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PERSPECTIVE

The role of interleukin-6 in central nervous system demyelination

Demyelination of the central nervous system (CNS) is a hallmark of multiple sclerosis (MS), chronic inflammatory and neurodegenerative disease. Chronic demyelination favors neurodegeneration of denuded axons, which is a major cause of irreversible neuronal deficits and disability in MS patients (Lucchinetti et al., 2000). MS remains an incurable disease, despite formidable global research efforts. The etiology of MS is unknown and the pathological mechanisms involved in its evolution are still incompletely understood. One of the various molecules with a potential role in MS pathology is a cytokine interleukin (IL)-6. Our latest research has focused on analyzing the role that chronic production of IL-6 within the CNS might exert in an experimental model of demyelination induced by treatment with cuprizone (Petković et al., 2016). For this purpose, transgenic mice with astrocyte-targeted production of IL-6 (GFAP-IL6Tg) along with their wild type (WT) littermates were fed with cuprizone. Our results demonstrated that, in comparison with cuprizone-fed WT, cuprizone-fed GFAP-IL6Tg mice showed a reduced astroglial and microglial activation in the corpus callosum (CC), upon primary oligodendrocyte (OL) injury, which consequently led to inefficient removal of damaged myelin and impaired OL regeneration. At the same time, axonal pathology was absent in transgenic mice. These results support the already recognized ambiguous effects of microglial activation in the injured brain.

Cuprizone-induced demyelination: An experimental animal model reproducing MS histopathological hallmarks-MS lesions shows remarkable heterogeneity. Currently, four distinct patterns of demyelination have been described in MS patients, all of which are characterized by varying degrees of T cell infiltration and macrophage/microglia activation within the lesion (Lucchinetti et al., 2000). Patterns I and II are proposed to be autoimmune mediated, as they are characterized by prominent perivascular T cell and macrophage infiltration and demyelination, as well as by the presence of antibodies and C9 complement depositions within the lesion. Patterns III and IV are distinct in their appearance, as these lesions show prominent OL apoptosis, unlike patterns I and II. In addition to the presence of OL damage, pattern III lesions do not show demyelination around the inflamed blood vessels, suggesting that neurodegeneration might be a primary event in the pathogenesis of these types of lesions. In addition, certain newly-formed MS lesions, devoid of T lymphocytes, show extensive OL apoptosis, which supports the hypothesis that OL stress could be the disease initiating event (Barnett and Prineas, 2004). In the context of these histopathological differences, patterns I and II could be effectively studied with experimental autoimmune encephalomyelitis (EAE), a T cell-mediated MS model, while the cuprizone-induced demyelination model might be a better experimental approach for studying MS-pattern III lesions. Cuprizone feeding induces early OL apoptosis, preferentially in the CC, which is followed by astroglial and microglial activation and demyelination. Additionally, the blood-brain barrier remains intact and there is no T cell infiltration (Kipp et al., 2009). In this paper we will summarize our latest research and try to frame it in the context of the histopathology of pattern III lesions and chronic neuroinflammation in MS (**Figure 1**).

IL-6 promotes Th lymphocyte pathogenicity in the periphery, but what about its effects within the CNS? IL-6 is a multifunctional cytokine, capable of affecting a wide range of cells outside and inside of the CNS. In EAE, IL-6 aggravates clinical manifestations and spinal cord pathology, principally by promoting pathogenic T helper (Th) 17 cell generation in the peripheral lymphoid organs, which initiate and perpetuate neuroinflammation and demyelination in this model (Samoilova et al., 1998). The major effect of IL-6 on auto-reactive effector T cells has also been demonstrated in MS. In patients with active relapsing-remitting MS, IL-6 signaling was shown to support T effector cell resistance to regulation by regulatory T cells, which may contribute to disease aggravation (Schneider et al., 2013). In both EAE and MS, IL-6 seems to affect the disease pathogenesis through its activity in the peripheral lymphoid organs. Much less is known about CNS-restricted IL-6 actions in EAE and MS. It has recently been shown that mice with an IL-6 deficiency in astrocytes (Ast-IL6 KO) showed modest amelioration of EAE symptomatology and histopathology (Erta et al., 2016). In female Ast-IL6 KO, but not in male mice, clinical scores were slightly lower than in WT, which coincided with reduced demyelination and lower numbers of cellular infiltrates in the spinal cord. These results imply that IL-6 might exert CNS-restricted modulation of neuroinflammation in EAE, in addition to its effects on T lymphocytes in the periphery. However, within the CNS different cell populations appear to be involved in the MS pathology, thus making it more difficult to unravel the potential effect of IL-6 on distinct CNS-resident cell types. Within the MS lesions, IL-6 is produced by both astrocytes and microglia (Schonrock et al., 2000). In the lesions with significant OL preservation, there is high IL-6 expression, suggesting a possible protective role of IL-6 towards OL (Schonrock et al., 2000). Nevertheless, in our experiments, astrocyte-targeted production of IL-6 did not protect OL from the cuprizone-induced apoptosis. We also did not observe acceleration of the subsequent oligodendrocyte precursor cell (OPC) repopulation of the lesioned area in cuprizone-fed GFAP-IL6Tg mice. We found that the major difference between cuprizone-fed WT and cuprizone-fed GFAP-IL6Tg mice was in the activation of astrocytes and microglia. In the cuprizone model, and supposedly in type III MS lesions, the primary neurodegenerative event is OL damage, which triggers activation of astrocytes and microglia. As a potent source of chemokines, astrocytes are capable of influencing microglial activation and their attraction to the sites of OL damage. Activated microglial cells phagocytose damaged myelin, which had previously lost the metabolic support of apoptotic OL, producing zones of demyelination. We found that astrocyte-targeted production of IL-6 reduced astroglial and especially microglial activation to primary OL damage. Lower levels of chemokines CXCL10, CXCL1 and CCL4 in cuprizone-fed GFAP-IL6Tg mice could be responsible for the decrease in microglial attraction to a lesioned site. Previous studies in the cuprizone model have suggested that astrocyte-derived CXCL10 plays an essential role in attracting microglia to the demyelinating CC (Skripueltz et al., 2013). As a consequence of reduced microglial activation in cuprizone-fed GFAP-IL6Tg mice, damaged myelin could not be removed effectively, which is a necessary prerequisite for proper differentiation of OPCs into maturing OL and ultimately remyelination. Indeed, although $Olig2^+$ cells, representing the entire OL lineage population, were present in the CC of cuprizone-fed GFAP-IL6Tg, they failed to properly initiate their maturation, as assed by expression of adenomatous polyposis coli (APC), a mature OL marker. At the same time, we observed a beneficial effect of reduced microglial activation in cuprizone-fed GFAP-IL6Tg mice, as the axonal pathology was practically absent in these mice, unlike in WT littermates. Microglial cells are the major source of reactive nitrogen and oxygen species, which can damage demyelinated axons. Overall observations suggest that chronic production of IL-6 did not have any influence on activated microglia shifting towards the neurotoxic M1 phenotype or anti-inflammatory

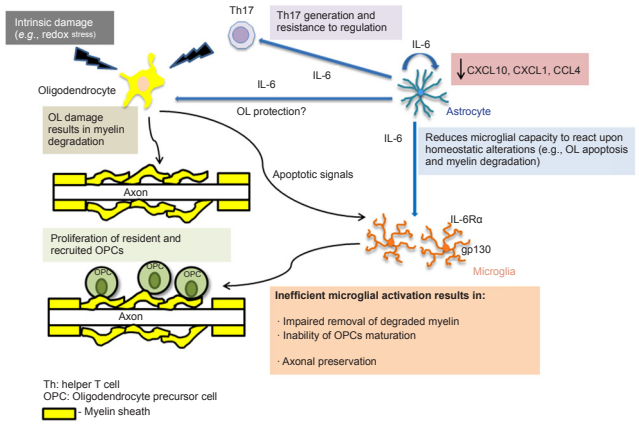

Figure 1 Potential effects of interleukin (IL)-6 in central nervous system (CNS) demyelination.
gp130: Glycoprotein 130; OL: oligodendrocyte; OPCs: oligodendrocyte precursor cells; Th17: T helper 17.

M2, but, rather, that it reduced microglial response in general.

Different modes of IL-6 signaling promote distinct cell response: Several members of the IL-6 cytokine family have shown a modulating effect in the cuprizone model, such as oncostatin M, IL-11, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). All these cytokines signal through the ubiquitously present membrane-bound β-receptor glycoprotein 130 (gp130), upon binding to their respective receptors. For this reason, it is not surprising that different members of the IL-6 family exert partially similar effects. Focusing on IL-6, two distinct modes of signaling have been described, depending on whether IL-6 binds to the membrane-bound or soluble form of IL-6Rα, named classical (canonical) and trans-signaling (non-cannonical), respectively. Among CNS-resident cells, microglia express the membrane-bound IL-6Rα, thus being able to respond to classical signaling, unlike astrocytes or neurons (Campbell et al., 2014). This feature makes microglial cells an interesting potential target for classical IL-6 signaling, which is often associated with their anti-inflammatory and regenerative function, unlike trans-signaling. However, in our study it was not possible to study these two modes of IL-6 signaling separately, thus, in the experiments presented in Petković et al. (2016), the modulating effect on microglia was attributed to the overall effects of IL-6 signaling.

Chronic neuroinflammation might induce microglial senescence: Another relatively recent concept of microglial biology in the chronic neuroinflammation and aging associated with CNS diseases has been proposed by Streit et al. (2014), suggesting that chronic neuroinflammation could lead to dysfunctional or senescent microglia. GFAP-IL6Tg mice might partially reflect this situation, as they are characterized by chronic, localized production of IL-6, which causes a chronic state of low level neuroinflammation and reactive gliosis (Chiang et al., 1994). We speculate that the constant presence of IL-6 might exhaust microglia, rendering them partially dysfunctional or senescent, which could result in their reduced response to cuprizone-induced OL damage.

Conclusion: Bearing in mind the vast range of possible actions of IL-6 in the inflamed CNS, additional studies are necessary to clarify its functions. To gain deeper knowledge on this matter, further research should focus on modes of IL-6 signaling on distinct cell populations within the CNS, with special emphasis on how such actions could affect CNS demyelination, in both acute and chronic states of neuroinflammation.

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Filip Petković*, Bernardo Castellano

Department of Cell Biology, Physiology and Immunology, Institute of Neuroscience, Universitat Autònoma de Barcelona, Bellaterra, Spain (Petković F, Castellano B)
Department of Immunology, Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia (Petković F)

***Correspondence to:** Filip Petković, Ph.D., petkovicfilip@yahoo.com.*
***Accepted:** 2016-12-10*
***orcid:** 0000-0003-2306-443X (Filip Petković)*
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