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*Cytokines and the Brain*
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# *Immune Response in the Brain: Glial Response and Cytokine Production*

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ABSTRACT

Although the brain has been considered as an immunologically privileged site, the evidence to date suggests that this is no longer the case. Cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-3 induce class I major histocompatibility complex (MHC) antigen expression on neural cells. IFN-γ, the most potent inducer of MHC antigen, also induces class II MHC antigen expression on microglia and astrocytes, which enable them to function as antigen-presenting cells. Thus, in some pathological conditions, invading T cells can interact with neural cells to induce central nervous system (CNS) damage. Glial cells have also been shown to produce various cytokines and chemokines. Almost all cytokines and chemokines known to occur in the immune system are also produced in the CNS. In this chapter, the glial responses contributing to neuroimmune interactions are reviewed, with a focus on production and functions of cytokines in the CNS.

## 1. INTRODUCTION

The brain has long been considered as an immunologically privileged site based on a large body of evidence: the lack of major histocompatibility complex (MHC) antigen expression on neural cells; the lack of lymphoid drainage in the central nervous system (CNS); and the presence of the blood–brain barrier (BBB), which blocks the invasion of immune cells or high molecular substances including antibodies into the brain. However, as has been shown by research published in the 1980s, some cytokines or viral infections induce MHC antigen expression on both neuronal and glial cells. Interferon-γ (IFN-γ), the most potent inducer of MHC antigen, also induces class II MHC antigen expression on microglia and some populations of astrocytes, which enable them to function as antigen-presenting cells (APCs). In order to effectively present antigens to T cells, APCs have to express other costimulatory molecules. Microglia and astrocytes have been shown to express these costimulatory molecules, and this expression is also enhanced by exposure to IFN-γ. Thus, if activated T cells enter the CNS, either microglia or some populations of astrocytes are able to present CNS antigens to expand a T-cell clone specific for a particular CNS antigen. In fact, it has also been shown that activated T cells can enter the brain through an intact BBB. Consequently, in certain pathological conditions, glial

cells may alter their functions to actively interact with immune cells. In most cases these glial changes are mediated by cytokines.

Another remarkable glial response in pathological conditions is the production of cytokines. In the late 1980s, many laboratories, including ours, have demonstrated the production of cytokines by glial cells. Almost all cytokines known to occur in the immune system were produced in the CNS. Thus, the brain should no longer be considered as an immunologically privileged site. In this chapter, I will review the glial responses in neuroimmune interactions in the CNS with a focus on the production and functions of cytokines.

## 2. GLIAL RESPONSE TO INTERACT WITH IMMUNE CELLS

### 2.1. Induction of major histocompatibility complex antigens

In normal or unstimulated conditions *in vivo* and *in vitro*, neuronal and glial cells do not usually express class I or class II MHC antigens on their surface, whereas microglia only weakly express class I MHC antigens *in vitro*. Consequently, in the normal brain, neural cells cannot interact with their own immune cells in a specific manner. However, it has been shown that both neuronal and glial cells can be induced to express class I MHC antigens in response to lymphokines [1,2]. As a result of this induction, the cytotoxic T cells acquire the capacity to lyse the CNS cells in a MHC-restricted manner. Although IFN-γ is a principal factor for the induction of MHC antigen expression, tumor necrosis factor-α (TNF-α) can also induce class I MHC antigen expression on astrocytes, but not on oligodendrocytes [3].

Lymphokines, especially IFN-γ, also induce the expression of class II MHC antigens on astrocytes [4] and microglia *in vitro* [5]. This expression is associated with the induction of mRNA for class II MHC antigens. Induction of class II MHC antigens is also observed *in vivo* in certain pathological conditions. In the brains of *experimental allergic encephalomyelitis (EAE)*, microglia near the infiltrating T cells are reported to be class II MHC antigen-positive [6–8], suggesting that a T cell-derived cytokine, most probably IFN-γ, can induce class II MHC antigen expression *in vivo* as well.

After axotomy there are increased numbers, relative to controls, of microglia in and around facial nerve nuclei. Moreover, these cells are reportedly class II MHC antigen-positive [9]. Since the BBB is not damaged in this experimental condition and since there is no definitive evidence that neural cells produce INF-γ in the CNS, it is unlikely that IFN-γ is responsible for the induction of class II MHC antigen expression in this model. Another candidate for the induction of class II MHC antigens in microglia is interleukin (IL)-3. We have shown that IL-3 induces, in a dose-dependent manner, surface expression and mRNA for class II MHC antigens in microglia, which is completely inhibited by anti-IL-3 antibody [10]. Although we do not detect IL-3 or IL-3 mRNA in either microglia or astrocytes in the mouse cellular system under study, it has been reported that rat microglia produce IL-3 *in vitro* [11], and that IL-3 mRNA is detected in some populations of astrocytes and neurons by *in situ* hybridization [12]. Therefore, it is possible that IL-3 derived from degenerating neurons, reactive astrocytes, or microglia *in vivo* may themselves induce class II MHC antigens on microglia in certain pathological conditions.

In contrast to IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF) down-regulates IFN-γ-induced class II MHC antigen expression in microglia. The suppression occurs in a dose-dependent manner and is neutralized by anti-GM-CSF antibody [10]. As we have

shown previously, GM-CSF is produced by astrocytes [13] and induces the proliferation of microglia *in vitro* [14,15]. It is possible that astrocytes downregulate immunoregulatory functions of microglia. However, so far, there is no evidence that GM-CSF contributes to the modulation of microglia proliferation and suppression of their Ia antigen expression in either physiological or pathological conditions *in vivo*.

All the macrophage deactivating cytokines, or inhibitory cytokines, such as IL-10, IL-4, and transforming growth factor-β (TGF-β) downregulate the INF-γ-induced class II MHC antigen expression in microglia [16–18]. As we and other groups have shown, astrocytes and microglia produce IL-10 [18] and TGF-β [19,20], but neither cells produce IL-4, although both cell types express IL-4 receptors [17,21]. Thus, microglia may downregulate their own immunoregulatory functions in an autocrine fashion, or the astrocyte may suppress the functions of microglia in a paracrine manner. It is also possible that invading T helper cells, especially T helper 2 (Th2), may downregulate class II MHC antigen expression in microglia by these inhibitory cytokines.

Induction of MHC antigen expression on glial cells occurs without breakdown of BBB, without invasion of immune cells. We, and another group, have shown that infection with neurotropic corona virus induces class I MHC antigen expression on oligodendrocytes and astrocytes [22,23] and class II MHC antigen on astrocytes [23]. These inductions permit glia to interact with invading immune cells to produce CNS pathology.

## 3. CYTOKINE PRODUCTION AND EXPRESSION OF RECEPTORS FOR CYTOKINES IN GLIA CELLS.

Microglia produce various cytokines, such as IL-1, IL-1 receptor antagonist (IL-1ra), IL-5, IL-6, IL-10, IL-12, IL-15, IL-18, IL-23, IL-27, TGF-β, TNF-α, IFN-γ, as shown in Table 1, and chemokines [18,19,24–33]. Rat microglia reportedly produce IL-3 in culture [11]. Only a trace amount of IL-1, but not the other cytokines, is detectable in the supernatant of unstimulated microglial culture. However, lipopolysaccharides (LPS), and/or IFN-γ in some cases, induce cytokine production. Since microglia express receptors for most of the cytokines produced (see Table 1), these components may function as an autocrine regulator. They also express receptors for cytokines, which are produced by other cells, but not by themselves, such as IL-2 or GM-CSF. Thus, the latter may function as paracrine mediators (for functions of these cytokines on microglia, refer to our previous review [34]). Unstimulated microglia do not express the IL-2 receptors (IL-2R); however, LPS treatment will induce IL-2R expression on these cells. Moreover, IL-2 can also induce the proliferation of LPS-stimulated microglia [35]. Although IL-2 treatment has been shown to induce the proliferation of oligodendrocytes as well [36], we could not confirm these effects [2]. Microglia also express the receptor for IL-4, the cytokine produced by T cells, but not in the CNS. Thus, IL-4 may be a paracrine mediator exerting its effects only in cases of an inflammatory process occurring in the CNS, but not in the normal brain. We have shown the production of IL-5 and the upregulation by IFN-γ in murine microglia by means of RT-PCR for mRNA expression and the bioassay to assess IL-5 activity [28]. However, since we have not detected IL-5 receptors on neural cells, the functions of IL-5 in the CNS remain to be elucidated. In contrast to murine microglia, Lee et al. [37] failed to detect IL-5 mRNA expression in human microglia as assessed by RT-PCR, while they detected mRNA for the IL-5 receptor.

Astrocytes produce cytokines very similar to those produced by microglia (Table 2). However, microglia, rather than astrocytes, seem to be a principal source of most critical cytokines,

Table 1. Cytokine production and receptor expression in microglia

| | Production | Receptor expression |
|---|---|---|
| IL-1 | Yes | Yes |
| IL-1ra | Yes | Yes |
| IL-2 | ND | Yes** |
| IL-3 | No (yes)* | Yes |
| IL-4 | No | Yes |
| IL-5 | Yes | No |
| IL-6 | Yes | Yes |
| IL-7 | ND | Yes |
| IL-10 | Yes | Yes |
| IL-12 | Yes | Yes |
| IL-13 | No | Yes |
| IL-15 | Yes | Yes |
| IL-18 | Yes | Yes |
| IL-23 | Yes | ND |
| IL-27 | Yes | ND |
| TNFα | Yes | Yes |
| IFNγ | Yes | Yes |
| TGFβ | Yes | Yes |
| M-CSF | Yes | Yes |
| GM-CSF | No | Yes |
| Chemokines | IL-8, IP-10, MIP-1α,β, MCP-1, RANTES, fractalkine | CCR2, CCR3, CCR5, CXCR4, CX3CR1 |

ND, not determined; ( ), most probably yes; *, reportedly yes, although we could not confirm in our mouse system; **, inducible.

Table 2. Cytokine production and receptor expression in astrocytes

| | Production | Receptor expression |
|---|---|---|
| IL-1 | Yes | Yes |
| IL-2 | No | No |
| IL-3 | (Yes) no | ND |
| IL-4 | No | Yes |
| IL-5 | Yes | Yes* |
| IL-6 | Yes | Yes |
| IL-10 | Yes | (Yes) |
| IL-12 | No | No |
| TNFα | Yes | (Yes) |
| IFNγ | No | (Yes) |
| TGFβ | Yes | ND |
| M-CSF | Yes | ND |
| G-CSF | Yes | ND |
| GM-CSF | Yes | ND |
| Chemokines | IL-8, MCP-1, MIP-1α,β, RANTES, fractalkine | CCR1, CCR2, CCR5, CXCR3, CXCR4, CX3CR1 |

ND, not determined; ( ), most probably yes; *, reportedly yes, although we could not confirm in our mouse system.

such as TNF-α, IL-1, and IL-12 as discussed later [26,29], in both pathological conditions and in culture systems. Astrocytes sometimes have suppressive effects on microglia or microglia-derived cytokines. For, example, in contrast to microglia after stimulation with LPS and IFN-γ, astrocytes produce IL-12 p40, but not p35 [29]. When the astrocyte-derived p40 forms a homodimer, it may suppress the functional heterodimer IL-12 p70 produced by microglia. In addition, the production of IL-12 p70 by activated microglia was inhibited by coculture with astrocytes [38]. Thus, it is possible that astrocytes suppress microglial cytokine production and/or the effects of produced cytokines. The suppression of IFN-γ-induced MHC class II expression on microglia by astrocyte-derived GM-CSF is another example of this type of interaction [10].

As discussed above, IFN-γ activates various functions of glial cells including the induction of cytokines. The production of IFN-γ was thought to be restricted to lymphoid cells. However, it has recently been shown that human fetal forebrain cells can be induced to express IFN-γ mRNA and produce IFN-γ protein when stimulated with *trypanosome lymphocyte-triggering factor* (TLTF) [39]. The authors claimed that astrocytes were the major producer of IFN-γ in response to TLTF. We have shown recently that microglia produce IFN-γ in response to IL-12 and/or IL-18 [32]. Thus, microglia may be another source of INF-γ production in the CNS. It has been shown that APCs such as macrophages and dendritic cells also produce IFN-γ in response to IL-12 [40].

## 4. CYTOKINE NETWORKS IN THE CENTRAL NERVOUS SYSTEM

### 4.1. Immunoregulatory cytokines, which affect the functions of antigen-presenting cells

The immune response is initiated when a protein antigen is presented to T cells by APCs within lymphoid organs. The APC processes antigen, either foreign or self, by internalizing and digesting it into peptide fragments. Subsequently, processed peptide fragments are expressed on the surface of APCs as a MHC–peptide complex. When the MHC–peptide complex interacts with T-cell receptors (TCRs), T-cell activation occurs. Class II MHC molecules present antigen to CD4-positive T cells, while class I MHC molecules present antigens to CD8-positive T cells. Binding of the MHC–peptide complex to the TCRs is critical, but not sufficient, for the activation of T cells. There should be several costimulatory molecules that interact with the ligands on T cells in order for sufficient activation to occur. These costimulatory molecules on APC are B7.1, B7.2, leukocyte function-associated molecule 3 (LFA-3), intercellular adhesion molecule-1 (ICAM-1), ICAM-2, and ICAM-3. The molecules bind to ligands on T cells to form ligand pairs such as B7.1–CD28, B7.2–CTLA4, LFA-3–CD2, ICAM-1, 2, or 3–LFA-1. Interaction of T cells and APCs occurs in a MHC-restricted manner. The T cells recognize a foreign antigen only when the antigen is complexed with self-MHC molecules on APCs. Therefore, the cells expressing class II MHC and costimulatory molecules constitutively are considered to be professional APCs. These include macrophages, B cells, dendritic cells, and Langerhans cells. Nonprofessional APCs differ from the professional APCs by expressing little or no MHC class II molecules constitutively, and by not having a complete set of costimulatory molecules. Candidates for nonfunctional APCs in the CNS are microglia, astrocytes, and endothelial cells [4,41–45]. They usually do not express class II MHC antigen constitutively, although some populations of microglia reportedly may express class II MHC antigens constitutively [46]. These cells induced the expression of class II MHC molecules after treatment with certain inflammatory cytokines, especially IFN-γ [4,5,41,44], in addition to expressing some of the

costimulatory molecules as well [47–49]. There is published evidence that endothelial cells [50], astrocytes [41], and pericytes [51] can process and present protein antigens to primed CD4-positive T cells *in vitro*, but the specific role of these cells as APCs *in vivo* is still unclear. Astrocytes do not usually express class II MHC antigens *in vivo*, even in the presence of inflammatory cells [42]. Since microglia have functional characteristics very similar to macrophages and can be induced to express class II MHC antigens as discussed above, *microglia* are the most possible candidates for APCs in the CNS. The expression of costimulatory molecules, such as B7, ICAM, LFA3, in microglia, but only a few in astrocytes, further supports this hypothesis. Menendez Iglesias et al. [49] detected B7-2, but not B7-1, in murine microglia only after stimulation with LPS and IFN-γ. Satoh et al. [48] have shown that human microglia, but not astrocytes, express both B7-1 and B7-2, suggesting that microglia is a much more suitable candidate for local APCs in the CNS. In fact, microglia when stimulated with IFN-γ reportedly presented antigen to ovalbumin-specific or myelin basic protein (MBP)-specific T cells *in vitro* [44,45]. In a carefully executed study, Hickey and Kimura [43] have shown that microglia function as APCs in pathological conditions *in vivo*. They used bone marrow chimeras of EAE-susceptible and -resistant animals, and found that EAE lesions developed only when the perivascular microglia were replaced with those of an EAE-susceptible strain, suggesting that antigen presentation by perivascular microglia is critical for the development of EAE lesions.

Professional APCs such as dendritic cells and macrophages produce IL-12 and IL-18. Both cytokines have been shown to be key cytokines in the development of autoimmune processes, regulating differentiation of naïve T cells into Th1. In order to exert its activity, IL-12 has to form a heterodimer of P35 and P40; the homodimer of P40 suppresses the functional heterodimer. Immature IL-18 is cleaved by caspase-1 to become a functionally mature IL-18 that induces the differentiation of Th1 and the cytotoxic activity of NK and T cells. It has been reported that both microglia and astrocytes produce IL-12 upon stimulation with LPS [38], while we detected functional IL-12 p70 production only in microglia, but not in astrocytes, after stimulation with LPS and IFN-γ [30]. Since soluble TNF receptors reportedly suppress IL-12 production by human microglia [52], TNF signal may also be involved in IL-12 production. Microglia and astrocytes also express IL-18 mRNA after stimulation with LPS [32,53]. LPS-stimulated microglia have enough IL-18 bioactivity to induce INF-γ production by thymocytes and splenocytes in synergism with IL-12. This suggests that microglia express caspase-1 as well. In fact, caspase-1 mRNA expression is elevated in microglia in multiple sclerosis (MS) plaques [54] where IL-18 is also reported to be elevated [55]. Interestingly, there is a group of microglia that produce only IL-12 P40, but not IL-12 P35, resulting in the failure to produce functional IL-12 p70 heterodimers [29]. The population did not produce IL-18 even after LPS stimulation (unpublished observation). Therefore, microglia may have subpopulations, which regulate the differentiation of T cells in a different manner.

### 4.2. The roles of microglia in the central nervous system cytokine network

Both microglia and astrocytes produce the same cytokines, such as IL-1, IL-6, TNF-α, and TGF-β. However, there are several differences in the response to stimulation in these two cell types. For example, microglia produce TNF-α in response to lower doses of LPS than are required for astrocytes and more rapidly than astrocytes as well. IL-6 production is induced by TNF-α in astrocytes, but not in microglia [27]. Similarly, GM-CSF produced by astrocytes induces IL-6 production in microglia, but not in astrocytes [56]. These observations indicate that microglia and astrocytes may mutually regulate their individual cytokine production. Since

Table 3. Effects of inhibitory cytokines on microglial functions

| | IL-4 | IL-10 | TGF-β |
|---|---|---|---|
| Proliferation | ↑ | → | ↓ |
| Enzyme activity | ↑↑ | ↓ | ↓ |
| IFN-γ-induced Ia expression | ↓ | ↓ | ↓ |
| LPS-induced cytokine production | → | ↓ | ↓ |
| GM-CSF-induced IL-6 production | ↓ | ↓ | ↓ |
| Cytokine receptor expression | →(↑)[a] | ↓ | → |

↑, Upregulate; →, no effect; ↓, downregulate.
[a] IL-4 upregulates IL-4 receptor, but does not affect the expression of other receptors on microglia.

microglia are activated in the earlier phase than are astrocytes under various pathological conditions, microglia may initiate the cascade of cytokine actions in the CNS cytokine network. Inhibitory signals are also included in the network (Table 3). TGF-β, produced by astrocytes and microglia, suppresses all the functions of microglia. It suppresses M- and GM-CSF-induced proliferation of microglia, LPS-induced activation of enzymatic activity in microglia, IFN-γ-induced class II MHC antigen expression and cytokine production by microglia. TGF-β along with IL-4 and IL-10 is known to be a *macrophage-deactivating* factor. Therefore, these cytokines may function as negative regulators in the CNS cytokine network by suppressing cytokine production and activation of microglia. In fact, it has been found that these inhibitory cytokines exert their influence on microglia differently. TGF-β functions as if it is a total inhibitory factor [16]. IL-10 suppresses cytokine production and IFN-γ-induced class II MHC antigen expression in microglia, but does not suppress the proliferation or the activation of lysosomal enzymes in microglia [18]. IL-4 also suppresses IFN-γ-induced class II MHC antigen expression in microglia [17]. However, unlike other inhibitory cytokines, IL-4 induces the proliferation of microglia in either unstimulated or M-, or GM-CSF-stimulated conditions. IL-4 does not suppress LPS-induced cytokine production, though it suppresses GM-CSF-induced IL-6 production by microglia [56]. We also found that IL-10, but neither TGF-β nor IL-4, suppressed the expression of cytokine receptors [57]. Thus, it would appear that all these three inhibitory cytokines regulate the functions of microglia in a distinct manner, and that IL-10 may be the most potent inhibitor for the functions of cytokines on microglia because it suppresses both cytokine production and receptor expression.

## 5. CYTOKINES IN THE CENTRAL NERVOUS SYSTEM PATHOLOGIES

### 5.1. Demyelination

Several lines of evidences suggest that TNF-α plays a critical role in the pathogenesis of *inflammatory demyelination*, either directly or indirectly via induction of other cytokines, nitric oxide (NO), or free radicals (Fig. 1). Increased cerebrospinal fluid levels of TNF-α have been demonstrated in patients with MS [58]. TNF-α-positive microglia and astrocytes have been identified, especially in new active plaques. *In vitro* studies have demonstrated that TNF-α kills oligodendrocytes, myelin-forming cells in the CNS [59,60], and that microglia are the principal

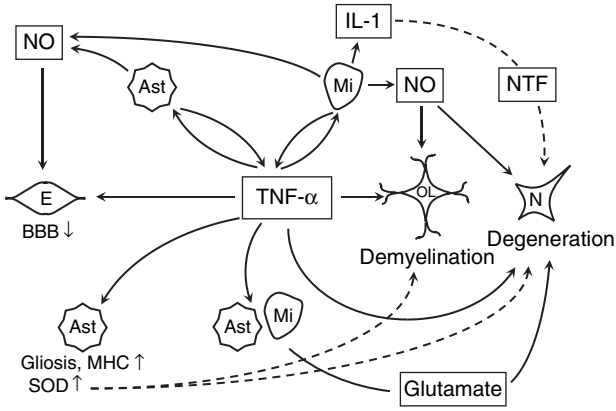


Figure 1. The roles of TNF-α in the development of CNS pathology. Ast, astrocytes; Mi, microglia; OL, oligodendrocytes; N, neuron; E, endothelial cells. NO, nitric oxide; NTF, neurotropic factors; BBB, blood–brain barrier. ———— destructive; - - - - - - protective.

effectors for oligodendrocyte killing [61]. It has also been shown that anti-TNF-α antibody suppresses the development of EAE, an animal model of MS [62,63]. Demyelination has been demonstrated to be much more severe in transgenic mice producing TNF-α in the CNS [64]. In addition, TNF-α induces inflammatory cytokines or chemokines in endothelial cells and impairs the tight junctions of the BBB [65]. Up until now, several substances that suppress TNF-α production have been used for the treatment of EAE and MS. Most of them, such as phosphodiesterase inhibitors, *N*-acetyl-L-cysteine, have been shown to effectively suppress the development of EAE and MS [66–68], further supporting the hypothesis that TNF-α is critical for the development of inflammatory demyelination. However, experimental demyelination could also be induced in TNF-α knockout mice, though EAE was delayed in the onset and inflammatory leukocytes failed to move normally into the CNS parenchyma [69]. More recently, TNF-α has been identified as a factor that promotes *remyelination* [70]. Thus, although TNF-α is an important cytokine, it may not be the sufficient effector molecule for inflammation and demyelination. It is also possible that TNF-α may exert different effects on inflammatory demyelination, depending on whether the TNF signaling through type 1 TNF receptor (TNFR1) or TNFR2 is dominant.

### 5.2. Gliosis

Gliosis is a rather common pathological finding observed as a glial scar following inflammation, demyelination, ischemia, and neuronal degeneration. It consists of astrocyte proliferation, hypertrophy, and increased synthesis of glial fibrillary acidic protein (GFAP), a phenotypic marker for astrocytes. Evidence to date suggests critical roles for cytokines in the development of *astrocytic gliosis*. Fontana et al. [71] first demonstrated that factors from activated lymphocytes stimulated astrocyte proliferation and designated the factor(s) as glial cell-stimulating factor (GSF). Merrill et al. [36] also demonstrated increased proliferation of astrocytes after treatment with lymphokines. Using enriched cultures of astrocytes and recombinant cytokines, Selmaj et al. [72] showed that TNF-α is a primary factor to bring about the proliferation of rat

astrocytes. However, Giulian et al. [73,74] have shown that IL-1 derived from microglia [24] is the principle factor to induce astrocyte proliferation in gliosis. In contrast, Yong et al. [75] claimed that the primary factor that induced the proliferation of human astrocytes was IFN-γ and not IL-1 or TNF-α. These differences in experimental results may be attributed to either species differences or redundancy of functions for these cytokines. Alternatively, it is possible that other factors induced by either IL-1, TNF-α, or IFN-γ may also play a role in the proliferation of astrocytes. In view of these diverse results, it can be concluded that cytokines contribute to the pathogenesis of gliosis. However, precise identification of individual cytokine contributions to the overall process will require additional experimental inquiries.

### 5.3. Neuronal degeneration

TNF-α has also been implicated as an effector for neuronal degeneration [76–78]. TNF-α exerts its cytotoxicity directly via TNFR1. Alternatively, it also induces NO or free radicals to form the toxic peroxinitrite. It has been shown that β-amyloid stimulates microglia to produce factors toxic to neurons. It is possible that neuronal apoptosis induced by β-amyloid is also mediated by glia-derived TNF-α [79]. Combs et al. [80] concluded that the most critical factor in β-amyloid-induced, microglia-mediated neuronal apoptosis might be NO, because neurotoxicity was decreased by the selective inhibitors against inducible nitric oxide synthase. Apoptosis of motor neurons and dorsal root ganglion neurons by peripherin aggregates is also reportedly mediated by TNF-α [81]. TNF-α also exerts its neurotoxicity by activating astrocytes to release glutamate [82]. Recently, we have shown that the most neurotoxic factor from activated microglia is glutamate [83]. TNF-α dose not exert direct neurotoxicity, but induces neurotoxicity via glutamate production by microglia. Glutamate disturbs the mitochondrial respiratory chain to cause energy depletion in neurons, which results in neuronal damage toward cell death [84]. In contrast, IL-1, but not TNF-α, may be involved in neurotoxicity during some variants of viral encephalitis [85]. The protein Fas associated with death domain (FADD) is an adaptor protein of the TNF receptor family death pathway. A number of FADD-positive dopaminergic neurons in the substantia nigra pars compacta have been shown to be significantly decreased in patients with Parkinson's disease (PD), as compared to levels in normal subjects [86]. This decrease correlated with the known selective vulnerability of nigral dopaminergic neurons in PD. On the basis of the latter, the authors concluded that the TNF–FADD pathway contributed to the susceptibility of dopaminergic neurons in PD to the effects of TNF-mediated apoptosis [86].

Interestingly, cytokines described above as toxic also have protective roles for neurons against oxidative stress. TNF-α and IL-1 have been shown to increase the level of manganese superoxide dismutase (Mn-SOD) in astrocytes, in a dose- and time-dependent manner [87]. Since SOD functions as protective against oxidative stress, and since the increased Mn-SOD activity has been demonstrated in the substantia nigra of parkinsonian patients [88], these cytokines may function to protect degenerating neurons, via induction of SOD. IL-1 reportedly increases the production of nerve growth factors by astrocytes [89]. Therefore, a balance between toxic and protective factors induced by cytokines may determine neuronal damage (see Fig. 1).

### 5.4. Other pathological conditions in the central nervous system

Microglia undergo various morphological changes to become either ramified, amoeboid, or rod-shaped. We have shown that all of these morphological changes could be reproduced *in vitro* with various cytokines [14,15]. Microglia also form a unique phenotype of multinucleated giant

cells (MNGC), which are observed in AIDS encephalopathy, tuberculosis, etc. Lee et al. [90] have demonstrated that treatment with IL-3, IL-4, IFN-γ, and GM-CSF induces MNGC in rat microglia, while addition of IL-1, IL-6, or TNF-α failed to form MNGC. In mouse experiments using microglia, there was no single cytokine that induced MNGC in culture. However, when stimulated with IL-4 or IL-13 in the presence of GM-CSF or M-CSF, MNGC formation occurred in the cultures of mouse microglia [91]. The different results between these studies may be attributable to species differences. Nevertheless, the results of these studies indicate that introduction of cytokines, most probably those that are T cell-derived, can induce MNGC formation without infectious agents.

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