Neuroinflammation: Ways in Which the Immune System Affects the Brain

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Abstract Neuroinflammation is the response of the central nervous system (CNS) to disturbed homeostasis and typifies all neurological diseases. The main reactive components of the CNS include microglial cells and infiltrating myeloid cells, astrocytes, oligodendrocytes, and the blood–brain barrier, cytokines, and cytokine signaling. Neuroinflammatory responses may be helpful or harmful, as mechanisms associated with neuroinflammation are involved in normal brain development, as well as in neuropathological processes. This review examines the roles of various cell types that contribute to the immune dysregulation associated with neuroinflammation. Microglia enter the CNS very early in embryonic development and, as such, play an essential role in both the healthy and diseased brain. B-cell diversity contributes to CNS disease through both antibody-dependent and antibody-independent mechanisms. The influences of these B-cell mechanisms on other cell types, including myeloid cells and T cells, are reviewed in

relationship to antibody-mediated CNS disorders, paraneoplastic neurological diseases, and multiple sclerosis. New insights into neuroinflammation offer exciting opportunities to investigate potential therapeutic targets for debilitating CNS diseases.

Keywords Central nervous system · immunology · inflammation · microglial cells · B cells · paraneoplastic syndromes · multiple sclerosis.

Introduction

The concept of neuroinflammation has widened over the last few decades to include the response of brain cells toward infections and other causes of cell death, as well as infiltration of the brain and spinal cord by cells of the innate and adaptive immune systems. Thus, neuroinflammation is defined as the response of the reactive central nervous system (CNS) elements to altered homeostasis, whether imposed from inside or outside the CNS, and characterizes all neurological diseases, including developmental, traumatic, ischemic, inflammatory, metabolic, infectious, toxic, neoplastic, and neurodegenerative diseases. Mechanisms reminiscent of neuroinflammation, such as the involvement of complement components and microglia in synapse pruning, also occur in healthy brain development. The CNS inflammatory response is also driven by processes as varied as aging, systemic infection, metabolic syndrome, and intrinsic CNS disease. Microglial cells and infiltrating myeloid cells, astrocytes, oligodendrocytes, and NG²⁺ glia (also termed polydendrocytes or oligodendrocyte progenitor cells), along with the blood–brain barrier (BBB), cytokines, and cytokine signaling, form the main reactive components of the CNS. Notably, all cells of the CNS appear to have the capacity to contribute to the inflammatory process.

Summary of a symposium presented at the 17th annual meeting of the American Society for Experimental NeuroTherapeutics (ASENT), Washington, DC, February 20, 2015.

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Microglial cells enter very early in embryonic development and play crucial roles in normal brain development, brain maintenance over the lifespan of the animal, and disease [1–3].

The adaptive immune system also affects neuroinflammation. T cells act in the periphery to initiate immune responses through interactions with antigen-presenting cells (such as dendritic cells). However, no cell exists in the normal brain parenchyma capable of taking up antigen for presentation, exiting the CNS, entering a local lymphatic en route to a lymph node, and initiating an immune response, as is seen in adaptive immune responses elsewhere in the body [4–6]. This fundamental difference represents the cellular basis of immune privilege of the CNS. Thus, T cells respond in the periphery and traffic to the CNS to respond to disease, as shown in Fig. 1 [6]. This “efferent” system by which immune cells respond to an antigen depot in the brain is efficient and implies immunosurveillance.

Central memory CD4⁺ T cells in the cerebral spinal fluid (CSF) carry out immunosurveillance. These cells enter the CSF across the choroid plexus and meningeal veins, and exit in large part via the cribriform plate to the deep cervical lymph nodes, which are accessed via lymphatics in the nasal mucosa [7–9], or as previously shown by Andres et al. [10] and recently revisited [7]. Immune cells cross the parenchymal BBB only under conditions of pathology and indicate the presence of an immune effector response [9]. Leukocyte migration is also an integral component of many neuroinflammatory reactions but has been only partially characterized. Leukocyte–endothelial cell interactions occur in an enormous number of different contexts (physiological, pathological) involving varied cells types and a diversity of vascular beds. This panoply of processes is mediated by molecular combinatorial diversity that includes selectins/carbohydrate ligands; chemokines or other G protein-coupled receptor ligands and their receptors; and integrins/cellular adhesion molecules, with some overlap in trafficking between physiological and pathological processes [11, 12].

B cells produce antibodies after injection of antigen into the CNS [13], indicating that humoral (unlike cellular) adaptive immune reactions can be initiated within the CNS. B cells can differentiate to plasmablasts or plasma cells that produce antibodies with varying functionalities. B cells can also contribute antibody-independent functions that may be disease relevant, including production of inflammatory cytokines and physical interaction and activation of T cells (including presentation of antigen). Positive results of clinical trials using B-cell depletion to treat multiple sclerosis (MS) have sparked renewed interest in B cells and their roles in CNS inflammatory diseases.

A deeper understanding of neuroinflammation can provide targets for development of new

neurotherapeutics. This review summarizes a symposium on neuroinflammation that was presented at the 17th annual meeting of the American Society for Experimental NeuroTherapeutics (ASENT) in Washington, DC, on February 20, 2015. The objectives of this review are to describe the role of microglia in neurodevelopment and neurodevelopmental disorders; examine the diversity of B-cell responses that contribute to CNS disease, including antibody-mediated encephalitis, paraneoplastic neurological diseases (PND), and MS; and understand the coordinated interactions between B cells, myeloid cells, and T cells that may contribute to immune regulation and dysregulation.

The Role of Microglia in CNS Development

Microglia belong to the hematopoietic lineage, as evidenced by microglia loss in *Pu.1* knockout mice, which lack a transcription factor required specifically in hematopoietic cells [14]. Microglia progenitors enter the CNS at embryonic day 9.5–10.5 [1, 2], prior to the emergence and differentiation of other nervous system glial-cell types, and consistent with their critical role in shaping CNS development. The role of microglia in brain development and function was suggested by investigation of Nasu-Hakola disease, a rare genetic dementing leukoencephalopathy caused by homozygous deficiency of triggering receptor on myeloid cells 2 (TREM2), which is only expressed in the CNS on microglia [15]. Moreover, using *in vivo* 2-photon imaging, the processes of cortical microglia can be shown to be constantly active, surveying the brain parenchyma every 4 h and interacting with synapses [16, 17]. It is worth considering whether these active functions may later be deployed in a maladaptive fashion during neurodegenerative processes. As examples of the recent direct implication of microglia in neurodegeneration, some rare polymorphic structural variants of *TREM2* were shown to be risk factors for Alzheimer disease [18, 19], while other *TREM2* variants were found to be associated to frontotemporal dementia [20].

Most recently, a number of critical roles for microglia in brain development and function have been characterized. For example, it was shown that microglia-derived insulin-like growth factor 1 was required for survival of layer V cortical neurons during the first week of post-natal life [21]. In addition, deletion of the fractalkine receptor, CX3CR1, which is enriched in microglia *versus* other resident brain cell types, resulted in delayed maturation of hippocampal synapses and abnormal circuit connectivity in adult mice [22, 23]. Microglia-associated functions are also evident in the adult. For example, by specifically deleting *BDNF* from microglia

Fig. 1 Central memory T cells of cerebral spinal fluid (CSF) are mediators of central nervous system immune surveillance. From *The New England Journal of Medicine*, Israel F. Charo and Richard M. Ransohoff, The many roles of chemokines and chemokine receptors in

inflammation, 354, pages 610–621. Copyright © 2006. Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society [6].

in adult mice, deficits in multiple learning tasks and a significant reduction in motor learning-dependent

synapses were observed, suggesting that microglial production of *BDNF* is important for learning and memory [24].

Synaptic Pruning and Neuronal Development

The brain is diverse and complex, yet precise, with billions of neurons that are connected through thousands of synapses per neuron. Initially, the brain has more neurons and synapses than are required for optimal network function in the mature animal. Redundant synapses are eliminated through a process called pruning, while remaining synapses are maintained and strengthened [25–27]. The pruning process is regulated by neural activity, with the less active synapses being more likely to be eliminated [25–28]. To determine if microglia were involved in synaptic pruning, the postnatal retinogeniculate system has been examined [29].

The retinogeniculate system is comprised of retinal ganglion cells (RGCs) that project and synapse on relay neurons within the lateral geniculate nucleus (LGN) of the thalamus. Synaptic inputs from the ipsilateral and contralateral eyes compete for territory [30–33]. To achieve mature projection patterns, synaptic remodeling occurs, including synapse elimination as well as stabilization and elaboration of remaining synapses [34, 35]. Using high-resolution imaging, microglia were found to engulf presynaptic inputs from both eyes during a peak period in early postnatal synapse remodeling within the LGN [postnatal day 5 (P5)] compared with older ages (P9 and P30) (Fig. 2). These findings suggest the microglia are involved in developmental regulation of synaptic circuit remodeling.

What specific mechanisms drive microglial synaptic phagocytosis? Microglia express many phagocytic surface receptors, including complement receptors [36–38]. In the innate immune system, invading pathogens and debris are eliminated by complement. This process is initiated by C1q to elicit a downstream cascade ultimately leading to the deposition of C3, which interacts with surface receptors on microglia to mediate phagocytosis. Complement deficiency (C1q and C3) has been shown to result in structural and functional deficits in synapse elimination in RGCs [39]. In addition, complement receptor 3, the high-affinity receptor for C3, is highly enriched in microglia during peak remodeling within the LGN (P5). In C3 and complement receptor 3 knockout mice, microglia exhibited a 50 % reduction in engulfment of presynaptic inputs [29], which demonstrates that engulfment of synapses by microglia is, in part, complement dependent. In addition, microglia preferentially engulf less active synapses, suggesting that microglia were, in some way, able to sense changes in neural activity and respond [29].

To assess how microglia respond to activity on a molecular level, a gene expression profile was performed. Following visual stimulation, microglia were isolated from the visual cortex and assessed for changes in gene expression. Preliminary results suggest that a host of genes change in microglia upon manipulation of neuronal activity in the visual system,

and one of the largest categories of genes is related to phagocytic function.

Results thus far demonstrate that microglia respond to changes in neural activity and participate in the process of synaptic remodeling by engulfing synaptic elements. Given that many neurodevelopmental and/or neuropsychiatric disorders (e.g., autism, schizophrenia) have now been associated with abnormalities in synaptic circuits and glial cells, it was hypothesized that microglia–synapse interactions in the developing brain could be disrupted and underlie abnormalities in the synaptic circuitry in these disorders. Rett syndrome, a neurodevelopmental disorder that primarily affects girls, is caused by mutations in a global regulator of chromatin remodeling and gene transcription, methyl CpG-binding protein 2, in >95 % of cases [40, 41]. In a mouse model of this disorder (*MeCP2*-null), RGC synapse remodeling is abnormal [42]. In addition, microglia have been implicated in the disorder by a study in which *MeCP2* was shown to contribute substantially to microglial gene expression phenotypes [43]. However, it was unknown precisely how microglia are contributing to the disorder and whether microglia could be contributing to synaptic abnormalities. In order to address these questions, microglia-mediated engulfment of synapses was assessed. In *MeCP2*-null animals, there was no difference in engulfment at P5 (prephenotypic), whereas at P60 (end stage) microglia are aberrantly upregulating phagocytic machinery (i.e., lysosomes) and engulfing synapses compared with littermate, wild-type control animals. Microglia may not initiate the synaptic abnormalities and phenotype, but contribute to the later destruction of synapses at end stages of disease.

Taken together, these data suggest that microglia contribute substantially to healthy CNS development and function. Understanding their involvement in developmental process will likely provide valuable insights into mechanisms of neurodevelopmental diseases, as well as other neurological disorders with underlying abnormalities in synaptic circuits and glial cell function, such as Alzheimer disease and Huntington disease.

Antibody-Mediated CNS Disorders

Our appreciation of autoantibody-mediated neurological diseases is derived primarily from work on myasthenia gravis (MG), in which the role of acetylcholine receptor antibodies has been well established by both *in vivo* and *in vitro* approaches (reviewed in Vincent [44]). The following criteria for an autoantibody-mediated disease are recognized: 1) detection of autoantibodies in patients and only very occasionally in controls; 2) antibody interaction with the extracellular domain of the target antigen can be detected bound to the target tissue; 3) passive transfer of antibody reproduces features of disease; 4) immunization with antigen produces a

Fig. 2 Microglia-mediated engulfment of retinal ganglion cell (RGC) inputs is developmentally regulated. (A) Schematic of retinogeniculate pruning and strategy used for assessing engulfment: contralateral (red) and ipsilateral (blue) inputs overlap at early postnatal ages [postnatal day 5 (P5)]. Inputs from both eyes prune throughout the dorsal lateral geniculate nucleus (dLGN) during the first postnatal week, which is largely complete by postnatal day 9/10 (P9/10). Engulfment was analyzed throughout the dLGN. (B) Engulfment of RGC inputs is significantly increased during peak pruning in the dLGN (P5).

* $p < 0.001$ by 1-way analysis of variance, $n = 3$ mice/age. (C) Engulfment in P5 dLGN occurs most significantly in synapse-enriched (contralateral and ipsilateral dLGN) versus nonsynaptic (optic tract) regions. * $p < 0.01$ by Student's t test, $n = 3$ P5 mice. All error bars represent SEM. From Dorothy P Schafer, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner, *Neuron*, 74(4), pages 691–705, 2012, Elsevier Inc., doi:10.1016/j.neuron.2012.03.026 [29].

model disease; and 5) reduction of antibody levels in patients correlates with clinical improvement. However, some criteria need modification upon their application to antibodies directed at CNS targets. As an example, because the CNS is protected from circulating antibodies by the BBB, the response to rapid antibody reduction (e.g., following plasma exchange) is usually slow, and transfer of antibody-mediated disease to the experimental animal is more complex. Hence, the identification of CNS disorders that are antibody-mediated currently relies mainly on the presence of an antibody in the serum and usually also in the CSF, the temporal relation to the disease state, and the success of immunological treatments.

MG also led to the hypothesis of a possible role of antibodies in neurodevelopmental disorders. A proportion of women with MG will have babies with neonatal, but usually reversible, myasthenia. However, a very small number have babies affected by arthrogryposis multiplex congenital. This is the result of paralysis *in utero* by antibodies that inhibit the fetal isoform of acetylcholine receptors [45]. A maternal-to-fetal passive transfer model demonstrated that these antibodies were pathogenic and caused arthrogryposis multiplex congenital in the offspring [46]. The possibility that some neurodevelopmental diseases were also due to maternal antibodies was proposed and investigated in one case. Serum

from a mother of 3 children (1 healthy, 1 with autism, and 1 with a specific language problem) was injected systemically into pregnant mice resulting in motor and behavioral abnormalities in the mouse offspring that persisted into adult life [47]. The role of maternal immune activation and so-far unidentified maternal antibodies in behavioral developmental abnormalities continues to be studied in autism and schizophrenia [48].

Antibodies to Identified CNS Proteins

Antibodies to CNS proteins were initially only thought to occur in PND. In the last decade, however, there have been growing reports of diseases with antibodies to CNS surface proteins such as ion channels, receptors, and related proteins. Patients with limbic encephalitis typically present with subacute memory loss, seizures, and personality change, and have magnetic resonance imaging changes in the medial temporal lobes. The most common form of non-PND limbic encephalitis was identified by antibodies to the voltage-gated potassium channel complex (VGKC complex), and found to respond to corticosteroids, plasma exchange, and intravenous immunoglobulin [49, 50]. Low plasma sodium levels are a frequent clue to this form of encephalitis. Subsequently, it became clear

that the antibodies bind to the proteins complexed with the VGKC, most frequently the secreted protein leucine-rich glioma inactivated protein 1 (LGI1) [51]. Patients with LGI1 antibodies often have a novel seizure type, faciobrachial dystonic seizures, during or even preceding the limbic encephalitis [52, 53]. Prompt recognition of these seizures and immunomodulatory treatment may prevent progression to limbic encephalitis [53]. Purified IgG from a patient with LGI1 increased neuronal excitability in hippocampal slices in a similar way to the snake toxin α -dendrotoxin, suggesting the antibodies act to reduce Kv1.1, 1.2 and/or 1.6 function and are epileptogenic [54]. LGI1 is thought to interact presynaptically with the VGKC complex and postsynaptically with α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), and the antibodies also reduce AMPAR expression *in vitro* [55]. Other forms of limbic encephalitis in adults with antibodies to the VGKC complex protein contactin-associated protein 2, γ -aminobutyric acid (b) receptor, or AMPAR may not be clinically distinguishable from this form of limbic encephalitis at presentation but can now be identified by specific antibody tests and are associated with tumors.

Two diseases with strong evidence of pathogenic antibodies are even more important to characterize. First, neuromyelitis optica with aquaporin 4 antibodies is a highly disabling relapsing condition with predominantly white matter lesions in the spinal cord, brain, and optic nerves that needs to be distinguished from MS. Because disability accrues with each relapse, it must be treated effectively. The antibodies lead to loss of aquaporin 4 from the astrocytes *in vitro*, but *in vivo* the predominant mechanism is complement-dependent damage (see Kimbrough et al. [56] for a review). Other patients, often with optic neuritis, have antibodies to myelin oligodendrocyte glycoprotein, which, if measured by IgG-specific assays, appear to distinguish these patients from MS [57].

Second, *N*-methyl-D aspartate receptor (NMDAR) antibodies were first described in young females presenting with severe encephalopathy, psychiatric features, movement disorder, and autonomic dysfunction associated with an ovarian teratoma [58]. Encephalitides with NMDAR antibodies are the most common CNS antibody-mediated diseases with an incidence exceeding that of any single viral etiology in young patients [59]. The characteristic clinical syndrome of a polysymptomatic encephalopathy affects both adults and children with approximately 40 % of the patients presenting under the age of 18 years and many below the age of 50 years [60]. The disease usually presents as a psychiatric/behavioral syndrome with cognitive changes and seizures, and progresses to a very distinctive movement disorder, often with autonomic instability and reduced consciousness. Despite the severity of the encephalopathy, with 69 % of the patients being admitted to intensive care units [60], the majority exhibit normal neuroimaging findings. Similarly, CSF analysis reveals only moderate lymphocytic pleiocytosis and often normal protein

concentration. Intrathecal oligoclonal bands (OCB) are present in up to 60 % of patients [61], but not necessarily at onset of the disease [50, 62]. The electroencephalogram is encephalopathic in the majority, with generalized rhythmic delta activity with or without epileptic discharges [61]. Treatments are similar to those for limbic encephalitis, but second-line therapy with rituximab and/or cyclophosphamide is often required, and many patients need longer-term immunosuppression with azathioprine or mycophenolate mofetil [60]. The clinical presentation of NMDAR antibody encephalitis resembles, to some extent, that of NMDAR agonists, such as phencyclidine and ketamine (which are psychotomimetic and cause stereotypic movements, autonomic instability, and seizures), and the antibodies reduce NMDAR expression in the hippocampus both *in vitro* and *in vivo* [63, 64]. A recent animal model using intraventricular infusion of pooled patients' CSF for 14 days resulted in progressive decrease in novel object recognition memory with depressed behavior, correlating over time with decrease of hippocampal NMDAR [65]. A single intraventricular injection of purified NMDAR IgG into mice increased seizures following the proconvulsant pentylenetetrazol and the number of seizures correlated with the antibodies bound to the hippocampus [66].

Intriguingly, relapses reported after herpes simplex virus infection, particularly in children, can be associated with NMDAR antibodies [67, 68], indicating a clear relationship between the infection and subsequent development of an autoimmune disease. Also surprising, NMDAR antibodies can also be associated with white matter inflammation. Preceding herpes simplex virus infection and white matter involvement are illustrated by the clinical course in 1 unusual case of a child who relapsed with a leukoencephalopathy (Fig. 3) [69]. The NMDAR antibodies were not detected until the relapse occurred.

Finally, a relatively rare antibody is directed to glycine receptors that are inhibitory receptors on the surface of motor neurons in the spinal cord and brainstem. Glycine-receptor antibody disease, or 'stiff-person syndrome plus', is a life-threatening disorder that involves progressive encephalomyelitis, rigidity, and myoclonus [70]. Immunotherapy is an effective treatment [71]. Transfer of antibodies intraperitoneally from patients to mice, with lipopolysaccharide to open the BBB, achieved impaired motor performance, and the antibodies were taken up into spinal cord and brainstem neurons (A Carvajal-González, L Jacobson, and A Vincent, in preparation).

Further work into the pathogenic mechanism of each auto-antibody may help researchers design specific targeted therapies to treat these diseases. For instance, eculizumab, a therapeutic monoclonal IgG that neutralizes the complement protein C5, was recently shown to reduce attack frequency, and stabilize or improve neurological disability in patients with aggressive neuromyelitis optica spectrum disorders [72].

Fig. 3 *N*-methyl-D-aspartate receptor (NMDAR) antibodies in a 2-year-old male following herpes simplex virus encephalitis (HSVE). An unusual case presenting with leukoencephalopathic magnetic resonance imaging changes arising post-HSVE. (A–C) Serial axial T2 fluid attenuation inversion recovery images show localized cortical changes in the left thalamus and occipital lobe resulting from HSVE (not shown). However, after improvement from HSVE the patient relapsed with worsening of cognition and behavior and motor regression. This was associated with bilateral white matter signal changes (leukoencephalopathy) evident in (A) and (B). (C) Following treatment

with intravenous immunoglobulin (IVIG), neuroimaging 2 months later demonstrated substantial resolution of the white matter change. (D) Neurologic relapse correlated with raised NMDAR antibodies in both serum and cerebrospinal fluid (CSF), and demonstrated a clinical response to immunotherapy with reduction of antibody levels. Ab = antibody. From *N*-methyl-D-aspartate receptor antibodies in post-Herpes simplex virus encephalitis neurological relapse, Yael Hacohen, et al., *Mov Disord.* 29(1). Copyright (c) 2014 [Movement Disorder Society] [69].

PND

PND are autoimmune neurodegenerative diseases triggered by an effective antitumor immune response against neuronal antigen expressed in cancer cells [73]. PNDs are rare complications of cancer, and, as such, few controlled trials have evaluated treatments. Animal models of PND provide evidence of mechanisms of both immune activation and insights for treatment.

Paraneoplastic cerebellar degeneration (PCD) is a PND that manifests with the loss of cerebellar function and is associated with autoantibodies named anti-Yo or PCA-1 against the intracellular protein, cerebellar degeneration-related protein 2 (CDR2) protein expressed in Purkinje neurons. CDR2 is expressed ectopically by tumor cells of breast, ovarian, and lung tissue. The signs and symptoms of PCD include saccadic eye movements; double vision; speech difficulties due to disturbances of orofacial muscles, tongue, lips, and throat; sudden changes in voice loudness; voice tremors; dysarthria; and proprioception problems. At autopsy, significant loss of Purkinje cells is detected in the cerebellum [74].

High titer autoantibody responses (IgG1) are observed in patients with PCD [75]. These immune responses are used to

diagnose PCD and imply cooperation between B cells and T helper cell responses. However, high titer antibodies alone do not appear to cause PCD. Transfer of high titer IgG antibodies to mice did not result in neuropathology [76]. As a result, it was hypothesized that the IgG part of the immune response was not sufficient to cause PCD but that a cellular immune response may be contributing in the pathology of PCD. Thus, in addition to examining the IgG responses in PCD, a cellular immune response to CDR2 was investigated and identified [77]. Patients with PCD have CDR2-specific cytotoxic T lymphocytes (CTLs) in blood that may subsequently result in autoimmune neurodegeneration in the CNS. These data are among the first to demonstrate a CTL response to a neuronal antigen, and indicate that both T cells and antibodies are observed in the immune response of PCD.

A transgenic mouse model was developed to better understand immune responses to CNS antigens [78]. The N2-LacZ model was designed to express the intracellular model protein β -galactosidase (β gal) under the control of the *Nov. 2* promoter, which allowed restriction of expression to the CNS. *Nov. 2* is the antigenic target of paraneoplastic opsoclonus–myoclonus ataxia. Immunization with β gal protein resulted in high antibody titers to β gal in both N2-LacZ and control mice and

these titers were maintained for over 6 months, yet no neurological disorders were observed over 1 year of follow-up. However, when adenovirus-expressing β gal was used to induce a CTL response, responses in N2-LacZ mice did not kill as efficiently as controls, demonstrating that the autoreactive CD8 T cells of N2-LacZ mice had become tolerant. Consistent with this interpretation, when β gal-immunized mice were challenged with β gal-expressing tumor, immune responses from control mice prevented tumor growth while N2-LacZ mice had only partial immunity to tumors and none of the mice developed neurologic symptoms.

The results from this model suggest that β gal expression in the CNS affected peripheral T-cell responses. Subsequently, high-affinity T cells from transgenic mice whose T cells were engineered to recognize β gal (CD8-BG1 and CD4-BG2) were transferred to provide a large population of high-affinity T cells specific for β gal. After the T-cell transfer, antitumor immunity was observed in both N2-LacZ and wild-type mice, which was dependent on the presence of both CD4 and CD8 cells. Despite the targeting of β gal by T cells in the periphery, none of the animals developed neurological symptoms or antibodies to β gal. When these mice (harboring the β gal-specific T cells) were subjected to the antibody-producing immunization scheme, 25 % of mice became symptomatic and had evidence of neuronal death and intraparenchymal neutrophils (Fig. 4). These data demonstrate that autoantibodies or T cells alone directed to neuronal antigens do not cause autoimmune disease; rather, both work together to target neurons and induce neuronal cell death.

Targeted Therapy for PND

Anti-B-cell therapeutics have been tested as treatment for PND because antibodies are required for disease manifestation. Rituximab (anti-CD20) was administered to 9 patients with newly diagnosed anti-Hu- or anti-Yo-associated PND at a dose of 375 mg/m² for up to 4 infusions [79]. In 3/9 patients, symptoms improved by a >1 point decrease in the Rankin Scale. Interestingly, not all responding patients showed decreased serum IgG, which suggests that antibody depletion is insufficient to explain the symptomatic improvement associated with treatment. The antibody independent role of B cells in PND requires further study. Another group has tried targeting T-cell responses in patients with PND using tacrolimus, which inhibits calcineurin and blocks T-cell activation [80]. In 26 patients with PND with high titer ($\geq 1:1000$) autoantibodies and worsening neurological symptoms, tacrolimus 0.15–0.3 mg/kg/day plus prednisone provided a median survival of 52 months from diagnosis. Five patients had improvement, but experienced worsening of symptoms and returned for additional treatment. In 2 of these patients, deterioration

was shown to be associated with increased white blood cells in the CSF. Additional analyses showed correlation between neurologic symptoms and white blood cell count in the CSF, suggesting a role for targeting T cells as a means of preventing PND.

B Cells in MS

B cells are now recognized to have both antibody-dependent and antibody-independent actions, with functional heterogeneity existing within the broad B-cell population.

The early MS clinical course involves bouts of focal CNS inflammation and breach in the BBB (the substrate of disease relapses) thought to be mediated by waves of peripherally activated immune cells that target the CNS. Later in the disease course, neuroinflammation appears to be predominantly CNS compartmentalized. Clinical relapses tend to diminish, while ongoing injury is now thought to involve smoldering local CNS responses that are potentially propagated by inflammatory cells within the CNS (the substrate of nonrelapsing disease progression), which are relatively independent of infiltrating waves of peripheral inflammation. The mechanisms by which B cells may participate in both of these disease processes, the peripherally triggered and the compartmentalized CNS, is of interest.

B cells and their products are long recognized as abnormally present in the CNS of patients with MS. The synthesis rate of immunoglobulins, both IgG and IgM, in the CNS is abnormally increased in 95 % of patients with MS. Oligoclonal expansion of B cells and antibodies results in the presence of OCBs in the CSF. Family trees of B cells can be reconstructed using somatic hypermutation analysis of the Ig genes of cells isolated from MS brain tissue and CSF. Such analysis reveals that clonally related B cells populate the CSF and are shared within distinct regions of the CNS of the same patients (though different across patients) [81]. A relationship is seen between the CSF B-cell clones and the IgG/OCB produced in the CSF of patients [82]. The significance, including antigenic specificities of the abnormal CSF antibodies and OCB in patients with MS, remains largely unknown. Circulating anti-CNS antibodies have also been described, and investigations are ongoing to determine the roles of anti-myelin oligodendrocyte glycoprotein and anti-inward rectifier-type potassium channel (KIR4.1) serum antibodies. Overall, contributions of intrathecal antibodies in MS likely vary and may include 1) causing injury or modulating disease expression [83]; 2) occurring as epiphenomenon of B-cell activation (while such antibodies may not be pathogenic, they may still be worthwhile investigating for their potential as biomarkers of disease activity and injury); and 3) having a potential to act beneficially, such as may be the case for anti-Nogo and anti-Lingo antibodies.

Fig. 4 Central nervous system (CNS) autoimmunity demonstrated in N2-LacZ and wild-type (WT) mice. (A) T cells from transgenic mice (CD8-BG1 and CD4-BG2) reject tumor but do not cause autoimmune brain disease. Experimental design: 5×10^6 million CD8⁺ T cells with and without 5×10^6 CD4⁺ T cells from BG1, BG2, or WT mice were transferred into WT or N2-LacZ hosts and challenged with β -galactosidase (β gal)-expressing WP4 cells. After 30 days, mice were secondarily challenged with AdV- β -gal and pertussis toxin (PTx). Tumor growth in N2-LacZ or WT mice was assessed every 2–3 days. (B) T and B cells collaborate to generate neuronal targeting. Brain sections from a neurologically ill mouse were stained and arrows indicate dying neurons in the dentate gyrus. Arrowheads indicate normal reference neurons. H&E = hematoxylin and eosin; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling. From T cells targeting a neuronal paraneoplastic antigen mediate tumor rejection and trigger CNS autoimmunity with humoral activation. Nathalie E. Blachère, et al., *Eur J Immunol.* 44. Copyright (c) 2014 [WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim] [78].

B-Cell Depletion in MS

Several drugs target CD20 and have been shown through phase II clinical trials to decrease new MS disease activity: rituximab [84–86], ocrelizumab [87], and ofatumumab (A. Bar-Or et al., in preparation). The effect of these drugs on B-cell depletion does not appear to affect significantly the abnormal antibodies (IgG), OCB number and pattern, or antibody synthesis rates in the CSF of treated patients [88–90]. Together with the rapid onset of action, these findings suggest that the benefit of B-cell depletion targets antibody-independent functions of B cells. Rituximab treatment resulted not only in near complete depletion of B cells in the circulation, but also in partial depletion of B cells in the CSF (75–80 %), and notably, T cells were also partially depleted (50 %). This observation indicates that the presence of T cells in the CNS of patients with MS may be somehow influenced by B cells. Thus, B-cell

depletion studies in MS suggest that disease-relevant B-cell functions may extend beyond antibody production to include effects on T cells. This may relate to the emerging capacities of B cells to function through antigen presentation, cytokine production, and contribution to the formation of lymphoid architecture.

B-Cell Cytokines and B-Cell/T-Cell Interactions in MS

Several studies have shown that the B cells of patients with MS have defects in the balance of their cytokine expression, with a propensity for overproduction of inflammatory cytokines [e.g., lymphotoxin, tumor necrosis factor alpha (TNF- α), interleukin (IL)-6] and a deficit in production of anti-inflammatory cytokines (e.g., IL-10) [91–93]. In studies of patients with MS who underwent B-cell depletion, treatment resulted in

decreased proinflammatory CD4 responses, including responses of T helper (Th) 1 cells (CD4 T cell or interferon- γ), Th17 cells (CD4 T cell, IL-17), as well as CD8 T-cell responses. Soluble products of activated B cells of untreated patients could reconstitute the diminished T-cell responses observed following *in vivo* B-cell depletion, and this effect was mediated by B-cell lymphotoxin and TNF- α . IL-6 was identified as another inflammatory cytokine abnormally expressed by MS B cells that enhanced T-cell responses, including disease-implicated Th17 cell responses in both patients and the experimental autoimmune encephalomyelitis (EAE) model in which IL-6 was selectively removed only from B cells (Fig. 5) [94]. In addition to IL-10-producing regulatory B cells, another population of B-regulatory cells (and plasma cells) was found to express IL-35 and was able to downregulate T-cell responses. Knockout mice in which only B cells did not express IL-35 lost their ability to recover from EAE [95]. In another study, IgA⁺ plasma cells were shown

to produce TNF- α and inducible nitric oxide synthase, which enhance inflammatory responses in the local environment [96]. These findings suggest that not only B cells, but also plasma cells, may contribute to local inflammation through release of either inflammatory or anti-inflammatory factors.

With respect to the underlying therapeutic mechanism of action of B-cell depletion in MS, the substantial reductions in new MS relapses observed following depletion likely reflects removal of inflammatory B cells that, when present, can induce inflammatory T cells involved in mediating relapses. It is of interest to understand whether B cells can also affect responses of myeloid cells that are known to importantly shape T-cell responses.

Research has also pointed to potential B-cell contributions to CNS-compartmentalized propagation of neuroinflammation, which likely occurs throughout the MS disease process and predominates in the later stages of disease. Meningeal inflammation in MS may include B-cell-rich collections of immune cells [97], and secretory

Fig. 5 Direct implication of interleukin (IL)-6 from B cells mediating a proinflammatory T-cell response. (A) IL-6 production by B cells isolated from patients with multiple sclerosis (MS) is increased compared with healthy controls (HC) after *in vitro* stimulation. * $p < 0.05$. (B) IL-6 production from B cells from patients with MS before and after rituximab treatment (Rtx: 1000 mg $\times$ 2 infusions, 2 weeks apart). * $p < 0.05$; NS=not significant ($p > 0.05$). (C) Mice with a B-cell IL-6 deficiency (B-IL6^{-/-}) develop an attenuated form of experimental autoimmune encephalomyelitis (EAE), implying that B cells drive

disease exacerbation through the production of IL-6. EAE progression was monitored for 32 days after immunization with myelin oligodendrocyte glycoprotein (MOG) in B-WT (blue circles) and B-IL6^{-/-} mice (pink circles). (D) In the EAE model, IL-17 and interferon (IFN)- γ secretion by CD4 splenic T cells from B-WT (blue circles) and B-IL6^{-/-} mice (pink circles) shows impaired T helper 17 cell responses. Error bars represent SEM. ©Tom A. Barr, et al. 2009. Originally published in the *Journal of Experimental Medicine*. doi:10.1084/jem.20111675 [94].

products of MS B cells have been shown to be cytotoxic to oligodendrocytes [98]. Astrocytes have been implicated in support of B-cell survival in inflamed MS CNS, through expression of the B-cell-activating factor of the TNF family [99]. Ongoing research is directed at elucidating effects of glial cells on B-cell subsets and, in turn, effects of B-cell regulatory and effector (Breg and Beff, respectively) products on CNS cells. Emerging insight into complex dynamics of B-cell trafficking in and out of the MS CNS indicates that B-cell clones identified in the MS brain may actually mature in cervical lymph nodes, suggesting that the B cells traffic across the BBB after maturation in secondary lymphoid tissue [100]. This finding raises the interesting possibility that not all MS relapses are triggered from the periphery; rather, they may be “invited” from within the CNS by B cells that exit the CNS to draining lymph nodes where they may act as antigen-presenting cells to activate T cells. There are broad implications for understanding the dynamic interactions of these various cell types, including elucidating the contributions of distinct B-cell subsets in the different MS compartments.

Conclusions

As our understanding of CNS immune function and pathology increases, the roles of individual cell types, particularly microglia and B cells, has expanded. Microglia are crucially involved in CNS development and synaptic pruning, and, as such, they likely affect the incidence and severity of neurodevelopmental disorders. CNS immune privilege in healthy states is different for T cells and B cells and it now appears that the peripheral immune system can generate disease-causing antibodies to CNS-specific surface proteins, as well as generating antibodies to intracellular PND proteins when tumor antigens break tolerance. In the latter, animal models of PND suggest that antibodies and T cells work in concert to target and kill neuronal cells. Targeting B-cell or T-cell immune responses may be effective in preventing these diseases, but long-term immunotherapy entails risks associated with broad depletion or suppression. Ongoing research supports both antibody-dependent and antibody-independent functions of B cells and plasma cells. Antibody-independent functions include cytokine-mediated up- or downregulation of T-cells response. Of interest will be to elucidate how distinct B-cell subsets may contribute differently in MS, both in the periphery and within the CNS (including effects on other immune cells and CNS-resistant cells), over the course of disease to affect both relapsing and progressive disease biology. Taken together, the interactions between B cells, T cells, and myeloid cells will have broad implications in

therapeutic targeting of neuroinflammation and many different types of neurological disease.

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