

# The Effects of Interferon- $\gamma$ on the Central Nervous System

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## Abstract

Interferon-gamma (IFN- $\gamma$ ) is a pleotropic cytokine released by T-lymphocytes and natural killer cells. Normally, these cells do not traverse the blood-brain barrier at appreciable levels and, as such, IFN- $\gamma$  is generally undetectable within the central nervous system (CNS). Nevertheless, in response to CNS infections, as well as during certain disorders in which the CNS is affected, T-cell traffic across the blood-brain barrier increases considerably, thereby exposing neuronal and glial cells to the potent effects of IFN- $\gamma$ . A large portion of this article is devoted to the substantial circumstantial and experimental evidence that suggests that IFN- $\gamma$  plays an important role in the pathogenesis of the demyelinating disorder multiple sclerosis (MS) and its animal model experimental allergic encephalomyelitis (EAE). Moreover, the biochemical and physiological effects of IFN- $\gamma$  are discussed in the context of the potential consequences of such activities on the developing and mature nervous systems.

**Index Entries:** Cytokines; demyelination; neural development.

## Introduction

Interferon-gamma (IFN- $\gamma$ ) was originally discovered by Wheelock (1965) as an activity that interfered with viral replication. T-lymphocytes and natural killer cells are the only cells known to be capable of expressing this cytokine (reviewed in Trinchieri and Perussia, 1985). Because under normal condi-

tions T-cell traffic within the CNS is minimal, this cytokine is not generally detectable in the CNS (reviewed in Fabry et al., 1994). Nevertheless, T-cells cross the blood-brain barrier at significantly increased levels in response to CNS infections (reviewed in Hickey, 1991). Moreover, during certain autoimmune disorders in which CNS antigens are targeted, immune cell traffic within the CNS increases considerably.

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Under these conditions, neuronal and glial cells that normally do not encounter IFN- $\gamma$  are exposed to the potent, pleotropic effects of this cytokine. There is considerable evidence that suggests that the effects of IFN- $\gamma$  are important in a variety of CNS disorders (reviewed in Ransohoff and Benveniste, 1996). In this article, we discuss various molecular, biochemical, cellular, and physiological properties of IFN- $\gamma$  and its evoked response. Moreover, the potential role this cytokine plays in CNS disorders is discussed.

## General Properties of IFN- $\gamma$

A considerable amount of information has been learned concerning the molecular biology and biochemistry of IFN- $\gamma$  since the human and mouse cDNAs were cloned over a decade ago (Gray et al., 1982; Gray and Goeddel, 1982, 1983). The human and mouse proteins are 143 and 134 amino acids in length, respectively, and are encoded for by four exons that reside on 6 kb of DNA. Interestingly, the human and mouse proteins share only limited homology, approx 40% at the amino acid level, and do not bind to the other's receptor to an appreciable extent (reviewed by Farrar and Schreiber, 1993). Both proteins contain two glycosylation sites (Gray and Goeddel, 1982; Ealick et al., 1991), although glycosylation is not essential for IFN- $\gamma$  activity (Kelker et al., 1983). One factor that may play a role in regulating the biological activity of IFN- $\gamma$  is message stability, since the IFN- $\gamma$  mRNA contains an AU rich sequence in the 3'-untranslated region that has been shown to reduce the mRNA half-life of other cytokines dramatically (Shaw and Kamen, 1986). Biologically active IFN- $\gamma$  from both human and mouse is a noncovalently bound homodimer that contains two receptor binding sites (Farrar and Schreiber, 1993).

The IFN- $\gamma$  receptor is a multimeric receptor complex composed of a ligand binding subunit ( $\alpha$ -chain) (Aguet et al., 1988) and a transmembrane, accessory factor ( $\beta$ -chain) (Soh et al., 1994; Hemmi et al., 1994). The  $\alpha$ -chain is a ubiqu-

itously expressed, glycosylated, cell-surface protein that binds IFN- $\gamma$  with high affinity (reviewed by Farrar and Schreiber, 1993). The extent of glycosylation is variable depending on cell type and accounts for the range of sizes of the  $\alpha$ -chain, between 80 and 95 kDa. On binding, IFN- $\gamma$  induces  $\alpha$ -chain dimerization, which is believed to be critical to the initiation of the signal transduction cascade (Greenlund et al., 1993; Fountoulakis et al., 1992). This feature of the IFN- $\gamma$  receptor has been exploited to generate mutant forms that confer IFN- $\gamma$  unresponsiveness to cells in a dominant manner (Dighe et al., 1993, 1995). The crystal structure of the complex between IFN- $\gamma$  and the  $\alpha$ -chain of its receptor has recently been reported (Walter et al., 1995). The  $\beta$ -chain of the receptor, which associates with the  $\alpha$ -chain in an IFN- $\gamma$ -dependent manner, is essential for the induction of the IFN- $\gamma$  signaling cascade (Bach et al., 1996).

Recently, considerable progress has been made in the elucidation of the IFN- $\gamma$ -induced signal transduction pathway (Sadowski et al., 1993; Darnell et al., 1994; Shuai, 1994; Vilcek and Oliveira, 1994; David, 1995; Schindler, 1995). The Janus kinases JAK1 and JAK2 become phosphorylated following binding of IFN- $\gamma$  to its receptor, with which the JAK kinases associate (Muller et al., 1993; Watling et al., 1993). The JAK1 kinase appears to associate with the  $\alpha$ -chain, and the JAK2 kinase associates with the  $\beta$ -chain (Sakatsume et al., 1995). Following activation, the JAK kinases phosphorylate the transcriptional factor known as signal transducer and activator of transcription (STAT-1 $\alpha$ ), which binds to a specific DNA element referred to as  $\gamma$ -activation site (GAS) (Shuai et al., 1993). STAT-1 $\alpha$  binding results in the rapid transcriptional induction of genes containing the GAS element. For example, the genes encoding the gaunylate binding protein and the IgG Fc receptor are induced in this way. Other genes that are stimulated by IFN- $\gamma$ , such as the class I and class II molecules of the major histocompatibility complex (MHC), require a longer period (several hours) before transcriptional induction is detected.

These genes lack the GAS element and are likely activated through IFN- $\gamma$ -induced intermediates (Benveniste and Benos, 1995).

### Activities of IFN- $\gamma$ (Summarized in Table 1)

IFN- $\gamma$  plays a critical role in the regulation of the immune response (Young and Hardy, 1995). For this reason, it is often referred to as immune interferon. IFN- $\gamma$  facilitates the stimulation of the Th1 subpopulation of T-lymphocytes, which control cell-mediated immunity and which, in fact, express IFN- $\gamma$ ; whereas it impedes the stimulation and activity of the Th2 subpopulation of T-cells, which express IL-4 and are involved in regulating humoral immunity (Gajewski et al., 1989; Swain et al., 1991; Paul and Seder, 1994; Seder and Paul, 1994; Reiner and Seder, 1995). Cytotoxic T-cells and natural killer cells are also activated by IFN- $\gamma$  (Trinchieri and Perussia, 1985). IFN- $\gamma$  also plays a role in regulating antibody production, promoting an IgG<sub>2a</sub> response through a direct effect on B-cells (Snapper and Paul, 1987). Importantly, IFN- $\gamma$  is a potent stimulator of expression of the antigen-presenting components of the immune system. MHC class I and II molecules are dramatically upregulated in the presence of IFN- $\gamma$  on a variety of cell types (reviewed in Vilcek et al., 1985; Benveniste and Benos, 1995).

IFN- $\gamma$  is also a powerful effector molecule of the inflammatory response (reviewed in Schreiber and Celada, 1985). IFN- $\gamma$ -stimulated macrophages release a number of inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1, IL-6, and IL-8. In addition, IFN- $\gamma$  increases the microbicidal activities of macrophages through the induction of the synthesis of hydrogen peroxide and nitric oxide (Farrar and Schreiber, 1993). IFN- $\gamma$  is also a potent stimulator of the expression of the Fc receptors for IgG immunoglobins on macrophages (Erbe et al., 1990). All of these actions of IFN- $\gamma$  on macrophages serve to stimulate the cytotoxic activity of these cells.

Table 1  
Immune Activities of IFN- $\gamma$

Facilitates the stimulation of Th1 T-cells
Inhibits the stimulation of Th2 T-cells
Activates cytotoxic T-cells and natural killer cells
Regulates antibody production
Stimulates MHC expression
Stimulates microbicidal activity of macrophages

### Immune-Mediated Demyelinating Disorders

Multiple sclerosis (MS) is the most common human demyelinating disease that affects the CNS. Although the etiology of MS has not been established, it is widely believed that immunological mechanisms are involved (reviewed in Martin et al., 1992; Raine, 1994a,b). The CNS is immunologically distinct owing to the presence of the blood-brain barrier (Crone, 1986), which inhibits entry of both immune cells and humoral factors. In MS patients, this barrier is disrupted (Martin et al., 1992) allowing increased access of blood components (cellular and noncellular) to the CNS. The enhanced permeability of the blood-brain barrier is thought to be a contributing factor in immune-mediated demyelinating disorders (reviewed in Poser, 1987, 1993). Importantly, there is an increased infiltration of T-lymphocytes (CD4+ and CD8+) into the CNS of MS patients (Martin et al., 1992; Utz and McFarland, 1994), with CD4+ (T-helper) T-cells being predominant at the sites of active demyelination (Booss et al., 1983; Traugott et al., 1983). T-cell responses to a variety of myelin antigens, including myelin basic protein (MBP) and proteolipid protein (PLP), have been identified in MS patients (reviewed in Miller et al., 1995).

Experimental allergic encephalomyelitis (EAE) is the primary animal model used in the study of MS (reviewed in Alvord et al., 1984; Zamvil and Steinman, 1990; Martin and McFarland, 1995). EAE can be induced in a variety of laboratory animal species by immunization with either CNS tissue, myelin, or

myelin components. As demonstrated through passive transfer studies, EAE is a T-cell (CD4+, Th1) mediated disease model (Zamvil and Steinman, 1990; Voskuhl et al., 1993). A relapsing/remitting form of EAE is induced at high incidence in SJL mice immunized with an encephalitogenic epitope of PLP (Tuohy et al., 1988a,b, 1989, 1992; McRae et al., 1992). This model of EAE exhibits many of the clinical, pathological, and immunological features of MS.

### IFN- $\gamma$ in MS and EAE

The T-cells that infiltrate into the CNS in MS and EAE are activated and produce IFN- $\gamma$ , which is believed to play a role in the pathogenesis of immune-mediated demyelinating disorders (reviewed in Hartung et al., 1992; Olsson, 1992; Panitch, 1992). There is considerable circumstantial evidence that suggests that IFN- $\gamma$  is a key mediator of inflammation in MS and EAE. Many of the pathological events observed in MS and EAE, such as increased MHC expression, macrophage activation, increased expression of leukocyte adhesion molecules on blood-brain barrier endothelial cells (Olsson, 1992), and reactive gliosis (Balasingam et al., 1994), are consistent with the known effects of IFN- $\gamma$ . IFN- $\gamma$  is detected in active MS lesions (Traugott and Lebon, 1988), and increased numbers of IFN- $\gamma$ -secreting T-lymphocytes are present in the cerebrospinal fluid of MS patients (Olsson et al., 1990). Beck et al. (1988) reported a correlation between the onset of clinical symptoms of MS and mitogen-stimulated IFN- $\gamma$  production. Moreover, EAE can be transferred to naive animals using IFN- $\gamma$ -secreting T-lymphocytes isolated from animals experiencing EAE (Zamvil and Steinman, 1990), and a significant increase in the level of IFN- $\gamma$  mRNA is observed in the CNS of animals with severe EAE compared to animals with mild EAE (Renno et al., 1994).

Perhaps the best direct evidence for the deleterious effects of IFN- $\gamma$  in MS came inadvertently from a small clinical trial in the early 1980s. Of 18 MS patients receiving IFN- $\gamma$ , seven

experienced exacerbations during the 4-wk treatment period, which was significantly higher than the pretreatment exacerbation rate (Panitch et al., 1987; Panitch, 1992). Not only did this study clearly eliminate IFN- $\gamma$  as a potential therapeutic agent for MS, but it also demonstrated that IFN- $\gamma$  activity can lead to a worsening of the disease course, suggesting a role for this cytokine in normal disease progression.

There are, however, contradictory experimental data concerning the role of IFN- $\gamma$  in EAE. The administration of antibodies to IFN- $\gamma$  enhanced the severity of EAE in mildly susceptible mice (Billiau et al., 1988; Voorthuis et al., 1990; Duong et al., 1992), and resulted in EAE susceptibility in resistant strains (Duong et al., 1994). Moreover, treatment of SJL/J mice, which are very sensitive to EAE, with IFN- $\gamma$  results in enhanced survival (Billiau et al., 1988). Nevertheless, the protective effect of IFN- $\gamma$  is not observed when EAE is passively transferred. Voorthuis et al. (1990) reported that intraventricular administration of IFN- $\gamma$  into EAE-induced rats resulted in complete suppression of clinical signs, and Willenborg et al. (1995), using a viral delivery system, suggested that IFN- $\gamma$  had no effect on disease. Recently, Ferber et al. (1996) examined the EAE susceptibility of B10.PL mice that contained an experimentally inactivated IFN- $\gamma$  gene (Dalton et al., 1993). B10.PL mice are normally very susceptible to MBP-induced EAE, and the IFN- $\gamma$  knockout mice appear equally sensitive to disease induction. Although morphological data were not presented, this work clearly demonstrates that IFN- $\gamma$  is not essential for EAE induction, at least not in B10.PL mice.

### Experimental Analysis of the Effects of IFN- $\gamma$ on the CNS

To understand better the effects of IFN- $\gamma$  on CNS cells in vivo, in the absence of other T-cell-derived factors, a variety of approaches have been used to deliver this cytokine to the CNS. The direct injection of IFN- $\gamma$  into various animal models has been shown to upregulate the

expression of both MHC class I and class II glycoproteins on various neuronal cell types that do not normally express these proteins at detectable levels. Wong et al. (1984) demonstrated a dramatic increase in the expression of the MHC class I glycoprotein in astrocytes, neurons, oligodendrocytes and microglia following the delivery of IFN- $\gamma$  to the CNS of 2-d-old mice. Direct injection of IFN- $\gamma$  into the CNS has also been demonstrated to increase the expression of MHC class I on rat endothelial and ependymal cells and class II on microglia, ependymal, and perivascular cells (Sethna and Lampson, 1991). Continuous iv infusion of IFN- $\gamma$  into Lewis rats for a period of 3 d induced MHC class II expression on microglia, ependymal and endothelial cells of large blood vessels (Steiniger and van der Meide, 1988). These findings support the earlier work of Momburg et al. (1986) who reported that the iv infusion of IFN- $\gamma$  into mice resulted in class II expression in many organs, including the brain, where "round cells in the vicinity of meningeal blood vessel," presumably microglia, were class II immunoreactive.

IFN- $\gamma$  has also been suggested to play a role in the breakdown of the blood-brain barrier and the recruitment of inflammatory cells into the CNS. Sethna and Lampson (1991) reported that a single injection of IFN- $\gamma$  into the rat basal ganglia induced the infiltration of CD4<sup>+</sup> T-cells into the perivascular space and monocytes/macrophages, and presumptive natural killer cells into the brain parenchyma. These authors suggested that such cellular recruitment may be facilitated by IFN- $\gamma$ -induced changes in the endothelial cells, resulting in an alteration in the permeability of the blood-brain barrier. Moreover, Simmons and Willenborg (1990), using direct injection of IFN- $\gamma$  into the spinal cord of rats, reported an inflammatory response similar in pattern to that observed in early EAE with the accumulation of inflammatory cells in the meninges and around spinal cord veins also suggestive of a compromised blood-brain barrier. Tjuvajev et al. (1995) reported an IFN- $\gamma$ -induced breakdown of the blood-brain barrier in an *in vivo* brain tumor

model, and Huynh and Dorovini-Zis (1993) demonstrated that brain endothelial cells undergo dramatic morphological, functional, and permeability changes in response to IFN- $\gamma$ , suggesting that this cytokine alters blood-brain barrier function. Steffen et al. (1994) demonstrated increased expression of vascular cell adhesion molecules (VCAM) and intercellular adhesion molecule-1 (ICAM-1) on the brain endothelium of SJL mice experiencing EAE, and antibodies to these molecules inhibited binding of peripheral lymphocytes to inflamed brains. Furthermore, McCarron et al. (1993) showed that IFN- $\gamma$  significantly increased the adhesion of MBP-specific encephalitogenic T-cells to mouse cerebrovascular endothelial cells, and that this effect correlated with the induction of ICAM-1 expression by the endothelial cells.

The studies discussed above consistently indicate a role for IFN- $\gamma$  in the inducement of expression of MHC and cell adhesion molecules, and the recruitment of inflammatory cells; however, the infusion of IFN- $\gamma$  into the CNS has also been implicated in a variety of other processes. For example, Yong et al. (1991) reported that the direct administration of IFN- $\gamma$  into the adult mouse brain following corticectomy resulted in an increase in trauma-initiated gliosis. Brosnan et al. (1989), using the visual pathway of the rabbit, demonstrated that the injection of IFN- $\gamma$  significantly reduced neural conduction and induced subtle axonal pathology. In addition, the simultaneous injection of IFN- $\gamma$  and a myelin/oligodendrocyte glycoprotein antibody significantly delayed cervical somatosensory evoked potentials, and caused massive demyelination in the spinal cord (Vass et al., 1992). Vass et al. (1992) also reported an IFN- $\gamma$ -induced increase in MHC expression, which accompanied spinal cord demyelination.

In complement with various delivery systems previously discussed, we have used a transgenic approach to examine the effects of IFN- $\gamma$  on the developing nervous system (Corbin et al., 1996). Transgenic mice were generated in which the expression of IFN- $\gamma$  was

placed under the transcriptional control of the MBP gene (MBP/IFN- $\gamma$  transgenic animals). Transgenic mice generated with this construct have a shaking/shivering phenotype that is similar to that observed in naturally occurring mouse models of hypomyelination (e.g., shiverer, jimpy, quaking), and these transgenic animals have dramatically less CNS myelin than control animals. Reactive gliosis and increased macrophage/microglia F4/80 immunostaining were also observed. Additionally, MHC class I and class II mRNA levels were increased in the CNS of MBP/IFN- $\gamma$  transgenic mice, and the increase in MHC class I mRNA expression was detected in both white and gray matter regions. Surprisingly, cerebellar granule cell migration was also disrupted in these animals. These results strongly support the hypothesis that IFN- $\gamma$  is a key effector molecule in immune-mediated demyelinating disorders, and suggest that the presence of this cytokine may also disrupt the cytoarchitecture of the developing nervous system.

### Cellular Sites of IFN- $\gamma$ Action in Immune-Mediated Demyelination

The mechanism by which IFN- $\gamma$  exerts its action within the CNS remains unresolved. One idea concerning the potential cellular site of action of IFN- $\gamma$  in CNS demyelinating disorders is that IFN- $\gamma$  activates macrophages/microglial cells, which in turn mediate cytotoxic effects on oligodendrocytes (Fig. 1). TNF- $\alpha$ , which has previously been shown to be toxic to oligodendrocytes *in vitro*, is expressed by IFN- $\gamma$ -activated macrophages/microglia (Selmaj et al., 1991). More recent *in vitro* experiments suggest that the microglial cell toxicity to oligodendrocytes is mediated through cell-surface expression of TNF- $\alpha$  and the production of nitric oxide (Merrill et al., 1993). Nitric oxide is very labile, such that to elicit their cytotoxic effects, the activated microglia need to be in intimate contact with the target oligodendrocytes. Oligodendrocytes undergo necrotic cell death following exposure to nitric oxide

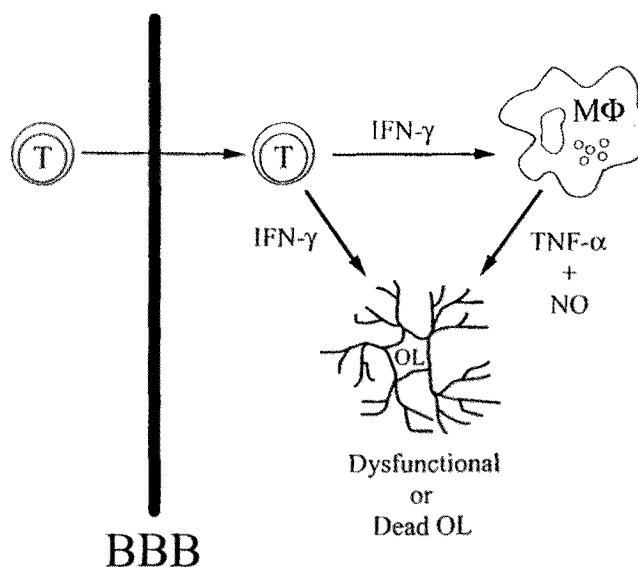


Fig. 1. Potential effects of IFN- $\gamma$  on oligodendrocytes. A schematic diagram is presented showing a possible direct effect of IFN- $\gamma$  on oligodendrocytes. An indirect effect is also shown in which macrophages, or microglia, are activated to release cytotoxic substances. BBB, blood-brain barrier; T, T-cell; M $\phi$ , macrophage; OL, oligodendrocyte.

(Mitrovic et al., 1995). In support of the hypothesis that nitric oxide is important in the pathogenesis of immune-mediated demyelinating diseases, it has been shown that nitric oxide synthase mRNA levels are increased in mice experiencing EAE (Koprowski et al., 1993), and NADPH-diaphorase histochemical staining, a marker for nitric oxide production, as well as nitric oxide synthase mRNA levels were found to be elevated in the brains of MS patients (Bö et al., 1994).

IFN- $\gamma$  may also have a direct deleterious effect on oligodendrocytes (Fig. 1), which have been shown to express IFN- $\gamma$  receptors (Torres et al., 1995). We have examined the effects of IFN- $\gamma$  on oligodendrocytes using the MOCH-1 cell line, which was derived from an oligodendroglioma that developed in a transgenic line of mice and expresses a variety of features of oligodendrocytes (Hayes et al., 1992; Li et al., 1995). When MOCH-1 cells are cultured in medium containing low concentrations of

serum (1% fetal bovine serum [FBS]) they extend multiple, thin, branched processes and have phase-bright cell bodies, which are typical features of cultured primary oligodendrocytes (reviewed in Popko et al., 1994). Furthermore, these cells are immunoreactive against antibodies to galactocerebroside, a glycolipid marker of mature oligodendrocytes, and abundantly express myelin protein genes (Hayes et al., 1992). MOCH-1 cells cultured in 1% FBS or chemically defined medium do not express detectable levels of the astrocyte marker glial fibrillary acidic protein (GFAP).

When MOCH-1 cells are cultured in 1% FBS medium containing IFN- $\gamma$ . However, they transform to flat, astrocyte-like cells with enlarged cell bodies and appear more adhesive (Li et al., 1995). In addition to these morphological changes, IFN- $\gamma$  stimulates the expression of abundant levels of the astrocyte marker GFAP, as well as slightly elevated levels of MBP and PLP mRNA. IFN- $\gamma$  also induces an enormous increase of MHC class I expression, as well as a comparatively modest increase of MHC class II mRNA expression in MOCH-1 cells. The morphological and molecular changes MOCH-1 cells undergo in response to IFN- $\gamma$  suggest that IFN- $\gamma$  may induce a direct effect on oligodendrocytes, or a subpopulation of these cells, *in vivo* (Li et al., 1995).

Recent studies further support the hypothesis that IFN- $\gamma$  has a direct, deleterious effect on oligodendrocytes. Using a morphological assay, oligodendrocytes *in vitro* were shown to undergo apoptotic cell death at very high frequency following exposure to relatively low concentrations of IFN- $\gamma$  (Vartanian et al., 1995). The addition of microglia to the culture did not significantly increase the frequency of oligodendrocyte death, further supporting a direct effect of the cytokine. Interestingly, Vartanian et al. (1995) also detected apoptotic oligodendrocytes, along with IFN- $\gamma$ , in the advancing margin of active MS plaques, but not in the chronic portion of the plaque or in adjacent unaffected white matter. Agresti et al. (1996) have also observed deleterious effects of IFN- $\gamma$  on cultured oligodendrocytes. In the absence

of cell death, they observed a decrease in the ability of oligodendrocyte progenitors to divide and differentiate, as well as changes in myelin protein gene mRNAs and a general metabolic depression. These effects were shown to be fully reversible following cytokine withdrawal (Agresti et al., 1996).

The harmful effects of IFN- $\gamma$  on myelination might be mediated through the induction of MHC expression in oligodendrocytes, which normally do not express these molecules at an appreciable level. In support of this possibility, transgenic mice in which MHC class I expression has been targeted to oligodendrocytes are severely hypomyelinated (Turnley et al., 1991b; Yoshioka et al., 1991; Turnley and Morahan, 1995). This hypomyelination occurs in the absence of immune cell infiltration, suggesting that MHC class I expression in oligodendrocytes is sufficient to disrupt myelination. Recently, Power et al. (1996) examined these transgenic animals in more detail and demonstrated that cell-surface expression of MHC class I in oligodendrocytes of these mice was minimal. Moreover, immunocytochemistry for  $\beta$ 2-microglobulin, which is required for cell-surface expression of MHC class I, revealed that expression of  $\beta$ 2-microglobulin was undetectable in oligodendrocytes and limited to microglia in the CNS. Power et al. (1996) suggest that the accumulation of MHC class I protein in the cytoplasm of the oligodendrocytes, owing to the absence of  $\beta$ 2-microglobulin, may interfere with normal myelin protein trafficking, leading to the observed hypomyelination in these mice.

*In vitro*, oligodendrocytes at all stages of differentiation can be induced by IFN- $\gamma$  to express cell-surface MHC class I (Suzumura et al., 1986; Turnley et al., 1991a; Massa et al., 1993). Nevertheless, it is unclear what the consequences of oligodendroglial expression of MHC class I are. As mentioned previously, CD8+ T-cells are present in the CNS of MS patients (Martin et al., 1992; Utz and McFarland, 1994). It is, however, unknown whether oligodendrocytes *in vivo* can present antigen to these cells.

In contrast, many investigators have searched for, but failed to demonstrate, IFN- $\gamma$ -mediated induction of MHC class II on oligodendrocytes in culture (Wong et al., 1984; Suzumura et al., 1986; Turnley et al., 1991a; Satoh et al., 1991b), suggesting that oligodendrocytes are refractory to MHC class II induction. Calder et al. (1988) demonstrated that oligodendrocyte precursors (O-2A progenitor cells) can be induced to express MHC class II by IFN- $\gamma$ , but that maturation into oligodendrocytes leads to loss of inducibility. Nevertheless, Bergsteinsdottir et al. (1992) were able to induce MHC class II expression in a subset of mature oligodendrocytes in culture with IFN- $\gamma$  in the presence of the synthetic glucocorticoid dexamethasone. Although glucocorticoids are normal CNS constituents (Birmingham et al., 1984; Kumar et al., 1989), the *in vivo* significance of these *in vitro* observations is unclear.

Another activity of IFN- $\gamma$  that might be relevant to the pathogenesis of immune-mediated demyelinating disorders concerns the ability of this cytokine to stimulate the expression of heat-shock (stress) proteins. These are ubiquitously expressed proteins that are believed to play a role as chaperones in normal protein synthesis and degradation (reviewed in Jindal, 1996). The expression of these proteins increases following cell stress, and this response is thought to be protective. Heat-shock proteins are highly conserved across divergent species and are very immunogenic. It has been demonstrated that there is increased heat shock protein expression in MS and EAE lesions, and importantly, that there is an immune response to the stress proteins in these disorders (Selmaj et al., 1992; Prabhaker et al., 1994; Gao et al., 1995; van Noort et al., 1995). It has also been demonstrated that IFN- $\gamma$  is capable of potentiating the induction of stress protein synthesis (Morange et al., 1986; Ferm et al., 1992), such that this effect might facilitate the induction of the immune response to the heat-shock proteins in immune-mediated disorders. The characterization of the heat-shock response in these disorders should provide further insight into the role these proteins play in the pathogenesis of disease.

A potential direct effect of IFN- $\gamma$  on oligodendrocytes in immune-mediated demyelinating disorders is consistent with recent studies that examined biopsy specimens from patients with acute MS (Rodriguez and Scheithauer, 1994). They observed degeneration of inner myelin loops with preservation of outer loops and axons in early plaques, as well as areas in which well-myelinated and demyelinated axons were in close proximity. These observations suggest that the early pathological lesions that occur in MS are compatible with a primary disturbance in the myelinating function of oligodendrocytes, not a nonspecific inflammatory reaction that affects the myelin sheath, as might occur if microglia were activated subsequently to phagocytose the myelin sheath.

### The Effects of IFN- $\gamma$ on Astrocytes

Astrocytes, which have been shown to express the receptor for IFN- $\gamma$  (Rubio and de Felipe, 1991), respond to the presence of this cytokine in a variety of ways. As mentioned previously, MHC class I expression on astrocytes increases substantially in the presence of IFN- $\gamma$  (Wong et al., 1984; Mauerhoff et al., 1988; Massa et al., 1993). This expression appears functional in that astrocytes are susceptible to lysis by cytotoxic T-cells in an antigen-specific manner (Skias et al., 1987). IFN- $\gamma$ -induced MHC class II expression by astrocytes has also been demonstrated *in vitro* and *in vivo* (Fontana et al., 1984; Fierz et al., 1985; Pulver et al., 1987), although the *in vivo* levels are low relative to that detected on microglial cells (reviewed in Benveniste, 1992). The biological significance of the antigen-presenting potential of astrocytes remains to be resolved *in vivo*.

There is also evidence that suggests that IFN- $\gamma$  plays a role in the reactive astrogliotic response, particularly in immune-mediated disorders. Reactive astrogliosis is the response in which astrocytes express increased levels of GFAP, undergo hypertrophy, and proliferate (Eng, 1988). There are dramatically elevated levels of GFAP expression in the CNS of the



MBP/IFN- $\gamma$  transgenic animals, although it is unclear whether this represents a direct response of astrocytes to IFN- $\gamma$  or an indirect response elicited by the myelin abnormalities that occur in these mice (Corbin et al., 1996). Moreover, as mentioned, Yong et al. (1991) showed that IFN- $\gamma$ , as well as a number of other cytokines (Balasingam et al., 1994), increased trauma-induced gliosis in mice. These investigators also demonstrate that IFN- $\gamma$  promotes the proliferation of adult human astrocytes in vitro (Yong et al., 1991). Nevertheless, in a follow-up study, Yong et al. (1992) were unable to reproduce these results using mouse astrocytes, suggesting that there are species differences in the astrocytic response to IFN- $\gamma$ .

IFN- $\gamma$  has also been shown to increase the expression of intercellular adhesion molecules on astrocytes. Human fetal (Frohman et al., 1989) and adult (Satoh et al., 1991a) astrocytes, as well as mouse astrocytes (Satoh et al., 1991b), have been shown to express ICAM-1 in the presence of IFN- $\gamma$ . Such expression might facilitate the interaction of these cells with lymphocytes. IFN- $\gamma$ -stimulated human and rat astrocytes have also been shown to express VCAM-1, possibly suggesting a role in lymphocytic infiltration across the blood-brain barrier into the CNS (Rosenman et al., 1995).

### The Effects of IFN- $\gamma$ on Neurons

Although IFN- $\gamma$  readily induces the expression of MHC molecules in astrocytes, oligodendrocytes, and resident CNS microglia, IFN- $\gamma$ -induced expression of MHC molecules in neurons is under tighter control. Early evidence demonstrating the ability of brain cells, including neurons, to express MHC molecules includes experiments in which IFN- $\gamma$  was either added to mixed brain cultures or injected directly into the CNS (Wong et al., 1984, 1985). As detected by immunohistochemical analysis, the addition of IFN- $\gamma$  to brain cells in culture results in 9% of neurons expressing MHC class I, whereas direct injection into the CNS results in approx 15% of neurons expressing MHC class I. Moreover, the response of the OLB21

neuronal cell line to IFN- $\gamma$  has been investigated (Joly and Oldstone, 1992). The OLB21 cell line was developed by retroviral-mediated oncogene delivery to cells from the olfactory bulb (Ryder et al., 1990). In these cells, IFN- $\gamma$  stimulates the expression of MHC class I both at the mRNA level and on the cell surface. Furthermore, in the presence of IFN- $\gamma$ , the peptide transporters HAM 1 and HAM 2, which are involved in the processing of class I antigens, are induced in these cells, whereas an increase in the expression of  $\beta$ 2-microglobulin also occurs (Joly and Oldstone, 1992). In other studies, cell lines derived from two medulloblastomas, which are immunoreactive against neurofilament antibodies, but unreactive against GFAP and S-100 antibodies, have also been shown to express MHC class I and class II antigens following exposure to IFN- $\gamma$  (Tamura et al., 1989). Although these studies further demonstrated that cells of neuronal origin are intrinsically capable of expressing MHC molecules and suggest that this expression may serve a functional role, the physiological conditions under which functional neuronal MHC expression can occur have not yet been fully elucidated. Toward this goal, Neumann et al. (1995) have combined whole-cell patch-clamp recording techniques with single-cell RT-PCR analysis to demonstrate that in the presence of IFN- $\gamma$ , both MHC class I and  $\beta$ 2-microglobulin are significantly more inducible on electrically silent neurons compared to neurons spontaneously firing action potentials. This result suggests that functional MHC class I expression may occur only in neurons that are no longer biologically active. This would then allow these cells to be recognized and killed by cytotoxic T-lymphocytes, thus permitting active virus to be cleared from inactive neurons. This result also suggests that active neurons may not possess the potential to express functional MHC, thus allowing neurons to survive at the cost of continued viral infection (Joly et al., 1991). Future investigation in this area will likely yield additional insight into our understanding of the functional interactions between neurons and cells of the immune system.

Although the majority of the studies addressing the effect of IFN- $\gamma$  on neurons have been directed at examining the expression of MHC glycoproteins, several studies have reported other IFN- $\gamma$ -induced neuronal consequences. As previously discussed, Brosnan et al. (1989) reported a significant reduction in neural conduction and modest axonal pathology in the visual system of the rabbit following injection of IFN- $\gamma$ . Recently, McMillian et al. (1995) reported that the treatment of rat primary basal forebrain mixed cultures with IFN- $\gamma$  decreased the number of choline acetyl transferase (ChAT) immunopositive neurons, whereas the number of ChAT immunonegative neurons was unaffected. This selective neuronal killing was shown to be mediated through IFN- $\gamma$ -induced microglia activation. Moreover, Birdsall (1991) demonstrated that following exposure to IFN- $\gamma$ , two human neuroblastoma cell lines, but not cultured human cortical neurons, expressed ICAM. IFN- $\gamma$  also enhanced TNF- $\alpha$ -induced binding of these cells to neutrophils. Finally, Collins (1995) demonstrated that susceptibility to viral infection of a human cerebral cortical neuronal cell line was dramatically increased following treatment with human IFN- $\gamma$  and that this increased susceptibility was owing to membrane expression of HLA class I molecules.

IFN- $\gamma$  has also been shown to affect neuronal differentiation *in vitro*. Chang et al. (1990) demonstrated that IFN- $\gamma$  retarded the death of cultured rat sympathetic neurons following nerve growth factor (NGF) deprivation. Moreover, IFN- $\gamma$  has been shown to potentiate NGF-stimulated differentiation of PC12 cells (Improta et al., 1988). Barish et al. (1991) also demonstrated that IFN- $\gamma$  increased the differentiation of cultured cortical and hippocampal neurons. Nevertheless, it is unlikely that the developing nervous system is exposed to appreciable levels of IFN- $\gamma$  under normal conditions, such that the *in vivo* relevance of these observations is unclear.

In contrast, there is *in vivo* evidence that suggests that IFN- $\gamma$  might have a detrimental effect on the developing nervous system. As

mentioned earlier, transgenic mice in which IFN- $\gamma$  was targeted to the CNS demonstrate a disruption in cerebellar granule cell migration (Corbin et al., 1996). During development, cerebellar granule cells migrate from the external granule cell layer (EGL), through the molecular layer, to the internal granule cell layer (IGL) along processes extended by radial glial cells (Chuong, 1990). In transgenic mice expressing IFN- $\gamma$  within the CNS, many granule cells are still observed in the EGL and in the molecular layer. This observation is reminiscent of other mouse mutants (e.g., weaver, staggerer) in which the interaction between granule cells and radial glia is disrupted (Chuong, 1990). Perhaps the ectopic expression of IFN- $\gamma$  in the cerebellum of transgenic mice has a deleterious effect on the interaction between granule cells and radial glia, thus disrupting the migration of these cells from the EGL to the IGL. Further investigation is warranted to characterize the potentially deleterious developmental effects of IFN- $\gamma$  on the CNS in more detail.

Recently, Meda et al. (1995) suggested a role for IFN- $\gamma$  in Alzheimer's disease. The senile plaques that develop in Alzheimer's patients are characterized by the extracellular deposits of  $\beta$ -amyloid protein. Meda et al. (1995) demonstrated that IFN- $\gamma$  acted synergistically with  $\beta$ -amyloid in the activation of microglia, which produce TNF- $\alpha$  and nitric oxide. In coculture experiments, it was shown that microglia activated by IFN- $\gamma$  and  $\beta$ -amyloid caused neuronal injury. Clearly, these are exciting findings that lead to the speculation that IFN- $\gamma$  might also be involved in other neurodegenerative disorders.

## Summary

IFN- $\gamma$  is a multifunctional cytokine involved in regulating a variety of immune responses. This cytokine also has a multitude of effects in the CNS (Table 2). Although normally excluded from the CNS, this cytokine is present during many disorders in which the CNS is affected, including the most common human demyelinating disease, MS. The presence of

Table 2  
Effects of IFN- $\gamma$  on the CNS

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Disruption of myelination
Exacerbation of reactive gliosis
Disruption of the blood-brain barrier
Recruitment of inflammatory cells from the periphery
Induction of MHC expression
Stimulation of macrophage/microglial cells
Disruption of cerebellar granule cell migration

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this cytokine in the CNS leads to an abundance of pathological effects, including decreased myelination, increased gliosis, disruption of blood-brain barrier function, increased MHC expression, stimulation of macrophage/microglial activation, and disruption of cerebellar granule cell migration. Taken as a whole, these observations demonstrate that IFN- $\gamma$  can either directly or indirectly mediate many of the CNS pathological effects that are observed in disorders in which immune cell traffic in the CNS increases. Understanding the mechanism(s) by which IFN- $\gamma$  exerts its actions on CNS function will undoubtedly prove useful toward generating rational therapeutic approaches for the treatment of CNS disorders in which this cytokine plays a harmful role.

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