical disease activity, which are in turn relevant readouts of the immune system’s ability to present and process antigen in CNS autoimmunity. There is, however, a downside. As stated above, the primary biological role of leukocytes is not to cause autoimmunity, but to recognize and eliminate pathogens. Thus, the occurrence of opportunistic infections or neoplastic growth is perhaps the most meaningful biological readout of impairment of CNS immune surveillance. Not surprisingly, some of the more potent pharmacological agents that

have been utilized in MS carry with them side effects of CNS virus reactivation and in some extreme cases the development of progressive multifocal leukoencephalopathy (PML).

This article will outline the main cellular components of CNS immune surveillance, including its innate and adaptive components. It will also conceptualize experimental models that may allow the preclinical measurement of an impact that pharmacological interventions may have on host defense. These models are urgently required to estimate an acceptable risk-benefit ratio of individual therapies.

The innate immune system in CNS immune surveillance

Monocytes - Macrophages

In most tissues, the initial recognition of a pathogen is followed by activation of resident macrophages, and other tissue-resident cells, including DCs and mast cells. Tissue macrophages express numerous surface receptors that identify so-called pathogen-associated molecular patterns (PAMPs), as well as danger-associated molecular patterns (DAMPs). These receptors include lectins, scavenger receptors, Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, and retinoic acid-inducible gene (RIG)-I family receptors [12–14]. Following the initial microbe challenge, tissue macrophages attract and stimulate the extravasation of neutrophils and monocytes [15–17]. Monocyte-derived macrophages subsequently become the majority of myeloid cells within an inflammatory site. Experimental depletion of resident macrophages results in reduced host protection to infection, reduced expression of soluble inflammatory mediators, and diminished chemoattraction [15,17–19].

There is considerable heterogeneity with regard to the cellular composition and receptor usage between tissues. The central nervous system (CNS) constitutes a unique organ system in that it is confined entirely within a bony space. A robust inflammatory response and the ensuing edema could easily result in severe CNS damage or even a fatal outcome. Thus, the innate immune response within the CNS appears to be somewhat attenuated in most situations that present a threat to the host. Microglia are the tissue-intrinsic macrophages within the brain and spinal cord, and they are considered one of the key players in an initial immune response [20–22]. It is currently thought that microglia are a long-lived population of tissue macrophages, but it is unknown how cell populations of brain macrophages are maintained in homeostasis and during disease [21]. The somewhat immune-restrained basic phenotype of microglia may be explained at least partly by the fact that, in contrast to other tissue macrophages, these cells are shielded from serum proteins that can selectively and potently activate macrophages [23]. In addition, the cytokine composition within the healthy adult CNS is relatively anti-inflammatory with detectable levels of transforming growth factor(TGF)-β2 and TGF-β3 [24,25], and prostaglandin E2 (PGE2) [21].

The pathogenic role of microglia in CNS autoimmunity has been established in the EAE animal model of the human inflammatory disorder MS. In the EAE model, several investigators demonstrated the capability of microglia to initiate an adaptive (auto)immune response against target CNS antigens. It has been known that the induction of EAE after adoptive transfer of $CD4^+$ antigen-specific T cells requires restimulation with the cognate

autoantigen within the CNS compartment [26]. Experiments with radiation bone marrow chimeras, in which bone marrow–derived donor cells expressed a MHC II haplotype distinct from that present on recipient parenchymal microglia have provided clarity with respect to their role as APCs. Adoptive transfer of myelin-specific $CD4^+$ T cells restricted by the MHC II haplotype of the recipient parenchymal cells did not cause EAE. In contrast, myelin-specific $CD4^+$ T cells that were restricted by the MHC II haplotype of perivascular macrophages derived from the donor bone marrow inoculum led to clinical disease [7]. Other investigators demonstrated that the elimination of all potential APCs except for $CD11c^+$ perivascular macrophages did not affect disease susceptibility in experimental animals [9]. Another set of experiments, in which EAE was actively induced with one myelin epitope, followed by adoptive transfer of unprimed T cell receptor (TCR) transgenic T cells specific for a different antigenic determinant, showed that myelin-specific T cell proliferation occurs mainly in the CNS after disease onset [27].

In most instances, a self-limited inflammatory response is in the best interest of the host. Ongoing inflammation can have detrimental consequences on tissues. Thus, the fate of resident macrophage populations and their initial and ongoing role in the priming of adaptive immunity and tissue homeostasis has been under intense investigation. One group of investigators assessed macrophage subsets in mild zymosan peritonitis and observed a prolonged retention of inflammatory monocyte–derived macrophages in the lesion [28–30]. During acute inflammation, few resident $F4/80^{high}$ $Tim4^+$ macrophages can be recovered. Subsequently, substantial numbers of resident macrophages can be identified, which suggests that some resident macrophages remain at the inflammatory site.

It is unclear, however, whether observations made in other compartments can be translated to the CNS. For instance, a disappearance of alveolar macrophages from bronchoalveolar lavage fluid was shown by Lauder et al to occur during influenza infection [31]. The change in cellular composition may at least in part be due to the transport of antigen to the draining lymph node, as shown by Kirby et al. [32]. Many alveolar resident macrophages, however, remain throughout inflammation, suggesting that these cells impact inflammatory responses and subsequently return to homeostasis. It was shown that the number of tissue-resident macrophages is maintained by local proliferative self-renewal, and without the need for replenishment from the periphery, including in the brain [33]. However, it is not known what happens to inflammatory monocyte-derived macrophages in the CNS after the inflammatory response is resolved. In other compartments, these cells have been suggested to exit via the lymphatics or to recirculate [34–36]. The CNS, of course, does not have a lymphatic system. In addition, in experimental acute lung injury models, many inflammatory macrophages succumb to apoptotic cell death, while the resident alveolar cells persist [37]. It can also not be excluded that monocyte-derived macrophages in the CNS undergo *in situ* phenotypic conversion to become tissue-resident macrophages, which may occur during inflammation [38]. There is some knowledge on the kinetics of APC turnover in the CPVS. Lassmann et al demonstrated that meningeal and perivascular monocytes are replaced over the course of several weeks by hematogenous cells under normal conditions, and this turnover is accelerated during EAE [39]. Other investigators showed an ongoing migration of macrophages from peripheral blood into CPVS [40].

Dendritic cells

Mature DCs are established as the most competent APCs in the initiation of immune responses. DCs are currently classified into five distinct populations based on phenotypic, functional and developmental analysis [41], and include Langerhans cells, monocyte-derived DCs, plasmacytoid DCs, migratory and lymphoid tissue-resident $CD8^+$ DC-like DCs, and migratory and lymphoid tissue-resident $CD11b^+$ DCs. There is overwhelming data that contradict an old dogma that the healthy CNS parenchyma contains no DCs. Prodinger et al., using mice transcribing the green fluorescent protein (GFP) under the promoter of the DC marker CD11c, determined the distribution, phenotype, and source of $CD11c^+$ cells in non-diseased brains [42]. DCs were identified in periventricular areas, adjacent fiber tracts, and optical nerves. The majority of $CD11c^+$ cells were located within the juxtavascular parenchyma rather than the perivascular spaces.

In MS and EAE, DCs are readily detectable in the brain [42–48]. Fischer and Reichmann showed that CNS inflammation in EAE and toxoplasmic encephalitis was associated with the proliferation of $CD11b^+$ $CD11c^+$ cells [49]. These cells constituted up to 30% of the total $CD11b^+$ population of myeloid cells isolated from the brain. In both disease models, $CD11c^+$ cells were located in perivascular spaces and intraparenchymatic inflammatory sites, and displayed the surface phenotype of myeloid DCs. Morphologically, $CD11c^+$ cells from inflamed brain resembled bone marrow-derived DCs, and thus were identified as such. These cells secreted high levels of IL-12, and were more potent stimulators of naive or allogeneic T cell proliferation than microglia cells. Furthermore, DCs isolated from inflamed brain primed naive T cells from DO11.10 TCR transgenic mice to produce IFNγ and IL-2. A functional maturation of brain DCs occurred following the onset of encephalitis [49]. The role of DCs as relevant innate immune cells of the CNS was underscored by work by Greter et al, who showed that $CD11c^+$ DCs alone are sufficient to present antigen *in vivo* in EAE to primed myelin-reactive T cells in order to mediate CNS inflammation and clinical disease [9].

It was also recently shown that steady-state DCs are capable of inducing peripheral T cell tolerance, and of regulating CNS inflammation. Yogev and colleagues used genetic approaches to deplete $CD11c^+$ DCs in mice [50]. Experimental animals that lack DCs developed more severe EAE than control mice. DCs engineered to present a CNS-associated autoantigen in an inducible manner were capable of inducing peripheral T cell tolerance and EAE resistance. This tolerance coincided with an upregulation of the PD-1 receptor on antigen-specific T cells. PD-1 was necessary for DC-mediated induction of regulatory T cells [50]. These findings were supported by the work of Zozulya et al, who demonstrated that intracerebral microinjection of DCs modified by tumor necrosis factor resulted in relative EAE disease resistance, and triggered the generation of IL-10-producing antigen-specific T cells in the periphery, and restricted IL-17 production within the CNS [47].

Thus, while DCs are clearly critical cellular players in innate and adaptive immune responses within the CNS, DC responses can also be modulated to downregulate inflammation.

The adaptive immune system in CNS immune surveillance

T cells

There are numerous line of evidence that suggest a prominent role for T cells in MS. Experimental autoimmune encephalomyelitis (EAE) is considered by many the archetypal animal model of MS [51–53]. Specifically, EAE can be adoptively transferred to naïve recipient mice by injection of myelin-specific $CD4^+$ T cells [54]. MS susceptibility is associated with HLA-DR1*15:01. Among cells isolated from the inflammatory infiltrate in actively demyelinating MS lesions, approximately 10% are $CD4^+$ and $CD8^+$ T cells, whereas the remainder are monocyte-derived macrophages and CNS-intrinsic microglia [55].

More recent research has focused on the pathogenic role of different $CD4^+$ T cell phenotypes in MS and other autoimmune diseases. $CD4^+$ Th cell subsets are currently distinguished by the cytokines they express [56]. Th1 cells are defined as expressing IFNγ, TNF-β, IL-2, and NO [57]. Th1 cells activate myeloid cells to stimulate and maintain cell-mediated immunity. In contrast, Th2 cells secrete IL-4, IL-5, IL-6, IL-10, IL-13 and TGF-β. While Th1 cells are considered predominantly pro-inflammatory in autoimmunity, Th2 immune responses have been associated with immune regulation and disease recovery in patients with MS. Both, IL-10 and IL-4, are capable of suppressing various aspects of the Th1 inflammatory response. TGF-β also plays a regulatory role in immune responses. The Th1-Th2 paradigm is insufficient, however, to associate one $CD4^+$ T cell phenotype with disease activity, and another with remission [58,59]. While Th1 and Th2 polarized cells can be observed in MS and other human autoimmune disorders, a clear distinction between these populations is not always possible. For instance, $CD4^+$ Th1 cells are capable of producing IL-10 and IFNγ during chronic infection and in experimental settings in mice [60–62] and humans [63–65].

Th17 cells are a distinct, more recently discovered lineage of $CD4^+$ T cells that may be very relevant to autoimmune diseases [66]. Induction and maintenance of Th17 cells depend on IL-23 provided by macrophages and dendritic cells [67]. Th17 cells, in turn, express IL-17, which mediates proinflammatory responses, including allergic reactions [68].

$CD8^+$ T cells have also more recently been implicated in MS pathogenesis. Within the peripheral blood and CSF of MS patients, clonal and oligoclonal expansion of $CD8^+$ T cells has been demonstrated [69–71]. Compared with $CD8^+$ myelin-reactive T cells isolated from healthy individuals, $CD8^+$ T cells from patients with MS express a less naïve, more activated phenotype ($CD8^{hi}$ $CD28^-$ $CD57^+$) [72,73]. In some histopathological studies, $CD8^+$ T cells outnumber $CD4^+$ T cells, perhaps suggesting that cytotoxic T cells are driving the inflammatory process. $CD8^+$ T cells may also directly mediate axonal damage observed in MS lesions. Neurons were not thought to be vulnerable to T-cell-mediated injury. However, the observation that *in vitro* exposure to IFNγ induces neurons to express MHC class I molecules provides a plausible explanation for $CD8^+$ T-cell–mediated neuronal damage [74]. Furthermore, activated $CD4^+$ and $CD8^+$ T cells were shown *in vitro* to align along axons and cause neuronal cell death by a cell contact dependent mechanism independent of MHC [75]. $CD8^+$ T cells directly establish contact with demyelinated axons,

upon which cytotoxic mediators are released [76]. *In vitro* experiments have further demonstrated that $CD8^+$ T lymphocytes are capable of transecting neurites [77].

Most T cell receptors (TCRs) consist of two linked polypeptides, α and β, which participate in the co-recognition of foreign antigen in the context of self-MHC[78]. As stated above, this is a critical step in immune surveillance. However, a small subpopulation of circulating lymphocytes expresses γδ TCRs, which also mediate T cell activation. Clonal expansion of activated lymphocytes bearing the γδ TCR was demonstrated in the CSF of patients with recent-onset MS, but not from patients with chronic MS or other neurological disorders [79]. With the EAE model, mice deficient in γδ T cells have reduced CNS mononuclear cell infiltrates [80–82]. γδ T cells may also downregulate CNS inflammation by promoting apoptosis in encephalitogenic αβ T cells. Interestingly, it was also recently shown that γδ T-cell–deficient mice were unable to recover from EAE [83,84]. Histopathologically, monocytes and lymphocytes persisted substantially longer in the CNS than in wild-type mice.

Again, while all of the T cells subsets discussed above appear to play a pathogenic role in MS and its animal model EAE, it is obvious that the primary biological function of these cells is immune surveillance. Thus, targeting them pharmacologically has potential risks and benefits that are discussed below in detail.

B cells

The EAE model has recently led to numerous insights into B cell function in the pathogenesis of MS. Early investigations into the role of B cells in EAE utilized injections of anti-IgM antibodies to deplete B cells in rats prior to EAE induction. In these early studies, depletion of B cells prevented the induction of EAE [85]. In follow-up experiments, EAE could be induced if experimental animal received MBP-specific antiserum in addition to anti-IgM antibodies [86]. Anti-IgM treated animals were also refractory to EAE induction by active immunization with MBP emulsified in complete Freund's adjuvant (CFA) [87]. However, EAE could be induced in a third of B cell depleted mice by adoptive transfer of MBP-specific encephalitogenic T cells, an incidence that was increased by the simultaneous administration of anti-MBP antibodies [87]. A pathogenic role for Ig was further shown by the exacerbation of mouse and rat EAE following the administration of anti-MOG mAb [88].

Recent studies indicate that B cells are also functioning as APCs that facilitate the priming of myelin-specific T cells. Using a transgenic mouse expressing a MOG-specific TCR on the SJL/J (H-2s) background, a mouse strain that exhibits a relapsing–remitting EAE disease course, a high rate of spontaneous EAE was observed [89]. Clinical disease activity in these mice was associated with a strong MOG-specific B cell response, and deposits of Ig and complement in CNS lesions [89]. In addition, pathogenesis of the MOG-specific Ig was demonstrated by its enhancement of the severity of suboptimal EAE. Finally, depletion of B cells with an anti-CD20mAb reduced the incidence of spontaneous EAE [89].

Susceptibility of B cell deficient mice to EAE has been shown by several investigators. B cell deficient mice were first tested in the B10.PL (H-2u) mouse strain by disruption of the

Ig μ heavy chain transmembrane exon (μMT). The mouse was subsequently backcrossed onto the C57BL/6 background (H-2b), in which active immunization with myelin oligodendrocyte glycoprotein peptide 35–55 ($MOG_{p35–55}$) led to clinical EAE [90,91]. Interestingly, the dependence on B cells for the induction of EAE in the C57BL/6 mouse appears to be dependent on the source and nature of the MOG immunogen. In a study by Lyons et al., both C57BL/6 wild-type and B cell-deficient mice were susceptible to EAE following immunization with $MOG_{p35–55}$ [92]. In contrast, only wild-type mice were susceptible to EAE induced with recombinant MOG (rMOG) protein [92]. Although the species source of the rMOG in this study was not indicated, subsequent studies demonstrated that the B cell dependence only occurred when human rMOG was used. Induction of EAE following immunization with rMOG from mouse or rat was shown to be B cell-independent [90,93]. Dittel et al. reported that the relapsing–remitting disease course in (B10.PLxSJL/J)F1 B cell-deficient mice was not altered as compared to wild-type control mice [94]. These data indicate a complex role for B cells in the pathogenesis and regulation of CNS autoimmunity and that the experimental outcome is highly dependent upon the EAE model used.

Anti-CD20 therapy has also been extensively examined in the EAE model. Using anti-mouse CD20 mAb, it was shown that B cell depletion in C57BL/6 mice prior to induction of EAE by active immunization with $MOG_{p35–55}$ exacerbates clinical disease [95]. These data support prior observations of regulatory B cells in controlling CNS autoimmunity [90,96,97]. Interestingly, clinical effects appear to depend on the timing of the B cell depletion in this EAE model: When B cells were depleted just prior to disease onset, or after peak disease was established, no change in disease severity was noted [95]. In contrast, EAE disease severity was reduced if B cells were depleted shortly after EAE onset [98]. In a study utilizing human CD20 transgenic mice, B cell depletion was achieved using an anti-human CD20 mAb [99]. B cell depletion prior to active EAE induction with $MOG_{p35–55}$ exacerbated EAE [99]. In contrast, in the same experimental design, immunization with recombinant (r)MOG, which requires B cell recognition and processing, resulted in less severe EAE [99]. Similar results were obtained if the B cells were depleted after the onset of EAE. Differences in the two outcomes were attributed to a reduction in MOG-specific Th1 and Th17 cells in the rMOG model, as compared to a relative lack of impediment of Th1 or Th17 differentiation in mice immunized with $MOG_{p35–55}$ [99]. Monson et al also showed that B cell depletion with rituximab in human CD20 transgenic mice was associated with diminished Delayed Type Hypersensitivity (DTH) and a reduction in T cell proliferation and IL-17 production during recall immune response experiments [100].

Pharmacological strategies that interrupt CNS immune surveillance

A reduction in the absolute number of leukocytes in the CNS can be achieved by reducing the number of cells in primary and secondary lymphoid organs and the peripheral blood, which will subsequently also diminish their numbers within the brain and spinal cord through a decrease in leukocytes that can be recruited. Both strategies work in patients with MS. However, both strategies also have side effects that are almost certainly related to their effects on CNS immune surveillance [101]. Mechanisms of action and potential side effects

of some of the relevant agents that are currently approved or frequently utilized in clinical practice are discussed below.

Pharmacological cell-depleting strategies

Anti-proliferative agents have been used to treat patients with multiple sclerosis for more than four decades. Many of these agents are nucleotide analogues that were often developed to treat lympho- or leukoproliferative disorders. Teriflunomide was approved in 2012 by the Food and Drug Administration (FDA) for treatment of relapsing forms of MS [102,103]. Teriflunomide is a pyrimidine synthesis inhibitor through the inhibition of dihydroorotate dehydrogenase. Proliferation of cells is reduced, including that of leukocytes. Additionally, the expression of cytokines by lymphocytes is impacted (reviewed in [104,105]). Until now, no opportunistic infections or neoplastic growths within the CNS have been reported. However, because of documented side effects with a known analog of teriflunomide, leflunomide, screening for latent tuberculosis has to be performed prior to the initiation of therapy. It is also recommended to not treat patients with a known immunodeficiency with teriflunomide.

Dimethyl fumarate was approved by the FDA [106] for treatment of RRMS based on the results of two phase III trials [107,108]. Dimethyl fumarate and its metabolites monomethyl fumarate are clearly anti-proliferative, as shown in studies on patients with psoriasis [109,110]. In addition, dimethyl fumarate appears to modulate adaptive immune-cell responses by shifting DC differentiation. The suppression of proinflammatory cytokine production and the inhibition of proinflammatory pathways are the biological results. Thus far, there have been two reports of PML in a patient with psoriasis and a patient with a diagnosis of MS who received fumaric acid preparations containing dimethyl fumarate [111,112]. Prolonged lymphopenia may have contributed to a higher risk for PML in these cases. Another contributing risk factor may have been prior immunosuppressant use [114].

Alemtuzumab is a humanized IgG_1 kappa recombinant monoclonal antibody (mAb) that targets CD52, which is expressed on the surface of mature lymphocytes, monocytes, DCs, and granulocytes. Alemtuzumab was originally developed as a rat mAb to reduce the number of circulating T cells in order to prevent graft-versus-host disease following allogeneic hematopoietic stem cell transplantation [113]. Death of the cellular target is achieved by antibody-mediated cell cytolysis (ADCC), complement-dependent-cytolysis (CDC), and apoptosis [114,115]. Alemtuzumab is currently not approved for the treatment of patients with MS in the United States of America. However, a substantial amount of efficacy data and safety data was accumulated in an extensive clinical trial program that included two phase III studies [116,117]. The proportion of serious adverse events was not significantly higher in the alemtuzumab treatment arms than in the control arms in any of the phase II or phase III trials [116–118]. However, in both phase III studies, herpetic viral infections were more frequent in the alemtuzumab treatment arms [116,117]. Furthermore, one alemtuzumab treated patient with disseminated tuberculosis was reported [117]. One patient in a phase II trial on alemtuzumab fell ill with *Listeria monocytogenes* meningitis after consumption of unpasteurized cheese [118].

Another mAb that was evaluated in clinical trial and has been widely used in clinical practice in patients with MS is rituximab. Originally, this agent was developed for the treatment of B cell lymphoma [67, 68]. Rituximab is a chimeric IgG_1 mAb that targets CD20 on large pre-B cells, small pre-B cells, immature B cells, naïve B cells, and mature B cells [119–122] Thus, rituximab depletes the vast majority of cells belonging to the B cell lineage, and likely prevents the *de novo* generation of plasma cells and memory cells from their precursors. Its cytotoxic effect is the result of apoptosis, ADCC and CDC [65, 66]. Hauser et al successfully tested rituximab in a phase II trial in patients with RRMS [123]. A retrospective analysis of patients with different autoimmune diseases and rituximab treatment showed that infections may occur in up to 13% of the patients [124]. It is also noteworthy that there are more than 20 reported cases of progressive multifocal leukoencephalopathy (PML) in patients with malignancies and autoimmune disorders who received rituximab as monotherapy or in combination with other agents. PML is caused by the human polyoma virus JC, which is highly prevalent and potentially neuroinvasive. After the initial infection, which typically occurs in the first two decades of life, JCV becomes latent in the bone marrow or kidney after exposure, and appears to reactivate during prolonged and severe immunosuppression.

Pharmacological sequestration of immune-competent cells out of the CNS

Fingolimod is an agent with a relatively novel mode of action that is currently approved in the United States of America to treat patients with relapsing-remitting multiple sclerosis (RRMS) [125], and in Europe to treat patients with RRMS who failed first line treatment, or who display an evolving severe RRMS clinical phenotype [126].

Fingolimod engages the sphingosine-1-phospate (S1P) receptors on leukocytes. Consequently, these immune cells, and mostly $CD4^+$ T helper cells, are unable to egress from lymphatic tissues [127] [71]. Specifically, fingolimod prevents the efflux of $CCR7^+$ naive and central memory lymphocytes from lymph nodes, but not that of $CCR7^-$ effector memory T cells [128,129]. CCR7 induces homing of T cells to lymph nodes. Interstingly, lymphocytes that reside within lymph nodes account for approximately 2% of the entire lymphocyte pool, whereas effector memory cells represent the major T cell population in the peripheral blood.

The biological functions of circulating lymphocytes do not seem to be affected by fingolimod. The most common side effects of fingolimod are not related to its effects on the immune system, but are the result of the almost ubiquitous distribution of S1P receptors. However, neoplastic growth has been reported. Specifically, in clinical trials the incidence of cutaneous cancerous growths was increased in the fingolimod group compared to the interferon beta or placebo control groups [130,131]. Both, basal cell carcinomas and melanomas (*in situ*) were identified [130,131]. In addition, the Food and Drug Administration (FDA) is currently investigating a possible case of PML in a patient with a diagnosis of MS [132].

The poster child for pharmacotherapies that prevent the migration of leukocytes into the CNS is natalizumab, a monoclonal recombinant humanized IgG_4 mAb targeting the α4-chain of $\alpha4\beta1$ integrin and other α4-integrin-containing adhesion molecules. The

development of natalizumab is the result of rational drug design based on the knowledge of cell migration and adhesion molecules that was accumulated over the past three decades. Migration of leukocytes from the blood into the CNS involves multiple steps [133]. It is believed that slow rolling on endothelial walls allows leukocytes to identify proper arrays of chemoattractants and integrin ligands. Prolonged selectin-mediated rolling of neutrophils and lymphocytes may also lead to integrin activation [134–136]. Once firmly arrested, integrins facilitate the binding of leukocytes to other leukocytes and platelets. Activated T cells and B cell blasts express highly adhesive integrins [137]. All other circulating leukocytes maintain their integrins in mostly inactive states and must undergo *in situ* modulation to develop high avidity for their specific ligands [138]. Following rolling adhesion, the arrest of lymphocytes and myeloid cells in venules is mediated by the *in situ* activation of at least one of the four main integrins: α4β1, LFA-1, Mac-1, or VLA-4 [133]. Integrins of the β1 subfamily, specifically VLA-1, VLA-2, VLA-4, VLA-5 and VLA-6, have been shown to facilitate leukocyte migration across the basement membrane of blood vessels [139], and across extracellular matrix (ECM). There is considerable redundancy: Multiple ligands have been identified for a single receptor, and multiple receptors bind a single ligand [139]. The proadhesive properties of integrins is overlapping and additive, and depend on specific cytoskeletal and transmembrane associations with cytoskeletal adaptor molecules [140]. Integrins are activated bidirectionally: (1) Cytoplasmic rearrangements of their subunit tail, and (2) extracellular binding by their ligands [141]. A specific combination of chemokines and G protein–coupled receptors (GPCRs) is required for activation of integrin-dependent arrest under shear flow. Engagement of α4-integrin by natalizumab impairs its ability to bind to vascular cell adhesion molecule-1 (VCAM-1) and its other ligands, including fibronectin. As a consequence, leukocytes are diminished in their ability to adhere to the inner lining of cerebral vascular walls, and to subsequently migrate through the blood-brain-barrier (BBB) into the CNS [142–145]. In 2004, natalizumab received accelerated approval from the FDA on basis of the interim results of two ongoing phase III clinical trials [146,147]. However, the occurrence of three PML cases in patients with MS and Crohn's disease led to the voluntary withdrawal of natalizumab by its manufacturers in 2005. Following an evaluation period, natalizumab was re-introduced in 2006. Currently, more than 400 cases of PML have been reported. These numbers illustrate the dichotomy between the potential benefits and risks of leukocyte sequestration out of the CNS as an interventional strategy in MS.

Establishing and experimental model to test CNS immune surveillance

General considerations

There is currently no experimental model that is universally accepted to assess CNS immune surveillance. Consequently, no such model is currently being applied to test pharmacological agents for their effects on leukocyte homeostasis or function within the CNS. The lack of such a model is a substantial shortfall in drug development as it may prevent the occurrence of CNS infections and neoplastic growth, or may at least lead to the implementation of a pharmaco-vigilance program that could detect these events.

In order to implement an experimental animal model for CNS immune surveillance, the following prerequisites should be met:

- The experimental animal strain should be readily available.
- The experimental animal should be susceptible to an infection of the CNS by the pathogen under study.
- MHC-restricted determinants of the pathogen should be well characterized.
- Ideally, $CD4^+$ T cell and $CD8^+$ T cell responses should be established.
- The median lethal dose, or "Lethal Dose, 50%" (LD50) per bodyweight of the pathogen should be known.
- The median survival, net survival, and relative survival after exposure to the pathogen need to be determined.
- A tissue marker that results from pathogen exposure should be quantified and should correlate with host survival.

Toxoplasma gondii encephalitis

We recently devised an experimental model of CNS immune surveillance that meets all of the above-mentioned prerequisites (Castro-Rojas et al). The protozoan *Toxoplasma gondii* (*T. gondii*) is a highly prevalent obligate intracellular pathogen. While many mammalian species are susceptible, the main host is the felid (cat) family. The transmission of toxoplasmic trophozoites, or tachyzoitis occurs via the fecal-oral route, and leads to an acute infection that is characterized by flu-like symptoms. Immunocompetent hosts may not even know that they were infected. In individuals with a fully functional immune system, *T. gondii* becomes latent after two to four months. During this stage, only cyst-forming bradyzoites are present within the infected tissue. In immunocompromised hosts, an acute infection can cause encephalitis and necrotizing retinochoroiditis.

Virulence of *T. gondii* in mice is clone-specific: Type I clones are considered very virulent, type II clones possess intermediate virulence, and type III clones are thought of as avirulent. Type II clones are most frequently associated with human disease [148–150]. Systemic immune responses evoked by *T. gondii* have been extensively studied and involve the humoral and cellular arm of the adaptive immune system [151]. $CD4^+$ T cells, $CD8^+$ T cells, IL-12, and IFNγ are effectors considered highly significant in mediating resistance to acute and chronic *T. gondii* infection [152,153].

The ME49 strain of *T. gondii* is a type II clone. Like other mouse strains, the C57BL/6 mouse strain is susceptible to the ME49 strain of *T. gondii*. The C57BL/6 mouse (H-2b) is of great interest to experimental and clinical neuroimmunologists for several reasons: (1) It is the most frequently utilized mouse strain in EAE experiments; (2) most genetically-modified mouse strains are on the C57BL/6 background; (3) C57BL/6 mice are commonly backcrossed with transgenic mice or gene-deficient mice on the C57BL/6 background. Infection of C57BL/6 mice with *T. gondii* strain ME49 is characterized by a rapid expansion of tachyzoites in the host. Following the acute disease phase, tachyzoites differentiate into

bradyzoites, which form tissue cysts predominantly in the CNS. The development of tissue cysts defines the chronic stage of the infection. In C57BL/6 mice, the dominant epitopes of the ME49 strain of *T. gondii* have only been identified for the MHC II pathway [154,155]. Nevertheless, depletion of either $CD4^+$ or $CD8^+$ T-cell results in reactivation of the parasite, and is associated with rapid mortality of infected animals in this mouse strain [152]. The host survival of C57BL/6 mice following an infection with the ME49 strain of *T. gondii* is well established [152–154].

Conclusion

Infection of C57BL/6 mice with the ME49 strain of *T. gondii* is a valid experimental model to test CNS immune surveillance for MS pharmacotherapies. C57BL/6 mice are susceptible to *T. gondii* infection and develop encephalitis. The active infection eventually becomes latent. Innate and adaptive immune responses against the pathogen are well characterized in this mouse strain, and the survival rate is established. Parasite cysts accumulate within the brain, and there is an association between parasite numbers and clinical outcomes. Thus, the effect on any pharmacological intervention on survival, parasite numbers, and the composition on immune cells can be assessed.

We believe that a CNS immune surveillance experimental model that involves infection of C57BL/6 mice with the ME49 strain of *T. gondii* provides meaningful data on the composition and homeostasis of immune-competent cells. Knowledge derived from this model will enhance our knowledge on potential adverse events of monotherapies and combination therapies in MS and related disorders.

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Reference List

1. Shrikant P, Benveniste EN. The central nervous system as an immunocompetent organ: role of glial cells in antigen presentation. JImmunol. 1996; 157:1819–1822. [PubMed: 8757296]
2. Aloisi F, Ria F, Penna G, Adorini L. Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. JImmunol. 1998; 160:4671–4680. [PubMed: 9590212]
3. Weber F, Meinl E, Aloisi F, Nevinny-Stickel C, Albert E, et al. Human astrocytes are only partially competent antigen presenting cells. Possible implications for lesion development in multiple sclerosis. Brain. 1994; 117(Pt 1):59–69. [PubMed: 7511974]
4. Sedgwick JD, Mossner R, Schwender S, ter MV. Major histocompatibility complex-expressing nonhematopoietic astroglial cells prime only CD8+ T lymphocytes: astroglial cells as perpetuators but not initiators of CD4+ T cell responses in the central nervous system. JExpMed. 1991; 173:1235–1246.
5. Stuve O, Youssef S, Slavin AJ, King CL, Patarroyo JC, et al. The role of the MHC class II transactivator in class II expression and antigen presentation by astrocytes and in susceptibility to central nervous system autoimmune disease. JImmunol. 2002; 169:6720–6732. [PubMed: 12471103]

6. Heppner FL, Greter M, Marino D, Falsig J, Raivich G, et al. Experimental autoimmune encephalomyelitis repressed by microglial paralysis. NatMed. 2005; 11:146–152.
7. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science. 1988; 239:290–292. [PubMed: 3276004]
8. Huitinga I, van Rooijen N, De Groot CJ, Uitdehaag BM, Dijkstra CD. Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages. JExpMed. 1990; 172:1025–1033.
9. Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, et al. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. NatMed. 2005; 11:328–334.
10. Anthoons JA, Van Marck EA, Gigase PL, Stevens WJ. Immunohistochemical characterization of the mononuclear cells in the brain of the rat with an experimental chronic Trypanosoma brucei gambiense infection. ParasitolRes. 1989; 75:251–256.
11. Kivisakk P, Imitola J, Rasmussen S, Elyaman W, Zhu B, et al. Localizing central nervous system immune surveillance: meningeal antigen-presenting cells activate T cells during experimental autoimmune encephalomyelitis. Ann Neurol. 2009; 65:457–469. [PubMed: 18496841]
12. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, et al. Macrophage receptors and immune recognition. Annu Rev Immunol. 2005; 23:901–944. [PubMed: 15771589]
13. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001; 2:675–680. [PubMed: 11477402]
14. Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol. 2003; 3:371–382. [PubMed: 12766759]
15. Cailhier JF, Partolina M, Vuthoori S, Wu S, Ko K, et al. Conditional macrophage ablation demonstrates that resident macrophages initiate acute peritoneal inflammation. J Immunol. 2005; 174:2336–2342. [PubMed: 15699170]
16. Maus UA, Koay MA, Delbeck T, Mack M, Ermert M, et al. Role of resident alveolar macrophages in leukocyte traffic into the alveolar air space of intact mice. Am J Physiol Lung Cell Mol Physiol. 2002; 282:L1245–L1252. [PubMed: 12003780]
17. Ajuebor MN, Das AM, Virag L, Flower RJ, Szabo C, et al. Role of resident peritoneal macrophages and mast cells in chemokine production and neutrophil migration in acute inflammation: evidence for an inhibitory loop involving endogenous IL-10. J Immunol. 1999; 162:1685–1691. [PubMed: 9973430]
18. Kolaczkowska E, Goldys A, Kozakiewicz E, Lelito M, Plytycz B, et al. Resident peritoneal macrophages and mast cells are important cellular sites of COX-1 and COX-2 activity during acute peritoneal inflammation. Arch Immunol Ther Exp (Warsz). 2009; 57:459–466. [PubMed: 19885646]
19. Kolaczkowska E, Lelito M, Kozakiewicz E, van Rooijen N, Plytycz B, et al. Resident peritoneal leukocytes are important sources of MMP-9 during zymosan peritonitis: superior contribution of macrophages over mast cells. Immunol Lett. 2007; 113:99–106. [PubMed: 17826846]
20. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312–318. [PubMed: 8843599]
21. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol. 2009; 27:119–145. [PubMed: 19302036]
22. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev. 2011; 91:461–553. [PubMed: 21527731]
23. Adams RA, Bauer J, Flick MJ, Sikorski SL, Nuriel T, et al. The fibrin-derived gamma377–395 peptide inhibits microglia activation and suppresses relapsing paralysis in central nervous system autoimmune disease. J Exp Med. 2007; 204:571–582. [PubMed: 17339406]
24. Flanders KC, Ludecke G, Renzing J, Hamm C, Cissel DS, et al. Effects of TGF-betas and bFGF on Astroglial Cell Growth and Gene Expression in Vitro. Mol Cell Neurosci. 1993; 4:406–417. [PubMed: 19912947]
25. Unsicker K, Flanders KC, Cissel DS, Lafyatis R, Sporn MB. Transforming growth factor beta isoforms in the adult rat central and peripheral nervous system. Neuroscience. 1991; 44:613–625. [PubMed: 1754055]

26. Slavin AJ, Soos JM, Stuve O, Patarroyo JC, Weiner HL, et al. Requirement for endocytic antigen processing and influence of invariant chain and H-2M deficiencies in CNS autoimmunity. JClinInvest. 2001; 108:1133–1139.
27. 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; 11:335–339. [PubMed: 15735651]
28. Davies LC, Rosas M, Smith PJ, Fraser DJ, Jones SA, et al. A quantifiable proliferative burst of tissue macrophages restores homeostatic macrophage populations after acute inflammation. Eur J Immunol. 2011; 41:2155–2164. [PubMed: 21710478]
29. Davies LC, Rosas M, Jenkins SJ, Liao CT, Scurr MJ, et al. Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. Nat Commun. 2013; 4:1886. [PubMed: 23695680]
30. Rosas M, Thomas B, Stacey M, Gordon S, Taylor PR. The myeloid 7/4-antigen defines recently generated inflammatory macrophages and is synonymous with Ly-6B. J Leukoc Biol. 2010; 88:169–180. [PubMed: 20400676]
31. Lauder SN, Taylor PR, Clark SR, Evans RL, Hindley JP, et al. Paracetamol reduces influenza-induced immunopathology in a mouse model of infection without compromising virus clearance or the generation of protective immunity. Thorax. 2011; 66:368–374. [PubMed: 21310755]
32. Kirby AC, Coles MC, Kaye PM. Alveolar macrophages transport pathogens to lung draining lymph nodes. J Immunol. 2009; 183:1983–1989. [PubMed: 19620319]
33. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci. 2011; 14:1142–1149. [PubMed: 21804537]
34. Bellingan GJ, Xu P, Cooksley H, Cauldwell H, Shock A, et al. Adhesion molecule-dependent mechanisms regulate the rate of macrophage clearance during the resolution of peritoneal inflammation. J Exp Med. 2002; 196:1515–1521. [PubMed: 12461086]
35. Leuschner F, Rauch PJ, Ueno T, Gorbatov R, Marinelli B, et al. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. J Exp Med. 2012; 209:123–137. [PubMed: 22213805]
36. Cao C, Lawrence DA, Strickland DK, Zhang L. A specific role of integrin Mac-1 in accelerated macrophage efflux to the lymphatics. Blood. 2005; 106:3234–3241. [PubMed: 16002427]
37. Janssen WJ, Barthel L, Muldrow A, Oberley-Deegan RE, Kearns MT, et al. Fas determines differential fates of resident and recruited macrophages during resolution of acute lung injury. Am J Respir Crit Care Med. 2011; 184:547–560. [PubMed: 21471090]
38. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity. 2013; 38:79–91. [PubMed: 23273845]
39. Lassmann H, Schmied M, Vass K, Hickey WF. Bone marrow derived elements and resident microglia in brain inflammation. Glia. 1993; 7:19–24. [PubMed: 7678581]
40. Bechmann I, Kwidzinski E, Kovac AD, Simburger E, Horvath T, et al. Turnover of rat brain perivascular cells. ExpNeurol. 2001; 168:242–249.
41. Heath WR, Carbone FR. The skin-resident and migratory immune system in steady state and memory: innate lymphocytes, dendritic cells and T cells. Nat Immunol. 2013; 14:978–985. [PubMed: 24048119]
42. Prodinger C, Bunse J, Kruger M, Schiefenhovel F, Brandt C, et al. CD11c–expressing cells reside in the juxtavascular parenchyma and extend processes into the glia limitans of the mouse nervous system. Acta Neuropathol. 2011; 121:445–458. [PubMed: 21076838]
43. DeBoy CA, Rus H, Tegla C, Cudrici C, Jones MV, et al. FLT-3 expression and function on microglia in multiple sclerosis. Exp Mol Pathol. 2010; 89:109–116. [PubMed: 20566414]
44. Jain P, Coisne C, Enzmann G, Rottapel R, Engelhardt B. Alpha4beta1 integrin mediates the recruitment of immature dendritic cells across the blood-brain barrier during experimental autoimmune encephalomyelitis. J Immunol. 2010; 184:7196–7206. [PubMed: 20483748]

45. Israelsson C, Bengtsson H, Lobell A, Nilsson LN, Kylberg A, et al. Appearance of Cxcl10-expressing cell clusters is common for traumatic brain injury and neurodegenerative disorders. Eur J Neurosci. 2010; 31:852–863. [PubMed: 20374285]

46. King IL, Dickendesher TL, Segal BM. Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. Blood. 2009; 113:3190–3197. [PubMed: 19196868]

47. Zozulya AL, Ortler S, Lee J, Weidenfeller C, Sandor M, et al. Intracerebral dendritic cells critically modulate encephalitogenic versus regulatory immune responses in the CNS. J Neurosci. 2009; 29:140–152. [PubMed: 19129392]

48. Cravens PD, Kieseier BC, Hussain R, Herndon E, Arellano B, et al. The neonatal CNS is not conducive for encephalitogenic Th1 T cells and B cells during experimental autoimmune encephalomyelitis. J Neuroinflammation. 2013; 10:67. [PubMed: 23705890]

49. Fischer HG, Reichmann G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. J Immunol. 2001; 166:2717–2726. [PubMed: 11160337]

50. Yogev N, Frommer F, Lukas D, Kautz-Neu K, Karram K, et al. Dendritic cells ameliorate autoimmunity in the CNS by controlling the homeostasis of PD-1 receptor(+) regulatory T cells. Immunity. 2012; 37:264–275. [PubMed: 22902234]

51. Zamvil SS, Steinman L. The T lymphocyte in experimental allergic encephalomyelitis. AnnuRevImmunol. 1990; 8:579–621.

52. Hemmer B, Cepok S, Zhou D, Sommer N. Multiple sclerosis -- a coordinated immune attack across the blood brain barrier. Curr Neurovasc Res. 2004; 1:141–150. [PubMed: 16185190]

53. Delgado S, Sheremata WA. The role of CD4+ T-cells in the development of MS. Neurol Res. 2006; 28:245–249. [PubMed: 16687048]

54. Cravens PD, Hussain RZ, Zacharias TE, Ben LH, Hernden E, et al. Lymph node-derived donor encephalitogenic CD4+ T cells in C57BL/6 mice adoptive transfer experimental autoimmune encephalomyelitis highly express GM-CSF and T-bet. JNeuroinflammation. 2011; 8:73. [PubMed: 21702922]

55. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med. 2006; 354:610–621. [PubMed: 16467548]

56. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. JImmunol. 1986; 136:2348–2357. [PubMed: 2419430]

57. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986; 136:2348–2357. [PubMed: 2419430]

58. Allen JE, Maizels RM. Th1-Th2: reliable paradigm or dangerous dogma? Immunol Today. 1997; 18:387–392. [PubMed: 9267081]

59. Gor DO, Rose NR, Greenspan NS. TH1-TH2: a procrustean paradigm. Nat Immunol. 2003; 4:503–505. [PubMed: 12774069]

60. Assenmacher M, Schmitz J, Radbruch A. Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. Eur J Immunol. 1994; 24:1097–1101. [PubMed: 7910138]

61. Anderson CF, Oukka M, Kuchroo VJ, Sacks D. CD4(+)CD25(−)Foxp3(−) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J Exp Med. 2007; 204:285–297. [PubMed: 17283207]

62. Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, et al. Conventional T-bet(+)Foxp3(−) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J Exp Med. 2007; 204:273–283. [PubMed: 17283209]

63. Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, et al. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol. 1993; 150:353–360. [PubMed: 8419468]

64. Gerosa F, Paganin C, Peritt D, Paiola F, Scupoli MT, et al. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon-gamma and interleukin-10. J Exp Med. 1996; 183:2559–2569. [PubMed: 8676077]

65. Pohl-Koppe A, Balashov KE, Steere AC, Logigian EL, Hafler DA. Identification of a T cell subset capable of both IFN-gamma and IL-10 secretion in patients with chronic Borrelia burgdorferi infection. J Immunol. 1998; 160:1804–1810. [PubMed: 9469440]

66. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005; 6:1123–1132. [PubMed: 16200070]

67. Gocke AR, Cravens PD, Ben LH, Hussain RZ, Northrop SC, et al. T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J Immunol. 2007; 178:1341–1348. [PubMed: 17237380]

68. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. NatMed. 2007; 13:139–145.

69. Jacobsen M, Cepok S, Quak E, Happel M, Gaber R, et al. Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients. Brain. 2002; 125:538–550. [PubMed: 11872611]

70. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, 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. [PubMed: 10934227]

71. Crawford MP, Yan SX, Ortega SB, Mehta RS, Hewitt RE, et al. High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. Blood. 2004; 103:4222–4231. [PubMed: 14976054]

72. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. N Engl J Med. 2006; 354:942–955. [PubMed: 16510748]

73. Ratts RB, Karandikar NJ, Hussain RZ, Choy J, Northrop SC, et al. Phenotypic characterization of autoreactive T cells in multiple sclerosis. J Neuroimmunol. 2006; 178:100–110. [PubMed: 16901549]

74. Neumann H, Cavalie A, Jenne DE, Wekerle H. Induction of MHC class I genes in neurons. Science. 1995; 269:549–552. [PubMed: 7624779]

75. Giuliani F, Goodyer CG, Antel JP, Yong VW. Vulnerability of human neurons to T cell-mediated cytotoxicity. J Immunol. 2003; 171:368–379. [PubMed: 12817020]

76. Skulina C, Schmidt S, Dornmair K, Babbe H, Roers A, et al. Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proc Natl Acad Sci U S A. 2004; 101:2428–2433. [PubMed: 14983026]

77. Medana I, Martinic MA, Wekerle H, Neumann H. Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. Am J Pathol. 2001; 159:809–815. [PubMed: 11549572]

78. Marrack P, Kappler J. The T cell receptor. Science. 1987; 238:1073–1079. [PubMed: 3317824]

79. Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. Proc Natl Acad Sci U S A. 1993; 90:923–927. [PubMed: 8430106]

80. Rajan AJ, Asensio VC, Campbell IL, Brosnan CF. Experimental autoimmune encephalomyelitis on the SJL mouse: effect of gamma delta T cell depletion on chemokine and chemokine receptor expression in the central nervous system. J Immunol. 2000; 164:2120–2130. [PubMed: 10657666]

81. Rajan AJ, Gao YL, Raine CS, Brosnan CF. A pathogenic role for gamma delta T cells in relapsing-remitting experimental allergic encephalomyelitis in the SJL mouse. J Immunol. 1996; 157:941–949. [PubMed: 8752949]

82. Rajan AJ, Klein JD, Brosnan CF. The effect of gammadelta T cell depletion on cytokine gene expression in experimental allergic encephalomyelitis. J Immunol. 1998; 160:5955–5962. [PubMed: 9637509]

83. Ponomarev ED, Dittel BN. Gamma delta T cells regulate the extent and duration of inflammation in the central nervous system by a Fas ligand-dependent mechanism. J Immunol. 2005; 174:4678–4687. [PubMed: 15814692]

84. Ponomarev ED, Novikova M, Yassai M, Szczepanik M, Gorski J, et al. Gamma delta T cell regulation of IFN-gamma production by central nervous system-infiltrating encephalitogenic T cells: correlation with recovery from experimental autoimmune encephalomyelitis. J Immunol. 2004; 173:1587–1595. [PubMed: 15265886]

85. Willenborg DO, Prowse SJ. Immunoglobulin-deficient rats fail to develop experimental allergic encephalomyelitis. JNeuroimmunol. 1983; 5:99–109. [PubMed: 6194180]

86. Willenborg DO, Sjollema P, Danta G. Immunoregulation of passively induced allergic encephalomyelitis. JImmunol. 1986; 136:1676–1679. [PubMed: 2936807]

87. Myers KJ, Sprent J, Dougherty JP, Ron Y. Synergy between encephalitogenic T cells and myelin basic protein-specific antibodies in the induction of experimental autoimmune encephalomyelitis. JNeuroimmunol. 1992; 41:1–8. [PubMed: 1281165]

88. 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. JImmunol. 1987; 139:4016–4021. [PubMed: 3500978]

89. Pollinger B, Krishnamoorthy G, Berer K, Lassmann H, Bosl MR, et al. Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit endogenous MOG-specific B cells. JExpMed. 2009; 206:1303–1316.

90. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. NatImmunol. 2002; 3:944–950.

91. Hjelmstrom P, Juedes AE, Fjell J, Ruddle NH. B-cell-deficient mice develop experimental allergic encephalomyelitis with demyelination after myelin oligodendrocyte glycoprotein sensitization. JImmunol. 1998; 161:4480–4483. [PubMed: 9794370]

92. 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. EurJImmunol. 1999; 29:3432–3439.

93. Oliver AR, Lyon GM, Ruddle NH. Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice. JImmunol. 2003; 171:462–468. [PubMed: 12817031]

94. Dittel BN, Urbania TH, Janeway CA Jr. Relapsing and remitting experimental autoimmune encephalomyelitis in B cell deficient mice. JAutoimmun. 2000; 14:311–318. [PubMed: 10882057]

95. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. JClinInvest. 2008; 118:3420–3430.

96. Mann MK, Maresz K, Shriver LP, Tan Y, Dittel BN. B cell regulation of CD4+CD25+ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. JImmunol. 2007; 178:3447–3456. [PubMed: 17339439]

97. Wolf SD, Dittel BN, Hardardottir F, Janeway CA Jr. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. JExpMed. 1996; 184:2271–2278.

98. Tedder TF, McIntyre G, Schlossman SF. Heterogeneity in the B1 (CD20) cell surface molecule expressed by human B-lymphocytes. MolImmunol. 1988; 25:1321–1330.

99. Weber MS, Prod'homme T, Patarroyo JC, Molnarfi N, Karnezis T, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann Neurol. 2010; 68:369–383. [PubMed: 20641064]

100. Monson NL, Cravens P, Hussain R, Harp CT, Cummings M, et al. Rituximab therapy reduces organ-specific T cell responses and ameliorates experimental autoimmune encephalomyelitis. PLoSONE. 2011; 6:e17103.

101. Rommer PS, Zettl UK, Kieseier B, Hartung HP, Menge T, et al. Requirement for safety monitoring for approved MS therapies - An overview. Clin Exp Immunol. 2013

102. http://www.fda.gov/downloads/Drugs/DrugSafety/UCM322204.pdfUFaDAMg.

103. O'Connor P, Wolinsky JS, Confavreux C, Comi G, Kappos L, et al. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. N Engl J Med. 2011; 365:1293–1303. [PubMed: 21991951]

104. Claussen MC, Korn T. Immune mechanisms of new therapeutic strategies in MS: teriflunomide. Clin Immunol. 2012; 142:49–56. [PubMed: 21367665]

105. Fox EJ, Rhoades RW. New treatments and treatment goals for patients with relapsing-remitting multiple sclerosis. Curr Opin Neurol. 2012; 25(Suppl):S11–S19. [PubMed: 22398660]
106. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm345528.htm
107. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med. 2012; 367:1098–1107. [PubMed: 22992073]
108. Fox RJ, Miller DH, Phillips JT, Hutchinson M, Havrdova E, et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med. 2012; 367:1087–1097. [PubMed: 22992072]
109. Schweckendieck W. Treatment of psoriasis vulgaris. Medizinische Monatsschrift. 1959; 13:103–104. [PubMed: 13643669]
110. Mrowietz U, Christophers E, Altmeyer P. Treatment of severe psoriasis with fumaric acid esters: scientific background and guidelines for therapeutic use. The German Fumaric Acid Ester Consensus Conference. Br J Dermatol. 1999; 141:424–429. [PubMed: 10584060]
111. Ermis U, Weis J, Schulz JB. PML in a patient treated with fumaric acid. N Engl J Med. 2013; 368:1657–1658. [PubMed: 23614603]
112. van Oosten BW, Killestein J, Barkhof F, Polman CH, Wattjes MP. PML in a patient treated with dimethyl fumarate from a compounding pharmacy. N Engl J Med. 2013; 368:1658–1659. [PubMed: 23614604]
113. Hale G, Bright S, Chumbley G, Hoang T, Metcalf D, et al. Removal of T cells from bone marrow for transplantation: a monoclonal antilymphocyte antibody that fixes human complement. Blood. 1983; 62:873–882. [PubMed: 6349718]
114. Rommer P, Dudesek A, Stuve O, Zettl U. Monoclonal Antibodies in Treatment of Multiple Sclerosis. Clin Exp Immunol. 2013
115. Crowe JS, Hall VS, Smith MA, Cooper HJ, Tite JP. Humanized monoclonal antibody CAMPATH-1H: myeloma cell expression of genomic constructs, nucleotide sequence of cDNA constructs and comparison of effector mechanisms of myeloma and Chinese hamster ovary cell-derived material. Clin Exp Immunol. 1992; 87:105–110. [PubMed: 1339322]
116. Coles AJ, Twyman CL, Arnold DL, Cohen JA, Confavreux C, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. Lancet. 2012; 380:1829–1839. [PubMed: 23122650]
117. Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, 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; 380:1819–1828. [PubMed: 23122652]
118. Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. NEnglJMed. 2008; 359:1786–1801.
119. Stashenko P, Nadler LM, Hardy R, Schlossman SF. Characterization of a human B lymphocyte-specific antigen. J Immunol. 1980; 125:1678–1685. [PubMed: 6157744]
120. Stashenko P, Nadler LM, Hardy R, Schlossman SF. Expression of cell surface markers after human B lymphocyte activation. Proc Natl Acad Sci U S A. 1981; 78:3848–3852. [PubMed: 6973760]
121. Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow. II. Normal B lymphocyte development. Blood. 1987; 70:1316–1324. [PubMed: 3117132]
122. Uchida J, Lee Y, Hasegawa M, Liang Y, Bradney A, et al. Mouse CD20 expression and function. Int Immunol. 2004; 16:119–129. [PubMed: 14688067]
123. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. NEnglJMed. 2008; 358:676–688.
124. Palazzo E, Yahia SA. Progressive multifocal leukoencephalopathy in autoimmune diseases. Joint Bone Spine. 2012; 79:351–355. [PubMed: 22281228]
125. http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022527s000lbl.pdf
126. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002202/WC500104528.pdf

127. Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, et al. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. JBiolChem. 2002; 277:21453–21457.

128. Mehling M, Brinkmann V, Antel J, Bar-Or A, Goebels N, et al. FTY720 therapy exerts differential effects on T cell subsets in multiple sclerosis. Neurology. 2008; 71:1261–1267. [PubMed: 18852441]

129. Mehling M, Lindberg R, Raulf F, Kuhle J, Hess C, et al. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology. 2010; 75:403–410. [PubMed: 20592255]

130. Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N Engl J Med. 2010; 362:402–415. [PubMed: 20089954]

131. Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. N Engl J Med. 2010; 362:387–401. [PubMed: 20089952]

132. http://www.fda.gov/Drugs/DrugSafety/ucm366529.htm

133. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. NatImmunol. 2005; 6:1182–1190.

134. Kunkel EJ, Dunne JL, Ley K. Leukocyte arrest during cytokine-dependent inflammation in vivo. JImmunol. 2000; 164:3301–3308. [PubMed: 10706723]

135. Atarashi K, Hirata T, Matsumoto M, Kanemitsu N, Miyasaka M. Rolling of Th1 cells via P-selectin glycoprotein ligand-1 stimulates LFA-1-mediated cell binding to ICAM-1. JImmunol. 2005; 174:1424–1432. [PubMed: 15661900]

136. Smith ML, Olson TS, Ley K. CXCR2- and E-selectin-induced neutrophil arrest during inflammation in vivo. JExpMed. 2004; 200:935–939.

137. 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. JClinInvest. 2001; 108:557–565.

138. Carman CV, Springer TA. Integrin avidity regulation: are changes in affinity and conformation underemphasized? CurrOpinCell Biol. 2003; 15:547–556.

139. Shimizu Y, van Seventer GA, Horgan KJ, Shaw S. Roles of adhesion molecules in T-cell recognition: fundamental similarities between four integrins on resting human T cells (LFA-1, VLA-4, VLA-5, VLA-6) in expression, binding, and costimulation. ImmunolRev. 1990; 114:109–143.

140. Liu S, Calderwood DA, Ginsberg MH. Integrin cytoplasmic domain-binding proteins. JCell Sci. 2000; 113(Pt 20):3563–3571. [PubMed: 11017872]

141. Kim M, Carman CV, Yang W, Salas A, Springer TA. The primacy of affinity over clustering in regulation of adhesiveness of the integrin {alpha}L{beta}2. JCell Biol. 2004; 167:1241–1253. [PubMed: 15611342]

142. Stuve O, Bennett JL. Pharmacological properties, toxicology and scientific rationale for the use of natalizumab (Tysabri) in inflammatory diseases. CNSDrug Rev. 2007; 13:79–95.

143. Stuve O, Marra CM, Jerome KR, Cook L, Cravens PD, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. AnnNeurol. 2006; 59:743–747.

144. Stuve O, Marra CM, Bar-Or A, Niino M, Cravens PD, et al. Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. ArchNeurol. 2006; 63:1383–1387.

145. Stuve O, Cravens PD, Frohman EM, Phillips JT, Remington GM, et al. Immunologic, clinical, and radiologic status 14 months after cessation of natalizumab therapy. Neurology. 2009; 72:396–401. [PubMed: 18987352]

146. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. NEnglJMed. 2006; 354:899–910.

147. Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. NEnglJMed. 2006; 354:911–923.

148. Howe DK, Sibley LD. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis. 1995; 172:1561–1566. [PubMed: 7594717]

149. Howe DK, Honore S, Derouin F, Sibley LD. Determination of genotypes of Toxoplasma gondii strains isolated from patients with toxoplasmosis. J Clin Microbiol. 1997; 35:1411–1414. [PubMed: 9163454]

150. Sibley LD, Howe DK. Genetic basis of pathogenicity in toxoplasmosis. Curr Top Microbiol Immunol. 1996; 219:3–15. [PubMed: 8791684]

151. Denkers EY, Gazzinelli RT. Regulation and function of T-cell-mediated immunity during Toxoplasma gondii infection. Clin Microbiol Rev. 1998; 11:569–588. [PubMed: 9767056]

152. Gazzinelli R, Xu Y, Hieny S, Cheever A, Sher A. Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with Toxoplasma gondii. JImmunol. 1992; 149:175–180. [PubMed: 1351500]

153. Gazzinelli RT, Hakim FT, Hieny S, Shearer GM, Sher A. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated Toxoplasma gondii vaccine. J Immunol. 1991; 146:286–292. [PubMed: 1670604]

154. Yarovinsky F, Kanzler H, Hieny S, Coffman RL, Sher A. Toll-like receptor recognition regulates immunodominance in an antimicrobial CD4+ T cell response. Immunity. 2006; 25:655–664. [PubMed: 17000122]

155. Goldszmid RS, Coppens I, Lev A, Caspar P, Mellman I, et al. Host ER-parasitophorous vacuole interaction provides a route of entry for antigen cross-presentation in Toxoplasma gondii-infected dendritic cells. JExpMed. 2009