The Role of Cytokines in Schwann Cell Damage, Protection, and Repair

Robert P. Lisak, Dusanka Skundric, Beverly Bealmear, and Samia Ragheb

Cytokines, proteins that are secreted by many cells, including inflammatory and glial cells, mediate interactions between cells, generally through paracrine and autocrine networks. Their effects are highly pleiotropic, with overlap of some activities. The pathogenesis of Guillain-Barré syndrome (GBS), especially the classic inflammatory demyelinating polyneuropathy form, seems to involve lymphocytes and macrophages, which are rich sources of cytokines. Macrophages likely have a role in the pathogenesis of the primarily axonal, less inflammatory forms of GBS. Cytokines appear to be involved in damage to Schwann cells, myelin, and axons, although the exact roles of the different cytokines is uncertain. There is increasing evidence that cytokines, including some proinflammatory cytokines that ordinarily cause damage, may also protect the cells of the peripheral nervous system and aid in its repair. The evolution of inflammatory and demyelinating disorders, including the degree of recovery, is probably dependent on the interactions of the different cytokines.

There is increasing interest in the effect of cytokines, proteins secreted by cells that act at short distances on the same or other cell types (paracrine) or on the same cell (autocrine) and glial cells [1–3]. There is extensive literature about cytokine interactions with central nervous system (CNS) glia, including the macroglia (oligodendrocytes and astrocytes) and microglia; the data were determined on the basis of in vitro and in vivo techniques and the study of pathologic material from the brains and spinal cords of patients with neurologic disease [3–6]. These studies have increased our understanding of the events occurring in target tissue in diseases such as multiple sclerosis, in CNS manifestations of human immunodeficiency virus infection, and in degenerative diseases, such as Alzheimer’s disease. In turn, this increased understanding has provided new and imaginative therapeutic strategies and increased the understanding of current therapies even though the etiology of these disorders is unknown.

There have been far fewer studies on the effects of cytokines and the lower-molecular-mass chemokines [7] on the glial cell of the peripheral nervous system (PNS), the Schwann cell. The Schwann cell is the PNS cell that makes and maintains myelin as a modification of its plasma membrane. It has similarities to the myelin-forming cell of the CNS, the oligodendrocyte; however, there are many differences, including the chemical composition of the myelin and the relationship of axons and neurons to glial cell differentiation and synthesis of myelinotypic proteins and glycolipids.

There is interest in the relation of cytokines, many of which are produced by inflammatory and immune system cells (although clearly not exclusively by these cells), and Schwann cells in inflammatory demyelinating neuropathies, such as the common demyelinating form of Guillain-Barré syndrome (GBS), acute inflammatory demyelinating polyneuropathy with or without significant secondary axonal degeneration, and chronic inflammatory demyelinating polyneuropathy [8]. However, because inflammatory cells, especially macrophages [9–12], are important in traumatic injuries of nerves, in degenerative neuropathies and in inflammatory and immune axonal neuropathies (e.g., the axonal forms of the GBS: acute motor-sensory axonal neuropathy and acute motor axonal neuropathy), cytokines may also be important in many diseases of the PNS. The macrophage and its products are important even if they are in response to an antibody-mediated immune reaction or to a chemotactic signal that has no antigen specificity instead of to a cell-mediated immune process. For example, complement-mediated myelin phagocytosis by macrophages has the potential to increase production of tumor necrosis factor (TNF)-α and nitric oxide (NO) [13], both of which could lead to additional damage in peripheral nerve.

Cytokines and Schwann Cell Damage

Damage to Schwann cells and demyelination are important features of many types of neuropathies, and cytokines released by inflammatory cells and perhaps by Schwann cells may be critical in mediating these pathologic changes. Cytokines induce several changes in Schwann cells in vitro that have potentially deleterious effects on the Schwann cells, myelin, and axons. Cytokines, such as interferon (IFN)-γ, can induce class II major histocompatibility (MHC) molecules on Schwann cells [14–17], which allow these cells, at least in vitro, to present antigen to...
CD4+ antigen-specific T cells [18–21]. Schwann cells are not “professional” antigen-presenting cells and it is not clear if they can contribute to propagation of a T cell immunopathologic reaction or can suppress such a reaction in vivo, as has been reported in vitro [22]. It is also not clear that there is an in vivo correlate of Schwann cell MHC class II induction and antigen presentation. In addition, IFN-γ and IFN-α/β also up-regulate MHC class I antigens [23], which allows Schwann cells to be targets of cytotoxic antigen-specific T cell reactions, either to a self-antigen or a microbial antigen infecting the Schwann cell [24]. One difference between purified Schwann cells in vitro and the in vivo situation may be the potentially suppressive effect on MHC up-regulation of intact electrophysiologically functional neurons [25]. It is also possible that inhibition of glial cell ion channels could inhibit MHC induction by Schwann cells [26]. Although studies in animal models do not usually demonstrate MHC class II molecules on Schwann cells [9, 27–32], MHC class II molecules have been reported on Schwann cells in diseased human PNS [33–37]. MHC antigen induction may potentiate nonimmune damage in the nervous system [38].

It has been shown that IFN-γ, interleukin (IL)-1, and TNF-α up-regulate or induce intercellular adhesion molecule-1 (ICAM-1) [20, 39], which would (as an accessory molecule) enhance the role of Schwann cells as antigen-presenting cells for CD4+ T helper cells responding to either a self-antigen or a microbial antigen. In addition, the presence of ICAM-1 on the Schwann cell surface would also allow a CD8+ T cell to mediate an antigen-specific cytotoxic reaction [24]. Moreover, adhesion molecules, including ICAM-1, can act as receptors for microbes and parasites [40–42]. It is clear that cytokine induction of adhesion molecules on endothelial cells are critical in allowing inflammatory cells to cross the blood-nerve barrier and trigger immune-mediated demyelinating diseases, such as experimental autoimmune neuritis and, presumably, GBS. It has been reported that in experimental autoimmune neuritis very little, if any, of the ICAM-1 detected in lesions is on Schwann cells [43], but human peripheral neuropathies have not yet been studied extensively.

TNF-α, which is found in lesions of animals with experimental autoimmune neuritis [44] and in the CNS in experimental autoimmune encephalomyelitis and multiple sclerosis [1, 4, 6, 45], is widely viewed as being important in inducing demyelination and toxic effects (including direct toxic effects) on oligodendrocytes in CNS diseases [4, 6, 46, 47]. In addition, inhibition of TNF-α inhibits the development of experimental autoimmune encephalomyelitis and experimental autoimmune neuritis [8, 48, 49]. TNF-α can act in the afferent phase of an immune reaction and likely contributes to development of inflammatory and immunopathologic lesions by activating the vascular endothelium [8, 50, 51] and other cells [52] important to the breakdown of the blood-brain or blood-nerve barrier; therefore, a major role for TNF-α may be via changes in the blood vessels rather than by direct or indirect toxic effects on glial cells and myelin.

There is no current evidence of a direct toxic effect of TNF-α on Schwann cells in vitro, and no toxic effect was noted on Schwann cells in cocultures of Schwann cells and neurons by Mithen et al. [53]. In our culture system, we have not seen any direct toxic effect of TNF-α on Schwann cells. On the other hand, intraneural injection of TNF-α has been reported to induce axonal damage or a mixture of damage to axons, myelin, and vascular cells [54]. It has also been reported that TNF-α down-regulates Schwann cell levels of myelin-associated glycoprotein [55] and inhibits Schwann cell proliferation to certain mitogenic stimuli [56]. IL-1, TNF-α, and IFN-γ are highly effective in inducing NO by up-regulating inducible NO synthase. NO has been reported to be synthesized by Schwann cells [22] and is also potentially toxic to Schwann cells and axons. Cytokines also up-regulate synthesis of prostaglandins and other eicosanoids [57], some of which are clearly toxic and proinflammatory and others of which inhibit some of the effects of some of these very same cytokines. As described later, we and others have evidence that Schwann cells themselves can synthesize certain cytokines, including IL-1 [58, 59]. The enzyme that converts the inactive form of IL-1β to the active form, IL-1β converting enzyme, is important in apoptosis. In addition, Skundric et al. [58, 59] have also found that IL-1 receptor antagonist (IL-1RA), which is known to be made by macrophages and inhibits the effect of both isoforms of IL-1 by competing for binding to the receptors for IL-1 [60], is also made by Schwann cells. Since there is evidence that IL-1 has the potential to enhance Schwann cell reparative processes (vide infra), production of IL-1RA could prove to be damaging to PNS attempting to repair itself. Cytokines may also be important in activating Schwann cells and other glia to secrete complement factors and receptors and proteins that protect cells from the damaging effects of complement [61, 62]. Thus, it is possible that cytokines could cause damage by contributing to complement production, although if some cytokines induce complement-modifying factors, these could protect Schwann cells from antibody complement-mediated reactions.

We and others are examining the effects of cytokines on myelin protein and glycolipid synthesis and myelination and the cellular and molecular mechanisms that result in demyelination or inhibition of remyelination caused by some of these proteins. Given the capacity of transforming growth factor (TGF)-β to down-regulate activated glial cells, inflammatory cells, and endothelial cells, one might expect TGF-β to be protective, as it well may be in experimental autoimmune models [8]. TGF-β has been detected in the PNS in experimental autoimmune neuritis [63]. However, TGF-β can inhibit cAMP-induced Schwann cell surface galactocerebroside [64], and it can down-regulate mRNA for P0 protein of myelin [64, 65] and up-regulate neural adhesion molecules [64, 66]. The effect of TGF-β on Schwann cells and the synthesis of TGF-β by Schwann cells depends in part on the relationship of the Schwann cells to axons and neurites [67–69]. We have observed that the ability of TGF-β to inhibit cAMP-
induced galactocerebroside is independent of the capacity of TGF-β to induce proliferation of isolated primary Schwann cells (unpublished data). Thus, the release of different cytokines during inflammatory, traumatic, and degenerative diseases of the PNS may contribute to Schwann cell toxicity, demyelination, and inhibition of remyelination.

**Cytokines and Schwann Cell Protection**

Cytokines can also serve as growth factors or protective factors, but very little is known about the role of cytokines in protecting Schwann cells from immunologically mediated damage. As discussed above, there are theoretically several activities of some cytokines that could lead to a down-regulation of an ongoing inflammatory or immune pathogenic process (or both) and thus protect Schwann cells and their myelin. Included among those cytokine-induced protective processes operating through the Schwann cell are induction of apoptosis of damaged or toxic inflammatory cells [8, 22], induction of surface proteins that protect against the effects of cytokines or complement-mediated damage [62], and induction of protective cytokines or growth factors (or both) by Schwann cells or inflammatory cells. In addition, while potentially damaging to Schwann cell production of NO and perinitrile (ONOO−), prostaglandins and other ecosanoids [57] could inhibit the inflammatory cells.

Little is known about such direct or indirect protective effects of cytokines directly involving Schwann cell responses, but certain growth factors, such as fibroblast growth factor and epidermal growth factor, have protective effects on NO-induced toxicity in certain neuronal populations [70]. If these mechanisms were active in vivo and could be further enhanced, they would limit damage to Schwann cells and presumably to myelin. It is well known that certain cytokines can inhibit the production and actions of other cytokines within the interactions of inflammatory cells. Thus, Schwann cells or inflammatory cells may protect Schwann cells and axons by inhibiting the production or effects of proinflammatory cytokines via the synthesis of other cytokines and other substances or even through potentially beneficial effects of the proinflammatory cytokines themselves.

**Cytokines and Schwann Cell Repair**

The PNS has the capacity for repair and regeneration, which partly explains the recovery, albeit variable, in patients with self-limited disorders (e.g., GBS or trauma) or diseases in which ongoing damage can be prevented or limited by treatment. Two important processes for recovery from damage to the PNS are proliferation of Schwann cells or Schwann cell precursors [71–74] and remyelination. These are important not only in a demyelinating neuropathy but in axonopathies as well since axonopathies are accompanied by secondary demyelination and Schwann cells produce cytokines (vide infra) and neuronal growth factors. Proliferation of Schwann cells also accompanies regeneration of nerve after injury-induced Wallerian degeneration. Our laboratory has been interested in the effects of different cytokines on Schwann cells and how these effects are involved in the pathogenesis of diseases of the PNS and, of equal importance, how these cytokines might be involved in repair in the PNS.

Eleven years ago our laboratory reported that unfractionated supernatants from activated inflammatory cells could induce proliferation of neonatal rat Schwann cells in vitro [75]. We have found that unfractionated supernatants (unfractionated cytokines) of rat [75, 76], mouse [77], and human [76, 77] origin all induce rat Schwann cell proliferation. We have found that while neither isoform of IL-1 seems to have a detectable direct mitogenic effect on Schwann cells in vitro, a finding reported by others [78], inhibition of IL-1 activity with antibodies to IL-1 inhibits the unfractionated cytokine-induced proliferation [76]. In most of our studies, we used human IL-1 and human unfractionated cytokines since purified or recombinant rat IL-1 or antibodies to or sufficiently cross-reactive with rat IL-1 of either isoform were not available. Neither isoform of mouse IL-1, a much closer species to that of the rat, induced rat Schwann cell proliferation [77]. Recently, we have begun to test rat IL-1β, which to date also has not been shown to directly induce significant Schwann cell proliferation.

Of interest, antibodies to human IL-1α inhibited proliferation, whereas those to IL-1β did not [77]. The explanation for this is not clear, although it is known that IL-1α, the more cell-bound isoform, binds with higher avidity to the type I IL-1 receptor [79, 80]. We also found that IL-1RA of human origin also inhibited unfractionated cytokine-induced proliferation [77]. This not only confirmed the role of IL-1 as a co-mitogen for Schwann cells but has important physiologic implications since IL-1RA, unlike antibodies to IL-1, is part of a regulatory pathway for IL-1 [60, 81]. Indeed it has been shown that inhibition of IL-1 activity by IL-1RA inhibits recovery of rat sciatic nerve subjected to traumatic injury in vivo [82]. Thus, IL-1, which is often thought of as a proinflammatory or deleterious cytokine, might actually contribute to repair of the PNS, and IL-1RA, its natural antagonist, might be harmful, at least at certain stages of a disease.

IL-1 can induce synthesis of neurotrophic factors (e.g., nerve growth factor) by Schwann cells [12, 83, 84]. Schwann cells also make ciliary neurotrophic factor [85], although they do not seem to respond to this growth factor/cytokines by proliferating. Ciliary neurotrophic factor and leukemia inhibitory factor [86] have some similarity to IL-6 and share some peptide chains of their receptors with IL-6 [87]. Schwann cells have been shown to synthesize IL-6 [88–91]. IL-1 has also been shown to up-regulate mRNA for leukemia inhibitory factor in axotomized sensory ganglia [92]. While IL-6 is not directly stimulatory of Schwann cell proliferation in vitro, it does seem to have co-mitogenic activity for Schwann cells [93].

Other cytokines/growth factors might also contribute to PNS recovery by inducing Schwann cell migration and differentia-
tion as well as proliferation. TGF-\(\beta\) has now been shown to induce proliferation of Schwann cells grown in the absence of neurons and axons [94–98]. In the presence of neurites, the response of Schwann cells to TGF-\(\beta\) is less clear [67, 68, 99]. Our group has recently found that TGF-\(\beta\) may act as a co-mitogen with IL-1 for Schwann cells [97]. Thus, TGF-\(\beta\), which is present in inflammatory lesions (e.g., in GBS [63]) and is developmentally regulated in the developing PNS [69], may prove to be an important factor in recovery of the PNS from damage by encouraging proliferation, migration, and differentiation of the Schwann cells and by limiting myelin protein and glycolipid production. Schwann cells proliferate in response to platelet-derived growth factor [78], which is an important mediator of the activities of other cytokines, including IL-1, in other cell types.

Synthesis of Cytokines by Schwann Cells

Although many cytokines, especially those associated with inflammatory and immune reactions, were originally thought to be synthesized exclusively by inflammatory cells, it is now well established that this is not the case. Glial cells, including the microglia (which are part of the macrophage lineage) and the macroglia (oligodendrocytes and especially astrocytes), have also been reported to synthesize many of these proteins when appropriately stimulated [1, 5]. It has been reported that Schwann cells can synthesize IL-1, although the isoform could not be specified [100], and more recently, they have been shown to synthesize IL-6 [88–91], TNF-\(\alpha\) [44, 91, 101], and TGF-\(\beta\) [63, 64, 69]. Our laboratory has shown that Schwann cells can also increase signal for IL-1RA [58, 59] and presumably also synthesize product. Thus, autocrine and paracrine IL-1 signaling and response to the IL-1 family can be seen in Schwann cells. Our laboratory has also demonstrated that Schwann cells up-regulate mRNA specific for type I IL-1 receptor [58, 59], and since exogenously supplied IL-1RA inhibits unfracionated cytokine-induced Schwann cell proliferation, we have evidence of the presence of functional product, the receptor itself. Therefore, the timing of the appearance of different cytokines secreted by Schwann cells, as well as by infiltrating cells, may be important in PNS repair.

Cytokines, Schwann Cells, and Axons

Damage to axons is a feature of many diseases of the PNS and is often the major limitation in recovery. Therefore, an important area for future research should be the effects of cytokines made by the infiltrating cells and Schwann cells on axons. There is compelling evidence that Schwann cell function is important in repair of axons damaged in Wallerian degeneration and presumably in axonal damage in immune-mediated and degenerative diseases of the PNS. Much less is known about toxic effects of cytokines on axons; however, as noted earlier, there are several substances produced by Schwann cells that could potentially be toxic to axons and perhaps explain some of the secondary demyelination seen in severe and recurrent demyelinating neuropathies. In addition, the damage to or loss of Schwann cells that are making important trophic factors for neurons and axons could also contribute to axonal loss.

It Is All a Matter of Timing

It is clear that we need to learn much more about the effects of cytokines on Schwann cells, using purified Schwann cell cultures, dorsal root ganglion cultures, and neuronal-Schwann cell co-cultures. Since single cytokines and chemokines do not occur in isolation in tissue in inflammatory lesions in humans and in animal models, the interactions between these cytokines and the effects of unfracionated cytokines also need to be studied. The pathways involved in signaling by these cytokines need to be studied in Schwann cells since important signaling pathways may differ for the same cytokine in different cell types. Thus, the pathways that are important in Schwann cells may be different than those that are important in inflammatory cells (lymphocytes and monocytes), oligodendrocytes, astrocytes, or microglia. The results from in vivo situations need to be compared with those of in vitro experiments. As pointed out by Lisak [102] in examining the effects of different cytokines on Schwann cells and in diseases of the PNS, a cytokine that may contribute to the pathogenesis of a lesion may at other times be protective and even contribute to repair. Lisak [2] and others [5] have found the same cytokine effects in diseases of the CNS and with CNS glia. The challenge is to sort out these relationships and then to work out paradigms to inhibit disease and encourage repair. This will not be easy since cytokines are so pleiotropic, share receptors, have overlapping but not identical properties on different cells, can induce or inhibit the induction of other cytokines, can synergize with or inhibit the actions of other cytokines, and in several instances, occur in more than one isoform. These overlapping relationships contribute to the difficulty in obtaining simple answers when using knock-out mouse models, although these relationships frequently are useful when taken in the context of data obtained in more classic in vitro and in vivo models.

Conclusions

GBS is an inflammatory disorder with various amounts of demyelination and secondary or primary axonal damage. Cytokines released by inflammatory cells and by Schwann cells likely contribute to the pathogenesis of the disorder but may also be involved in protection and recovery from this syndrome. These same proteins are also likely to prove important in other PNS disorders.

References


23. Lisak R, Bealmear B α/β interferon induces class I but not class II major histocompatibility complex (MHC) antigens on neonatal Schwann cells. Neurology 1991; 41(suppl):188.


42. Isberg R, Leong JM. Multiple β1 chain integrins are receptors for invasion, a protein that promotes bacterial penetration into mammalian cells. Cell 1990;60:861–71.


52. Dore-Duffy P, Balabanov R. Cytokine and the blood brain barrier. (in press).


58. Skundric D, Bealinear B, Lisak RP. Inducible IL-1α, IL-1β, IL-1R and IL-1 receptor antagonist (RA) expression in cultured rat Schwann cells (SC). J Neurochem 1995;64(suppl):136.


90. Gadient R, Otten U. Postnatal expression of interleukin-6 (IL-6) and IL-6 receptor (IL-6R) mRNAs in rat sympathetic and sensory ganglia. Brain Res 1996;724:41–6.
97. Lisak R, Bealmear B. Transforming growth factor β (TGF-β) is co-mitogenic for Schwann cells (SC) with interleukin-1α (IL-1α) [abstract]. Neurology 1995;45(suppl 4):A164–5.