The resolution of neuroinflammation in neurodegeneration: leukocyte recruitment via the choroid plexus

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Abstract

Inflammation is an integral part of the body's physiological repair mechanism, unless it remains unresolved and becomes pathological, as evident in the progressive nature of neurodegeneration. Based on studies from outside the central nervous system (CNS), it is now understood that the resolution of inflammation is an active process, which is dependent on well-orchestrated innate and adaptive immune responses. Due to the immunologically privileged status of the CNS, such resolution mechanism has been mostly ignored. Here, we discuss resolution of neuroinflammation as a process that depends on a network of immune cells operating in a tightly regulated sequence, involving the brain's choroid plexus (CP), a unique neuro-immunological interface, positioned to integrate signals it receives from the CNS parenchyma with signals coming from circulating immune cells, and to function as an on-alert gate for selective recruitment of inflammation-resolving leukocytes to the inflamed CNS parenchyma. Finally, we propose that functional dysregulation of the CP reflects a common underlying mechanism in the pathophysiology of neurodegenerative diseases, and can thus serve as a potential novel target for therapy.

Keywords choroid plexus; CNS; neuroinflammation; neurodegeneration; protective autoimmunity; T cells

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See the Glossary for abbreviations used in this article.

Introduction

The definition of 'inflammation' has changed dramatically since first described by the Roman scholar, Aulus Cornelius Celsus, 2000 years ago. This phenomenon, defined by Celsus's four cardinal signs of 'rubor et tumor cum calore et dolore' (redness and swelling with heat and pain), and later recognized by Matchnikoff in the 19th century as a productive phagocytic process (Scott *et al*, 2004), is known

today to reflect complex physiological interactions between resident and recruited immune cells, soluble factors and tissue-specific elements. This process, when properly orchestrated, results in protection from spread of infection or damage, followed by a resolution phase in which the affected tissues are restored to their original structural and functional state. However, as much as inflammation is a pivotal process in fighting off many threatening conditions, when it is unresolved, it forms the basis of a wide range of persistent/chronic diseases; while often not the primary cause of destruction in these diseases, secondary damage mediated by the inflammatory response constantly disrupts the return to homeostasis. Traditionally, resolution of inflammation was considered to be a passive process, through which inflammation spontaneously subsides. However, there is a growing appreciation that similarly to the initiation of inflammation, resolution of inflammation is an active process in which inflammation-resolving cells and their cytokines are pivotal for the termination of the inflammatory response (Nathan & Ding, 2010; Buckley *et al*, 2013).

Inflammation in the central nervous system (CNS), neuroinflammation, is common to all neurodegenerative conditions, and is frequently viewed as detrimental to neurological function. In many of these diseases, such as Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD) and Primary Progressive Multiple Sclerosis (PPMS), the etiology of the diseases is primarily sporadic, and no specific cellular or soluble components can account for the inflammatory response (Frank-Cannon *et al*, 2009). Importantly, while the mechanisms that ultimately lead to neurodegeneration are different in each disease, chronic neuroinflammation is typically a prominent feature in the progressive nature of neurodegeneration. Up until recently, it was believed that such local neuroinflammatory response reflects systemic inflammation. This contention, together with the immunologically privileged nature of the CNS, and the fact that neuroinflammation is often destructive to the neural parenchyma, led to the common view that entry of circulating immune cells to the CNS could only escalate the parenchymal damage, and therefore to the attempts to use systemic anti-inflammatory drugs to mitigate neuroinflammation in neurodegenerative diseases (Schwartz & Shechter, 2010b).

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Glossary

AD	Alzheimer's disease
ALS	Amyotrophic Lateral Sclerosis
Aβ	Amyloid beta
BBB	Blood-Brain Barrier
BCSFB	Blood-Cerebrospinal fluid Barrier
CCL	Chemokine (C-C motif) ligand
CCR	Chemokine (C-C motif) receptor
CNS	Central Nervous System
CP	Choroid plexus
CSF	Cerebrospinal fluid
EAE	Experimental Autoimmune Encephalomyelitis
GM-CSF	Granulocyte-macrophage colony-stimulating factor
ICAM-1	Intercellular Adhesion Molecule 1
IFN	Interferon
IL	Interleukin
PD	Parkinson's disease
PPMS	Primary-Progressive Multiple Sclerosis
RRMS	Relapsing-Remitting Multiple Sclerosis
SCI	Spinal Cord Injury
SPMS	Secondary-Progressive Multiple Sclerosis
Tregs	Regulatory T cells
VCAM-1	Vascular Cell Adhesion Molecule

Studies initiated by our group more than a decade ago, demonstrated that the recovery of the CNS from acute damage is non-tissue autonomous and requires the involvement of circulating leukocytes (Rapalino *et al*, 1998; Moalem *et al*, 1999; Yoles *et al*, 2001). These findings have led to subsequent studies which highlighted the possibility that infiltrating monocyte-derived macrophages are needed for fighting off neurodegenerative conditions (Butovsky *et al*, 2006, 2007) and brought to appreciation the pivotal role of CNS-specific T cells in CNS maintenance and repair (Kipnis *et al*, 2004c; Ziv *et al*, 2006), which led to the formulation of our theory of 'protective autoimmunity'. According to this theory, a T-cell response, involving CD4⁺ T cells that recognize CNS self-antigens, provides the body's mechanism of protection, maintenance and repair in health and disease (Box 1). How the CNS parenchyma can benefit from circulating leukocytes under the constraints of being an immune privileged site, and how the cross-talk between the CNS and circulating immune cells can take place, despite the complex barrier systems that separate the CNS from the circulation, are now becoming more fully understood.

Here, we will discuss the complexity of the immunological processes involved in chronic neuroinflammation and neurodegeneration, and emphasize that they are mediated by interactions of a physiological immune cell network, encompassing effector and regulatory, resident and infiltrating immune cells, which ultimately culminate in the recruitment of inflammation-resolving cells to the CNS via a designated 'gate' within the CNS territory but outside the parenchyma. Under physiological conditions, this immunological network maintains immune surveillance of the CNS from outside the parenchyma, and under pathological conditions, it participates in the resolution of neuroinflammation. We will suggest that this response is mediated by a unique neuro-immunological interface, the brain's choroid plexus (CP), which serves as a selective gateway for leukocyte entry. Accordingly, the fate of this interface under disease conditions can be viewed as a limiting factor in controlling the levels of systemic immune support provided to the CNS.

Box 1: Overview of "protective autoimmunity"

'Protective autoimmunity' (Moalem *et al*, 1999) has been proposed by our team as an essential physiological mechanism for CNS protection, repair and maintenance in both health and pathological disease. Accordingly, autoimmune T cells do not necessarily reflect immune system malfunction, as originally believed; a well-controlled generation and activation of CNS-specific T cells is a purposeful process, and only when it is dysregulated these cells become destructive. This model challenges the dogma that an organism needs to completely delete self-reacting cells, and suggests that the anti-self response is pivotal for CNS tissue maintenance, though it requires more rigorous control than the response to non-self. It remains to be established whether protective autoimmunity is a more general phenomenon which occurs in tissues other than the CNS.

Circulating immune cells fight off neuroinflammation in neurodegenerative diseases

The vicious cycle of non-resolved neuroinflammation

As briefly described above, for decades, neuroinflammation was viewed as a unified pathological phenomenon that should be completely eliminated regardless of its primary etiology. As a result of progress in understanding the pathophysiology of many neurodegenerative diseases, it has become slowly understood that the etiology of each disease has great impact on the nature of the local inflammatory response, and the role that is played by innate and adaptive immunity. Thus, the inflammatory component of the autoimmune disease Relapsing-Remitting MS (RRMS) differs from that of neurodegenerative diseases such as AD, ALS, and even other forms of MS [Secondary-Progressive (SPMS) and Primary-Progressive (PPMS)], with respect to both the local and the systemic immune response (Sospedra & Martin, 2005; Venken *et al*, 2008; He & Balling, 2013).

Most CNS pathologies are characterized by an early acute reparative phase of microglial activation, which is needed for the effective removal of threatening compounds, toxic agents, and misfolded proteins (Block *et al*, 2007). This response often fails to lead to complete removal of the threats, or even results in an escalating effect in the form of a vicious cycle of unresolved local cytotoxic inflammation. Such a phenomenon led our group to suggest the possibility that CNS pathologies emerge after a prolonged struggle between a pathological process unique to a given disease, and an attempt for local tissue restoration by the immune system, and that such a process is reminiscent of the 'competition' between tumors and immune cells (Schwartz & Ziv, 2008). The triumph of cancer and its rapid propagation occurs when the tumor ultimately escapes from immune control. Such a competition between the microglia and the local source of CNS pathology is seen for example in AD, in which amyloid beta (A β) deposits (amyloid plaques) locally activate the resident, 'resting', microglia. In their activated state, microglia can potentially restrict plaque formation by secreting proteolytic enzymes, or clear A β by receptor-mediated phagocytosis (Lai & McLaurin, 2012; Sierra *et al*, 2013). However, chronic microglial activation in neurodegenerative diseases is accompanied by the production of pro-inflammatory cytokines which might override the beneficial effect of these cells (Block *et al*, 2007; Hanisch & Kettenmann, 2007). Arresting such a

vicious cycle requires an active resolving immune response and the recruitment of systemic monocyte-derived macrophages (Simard *et al*, 2006; Butovsky *et al*, 2007), for which the unique structure of the CNS as an immune privileged site may pose an obstacle. As will be discussed below, we suggest that under acute injurious conditions microglial activation is among the first immune-related events at the lesion site (Fig 1), yet it seems that these cells either cannot acquire a resolving-phenotype, or fail to be skewed to this phenotype in a timely manner (Shechter *et al*, 2009; Shechter & Schwartz, 2013).

CNS-specific T cells: from offenders to protectors

The notion of the CNS as an immune privileged site (Ehrlich, 1885), from which immune cells should be excluded, was initially supported by the seminal observations of Shirai (Shirai, 1921) and Medawar (Medawar, 1948) who demonstrated that tissue grafts in the eye or brain survive longer than those implanted in other areas of the body. In the following decades, this notion was further substantiated by mounting experimental data demonstrating the spatial and immunological separation of the CNS from circulating immunity. Spatially, the CNS was found to be an enclosed compartment,

Figure 1. Local and systemic immune cell response to acute or chronic CNS damage.

Under neuroinflammatory situations, either acute (upper part) or chronic (bottom part), CNS parenchymal damage (black line) leads to glial cell activation and to local inflammatory response (red line). In response to acute CNS damage, circulating leukocytes are recruited to the CNS (blue line) and participate in the resolution of the innate inflammatory response. When such a response is not resolved it may lead to chronic neuroinflammation, associated with escalating toxicity and neuronal death, which is the case in chronic neurodegenerative diseases; the lack of resolution reflects insufficient recruitment of systemic inflammation-resolving immune cells to the CNS.

secluded from the circulation by physical barriers, and the fact that the CNS has its own population of phagocytic cells, the resident microglia, was used to support the view that it is an immunologically autonomous unit, which ideally functions in the absence of immune surveillance (Barker & Billingham, 1977). In apparent support of this view, whenever immune cells were found in the perivascular spaces of the CNS, in the cerebrospinal fluid (CSF), or in the parenchyma itself, it was almost always considered a sign of autoimmune disease or at least of the beginning of such a disease. Accordingly, adaptive immune cells inside the CNS were repeatedly described as the prime culprits in experimental autoimmune encephalomyelitis (EAE) (Swanborg, 2001), a murine model of MS, and were shown to directly attack CNS myelin, leading to neurodegeneration (Sospedra & Martin, 2005).

Several populations of immune cells were shown to be involved in autoimmune pathologies, among which are CNS-specific T cells (both CD4⁺ and CD8⁺) and monocyte-derived macrophages. Passive transfer of T cells specific for components of CNS myelin was shown to suffice for evoking EAE, and macrophages were shown to accumulate in the inflamed parenchyma (Martin *et al*, 1992; Steinman, 1996; Owens *et al*, 1998); such macrophages appeared morphologically identical to the activated microglia. It is thus perhaps not surprising that interactions between adaptive immune cells and the CNS have received a negative reputation, leading to widespread clinical attempts to use anti-inflammatory drugs to treat CNS pathologies, without necessarily differentiating between local neuroinflammation occurring under non-inflammatory neurodegenerative diseases, and inflammatory CNS diseases such as RRMS; in most of these cases, anti-inflammatory treatments failed (Cudkowicz *et al*, 2006; Gordon *et al*, 2007; Wolinsky *et al*, 2007; Fondell *et al*, 2012; Wyss-Coray & Rogers, 2012).

Over the past decades, the widely held view of autoimmune cells as an indiscriminately negative feature of the immune response has fundamentally changed. We now know that the CNS is constantly surveyed by circulating immune cells within the CSF (but not within the parenchyma), and that under physiological conditions, activated T cells patrol the CNS, without the appearance of autoimmune disease (Hickey, 1999; Engelhardt & Ransohoff, 2005; Kunis *et al*, 2013). In addition, CNS-specific T cells were shown to support brain plasticity, both in health and in response to CNS trauma [thoroughly reviewed in (Kipnis *et al*, 2012; Rook *et al*, 2011; Schwartz & Shechter, 2010a)]. Nevertheless, the fact that under homeostatic conditions, circulating immune cells are rarely found in the brain parenchyma raises several key questions regarding the locations from which CNS-specific T cells affect the healthy brain.

The theory of 'protective autoimmunity', which attributes a beneficial role to autoimmune CNS-specific CD4⁺ T cells in healthy CNS maintenance and repair, has provided insights to this enigma. The neuroprotective capacity of autoimmune cells was demonstrated across different models of CNS pathologies, including mechanical injuries (Moalem *et al*, 1999; Hauben *et al*, 2001; Kipnis *et al*, 2002b; Hofstetter *et al*, 2003; Ling *et al*, 2006), chronic neurodegenerative diseases (Benner *et al*, 2004; Butovsky *et al*, 2006; Laurie *et al*, 2007; Mosley *et al*, 2007), and imbalances in neurotransmitter levels (Schori *et al*, 2001). Moreover, in the healthy brain, autoimmune CD4⁺ T cells were found to play a role in maintenance of neuronal plasticity, including neurogenesis and spatial learning/memory (Kipnis *et al*, 2004c; Ziv *et al*, 2006; Radjavi

et al, 2013). Examining the potential mechanism by which these cells exert a neuroprotective role in models of CNS trauma and neurodegeneration has highlighted their ability to control CNS inflammation as part of a wider cellular network.

Circulating myeloid cells are recruited to the injured CNS by CNS-specific T cells

While the unexpected experimental findings that recovery from CNS injuries is impaired in immune compromised mice (Bakalash *et al*, 2002; Kipnis *et al*, 2001), and is boosted in animals vaccinated with CNS-specific antigens (Hauben *et al*, 2000, 2001), were well-documented, the underlying mechanism remained puzzling. A few years ago, an insight was obtained when it was shown that CNS-specific T cells have the ability to augment the recruitment of anti-inflammatory monocyte-derived macrophages to the injured spinal cord (Shechter *et al*, 2009). Through these studies, it became evident that infiltrating myeloid cells, which are virtually identical in morphology to the resident microglia and were often viewed as infiltrating 'microglia', have distinct and non-redundant activities from those of the resident cells (Shechter *et al*, 2009; Jung & Schwartz, 2012). Independent studies have revealed that each population of myeloid cells has a distinct origin (Ginhoux *et al*, 2010) (Box 2).

Box 2: Microglia and CNS-infiltrating monocyte-derived macrophages

Distributed throughout the various regions of the brain, the spinal cord and the retina, the microglia are the resident myeloid cells of the CNS (Río-Hortega, 1937); though long suspected (Alliot *et al*, 1991), these cells were only recently shown to have a distinct origin than monocyte-derived macrophages (Ginhoux *et al*, 2010), which infiltrate to the brain under pathological conditions. The microglia, which originate from the yolk sac (Ginhoux *et al*, 2010), populate the CNS during early development, and serve a sentinel role in maintaining adult brain homeostasis, with limited self-renewal capacity (Hanisch & Kettenmann, 2007; Saijo & Glass, 2011). Under both acute and chronic conditions of neuroinflammation, blood-borne myeloid cells which home to the damaged CNS share many morphological and phenotypical features with the activated microglia, a fact which indistinctly associated them with pathological CNS inflammation. Over the past two decades, intensive research has revealed that infiltrating blood-derived macrophages are not part of the microglia turnover, and that they can exhibit enhanced phagocytic capacity, neurotropic support, and anti-inflammatory characteristics, compared to the microglia (Shechter *et al*, 2009; Jung & Schwartz, 2012; London *et al*, 2013). Thus, the potential beneficial role of blood-derived macrophages was demonstrated in various CNS pathologies, ranging from acute insults to neurodegenerative diseases, and neurodevelopmental mental disorders [thoroughly reviewed in (Shechter & Schwartz, 2013)].

Evidence for a role of monocyte-derived macrophages in response to neuroinflammation was first obtained in experimental murine models of spinal cord injury, where it was demonstrated that 'alternatively activated' blood macrophages, when locally transplanted at the margin of a spinal cord lesion, resulted in improved recovery (Rapalino *et al*, 1998). The success of such macrophage transplantation was found to be dependent on the site of their injection (for example, no effect was found when cells were administered at the center of the lesion or far from its margins), the number of injected cells, and the time elapsed between the injury and the injection (Schwartz & Yoles, 2006). Using animal models of bone

marrow chimeric mice, which allowed distinction between activated microglia and CNS-infiltrating monocyte-derived macrophages, as well as their selective ablation, revealed that the infiltrating cells, display a local anti-inflammatory phenotype, which was critically dependent upon their expression of interleukin-10 (Shechter *et al*, 2009), a key factor in suppressing microglial activity (Taylor *et al*, 2006). This inflammation-resolving role of the recruited macrophages was further demonstrated and characterized in a model of retinal insult, in which monocyte-derived macrophages were shown to infiltrate the injured retina and support cell renewal and survival by skewing the local pro-inflammatory cytokine milieu (London *et al*, 2011).

Circulating myeloid cells were also shown to support the CNS under conditions of chronic inflammation and neurodegeneration. In ALS, a fatal neurodegenerative disease affecting motor neurons, a gradual increase in microglial activation at the spinal cord during the course of the disease leads to accumulation of toxic inflammatory compounds in a vicious cycle of microglial toxicity (Turner *et al*, 2004; Barbeito *et al*, 2010; Liao *et al*, 2012). *In vitro* studies have shown that microglia isolated from mice over-expressing human mutant superoxide dismutase (mSOD1), an ALS mouse model, produce higher levels of TNF- α when stimulated with LPS compared with wild-type microglia (Weydt *et al*, 2004), and microglia that express less mSOD1 can attenuate the local inflammatory response (Beers *et al*, 2006; Xiao *et al*, 2007). In line with the *in vitro* findings, animals whose bone marrow is replaced with bone marrow cells deficient in expression of myeloid differentiation

primary response protein (MyD88) have an earlier disease onset and a shorter lifespan than mSOD1 mice receiving normal bone marrow (Kang & Rivest, 2007). In murine models of AD, conditional ablation and reconstitution strategies demonstrated that amyloid beta (A β) plaque formation in the diseased brain can be attenuated by blood-borne macrophages (Simard *et al*, 2006; Butovsky *et al*, 2007; Town *et al*, 2008). This reduction in amyloid plaque load is correlated with arrest of the local neuroinflammatory response, reduction of pro-inflammatory cytokine levels, and local elevation of neurotrophic factors. The neuroprotective effect of circulating myeloid-derived cells was also demonstrated using various 'microglial replacement' strategies in *MECP2* mutant mice, a model for Rett syndrome (Derecki *et al*, 2012), and these cells were shown to correct abnormal behavior in *Hoxb8* mutant mice, a model for obsessive-compulsive disorder (Chen *et al*, 2010).

The brains choroid plexus: a selectively activated gate for leukocytes

From barrier to gate

Though the immune cell populations described above were repeatedly suggested to exert their beneficial effect on the diseased CNS by controlling neuroinflammation, their limited numbers within the healthy CNS parenchyma and the fact that their infiltration to the CNS upon injury is limited and carefully regulated, raised several key questions with regard to their trafficking routes and sites of interaction with the CNS.

Table 1. Structural and functional differences between the BBB and the BCSFB as neuro-immunological interfaces.

	BBB	BCSFB	References
Location	Brain parenchymal capillaries	Brain ventricles	Emerich <i>et al</i> (2005), Weiss <i>et al</i> (2009), Abbott <i>et al</i> (2010), Johanson <i>et al</i> (2011), Redzic (2011), Ransohoff & Engelhardt (2012)
Structure	Created by the endothelial cells that form the walls of the capillaries	A villous layer of modified cuboidal epithelium which surrounds an inner stroma, and is vascularized by capillaries	
Capillary type	Continuous	Fenestrated	
Barrier properties	Maintained at the level of specialized (extremely tight) endothelial tight junctions, and by the glia limitans	Maintained at the level of specialized (leaky) epithelial tight-junctions of the choroid plexus	
Main functions	Classically recognized for its barrier role and its disruption in CNS pathologies	Classically recognized for its secretory role as the main producer of the CSF	
Main roles in maintaining CNS biochemical homeostasis	Buffering passive diffusion and active transport of blood-borne solutes and nutrients to the CNS	Actively modulating the chemical exchange between the CSF and the brain parenchyma, including surveying the chemical and immunological status of the brain, detoxifying the CSF, and secreting a nutritive 'cocktail' of neurotrophic polypeptides	
Immune cell localization	Virchow–Robin perivascular spaces	At the choroid plexus stroma, and on the epithelial apical side (epiplexus/Kolmer cells)	Steffen <i>et al</i> (1996), Carrithers <i>et al</i> (2002), Kivisakk <i>et al</i> (2003), Szmydynger-Chodobska <i>et al</i> (2009, 2012), Kunis <i>et al</i> (2013), Shechter <i>et al</i> (2013b)
Expression of immune cell trafficking determinants	Induced under inflammatory conditions	Constitutively expressed and further induced in response to CNS "danger" signals	
Immune cell trafficking across the barrier	Mainly documented under inflammatory pathological conditions of the CNS	Immune surveillance of the CSF in the steady-state, and mediating trafficking of leukocytes to the CNS following parenchymal damage	

The CNS barrier system includes the blood–brain barrier (BBB), which is formed by tightly connected endothelium that surrounds parenchymal microvessels, and the blood–CSF barrier (BCSFB), which is formed by the CP, an epithelial monolayer that surrounds an inner stroma, and is vascularized by blood vessels (the structural and functional differences between the BBB and the BCSFB as neuro-immunological interfaces are summarized in Table 1). The classic role attributed to the CP is the production of the CSF, providing the brain with a nutritive metabolic milieu, and forming a protective mechanical cushion. Over the last decade, however, this compartment was reported to participate in various aspects of brain homeostasis, suggesting that it plays a much greater role than previously believed (Emerich *et al*, 2005; Johanson *et al*, 2011; Falcao *et al*, 2012; Baruch & Schwartz, 2013).

Structurally, in contrast to the BBB, the BCSFB lacks endothelial tight junctions or astrocytic glia limitans, and its barrier properties are mostly restricted to the tight junctions of the epithelial layer of the CP (Redzic, 2011). This relative structural permissiveness for immune cell trafficking, and the fact that the cellular composition of the ventricular and lumbar CSF differs from that of the blood, and is dominated by CD4⁺ memory T cells (Kivisakk *et al*, 2003; Provencio *et al*, 2005), led to the suggestion that T cells enter the CSF in a regulated manner via the choroid plexus. Unlike the BBB, the CP constitutively expresses adhesion molecules and chemokines, which support transepithelial leukocyte trafficking (Steffen *et al*, 1996; Kunis *et al*, 2013; Shechter *et al*, 2013b); the selective expression of integrin receptors, such as intercellular adhesion molecule (ICAM)-1, on the apical side of the CP was recently suggested to serve as a foothold for ‘basal to apical’ transepithelial migration of leukocytes across the CP (Kunis *et al*, 2013). Experimentally, leukocyte trafficking through the CP-CSF route is supported by findings that adoptively transferred T cell blasts are found in the CP (Carrithers *et al*, 2000, 2002), and that neutrophils (Szmydynger-Chodobska *et al*, 2009), monocytes (Szmydynger-Chodobska *et al*, 2012; Kunis *et al*, 2013; Shechter *et al*, 2013b) and T cells (Kunis *et al*, 2013) enter the injured CNS through this site in response to parenchymal damage. Interestingly, this route was also suggested to serve as a gateway for encephalitogenic cells entering the CNS via CCL20-CCR6 interactions between Th17 cells and CP-derived CCL20 (Ransohoff, 2009; Reboldi *et al*, 2009). Yet, as CCR6 is also expressed by other cell populations, including T regulatory cells (Tregs), CCL20 may also take part in facilitating CNS immune surveillance under non-pathological conditions. Importantly, encephalitogenic IL-17- or GM-CSF-producing T cells are scarcely found in the stroma of the CP in healthy mice (Kunis *et al*, 2013), and when CP epithelial cells are exposed to these cytokines, they do not upregulate the expression trafficking determinants (Kunis *et al*, 2013).

IFN- γ -dependent activation of the choroid plexus

Using high-throughput analysis of the T-cell receptor (TCR) repertoire, we recently demonstrated that the CP stroma is enriched with CD4⁺ T cells specific for CNS antigens (Baruch *et al*, 2013). These cells were found to express cellular markers of effector memory cells (Baruch *et al*, 2013), and to produce IL-4 and interferon (IFN)- γ (Kunis *et al*, 2013), indicating a constant presence of Th1 and Th2 cells in the naïve CP. *In vitro* studies of the response of the CP to

these effector cytokines, by co-culturing mouse primary CP epithelial cells with various cytokines, revealed that IFN- γ plays an essential role in the activation of the CP to enable leukocyte trafficking (Kunis *et al*, 2013). *In vivo* studies revealed that bone marrow chimeric mice lacking IFN- γ receptor (IFN- γ R) solely in the CNS, or lacking IFN- γ expression solely by circulating immune cells, as well as IFN- γ R knockout (IFN- γ R-KO) transgenic mice, all showed defects in the activation of the CP for leukocyte trafficking (Kunis *et al*, 2013).

These findings thus suggest that under physiological conditions, IFN- γ /IFN- γ R signaling by circulating cells and the CP epithelial cells is essential for leukocyte immune surveillance of the CNS. Moreover, this IFN- γ -dependent activation of the CP was also shown to be relevant in the context of CNS damage; following SCI, IFN- γ R-KO mice show reduced infiltration of both CD4⁺ T cells and monocytes to the CSF and to the spinal cord lesion site compared to injured non-transgenic mice, with detrimental effects on their recovery process (Kunis *et al*, 2013).

The CP-CSF route for monocyte-derived macrophage recruitment

A recent study by our group, in which monocyte trafficking routes to the CNS were examined following SCI, provided several important insights regarding the properties of the CP as a selective, as well as an educative gate, for monocyte entry to the CNS. The fact that following SCI, blood-derived macrophages appear at the lesion site of the CNS parenchyma relatively late (Shechter *et al*, 2009), suggested the possibility that their entry might take place through a remote gate, and not through breaches in the BBB, as was commonly assumed. Examining potential trafficking routes to the CNS revealed that following SCI, monocytes that locally become inflammation-resolving cells primarily traffic through the CP on their way to the lesion site (Shechter *et al*, 2013b). This route was found to be dependent on injury-induced expression of several trafficking molecules at the CP compartment, specifically, the integrin VCAM-1, expressed by the CP endothelial vasculature, and the enzyme CD73, expressed by the epithelial cells. Blocking of these homing molecules inhibited M2 macrophage recruitment to the injured parenchyma, and resulted in poor recovery following injury. Moreover, mechanical blocking of the CSF flow using a polymerizing agent (Matrigel) resulted in a similar observation of reduced recruitment of M2 macrophages to the lesion site following SCI (Shechter *et al*, 2013b).

The fact that leukocytes traffic through a remote ventricular gateway, located far from the lesion site at the spinal cord (Kunis *et al*, 2013; Shechter *et al*, 2013b), was suggested to serve a role in ensuring their cellular skewing capacity towards an anti-inflammatory phenotype. The composition of the CSF is largely immunosuppressive (Gordon *et al*, 1998), dominated by cytokines such as IL-13 and TGF- β 2 (Shechter *et al*, 2013b). This immunosuppressive environment is a common feature of many ‘immune privileged’ sites, such as the testis and the eye (Shechter *et al*, 2013a). As this soluble milieu is known to affect monocyte skewing towards M2/resolving activity, it was suggested that the extended migration route from the ventricular entry site at the CP to the spinal cord lesion site serves an ‘educative’ purpose for ensuring that those monocyte-derived macrophages that home to the injured spinal cord would be biased towards inflammation-resolving cells prior to their arrival at the inflamed site (Shechter *et al*, 2013b).

Protective autoimmunity: an inflammation-resolving immune cell network

As discussed above, under physiological conditions, T cells at the brain's territory are mainly found at the CSF, CP, and meningeal spaces (Engelhardt & Ransohoff, 2005). It is at these sites that T cells were suggested to encounter their cognate antigen, presented to them by tissue-resident APCs (Kivisakk *et al*, 2009; Derecki *et al*, 2010; Anandasabapathy *et al*, 2011; Baruch *et al*, 2013). The life-long presence of CNS-specific CD4⁺ T cells in the stroma of the healthy brain's CP (Baruch *et al*, 2013) suggests this compartment as a possible mediator through which they can affect the CNS. As such, the strategic location of the CP between the blood and the cerebrospinal fluid, makes it ideal for functioning as an active neuro-immunological interface that is constantly exposed to signals coming from both the CNS parenchyma and the circulation [thoroughly reviewed in (Baruch & Schwartz, 2013)]. Accordingly, the fate of this interface is likely to affect, on the one hand, healthy brain plasticity, and, on the other hand, aging, and neurodegenerative conditions, when its functioning is dysregulated.

Recognizing the CP as an important compartment for the life-long maintenance of the CNS, led us to examine its fate during aging. Studying the CP compartment in aged mice revealed that in terms of effector-cytokine balance, the CP stromal environment largely reflects peripheral immunity. Thus, the aged CP shows a strong bias towards the Th2 effector response (Baruch *et al*, 2013), a reflection of the situation in the circulation during aging, which exhibits a drastic shift of the T helper response towards a Th2 type (Shearer, 1997; Rink *et al*, 1998). The local cytokine balance shift of the aged CP was found to trigger the epithelial cells to produce the chemokine CCL11 (Baruch *et al*, 2013), associated with aged-dependent cognitive dysfunction (Villeda *et al*, 2011; Villeda & Wyss-Coray, 2013); IFN- γ levels at the CP were found to counter this effect (Baruch *et al*, 2013).

Our recent understanding of the essential role of circulating IFN- γ -producing cells in the activation of the CP to allow leukocyte trafficking (Kunis *et al*, 2013), together with the observation that this compartment is enriched with CNS-specific CD4⁺ cells, highlights the importance of these cells in the overall 'protective autoimmune' cellular network; yet, they do not function alone. An earlier study by our group demonstrated in a model of CNS injury (optic nerve crush) that myelin-specific Th1 cells cannot exert their beneficial effect on recovery when they are administered by passive transfer to immune compromised animals (neonatally thymectomized rats); the same cells are neuroprotective when administered to immune competent rats (Kipnis *et al*, 2002b). Importantly, *in vitro* studies of organotypic hippocampal slice cultures revealed that though both CNS-specific Th1 and Th2 cells are neuroprotective, Th2 cells are significantly more potent than Th1 cells in preventing neuronal death (Wolf *et al*, 2002). These results suggest that though Th1 cells are needed as part of the cellular network of protective autoimmunity, local parenchymal neuroprotection is mediated by other cell types.

Which cells are recruited to actually resolve the local neuroinflammatory response within the CNS? Examining the injured parenchyma using the model of SCI at the steady-state stage (not at the hyper-acute stage following the injury) shows that immune cells within the parenchyma mostly IL-10 and GATA3 (a Th2 transcription factor) and hardly any IFN- γ , which suggests that the injured parenchyma is populated at this stage by immune-resolving cells (Yoles

et al, 2001). These results are consistent with the accumulation of IL-10-producing cells in the CNS parenchyma following a local inflammatory response, whether these are M2 monocyte-derived macrophages (Shechter *et al*, 2009; Zhang *et al*, 2012) or T regulatory cells (Tregs) (O'Connor *et al*, 2007), and suggest such cells to be essential for the resolution stage of the inflammatory response. As systemic IFN- γ -producing cells are necessary for the activation of the CP to enable leukocyte trafficking (Kunis *et al*, 2013), it remains to be determined whether their sole role is in this compartment, or are they needed in the subsequent steps of this immunological cascade; specifically, whether Th1 effector cells are also actively recruited to the CNS parenchyma and are locally converted there, or *en route* within the CSF, to IL-10-producing cells; this phenotypic switch can occur under inflammatory conditions (Fujio *et al*, 2010; Cope *et al*, 2011).

The studies summarize above emphasize that it is imperative to distinguish between the need for local suppressive activity of immune cells within the inflamed CNS, and the levels of these cells in the circulation/lymphoid organs. Regulatory T cells are crucial for controlling the activity of self-reactive T cells (Costantino *et al*, 2008), and their dysfunction in MS (especially in RRMS) and EAE is mainly associated with their deficiency in the circulation (He & Balling, 2013). Therefore, regulatory T cells in the periphery were suggested to play a neuroprotective role under conditions of excessive inflammation or neuroinflammation (Liesz *et al*, 2009). Unlike the autoimmune inflammatory diseases, RRMS and possibly early stages of all types of MS, in neurodegenerative conditions and aging, Tregs levels are increased in the lymphoid organs and peripheral blood, affecting effector T cell activity and availability (Chiu *et al*, 2007;

Figure 2. A simplified diagram describing the relationship between peripheral immune suppression, recruitment of inflammation-resolving immune cells to the CNS, and disease progression in chronic neurodegenerative diseases versus that in relapsing-remitting MS (RRMS).

Notably, the distinct peripheral immunological states found in the different neurodegenerative conditions emphasize that opposite immunomodulatory approaches might be needed for resolution of local neuroinflammation under autoimmune inflammatory disease (blue line and dashed blue line) versus chronic neurodegenerative disease (red line and dashed red line).

Figure 3. The orchestration of inflammation-resolving leukocyte trafficking through the brain's choroid plexus.

(1) In the steady state, astrocytes and microglia serve as sentinels of tissue homeostasis, providing the neural parenchyma with a supportive neurotrophic environment. (2) Following CNS insult, dying cells and accumulation of cellular debris locally activate resting microglia and astrocytes. Activated microglia phagocytose cellular debris while concurrently secreting toxic compounds, including pro-inflammatory cytokines (such as IL-1 β , TNF- α and IL-6) and reactive oxygen and nitrogen species (ROS, NOS). (3) Parenchymal-derived signals (e.g., TNF- α) reach the choroid plexus (CP) through the cerebrospinal fluid (CSF) and are sensed by cytokine receptors and Toll-like receptors (TLRs) expressed by the CP epithelium. (4) These signals, together with IFN- γ from CP stromal Th1 cells, initiate a cellular trafficking cascade for T cells and monocytes entering the CNS. This cascade includes the upregulation of integrin receptors (e.g. ICAM-1), chemokines (e.g. CXCL10) and surface enzymes (e.g. CD73) by the CP epithelium, which enables selective recruitment of leukocytes to the CNS. (5) Entry through the CP-CSF serves an educative role in skewing infiltrating immune cells towards an anti-inflammatory/suppressor phenotype. (6) Along the repair process, monocyte-derived macrophages and regulatory T cells (Tregs) are recruited to the inflamed CNS parenchyma and suppress the inflammatory response by the secretion of anti-inflammatory cytokines such as IL-10 and TGF- β . (7) In chronic neurodegenerative diseases, circulating immune suppressor cells [such as Tregs and myeloid-derived suppressor cells (MDSCs)] maintain peripheral immune suppression and inhibit immune cell trafficking to the CNS. (8) Lacking the support of circulating inflammation-resolving leukocytes, dying cells, cellular debris and protein aggregates locally activate astrocytes and microglia in an escalating vicious cycle of local toxicity; neurons residing in this inflammatory microenvironment degenerate via apoptotic mechanisms.

Figure 4. A timeline in the understanding of the relationships between the brain and circulating immunity.

(1) Dating back to the 19th century, with the first observations of Paul Ehrlich regarding the anatomical separation of the CNS (Ehrlich, 1885), and continuing for the first half of the 20th century by the works of Shirai (1921) and Medawar (1948), the CNS was traditionally viewed as an immune privileged site. (2) At the time, according to the 'clonal selection theory' of Burnet (Burnet, 1959) and Lederberg (Lederberg, 1959), autoimmune T cells were considered to have only pathological roles. (3) Over the years, however, the presence of tissue-specific immune cells in healthy individuals raised the question of their nature and challenged the clonal deletion theory (Jerne, 1974; Cohen, 1992; Matzinger, 1994). (4) Studies showing that peripheral immunological activation can elevate T cell numbers in the CNS were the first to suggest a physiological immune surveillance mechanism (Hickey & Kimura, 1987; Hickey *et al*, 1991). Immune surveillance was found to constitutively take place at the 'borders' of the CNS – the CSF, the meningeal spaces, and the choroid plexus (Carrithers *et al*, 2002; Kivisakk *et al*, 2003; Ransohoff *et al*, 2003). (5) In the late 1990s, and early 2000s, a physiological role was attributed to autoimmune T cells in CNS repair (Moalem *et al*, 1999; Hauben *et al*, 2001; Wolf *et al*, 2002) and maintenance (Kipnis *et al*, 2004c; Ziv *et al*, 2006). (6) Our model suggests that CNS-specific CD4⁺ T cells constitutively reside at the brain's choroid plexus, controlling brain homeostasis from afar (Baruch & Schwartz, 2013; Baruch *et al*, 2013). This neuro-immunological interface serves as a tightly regulated entry gate into the CNS for immune surveillance by circulating leukocytes under physiological conditions, and for repair following CNS damage (Kunis *et al*, 2013; Shechter *et al*, 2013b).

Gruver *et al*, 2007; He & Balling, 2013). Under these conditions, Tregs in the periphery are likely to interfere with the ability of the immune system to cope with the neuroinflammatory response (Kipnis *et al*, 2002a, 2004a,b). Indeed, depletion of Tregs was shown to confer neuroprotection following CNS injury (Kipnis *et al*, 2002a). Notably, T cell immunity is improved in aged mice following depletion of Tregs by enhancing IFN- γ secretion by effector T cells in response to immunological challenge (Lages *et al*, 2008). Such a connection between enhanced Tregs levels in the periphery, the unresolved neuroinflammation in chronic neurodegenerative diseases, and the limited trafficking of inflammation-resolving cells to the parenchyma (Fig 2), is further discussed below. In contrast, in RRMS the chronic inflammation might be related to continuous invasion of inflammatory cells.

Additional circulating immune cell populations were shown to participate in neurodegeneration-associated immune suppression. Specifically, myeloid-derived suppressor cells (MDSCs) or alternatively activated macrophages (M2 macrophages), which share many characteristics of immune-suppressive tumor-associated macrophages (Luo *et al*, 2006), are elevated in the circulation of ALS patients (Vaknin *et al*, 2011) and aged individuals (Grizzle *et al*, 2007). When such cells (in the form of IL-4 activated myeloid cells) are administered intravenously to mSOD1 mice prior to the emergence of disease symptoms, ALS disease progression is accelerated, yet when the same cells are administered to mice following the

induction of EAE, disease progression is inhibited (Vaknin *et al*, 2011). Importantly, the adoptively transferred M2 myeloid cells home to the spleen (a peripheral lymphoid organ) and exhibit immune suppressive activity on CD4⁺ T cells (Vaknin *et al*, 2011). These studies highlight the distinct and even opposite roles of the different suppressor/regulatory cell populations in the circulation, under specific neuropathological conditions, in the overall process of inflammation-mediated resolution, and demonstrate that immune activity in the periphery does not necessarily reflect that within the CNS. Thus, it is becoming evident that suppression of adaptive immunity in the periphery does not necessarily arrest unresolved CNS local inflammation; as suppression in the periphery might reduce recruitment of inflammation-resolving circulating immune cells to the CNS (Fig 3). According to this view, suppressing systemic immunity in neurodegenerative diseases is not likely to be beneficial, but rather, counterproductive. These results may explain why Glatiramer acetate (GA; Copolymer-1; Copaxone[®], an FDA-approved drug, Teva Pharmaceutical Industries, Petah Tikva, Israel) is beneficial when given in a daily regimen to RRMS patients, but not to patients suffering from PPMS (Wolinsky *et al*, 2007) or ALS (Haenggeli *et al*, 2007). In RRMS patients, daily administration of GA was found to increase circulating Treg levels (Haas *et al*, 2009). However, it was proposed that GA administration, either in an infrequent regimen or with adjuvant, acts as a weak agonist of CNS antigens and thereby boosts, rather than suppresses, the T-cell response

to self antigens (Kipnis & Schwartz, 2002). Indeed, it was demonstrated in rats following acute CNS injury (intraocular pressure-induced retinal injury) that a single injection of GA, or of GA emulsified in complete Freund's adjuvant, can boost systemic immunity and confer functional neuroprotection (Schori *et al*, 2001; Bakalash *et al*, 2005). Similarly, infrequent administration of GA in a chronic model of neurodegenerative disease, AD double-transgenic (APP/PS1) mice, was found to be beneficial in reducing plaque formation and in improving cognitive performance, at least in part, through boosting recruitment of monocyte-derived macrophages (Butovsky *et al*, 2006, 2007). Daily injections of GA under such chronic conditions were either ineffective or even detrimental (Bakalash *et al*, 2005; Schwartz *et al*, 2008).

Concluding remarks: linking physiology to pathology through protective autoimmunity, with the CP at the interface

Overall, the results summarized above suggest that immune homeostasis in the circulation influences the ability of the CNS to fight off pathology. It appears that the immune cells that interact with the CP reflect the balance between effector and regulatory T cells in the circulation, affecting CP function, and in turn, trafficking of neuroprotective leukocytes to the CNS. The data also suggest that the CP trafficking route to the CNS facilitates the recruitment of inflammation-resolving/anti-inflammatory cells, needed for the resolution of neuroinflammation. Such immune resolution is dependent on the ability of the CSF to provide an educative milieu, in which the infiltrating immune cells are skewed to acquire a resolving phenotype. We propose that a transient neuroinflammatory response is inevitable prior to this phenotypic switch, and is an integral aspect of the repair. Thus, determining the specific impediment in harnessing inflammation-resolving cells for each neuropathology is pivotal for restoring homeostasis; an excess of circulating immune suppressor cells or insufficient circulating effector T cells can each lead to suppression of the CP or its lack of activation, respectively, resulting in insufficient recruitment of inflammation-resolving leukocytes to the CNS.

It is instructive to consider how the concepts presented here evolved along scientific history. It is evident that the perceptions of the CNS and the immune system have undergone drastic changes over the past decades (Fig 4). Independent advances in the understanding of these two complex systems have led to the current view that the brain is not completely refractory to immune cells, as was previously thought, and that self-reactive T cells are not only part of pathology. Accordingly, the presence of immune cells in the territory of the CNS is not necessarily a sign of escaping through barriers, or a sign heralding the beginning of an autoimmune disease. Likewise, the mere presence of autoimmune T cells in the circulation is not an outcome of escape from deletion during ontogeny. Our current understanding suggests that self-reactive T cells are integral part of the CNS territory, and that their presence is restricted to specific compartments. As such, CNS-specific T cells shape brain function from afar, via the choroid plexus, regulating CNS maintenance, and control this compartment so as to allow it to serve as a selective and educating gateway for the recruitment of circulating leukocytes to the CNS.

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Conflict of interest

The authors declare that they have no conflict of interest.

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