The Multifaceted Role of Interferon-γ in Central Nervous System Autoimmune Demyelination

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Abstract: Extensive research has been devoted to the study of IFN-γ function in several autoimmune diseases. Previously considered the hallmark of Th1 differentiation and pro-inflammatory responses, it has soon become evident that this pivotal cytokine plays a much more complex role than initially thought. These considerations have been particularly relevant to the understanding of the pathogenesis of autoimmune demyelination of the central nervous system (CNS). Evidence of the multifaceted effects of IFN-γ in this disease has been gathered mainly by studies in the animal model, experimental autoimmune encephalomyelitis (EAE). In this review we summarize the fundamental steps and examine the possible factors involved in the apparent dichotomy between pro-inflammatory and protective effects of IFN-γ in CNS autoimmune demyelination. A clear understanding of the heterogeneous functions of this key cytokine is paramount in order to fully explore the potential of manipulation of its pathways for the treatment of Multiple Sclerosis.

Keywords: Multiple sclerosis, experimental autoimmune encephalomyelitis, interferon-γ, central nervous system, autoimmune demyelination.

INTRODUCTION

Interferon-γ is a pivotal cytokine in the complex network of soluble mediators that are produced during the immune response. Its pleiotropic functions modulate different phases of the inflammatory response against pathogens and are intimately involved in the autoimmune processes underlying several systemic and organ-specific diseases.

Autoimmune demyelination of the central nervous system (CNS) is an organ-specific inflammatory disorder that affects the brain and spinal cord. Multiple Sclerosis (MS) is the most common clinical manifestation of CNS autoimmune demyelination in humans and is mimicked by experimental autoimmune encephalomyelitis (EAE) in animals (reviewed in [1]). Up to a few years ago the common view was that MS and EAE were CD4⁺ Th1 driven diseases and for several years IFN-γ has been considered the hallmark cytokine of the inflammatory process. However evidence coming mainly from experimental models progressively lead to consider a broader spectrum of action for this cytokine. The Th1 dominance of the autoimmune reactions in EAE may well be an artefact induced by the Th1-skewing effect of the bacterial antigens in the adjuvant used for formulation of the antigens [2]. In addition, recent research has highlighted the role of a further population of inflammatory T cells characterized by the secretion of the cytokine IL-17, i.e. Th17 cells. Overall these considerations have prompted a redefinition of the traditional role of IFN-γ in this and other autoimmune diseases.

Here we briefly review the current knowledge on the disease-promoting and disease-limiting effects of IFN-γ in autoimmune demyelination of the CNS in both animal models and humans. We will then consider possible factors involved in generating seemingly opposite roles of IFN-γ in this disease, such as the site of action and timing of the immune response as well as the interaction with other key cell populations.

OVERVIEW ON IFN-γ BIOLOGY

IFN-γ is the only member of type II IFN family cytokines and was originally discovered as an anti-viral agent (reviewed in [3]). IFN-γ can be produced by several immune cell types, mainly by CD4⁺ Th1 and CD8⁺ cytotoxic T-lymphocytes in antigen-specific responses, and in a non antigen-specific fashion by natural killer (NK) and natural killer T (NKT) cells. Also antigen-presenting cells (APC) such as macrophages, dendritic cells and B lymphocytes can secrete IFN-γ as well as being target of its effects. IFN-γ has a wide range of cellular functions that are exerted through the stimulation of its receptor located on the surface of the target cells. The IFN-γ receptor (IFN-γR) is made up of two subunits formed by two ligand-binding IFNγR1 (alpha) chains and two signal-transducing IFNγR2 (beta) chains. The IFNγR1:IFNγR2 chains dimerize upon binding of the cytokine to its receptor to form the IFN-γR complex.

The IFN-γR is expressed virtually on all cell types and drives its responses mainly through the Janus kinase (JAK)/Signal Transducer and Activator of Transcription 1 (STAT1) pathway, which is shared by many other cytokines. This pathway involves sequential recruitment and activation of members of both families of signalling molecules. Binding of IFN-γ to its receptor causes conformational changes in the IFN-γR complex such that JAK kinases undergo phosphorylation and activate STAT1 homodimers.
mers can then travel to the nucleus where they bind to promoter IFN-γ activation site (GAS) elements to promote/suppress the transcription of IFN-γ regulated genes. Many of these genes are transcription factors such as the members of the interferon regulatory factor (IRF) family IRF1, IRF2 and IRF9. Amongst the transcription factors induced by IFN-γ, T-box expressed in T cells (T-bet) was recognised in 2000 as a key factor for the induction of Th1 CD4+ T cells [4]. IFN-γ promotes its induction during T cell activation in a process that further amplifies IFN-γ production itself. Of note, it has been increasingly recognised that IFN-γ and STAT1 can cross-regulate signalling pathways induced by other inflammatory and anti-inflammatory cytokines as well as innate immune receptors (reviewed in [5]).

Interleukin (IL)-12 and IL-18 are produced by mature APCs as part of the innate immune response. They are the two main cytokines responsible of driving the production of IFN-γ, which further promotes its production by inducing IL-12 secretion by phagocytes. On the contrary, IL-4, IL-10, TGF-β and glucocorticoids inhibit IFN-γ secretion.

IFN-γ stimulates a wide range of cellular responses, most remarkably those that are pathognomonic of the inflammatory response (reviewed in [6]). The main pro-inflammatory effect is the up-regulation of both major histocompatibility complex (MHC) class I and II antigen presentation molecules as well as co-stimulatory and cell adhesion molecules on APCs. This represents a crucial step for the promotion of T cell-APC interaction and the activation of the adaptive immune system. Moreover, IFN-γ is able to orchestrate the trafficking of immune cell populations through regulating the expression of adhesion molecules and chemokines. It is well known that the cytokine milieu heavily influences the phenotype adopted by a naïve T cell during T cell activation. IFN-γ and IL-12 are the prototypic cytokines involved in driving Th1 differentiation and inhibiting Th2 cell development during the primary response to antigens. IFN-γ is also well known for its ability to promote B cell differentiation and IgG2a switching, therefore promoting antibody responses. Its pro-inflammatory actions include also the stimulation of the production of other pro-inflammatory factors, such as TNF-α and nitric oxide (NO), by macrophages. Moreover, NK cell effector functions are enhanced by IFN-γ, secreted both by NK cells and by APCs. IFN-γ functions are not limited to the promotion of inflammatory responses and some of these are indeed part of immunosuppressive/regulatory mechanisms. It has been shown that IFN-γ inhibits T cell proliferation and stimulates programmed cell-death by apoptosis leading to the elimination of effector Th1 cells [7-9]. Interestingly, Th1 cells have been shown to be less susceptible to this anti-proliferative effect due to lack of expression of the IFN-γ receptor beta chain [10]. Finally, the recently recognised effects of IFN-γ on Th17 and regulatory T cells (Treg), two immune populations that have shown to play a crucial role in autoimmune processes, have further increased our knowledge of the spectrum of action of this key cytokine (see below).

EVIDENCE FOR DISEASE-PROMOTING ROLE OF IFN-γ IN CNS AUTOIMMUNE DEMYELENATION

Disease-Promoting role in EAE

IFN-γ is a “signature” Th1 cytokine and has generally been considered the hallmark of the inflammatory process of many autoimmune diseases. Several early observations in EAE, the experimental model of CNS autoimmune demyelination, have lead to the paradigm of CNS autoimmune demyelination as Th1 (and IFN-γ) driven disease. EAE can be induced in several animal models including rodents and primates by immunisation with whole spinal cord or myelin proteins such as myelin basic proteins (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP) (reviewed in [11, 12]). The pathology of EAE is characterised by lymphocytic and mononuclear cell infiltration of the brain and spinal cord. The lesions are considered to result from a delayed-type hypersensitivity (DTH) inflammatory reaction elicited by antigen-specific CD4+ T cells. The infiltrating T lymphocytes have been shown to typically express IFN-γ as well as other Th1 cytokines leading to the paradigm of a Th1 cell mediated disease [13-15]. Th1 cytokine expression correlated with disease severity, therefore supporting the causative role of IFN-γ and Th1 cytokines in the inflammatory response. A further confirmation was provided by the demonstration that EAE can be induced by the adoptive transfer of encephalitogenic Th1 cells [16, 17]. The numerous studies showing the role of IL-12 in EAE induction reinforced the hypothesis of a fundamental role of the IL-12/IFN-γ axis in this and other models of autoimmune disease (reviewed in [18]). In addition, studies showing that T-bet knock-out (KO) mice were resistant to EAE further supported the crucial involvement of IFN-γ pathways in this disease model [19]. These mice did not develop Th1 cells or IFN-γ producing cells, although other inflammatory cytokines induced by T-bet may have been involved in sustaining the inflammatory process. Finally, investigations of the mechanisms of suppression of EAE by treatments such as copolymer-1 or induction of oral tolerance have shown the correlation between disease suppression and shift of the immune response from Th1 to either a Th2 or Treg profile [20, 21].

Several functions of IFN-γ are likely to facilitate disease, such as the activation of APCs and mononuclear phagocytes, the differentiation of Th1 and B cells, and the up-regulation of cell-adhesion molecules on endothelial cells. Moreover, IFN-γ has been shown to induce MHC class II antigens not only on conventional APCs but also astrocytes which play a role as presenters of MBP to encephalitogenic T cells in EAE [22]. Amongst others, the ability of IFN-γ to induce NO has been considered one of the detrimental mechanisms used by encephalitogenic T cells in the CNS [23].

IFN-γ has also been implicated in the progression of autoimmune inflammation of the CNS to chronic demyelination and neurological deficit, especially in studies with transgenic mice (Table 1). Horwitz et al reported that mice with transgenic expression of IFN-γ showed primary demyelination associated with several inflammatory features such as upregulation of MHC molecules, gliosis and lymphocytic infiltration [24]. Other authors confirmed the presence of chronic demyelination and neurological deficits in transgenic mice expressing IFN-γ in myelinating oligodendrocytes [25]. A damaging role for IFN-γ in the CNS was further supported by the findings that transgenic mice, in which the expression of IFN-γ was under the transcriptional control of an MBP promoter, displayed hypomyelination, reactive gliosis and abnormal cerebellar development [26]. All these findings were suggestive not only that IFN-γ was involved in CNS...
Table 1. Summary of the Effects of Intervention on IFN-γ in EAE Models

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Animal model</th>
<th>Main findings</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exogenous IFN-γ (systemic)</td>
<td>SJL mice</td>
<td>Reduced morbidity and mortality.</td>
<td>Disease-limiting</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delayed disease onset (no change in final outcome).</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Biozzi ABH</td>
<td>Early administration: partial protection not only against the first attack, but also against subsequent relapses. Administration during the remission phase: some protection against subsequent relapses.</td>
<td>Disease-limiting</td>
<td>[45]</td>
</tr>
<tr>
<td>Exogenous IFN-γ (CNS)</td>
<td>Rat</td>
<td>Spinal cord injection: encephalomyelitis-like inflammation</td>
<td>Disease-promoting</td>
<td>[61,62]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Subarachnoidal injection in combination with anti-myelin/oligodendrocyte glycoprotein antibody: demyelination</td>
<td>Disease-promoting</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice</td>
<td>Intratechal delivery (viral vector): earlier onset, but milder course and earlier recovery.</td>
<td>Disease-limiting</td>
<td>[47]</td>
</tr>
<tr>
<td>Neutralising mAbs against IFN-γ</td>
<td>C57BL/6 mice</td>
<td>Increase in morbidity rates and mortality.</td>
<td>Disease-limiting</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>SJL mice</td>
<td>No change.</td>
<td>Disease-limiting</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased disease mortality.</td>
<td></td>
<td>[44, 46]</td>
</tr>
<tr>
<td></td>
<td>A/J, BALB/c, C3H/HeJ, AKR, NZW, DBA/2</td>
<td>All resistant strains but AKR developed MBP induced EAE.</td>
<td>Disease-limiting</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>SJL, Biozzi ABH</td>
<td>Facilitation of spontaneous relapses in Biozzi mice as well as induced relapses in SJL/J mice.</td>
<td>Disease-limiting</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>C3H mice (MBP-specific CD8⁺ T cell clones)</td>
<td>Intratechal co-injection of MBP-specific CD8⁺ T cell clones and anti-IFN-γ antibodies reduced severity of disease</td>
<td>Disease-promoting</td>
<td>[49]</td>
</tr>
<tr>
<td>IFN-γ KO mice</td>
<td>B10.PL mice</td>
<td>Increased mortality, no differences in the cell infiltrate in the CNS.</td>
<td>Disease-limiting</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>Highly susceptible to MBP-induced EAE (wild type resistant), infiltration of mononuclear cells in the CNS.</td>
<td>Disease-limiting</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>Rapidly progressing lethal disease with predominant neutrophil infiltrate.</td>
<td>Disease-limiting</td>
<td>[52]</td>
</tr>
<tr>
<td>IFN-γ receptor KO mice</td>
<td>129/Sv mice</td>
<td>Highly susceptible to MOG-induced EAE (wild type resistant), infiltration of mononuclear cells in the CNS. IFN-γ down-regulated EAE by inducing nitric-oxide (NO) production both in the periphery and the target tissue.</td>
<td>Disease-limiting</td>
<td>[53, 54]</td>
</tr>
<tr>
<td>Transgenic mice</td>
<td>Expression of IFN-γ by using oligodendrocyte-specific promoter</td>
<td>Primary demyelination, upregulation of MHC molecules, gliosis and lymphocytic infiltration.</td>
<td>Disease-promoting</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Expression of IFN-γ in myelinating oligodendrocytes</td>
<td>No spontaneous CNS inflammation or demyelination, similar EAE incidence and disease course, but chronic demyelination and neurological deficits.</td>
<td>Disease-promoting</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Expression of IFN-γ under the transcriptional control of MBP</td>
<td>Hypomyelination, reactive gliosis and abnormal cerebellar development.</td>
<td>Disease-promoting</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Suppressed oligodendrocyte responsiveness to IFN-γ</td>
<td>Accelerated EAE onset, enhanced early inflammation, markedly increased oligodendrocyte apoptosis.</td>
<td>Disease-limiting</td>
<td>[55]</td>
</tr>
</tbody>
</table>

The recent consideration given to Th17 cells as an important pro-inflammatory population in EAE has somewhat altered the common thinking of IFN-γ as a mainly detrimental cytokine in this disease model. Recent evidence shows that both Th1 and Th17 cells are involved in the disease process (reviewed in [26]). The finding that Th1 cells are indeed chronic inflammation but also that it seemed to be disruptive for the nervous system.
required for the entry of Th17 cells into the CNS [27] supports a fundamental role of IFN-γ secreting cells in CNS autoimmune demyelination.

Disease-Promoting Role in MS

The paradigm of MS as a Th1/IFN-γ driven disease has found supportive findings in human studies. Leukocytes from MS patients showed increased IFN-γ production in vitro [29]. Moreover, MBP-specific T cell clones in the CSF of MS patients were found to produce IFN-γ and drive the inflammatory process [30]. In two studies it was confirmed that increased production of IFN-γ by in vitro stimulated blood cells preceded relapses in patients [31, 32], but this was not confirmed in another study where TNF-α and IL-4 levels were increased before exacerbations [33]. However IFN-γ along with other type 1 cytokines was augmented in CD4⁺, CD8⁺, and CD14⁺ cells of acute MS patients and of patients undergoing disease reactivation whilst they were normalised in stable patients or patients on Interferon-β treatment [34]. Positive correlation between IFN-γ producing cells and disability was found in a study of patients with primary relapsing MS course [35]. Increased IFN-γ production was also demonstrated at the mRNA level in blood and CSF mononuclear cells of MS patients, although to a lesser extent than IL-4 and TGF-β [36]. Other authors reported that treatment with IFN-β lead to decreased T cell activation and IFN-γ production, indirectly supporting the disease-promoting effect of this cytokine [37]. Some studies confirmed the reduction of CD4⁺ and CD8⁺ T cells producing IFN-γ but also IL4 in patients under treatment with IFN-β and glatiramer acetate [38, 39]. The findings of increased T-bet and pSTAT1 expression in MS patients as well as their correlation with disease activity and response to treatment with glucocorticoid further supported the pro-inflammatory role of IFN-γ pathways in MS [40].

Finally, a recent study, investigating both Th1 and Th17 cell cytokines in MS, found that IFN-γ levels were increased in both clinically isolated syndrome (CIS) and relapsing-remitting patients during exacerbations, whilst IL-17 levels were increased in only CIS patients [41]. This would suggest a role for IL-17 mainly in the early stages of the disease, whilst IFN-γ would be involved in both the early stages and subsequent exacerbations.

EVIDENCE FOR DISEASE-LIMITING ROLE OF IFN-γ IN CNS AUTOIMMUNE DEMYELINATION

Disease-Limiting Role in EAE

The main observations for a disease-limiting role of IFN-γ came from animal models and in particular the administration of antibodies against IFN-γ and the use of KO and transgenic mice (Table 1).

Different authors showed that treatment with antibodies against IFN-γ exacerbated the disease in different strains of mice, including SJL, C57BL/6 and Biozzi ABH [42-45]. C57BL/6 mice, which are relatively resistant to EAE, showed an increased incidence of the disease if treated with neutralizing monoclonal antibodies (mAbs) against IFN-γ. Conversely, SJL mice, which normally develop EAE in a high proportion, were not affected by the treatment with a neutralizing mAb against IFN-γ in this study [42]. However, EAE in these mice resulted in reduced morbidity and mortality after systemic administration of IFN-γ. In another study treatment of SJL mice with neutralizing mAbs against IFN-γ resulted in increased disease mortality whilst systemic administration lead to delayed disease onset although it did not alter the final outcome [46]. These results indicate that endogenous IFN-γ is formed during the induction or development of EAE and that it plays a disease-limiting role. In support of this hypothesis it was shown that intrathecal delivery of IFN-γ protects mice [47] and also Lewis rats [48] from EAE. In mice the protective mechanism was mediated by increasing apoptosis of CNS-infiltrating lymphocytes. Interestingly the administration of anti-IFN-γ mAbs resulted in amelioration of the disease in a CD8⁺ T cell mediated EAE model in contrast with findings in CD4⁺ T cell mediated EAE models [49]. This observation remarks the complex functions of IFN-γ depending on its cellular source and the main cell population involved in the autoimmune process.

Several observations in IFN-γ KO mice further confirmed a protective role of IFN-γ in EAE. IFN-γ KO mice (B10.PL strain) developed MBP-induced EAE similarly to their wild type littersmates and showed increased mortality although there were no differences in the cell infiltrate in the CNS [50]. BALB/c IFN-γ KO mice were highly susceptible to MBP-induced EAE, whilst wild-type mice were resistant. Diseased mice displayed infiltration of mononuclear cells and high levels of TNF-α mRNA in their CNS [51]. These findings were supported in C57BL/6 IFN-γ KO mice which developed rapidly progressing lethal disease with predominant neutrophil infiltrate and overexpression of neutrophil-attracting chemokines [52]. In addition, IFN-γ receptor (R) KO mice (129 strain) were highly susceptible to MOG-induced EAE whilst the wild type strain was resistant. The disease was characterised by extensive infiltration of mononuclear- and polymorphonuclear cells and increased production of TNF-α by MOG-stimulated splenocytes [53, 54]. Overall it was shown that myelin-specific CD4⁺ T cells were expanded in IFN-γ KO mice, suggesting a role for IFN-γ in regulatory pathways during EAE. Further confirmation came from the use of a transgenic mouse line with suppressed oligodendrocyte responsiveness to IFN-γ. EAE onset in these mice was accelerated and associated with enhanced early inflammation and markedly increased oligodendrocyte apoptosis. Moreover, IFN-γ pre-treatment of mature oligodendrocytes in vitro had a protective effect against oxidative stress [55].

Disease-Limiting Role in MS

Human data supporting a protective role of IFN-γ in MS have not been commonly reported. Therefore animal studies are important in providing a full perspective of the functional activities of IFN-γ and its possible relevance to the in vivo disease process.

It is important to note that the paucity of this type of data does not necessarily imply that a regulatory role of IFN-γ is not relevant to the human condition. Most of the available data in MS patients come from ex vivo or in vitro studies on peripheral blood samples. For obvious reasons it is not possible to differentiate between the local and systemic effects of IFN-γ in this type of samples. Moreover, studies in human subjects are usually performed when the disease process has been ongoing for some time and several immune factors may
concomitantly take part in the cytokine profile detected at the time of sampling.

**POSSIBLE FACTORS INVOLVED IN DETERMINING SEEMINGLY OPPOSITE ROLES OF IFN-γ**

Several factors could be involved in determining what appear to be opposite effects of IFN-γ in CNS autoimmune demyelination, such as the cellular source, the site and timing of action of the cytokine, its endogenous production versus exogenous administration and the interplay with other key populations involved in the autoimmune response (summarised in Fig. 1). Moreover, several mechanisms of action of IFN-γ result indeed in immnosuppression, such as its anti-proliferative effects on T cells and inhibitory role on myelopoiesis.

First of all, it has to be noted that several IFN-γ producing cell types may be involved in the autoimmune process. Conventional CD4+ αβ-Th1 lymphocytes are thought to drive the myelin-specific inflammatory response and usually considered to be the main target of any intervention on IFN-γ pathways. However, other immune populations, such as CD8+ conventional T cells, γδ-T lymphocytes, CD56bright NK cells and NKT cells exert their effects through IFN-γ, sometimes secreted in large amounts and not necessarily targeted to a specific antigen. CD8+ conventional T cells have been...
suggested to play both a pathogenic and regulatory role in MS and EAE. As discussed before, Huseby et al demonstrated a pathogenic role of IFN-γ in a mouse model of CD8+ T cell mediated EAE [49]. CD8\textsuperscript{bright} NK cells, NKT cells and γδ-T lymphocytes have all been hypothesised to play a regulatory role in autoimmune inflammation of the CNS [56, 57]. It is possible that some of the contradictory effects of the cytokine in experimental models are the result of altering IFN-γ pathways that are relevant to the regulatory function of these cell subtypes. Of note, it has been shown that CNS resident γδ-T cells promote the production of IFN-γ by encephalitogenic (and IFN-γ producing) T cells in the CNS and this production is ultimately required for the recovery from EAE [58].

One of the main factors possibly involved in determining opposite roles of IFN-γ is the site of action of the cytokine, i.e. the periphery or the target organ and in particular the CNS. It has been proposed that IFN-γ plays a pro-inflammatory role locally whilst its action at the systemic level would result in immunomodulation and protection from disease [59]. This hypothesis is supported by several findings in both non-CNS tissues and EAE. Local injections of lipopolysaccharide in the footpad of mice caused local IFN-γ production and inflammatory reaction whilst intraperitoneal administration of IFN-γ suppressed the inflammatory process [60]. The detrimental effect of IFN-γ at the local level in EAE was demonstrated by the induction of leukocyte recruitment and expression of MHC class II in the brain and spinal cord [61, 62]. Combined injection of antimyelin/oligodendrocyte glycoprotein antibody and IFN-γ in rats also enhanced demyelination [63]. The effects of IFN-γ overexpression in the CNS of transgenic mice further supported the disease-promoting role of local overexpression of IFN-γ [24-26]. On the contrary, some authors reported amelioration of EAE after intrathecal delivery of IFN-γ suggesting the local induction of protective mechanisms [47, 48]. It has to be noted that there may be different outcomes resulting from exogenous administration versus endogenous production of the cytokine, possibly related to the existence of feedback responses. Brok et al showed that exogenous administration of IFN-γ in the periphery suppressed the local production of the cytokine in lymphoid organs [64], although it is not known if similar feedback mechanisms are present in the CNS.

The specific CNS compartment in which the cytokine exerts its effects seems to determine different outcomes of CNS demyelination. Again valuable insight has been provided by the study of EAE in IFN-γ and IFN-γR deficient mice. TCR transgenic mice expressing an MBP-specific monoclonal TCR developed spontaneous EAE with classical spinal cord localisation whilst the IFN-γ KO equivalent strain showed a distinct phenotype with predominant inflammation in the brain [65]. Therefore IFN-γ seemed to be protective in the brain whilst exerting a damaging role in the spinal cord. Findings in the IFN-γR KO mice confirmed this hypothesis. MOG\textsubscript{35-55} specific T cells determined inflammation into the whole CNS of IFN-γR KO mice whilst the same cells induced only spinal cord inflammation when transferred into wild type mice [66]. The involvement of the balance between Th1 and Th17 cells has been suggested as the determining factor in this CNS region-specific action of IFN-γ [27]. Stromnes et al showed that brain inflammation was found only when Th17 cells outnumbered or equalled Th1 cells in the brain meninges of EAE induced with the specific MOG\textsubscript{97-114}, whilst the use of two other MOG peptides induced only spinal cord inflammation [67]. In this model, the neutralisation of IL-17 prevented inflammation in the brain but not the spinal cord, suggesting a possible dichotomy of the inflammatory effects IL-17 and IFN-γ, the former sustaining the damage in the brain and the latter in the spinal cord.

One mechanism that supports a protective effect of IFN-γ is its ability to inhibit expansion of activated T cells. IFN-γ KO mice have more activated and proliferating CD4+ T cells than wild type mice as well as reduced apoptosis [7, 8]. It was also shown that addition of IFN-γ to activated CD4+ T cells from IFN-γ KO mice induced apoptosis of these cells in response to antigen restimulation. In fact, the ability of IFN-γ to induce apoptosis has been demonstrated since IFN-γ can elicit inducible nitric oxide synthase (iNOS) in activated macrophages and lead to release of NO [68]. It has also been demonstrated that IFN-γ can promote caspase-dependent apoptosis and therefore control the expansion of T cells [69]. Moreover, IFN-γ can induce the tryptophan-catabolizing enzyme indolamine 2,3-dioxigenase (IDO), which is known to inhibit T-cell responses by depleting tryptophan [70]. It has been speculated that the local IDO expression induced by IFN-γ could be implicated in a negative feedback loop to self-limit inflammation in EAE [71].

The time of action of IFN-γ during the development of EAE is another factor that could be involved in the multivariate functions of this cytokine. Heremans et al showed that early administration of IFN-γ to a chronic-relapsing model of EAE (Biozzi ABH mice) provided partial protection not only against the first attack, but also against subsequent spontaneous relapses [45]. The protective effect of IFN-γ in the early phases of the disease was also suggested by studies on the effect of IL-12 administration in EAE. In fact, IL-12 suppressed disease in an IFN-γ dependent fashion when administered systemically to mice during the early phase of EAE induction [72]. In addition, later administration of IL-12 (and therefore induction of IFN-γ) during established EAE showed no protective effect or led to disease exacerbations [73]. Of note, this is in contrast with findings in an adoptive transfer TCR transgenic model of DTH which used ovalbumin as antigen. In this model IFN-γ producing Th1 cells accelerated the inflammation in the early phase of the disease, whereas in the late phase mediated the process of self-limitation by limiting the number of Th1 effector cells [74].

Interestingly Billiau et al hypothesized that the disease-limiting role played by IFN-γ could be related to the use of complete Freund’s adjuvant (FA) in this and other animal models of autoimmunity such as collagen induced arthritis (CIA) and uveitis [59]. Complete FA is a water-in-oil mixture that contains heat-killed mycobacteria and serves as a vehicle for the administration of the autoantigen. Since complete FA contains dead mycobacteria it has been suggested that the protective role of IFN-γ in EAE may be due to its critical role in eliminating dead mycobacteria from the organism. It has been shown that immunisation with complete FA is associated with enhanced myelopoiesis with augmented proportion of Mac-1+ cells (monocyte-macrophage...
and granulocytic lineages). This process is more pronounced in IFN-γ KO mice indicating that IFN-γ controls the expansion of Mac-1+ cells which are the effector cells of the DTH reaction [75]. This hypothesis is supported by several findings in CIA [59]. Several studies have indicated that the inflammatory infiltrate in IFN-γ KO mice is characterised by predominance of neutrophils rather than mononuclear cells [50, 53]. This finding is supported by the demonstration in IFN-γ KO mice of a shift in the chemokine pattern of the CNS lesions which favours neutrophil rather than T cell and monocyte infiltration [52]. The relevance of this phenomenon could be addressed in the non-human primate species common marmoset (Callithrix jacchus) EAE model, since EAE in mice relies on the use of complete FA. In this species inflammatory demyelinating EAE can be induced with a synthetic peptide (MOG34-56) in complete [76] as well as incomplete FA (Jagesar et al., submitted manuscript), resulting in the activation of T-cells with the capacity to induce without autoantibody involvement the pathological hallmarks of MS, i.e. inflammation, demyelination and progressive axonal injury. While MOG34-56 specific T-cell responses in the complete FA-dependent model were skewed in Th1 direction, those in the incomplete FA-dependent model were more Th17-prone. Nevertheless, the clinical and pathological outcome of both models was highly comparable. The fact that MS-like disease can be induced without using bacterial antigens removes a major bias of EAE as a model of MS and could provide further insight into the role of IFN-γ in a model that is closer to the human disease.

Finally, IFN-γ is able to influence two immune populations that have been increasingly recognised as important in EAE and MS, Tregs and Th17. It was shown that CD4+CD25+Foxp3+ Tregs were reduced in number and function in EAE induced in IFN-γ KO mice. In vitro treatment of mouse CD4+CD25+ T cells with IFN-γ converted these cells to Tregs which were able to suppress EAE when transferred to naïve animals [77]. Moreover, they showed the same ability of IFN-γ to convert in vitro CD4+CD25+ T cells to Tregs in human cells. In support of this mechanism, it has been shown that treatment with copolymer-I induced increased expression of Foxp3 through an IFN-γ dependent mechanism in both humans and mice [78]. In addition, Zehntner et al. showed that neutrophils derived from the CNS of mice with EAE suppressed T cell responses [79]. This suppressor ability was dependent on IFN-γ production by T cells which stimulated the release of NO by infiltrating neutrophils. IFN-γ plays also a critical role in driving naïve T cells towards a Th1 phenotype and inhibiting the differentiation of Th17 cells in mice [80]. However the reciprocal roles of IFN-γ and IL-17 are not completely characterised in humans, since it has been shown that IFN-γ drives human APCs to abate Th1 polarization and secrete cytokines that promote memory Th17 cell differentiation [81].

CLINICAL IMPLICATIONS

Two clinical studies tested the in-vivo effect of direct modulation of IFN-γ in MS patients and concluded that IFN-γ promotes disease exacerbation and progression in humans. In the eighties Panitch et al. conducted an open, randomised study on 18 patients with clinically definite, relapsing-remitting multiple sclerosis (MS) who received intravenous infusion of IFN-γ twice a week for four weeks [82]. At the end of the trial, 7 patients had exacerbations with a significantly increased relapse rate as compared to pre- and post-treatment observations. There was also a concomitant increase in circulating relapse rate bearing class II (HLA-DR) surface antigen which was interpreted as a sign of immune activation.

Similar conclusions have been reached by another trial conducted in MS patients. Skurkovich et al reported that treatment with antibodies against IFN-γ, but not against TNF-α, was beneficial in secondary progressive MS [83]. After a short course of antibody treatment patients showed improvement in EDSS score and decreased number of MRI active lesions. There was a reduction of IFN-γ, IL1-β, TNF-α and increase of TGF-β production by in-vitro polyclonally activated lymphocytes.

These two studies showed that exogenous administration of IFN-γ in the periphery enhanced the autoimmune process in MS patients whilst its neutralization exerted protective effects, which is in contrast with many observations in mice [42]. Several factors may be involved in this discrepancy between mice and human studies. Exogenous administration of IFN-γ may have induced pseudo relapses, due to the known side effects of all interferons of causing flu-like symptoms, especially in the first study in which MRI scanning was not available to confirm clinical observations. The time of administration during the pathogenic process may be another factor. MS patients are necessarily studied when the disease is ongoing and disease activity is not always clearly identifiable, whilst studies in animals have been performed both before and after EAE induction showing opposite effects of the cytokine or anti-cytokine administration depending on timing. Finally, the site of administration may have influenced negatively the functional effect of IFN-γ in patients. The possible effects of intrathecal administration of IFN-γ in humans have not been investigated probably because considered potentially harmful.

FUTURE PERSPECTIVES

Past investigations on the role of IFN-γ in animal models of CNS autoimmune demyelination have highlighted the multivariate aspects of its biology. Although the results of the only two clinical trials performed to date concluded against a protective role of IFN-γ in humans, this cytokine and its pathways have the potential to be manipulated for therapeutic purposes. In particular the recently recognised role of IFN-γ in activating regulatory cells and inhibiting development of Th17 cells could be exploited to promote protective functions in humans. In addition, IFN-γ ability to induce apoptosis of Th1 cells could be utilized to induce the elimination of myelin specific effector T cells. Finally, other therapeutic interventions could be directed to the promotion of IFN-γ production by specific cell populations with regulatory properties such as CD56bright NK cells, NKT and γδ-T cells. Perhaps the most important point is that any intervention aimed at exploiting the protective functions of IFN-γ should be designed to concomitantly limit the possible induction of its pro-inflammatory effects. Further studies are therefore needed to clarify the complex interactions of this cytokine with several immune and non-immune factors involved in the pathogenesis of CNS autoimmune demyelination.
CONFLICTS OF INTEREST

The authors do not report conflicts of interest.

REFERENCES

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