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Chapter II

Interferon-Gamma, Oligodendrocyte Injury and Inflammatory Demyelination

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Abstract

Interferon-gamma (IFN-γ) is a pleiotropic inflammatory cytokine produced by T cells and natural killer cells. It is critically involved in the pathogenesis of the human demyelinating disease multiple sclerosis (MS) and its animal model, the experimental autoimmune encephalomyelitis (EAE). These diseases are mediated by activated self-reactive T cells, which target the central nervous system (CNS) oligodendrocytes and their myelin sheaths. In addition to its immunoregulatory properties, IFN-γ exerts a direct biological effect on CNS cells, providing, thereby, a link between CNS inflammation and cell injury. In this review, we discuss the role of IFN-γ in MS and EAE, as well as its involvement in the processes of oligodendrocyte injury and inflammatory demyelination. We summarize the molecular mechanisms of IFN-γ-induced oligodendrocyte injury and analyze the various experimental models that have been used for their elucidation. Finally, we discuss the disease-promoting role of IFN-γ's IRF-1/Caspase 1 signaling in MS and EAE, and draw attention to the potential therapeutic significance of its suppression. In conclusion, studying IFN-γ - oligodendrocyte interactions is likely to provide new perspective on the pathogenesis of MS.

Keywords: Interferon-gamma, multiple sclerosis, oligodendrocytes, IRF-1, Caspase 1, experimental autoimmune encephalomyelitis, inflammation, demyelination, cell signaling

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Introduction

Interferon-gamma (IFN-γ) is an inflammatory cytokine that belongs to the interferon family [1]. It exists as a soluble dimer of two identical peptides. Each peptide comprises 146 amino acids, which are organized in six alpha-helices. Formally known as immune interferon, IFN-γ is classified as a type II interferon because it is produced exclusively by T cells and natural killer (NK) cells upon immune stimulation. In contrast, type I interferons (IFN-α, IFN-β) can be produced by all cells in response to a viral infection. The former also differs from type II interferons by being acid-labile. IFN-γ has anti-viral and anti-tumor activities, and plays a critical role in the normal immune responses. Humans deficient in IFN-γ are predisposed for developing chronic infectious and granulomatous diseases. Aberrant and/or uncontrolled expression of IFN-γ, on the other hand, is associated with autoimmune diseases, including multiple sclerosis (MS). As a pharmacological and a therapeutic agent, IFN-γ appears to be beneficial to patients with chronic granulomatous disease but detrimental to those with MS [2].

The role of IFN-γ in MS has been a focus of intense research since its original description in the 1980s [3]. The scientific interest in this cytokine continues to be very high because of its pleiotropic immunological effects. Phenomena related to non-immune cells were discovered as well. In particular, IFN-γ can elicit strong responses from cells residing beyond the blood-brain barrier, for instance oligodendrocytes, which do not normally interact with blood-born cytokines. Some of these responses, as we will see below, are critical for the occurrence of CNS inflammation. In addition, IFN-γ - oligodendrocyte interactions are important from a therapeutic prospective of developing cell protective strategies in MS. In this review we will discuss the role of IFN-γ in MS, the molecular mechanisms of IFN-γ - oligodendrocyte interactions, and the experimental models used for their analysis.

Oligodendrocyte Injury and Inflammatory Demyelination in MS

MS is a leading cause of neurological disability in young adults [4]. Pathologically, the disease is characterized by chronic recurrent inflammation of the central nervous system (CNS), primary inflammatory demyelination, oligodendrocyte injury and relative axonal preservation [5]. Although the clinical and pathological features of MS have been known since the XIXth century, its etiology is still unidentified. It is hypothesized that MS is a result of an autoimmune response directed against the CNS myelin and the myelin-producing oligodendrocytes [6]. Mechanistically, the disease is driven by activated self-reactive CD4 (+) T cells, which recognize their antigen in the CNS [7]. Self-reactive T cells produce cytokines, which activate local cellular elements, and facilitate recruitment of additional immune cells, as well as extravasation of antibodies and complement [8]. Accumulation of effector cells and molecules ultimately leads to myelin destruction and oligodendrocyte injury [9]. There is strong clinical and experimental evidence in support of this hypothesis, including presence of immune-mediated demyelination, immune dysregulation, and a beneficial effect of immunotherapy [5]. Additionally, MS shares many similarities with some animal models of CNS inflammation, including the experimental autoimmune encephalomyelitis (EAE) [10].

Inflammatory demyelination and impaired remyelination are unique characteristics of MS lesions [5]. Detailed examinations of pathological specimens demonstrate that demyelination and oligodendrocyte injury are not a single unified phenomenon, but rather a spectrum of processes determined by the predominant presence of activated macrophages, T cells, cytokines, antibodies, complement, reactive oxidative species and toxic-metabolic factors [11]. Demyelinating lesions in MS can vary from massive tissue and myelin destruction (secondary to the inflammatory process) to subtle degenerative changes of myelin and oligodendrocyte cell death in the absence of any significant inflammation [11-13]. Myelin repair following demyelinating injury occurs as a partial and inefficient process in acute lesions, and it is virtually absent in chronic lesions [14]. The underlying pathological mechanisms compromising the remyelination process and repair are poorly understood but inhibition of oligodendrocyte progenitor cell (OPC) proliferation and differentiation can be considered [14, 15]. Furthermore, extensive oligodendrocyte injury in acute lesions results in particularly low levels of myelin repair [11].

Clinically, loss of myelin in MS is a symptom-producing pathology triggering a cascade of electrophysiological and trophic alterations in the demyelinated axons. It compromises the saltatory propagation of the axonal action potential, and causes slowing in conduction velocity and conduction block [16]. Failure of axonal conduction results in a multitude of neurological deficits, and the chronic-recurrent bouts of inflammatory demyelination, in the relapsing pattern of the disease course. In addition, loss of myelin deprives the axons of the trophic effects of oligodendrocytes, which may also predispose them to more severe injury, physical transection and retrograde degeneration [17, 18]. At the present time, there is no oligodendrocyte-based treatment in clinical use and all the available MS therapies target exclusively immune mechanisms, providing little direct protection to oligodendrocytes and their myelin sheaths.

A number of experimental studies suggest that oligodendrocyte injury and demyelination are not due to simple target destruction, but require an active cellular response [19-21]. Inflammatory cytokines, such as IFN-γ and tumor-necrosis factor-alpha (TNF-α), affect the oligodendrocytes in a complex fashion and cause cell death directly by triggering injurious cell signaling, or indirectly, by upregulating the expression of surface receptors of cell-mediated cytotoxicity [19-26]. Importantly, these responses can be blocked or even reversed in the presence of anti-apoptotic or protective cell signaling [27-30]. In support, animals lacking pro-apoptotic or overexpressing anti-apoptotic molecules in their oligodendrocytes are resistant to demyelination and EAE [31-36]. Hypothetically, if we understand how oligodendrocytes respond to immune-mediated injury and to inflammatory cytokines in particular, then novel therapeutic strategies in MS can be developed.

The goal of this review is to discuss the role of IFN-γ in oligodendrocyte injury and inflammatory demyelination. IFN-γ is a pleiotropic cytokine produced by activated CD4 (+), CD8 (+), γ/δTCR (+) T cells and NK cells, and it is recognized as the principle mediator of their immune responses [1]. It is involved in nearly every step of the autoimmune process of MS [37]. On the other hand, oligodendrocytes are the myelin-forming cells of the CNS, and, in the case of MS, the principal autoimmune target [9]. They express receptors for IFN-γ and generate a complex response upon stimulation with this cytokine, associated with significant metabolic perturbations, cellular stress and death [21-26, 38]. Inflammatory demyelination is a process of immune destruction of oligodendrocytes' processes and their multilayered lipo-protein ensheathment (myelin) of the CNS axons.

Regulation of Oligodendrocyte Injury and Inflammatory Demyelination by IFN-γ

There are several lines of evidence indicating that IFN-γ contributes to the pathogenesis of MS and EAE, and can function as an independent effector molecule of oligodendrocyte injury and inflammatory demyelination. Abnormally high levels of IFN-γ are found in serum, cerebrospinal fluid, and brain lesions of patients with MS [39-43]. The production of IFN-γ by myelin-specific T cells correlates positively with the clinical measures of disease activity and progression [44-46]. Most importantly, experimental treatment of MS patients with IFN-γ leads to disease exacerbations, but its blockade by neutralizing antibody to clinical improvement [2, 48]. Pathologically, IFN-γ is present at the margins of actively demyelinating MS lesions, co-localizing with oligodendrocytes undergoing injury and cell death [23]. In addition, oligodendrocytes in MS lesions demonstrate increased expression of IFN-γ-dependent genes, including major histocompatibility (MHC) class I molecule, Fas, tumor-necrosis factor-alpha receptor (TNF-αR), etc [23-26].

IFN-γ plays a critical role in the pathogenesis of EAE as well. Activated T cells and clones that have the capacity to adoptively transfer EAE to naïve animals invariably express IFN-γ [49]. IFN-γ is also expressed by the majority of T cells in EAE lesions [50-52]. Recovery from acute EAE correlates with downregulation of IFN-γ expression, whereas chronic and relapsing disease course with its sustained expression [53, 54]. Direct administration of IFN-γ into the spinal cord of normal animals produces EAE-like pathology [55, 56]. IFN-γ also potentiates antibody-mediated demyelination and, if administered centrally prior to the disease onset, induces more relapses and severe disease [57, 58]. Paradoxically, absence or blockade of IFN-γ worsens CD4 (+) T cell-mediated, but ameliorates CD8 (+) T cell-mediated EAE [59-66]. Both, protection and amelioration are seen in adoptive EAE. These apparently disparate findings can be reconciled by certain immunoregulatory properties of IFN-γ, such as inhibition of adjuvant-induced myelopoesis, induction of T cell apoptosis, formation of the cytokine/chemokine repertoire, establishing Th1/Th2 balance and Th17 polarization [67-71].

Transgenic experiments provide additional insights in the direct CNS effect of IFN-γ. Overexpression of IFN-γ in the CNS of mice, particularly during development, results in an abnormal clinico-pathological phenotype associated, clinically, with ataxia and tremor, and, pathologically, with impaired myelination, and oligodendrocyte loss [72-74]. Similar abnormalities are observed in certain transgenic mouse lines when the cytokine is overexpressed later in life, or during myelin repair following a demyelinating insult [75-77]. Evidently, IFN-γ can cause myelin damage, injury and death of oligodendrocytes, even in the absence of immune cells. The demyelinating effect is dependent on the IFN-γ levels: phenotypic abnormalities are observed only in mice expressing relatively high levels IFN-γ in the CNS, whereas those with low levels remain normal. Almost paradoxically, low levels of IFN-γ appear to be protective to oligodendrocytes against toxic-metabolic injury [78, 79].

IFN-γ causes a direct injury on oligodendrocytes *in vitro.* This effect is also dose-dependent and closely linked to the stage of cell development. OPC and immature oligodendrocytes are more susceptible to IFN-γ injury and die from apoptosis, whereas mature cells are less susceptible and die from necrosis [21]. The injurious effect of IFN-γ on oligodendrocytes is associated with induction of stress responses and metabolic perturbations,

including MEK-ERK signaling, endoplasmic reticulum (ER) stress, downregulation of myelin protein gene expression, and/or upregulation of iNOS, p53, PKR and caspase expression [16, 21, 22, 29, 80, 81]. This effect, however, can be reversed upon withdrawal of the cytokine from the medium, addition of protective factors such as leukemia inhibitory factor (LIF), interleukin (IL) 10, LINGO 1 antagonist, corticosteroids, or p35 caspase inhibitor [29-30]. IFN-γ is also a potent inducer of surface receptors, such as MHC class I molecule, Fas, TNF-αR, which mediate oligodendrocyte injury by CD8 (+) cells, γ/δ TCR (+) cells and macrophages [24-26]. Interestingly, at low non-cytotoxic doses IFN-γ may interfere with the cell cycle of differentiating OPC and cause their trans-differentiation into astrocytes [82, 83].

Role of IFN-γ's IRF-1/Caspase 1 Signaling

Defining the molecular mechanisms of IFN-γ-mediated oligodendrocyte injury and demyelination is of major interest. However, such analysis is complicated by the pleiotropic nature of IFN-γ and the technical limitations of the traditional animal models that do not allow for the isolation of the oligodendrocyte-specific mechanisms of injury and protection [84]. In addition, IFN-γ's intracellular signaling is associated with activation of two distinct pathways: a STAT1-dependent and a STAT1-indendent pathway that have differential significance in cell death and survival. STAT1-dependent pathway, for instance, controls the expression of MHC molecules, Fas, and several pro-apoptotic molecules; STAT1-independent pathway promotes cell growth and survival [85] (Figure 1). In order to examine

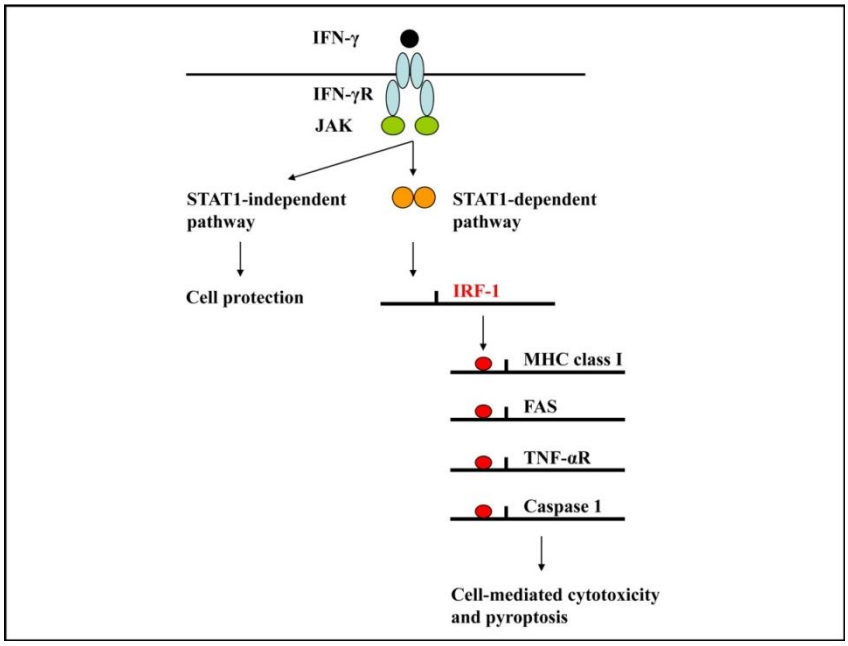

Figure 1. IRF-γ signaling in oligodendrocytes. IFN-γ triggers two signaling pathways with different biological significance: STAT-1-dependent pathway leads to cell injury, whereas STAT-1-independent pathway to cell protection. IRF-1, a STAT-1-dependent transcription factor, regulates the expression of genes involved in immune-mediated cell injury and death.

the mechanisms of IFN-γ injury on oligodendrocytes two methodological problems have to be resolved: differentiation of the direct from the pleiotropic mode of action of IFN-γ, and the STAT1-dependent from the STAT1-independent signaling pathway.

Our approach has been focused on identification of a signaling mechanism that can link the IFN-γ receptor to a specific mechanism of cell death. The initial work was performed *in vitro* using OPC since their responses are better understood and more consistent [86]. Additionally, since the majority of genes expressed in MS appear to be STAT1-dependent (MHC class I molecule, Fas, etc.) we focused on this pathway (Figure 1). First, we identified the expression of IFN-γ receptors on OPC cell surface and receptor-associated Janus kinases (Jak). Jak were activated (phosphorylated) within seconds of IFN-γ exposure, which was then followed by rapid STAT1 activation (phosphorylation) and translocation into the cell nucleus. Using microarray analysis we identified that IFN-γ upregulated the expression of a master transcription factor, interferon regulatory factor 1 (IRF-1), which controls the expression of a number of immune and pro-apoptotic molecules (MHC class I molecule, TNF-αR, Caspase 1) [26, 86]. Moreover we found that knocking down STAT1 and IRF-1 provided protection against IFN-γ, whereas overexpression of IRF-1 in OPC resulted in cell death. The latter was independently confirmed by others as well [87].

As we mentioned above, transgenic mice that overexpress IFN-γ in the CNS during postnatal age developed a clinical phenotype associated with hypocellular hypomyelination [73, 74]. In an effort to address the question of STAT1 signaling in this process we generated a transgenic mouse line, *PLP/SOCS1*. These mice overexpressed SOCS1 (suppressor of cytokine signaling 1), an inhibitor of Jak/STAT1 activation, in oligodendrocytes under the transcriptional control of the proteolipid protein (PLP) gene promoter [88]. *PLP/SOCS1* mice demonstrated normal phenotypic and morphological characteristics. However, the mice also displayed diminished oligodendrocyte responsiveness to IFN-γ, inability to activate STAT1 and to upregulate the expression of MHC class I molecule. IFN-γ mice were mated to the *PLP/SOCS1* mice, their F1 progeny examined and the results stratified according to genotype into four groups: wild-type/transgenic controls, mice overexpressing SOCS1, mice overexpressing IFN-γ, and mice overexpressing both, IFN-γ and SOCS1. The results of our experiments demonstrated that overexpression of SOCS1 in oligodendrocytes resulted in significant protection against IFN-γ [87]. Specifically, the IFN-γ-overexpressing mice developed, as expected, tremor and ataxia due to myelin and oligodendrocyte cell loss. In contrast, the transgenic mice that expressed both, IFN-γ and SOCS1, were essentially normal and displayed significant myelin and oligodendrocyte preservation. No abnormalities were observed in wild-type, single transgenic and *PLP/SOCS1* mice.

PLP/SOCS1 mice were further tested in EAE as well [89]. Strikingly, the *PLP/SOCS1* mice developed EAE earlier compared to the wild-type mice. However, despite the accelerated disease onset they recovered faster and had shorter disease duration. Histologically, the inflammatory infiltration in the *PLP/SOCS1* mice remained perivascular with little involvement of the myelinated tracts. These results suggested that IFN-γ exerted a protective, as well as a pro-injurious effect on oligodendrocytes (both blocked by SOCS1). This dual effect is in accord with previously published reports demonstrating that overexpression of IFN-γ in the CNS during the preclinical phase of EAE can be protective to oligodendrocytes, but injurious to them if overexpressed after the clinical onset [77, 80]. Our results also indicated that suppression of IFN-γ signaling at the level of the receptor and Jak/STAT1activation is not injury-specific - SOCS1 may also interfere with STAT1-

independent signaling - and that the intracellular factors responsible for its injurious effects should be sought downstream of the initial signaling step. This prompted us to look for other STAT1-dependent factors that are likely to be differentially involved in cell injury as opposed to cell protection.

As we mentioned, IRF-1 is a transcription factor whose expression is controlled by IFN-γ's STAT1-dependent pathway [86]. IRF-1 upregulates the expression of genes involved in oligodendrocyte response to immune stimuli and injury, including MHC class I molecule, TNF-αR, and Caspase 1 [26, 86]. It also interacts with NF-κB, a transcription factor that mediates the cellular effects of TNF-α [26]. To elucidate the role of IRF-1 signaling in oligodendrocytes *in vivo* we used two experimental models. The first one was based on generating bone-marrow chimera mice that differed in their IRF-1 expression in CNS. Such approach was necessary since IRF-1 (-/-) knockout mice cannot develop efficient immune (Th1) responses and are resistant to EAE [90, 91]. Wild-type C57Bl/6J and IRF-1 (-/-) knockout mice were used in these experiments as either donors of bone-marrow cells or transplanted hosts [92]. Transplants contained wild-type and knockout cells in different ratios, and were designed to produce mixed chimera mice with comparable peripheral immune system. The responses of WT/mixed (these mice express IRF-1 in the CNS) and KO/mixed (these mice do not express IRF-1 in the CNS) mice to EAE were compared. In these experiments, WT/mixed mice developed significant disease, but the KO/mixed mice were protected. The protection was clinical, in terms of decreased severity and duration of clinical signs, as well as pathological, in terms of preservation of white matter, myelin and oligodendrocytes [92].

The second experimental model was based on generating a transgenic mouse line with suppressed IRF-1 signaling in oligodendrocytes, *CNP/dnIRF-1* [93]. This was achieved by overexpressing the dominant negative form of IRF-1 (dnIRF-1) using the oligodendrocyte-specific 2', 3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) gene promoter. *CNP/dnIRF-1* mice, as designed, were phenotypically normal with the exception of their diminished IRF-1-depedendent gene expression. Specifically, these mice were not able to upregulate the expression of MHC class I molecule and Caspase 1 in their oligodendrocytes in the presence of IFN-γ neither *in vitro* nor *in vivo*. *CNP/dnIRF-1* mice were generated directly on C57Bl/6J background for the purpose of comparing their EAE response to our previous experiments. Similarly to the KO/mixed mice, *CNP/dnIRF-1* mice were protected against EAE compared to wild-type mice [92, 93]. In contrast to the wild-type mice, they developed minimal clinical signs with short duration and quickly recovered. Pathologically, the CNS inflammatory lesions were mainly meningeal and perivascular, causing little parenchymal and white matter infiltration. Myelin and oligodendrocytes were essentially preserved; axons were intact as well.

Our experiments indicated that IRF-1 mediates the injurious effect of CNS inflammation on oligodendrocytes. Moreover, oligodendrocyte expression of IRF-1 appeared to regulate EAE severity independently of the peripheral immune response. Potentially, such phenomenon can be explained by an early involvement of oligodendrocytes in the disease pathogenesis and by a major pro-inflammatory effect of oligodendrocyte injury and death (Figure 2). There is evidence in support of such a possibility. IFN-γ becomes detectable in the CNS during the preclinical phase of EAE, providing early immune signaling to the oligodendrocytes [89]. Some of the IFN-γ- and IRF-1-dependent genes, including MHC class

I molecule and TNF-αR, are also upregulated by the oligodendrocytes early in the disease course [36, 89].

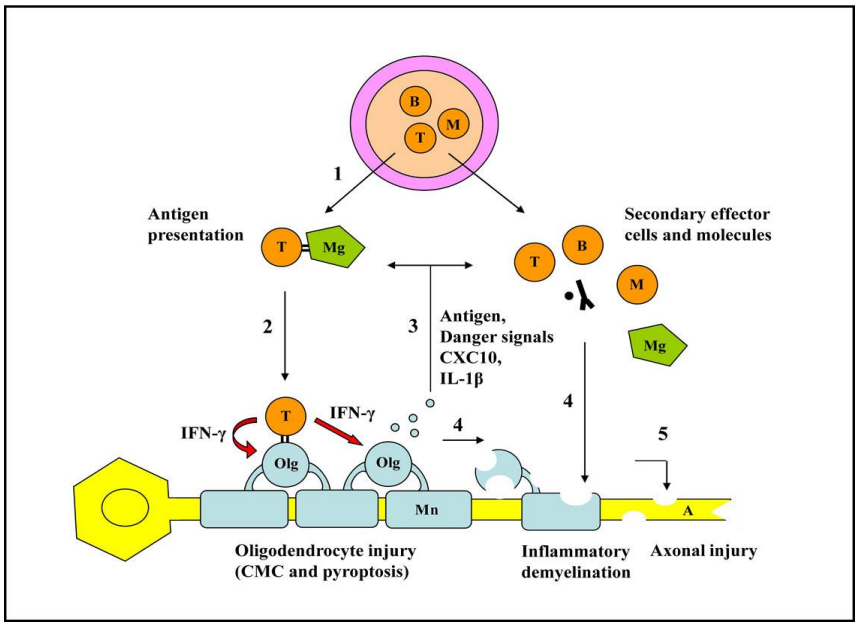

Figure 2. IFN-γ regulation of oligodendrocyte injury and inflammatory demyelination. Self-reactive T cells recognized their antigen in the CNS (1) and interact with oligodendrocytes (2). IFN-γ causes oligodendrocyte injury directly (cytopathic effect) or indirectly (CMC, cell-mediated cytotoxicity), which results in pro-inflammatory cell death (pyroptosis) and release of antigen, "danger signals", chemokines and cytokines (3). Oligodendrocyte-derived molecules accelerate the process of antigen presentation and recruitment of secondary effector cells and molecules (3). Inflammatory demyelination follows as a result of oligodendrocyte cell death and direct immune-mediated damage (4). Axonal injury occurs as well (5). A=axon, B=B cell, M=monocyte, Mg=microglial cell, Mn=myelin, Olg= oligodendrocyte, T=T cell.

Additionally, in response to IFN-γ oligodendrocytes can produce CXC10 (IP10), a chemokine that plays a critical role in the formation of EAE lesions [89]. Early oligodendrocyte cell death can also provide cell-free myelin antigens and accelerate the processes of antigen presentation and immune stimulation [93, 94]. For instance, transgenic mice with defective oligodendrocyte peroxisomes develop not only oligodendrocyte death and demyelination but also spontaneous T and B cell inflammation [95]. Finally, pyroptosis, a pro-inflammatory form of cell death mediated by Caspase 1 can be considered [96]. The pro-inflammatory effect of this form of cell death is related to the fact that Caspase 1 can activate IL1-β and induce membrane leakage of cellular content. Indeed, in the course of our experimentations we saw evidence supporting the occurrence of pyroptosis. Increased expression of Caspase 1 in oligodendrocytes was detected during EAE in wild-type mice. On contrary, protection against EAE, as seen in KO/mixed and *CNP/dnIRF-1* mice, was associated with absent or diminished expression of Caspase 1. Moreover, we found that IRF-1 positively regulated the expression of Caspase 1 in oligodendrocytes *in vivo* and *in vitro*, providing a mechanistic explanation of how oligodendrocyte response to IFN-γ can impact the extent of CNS inflammation and severity of EAE.

A single nucleotide polymorphism of IRF-1 gene is associated with progressive MS [97]. Prompted by our EAE experiments as well, we investigated whether IRF-1 and Caspase 1 are expressed in MS lesions [92]. We analyzed several active and chronic active lesions and the corresponding normal-appearing white matter using the methods of Western blot and immunohistochemistry.

The western blot analysis demonstated increased expression of both IRF-1 and Caspase 1 in active MS lesions, but not in the normal-appearing white matter. IRF-1 and Caspase 1 were further detected in CNPase (+) cells (oligodendrocytes) localized at the leading edge of lesional activity.

Nearly 20% of all counted oligodendrocytes were found to be positive for both molecules. Others have also reported increased expression of Caspase 1 by oligodendrocytes in MS lesions [98]. Thus, it appears that IRF-1/Caspase 1 signaling may serve as a common-end pathway regulating oligodendrocyte injury and inflammatory demyelination in MS and EAE. At a more global level, this signaling pathway may play a disease promoting role, creating a feet forward mechanism for accumulation of inflammatory cells at the side of lesions.

Conclusion

In summary, IFN-γ is critically involved in the pathogenesis of MS and EAE as an immunoregulatory, as well as an effector molecule. The hypothesis that IFN-γ exerts a direct injurious effect on oligodendrocytes and causes demyelination has a substantial experimental support.

Oligodendrocyte injury is not a passive process but requires active intracellular signaling and utilizes the STAT1-dependent signaling pathway. One of the downstream steps of this signaling pathway, IRF-1, is a master transcription factor that can amplify and diversify the initial receptor signal and trigger cell death. IRF-1 controls the expression of Caspase 1, which has the unique property to cause pro-inflammatory cell death (pyroptosis). In setting of autoimmunity pyroptosis can function as a disease-promoting mechanism by enriching the environment with self-antigens, cytokines, chemokines and “danger signals”.

Remarkably, most of the transgenic manipulations of oligodendrocytes leading to protection against EAE target genes that are involved in the STAT1-dependent signaling pathway. In the future, it will be important to identify the negative and the positive regulators of this pathway, as well as the molecules synergizing with or antagonizing its activities and downstream effects. Perhaps, therapeutic targeting of STAT1-dependent signaling may be of clinical interest in MS.

The significance of STAT1-indepedent pathway in oligodendrocyte survival should be examined, as it may represent an intrinsic signal of cell survival. In this regard, understanding the mechanisms balancing the STAT1-dependent and STAT1-independent signaling is of particular importance.

Other questions of how oligodendrocyte responsiveness to IFN-γ and to injury, in general, contributes to MS disease progression are of great scientific interest. Finally, in our opinion, studying IFN-γ - oligodendrocyte interactions are likely to provide new perspective on the pathogenesis of MS.

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