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Commentary

Leukemia inhibitory factor, a cytokine at the interface between neurobiology and immunology

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The growing appreciation of the active interface between the immune system and the nervous system includes recognition of the cell surface molecules and the transducing mechanisms that are shared between the two systems. Perhaps even more compelling is the identification of intercellular messengers that mediate active signaling between the two systems. Neurotransmitters and neuropeptides, well known for their role in the communication between neurons, are also capable of activating monocytes and macrophages and inducing chemotaxis in immune cells. Transmitters and neuropeptides released by local neuronal processes are, therefore, well suited for mediating the ability of neurons to participate in inflammatory reactions at sites of injury or infection, as discussed below. In addition, immune tissues such as the spleen and lymph nodes are innervated, and pharmacological manipulations indicate that transmitters and neuropeptides are likely to regulate immune functions (1, 2).

A different class of intercellular messengers is also likely to move in both directions between these two organ systems. Cytokines, many of which have been discovered in studies of the hematopoietic system, are well known to regulate immune development and function. Many cytokines have the ability to regulate neural cell proliferation and gene expression. Interleukin (IL) 1 and 2, γ-interferon, tumor necrosis factor α and β, and various members of the transforming growth factor β superfamily are immunoregulatory cytokines that affect survival, growth, and gene expression of various types of neurons and glia in culture. Moreover, several of these cytokines are either normally expressed in the nervous system or are up-regulated in the central or peripheral nervous system after injury or neurological disease. These findings indicate that a given cytokine could mediate "cross-talk" between the nervous and immune systems, in both directions.

Another, recently recognized and unusual cytokine family may play such a role. Results from overexpression studies presented in this issue and elsewhere (15, 45–48) and results from gene targeting experiments in mice (19, 44, 49) confirm that one of the members of this family, leukemia inhibitory factor (LIF), can regulate hematopoiesis and T-cell maturation, as well as neuronal survival and gene expression. The role of LIF in the interface between the two systems is particularly striking in inflammation. LIF is increased in a variety of inflammatory conditions, is produced by immune cell lines and primary glial cells, and is required for neurons to respond to injury.

LIF, IL-6, IL-11, ciliary neurotrophic factor (CNTF), growth promoting activity, and oncostatin M belong to an unusual family of proteins termed the neuropoietic cytokines (3–5). The unique aspect of this group of proteins is that although they share only very limited sequence homology, they exert very similar effects on a variety of tissues. Several of these proteins can, for instance, induce the same set of acute-phase response proteins in liver, support the self renewal of cultured embryonic stem cells, inhibit lipogenesis, and enhance the survival of cultured motor neurons (see ref. 6). Despite their limited homology, the neuropoietic cytokines are predicted to share a four antiparallel helix bundle secondary structure (7, 8), a prediction that is borne out by the recent structure determination of CNTF (N. McDonald, personal communication). These ligands of very similar structure bind to receptor subunits that share various degrees of homology (9, 10). The receptor complexes also contain an identical signal transducing subunit, gp130 (11), that leads to the induction of similar phosphorylation cascades by these cytokines (12–14). The overlaps in receptors and signal cascades offer a convenient molecular explanation for the often redundant biological activities of the neuropoietic cytokines.

Nervous System. Although extensively characterized in culture, much less is known about the effects of this cytokine family on the nervous system *in vivo*. In this issue, Bamber *et al.* (15) use the insulin promoter to induce ectopic expression of LIF in the pancreas and find that the normally noradrenergic sympathetic neurons that innervate this tissue switch transmitter phenotypes to become cholinergic. This result provides striking *in vivo* confirmation of early culture results with postmitotic sympathetic neurons, initially using conditioned medium and then pure LIF (called cholinergic differentiation factor in that body of work) (16–18). LIF does not, however, appear to be the cholinergic factor that is known to act during the normal development of sympathetic neurons innervating the sweat glands (19). Those neurons switch phenotype in response to a factor that appears to be similar but not identical to CNTF (20, 21). In addition to regulating neuronal gene expression, LIF can also act as a survival factor for neurons. When applied to peripheral nerves *in vivo*, the cytokine is retrogradely transported and rescues damaged sensory neurons (22, 23), as it does in culture (24). LIF also alters the phenotype of sensory neurons in culture (25, 26). Application of CNTF can prevent death of thalamic and motor neurons *in vivo*, both after lesion and during the normal cell death period (27–29). Exogenous CNTF also slows degeneration of motor neurons in a mouse model of progressive motor neuropathy (30) and induces sprouting of motor axons in muscle (31).

While overexpression reveals some of the capabilities of these cytokines *in vivo*, it does not necessarily demonstrate the normal roles of these proteins. For CNTF, gene targeting has provided evidence that this cytokine does not, in fact, regulate motor neuron survival during development. Rather, the results of the knockout of the CNTF gene point to a role for this protein in the maintenance of healthy motor neurons in adulthood (32). Null mutations in humans do not, however, lead to obvious motor symptoms (33). Disruption of the LIF gene, in contrast, demonstrates a role for this cytokine in neuronal response to injury. Prior results had suggested LIF as a candidate for mediating the induction of a set of neuropeptides after nerve transection. The model predicted that nerve section causes enhanced production or release of LIF, which could then act on neurons to alter their neuropeptide gene expression. While there was evidence that LIF can be produced by glial cells in culture (16, 34), recent Northern blot (35) and *in situ*

analysis shows that Schwann cells can dramatically up-regulate LIF expression in response to peripheral nerve section (36, 37). In addition, recombinant LIF can duplicate the effects of nerve injury in the induction of particular neuropeptides in sympathetic neurons (6, 37, 38). In striking confirmation of this model, when sympathetic nerves are cut in LIF$^-$ mice, the neuropeptide induction is greatly diminished compared to the neuronal response in wild-type mice (19).

In contrast to the induction of LIF, nerve transection in normal animals causes CNTF mRNA and protein levels in Schwann cells to drop (39–42). Thus, these two cytokines, which have identical effects on many types of neurons, are regulated in complimentary fashion in peripheral nerve. Moreover, the knockout mice for these cytokines display complimentary phenotypes that parallel those distinct regulatory controls. CNTF is high in normal Schwann cells and is required for maintenance of healthy motor neurons in adults, while LIF is induced by injury and is required for the neuronal response to injury. Similar studies are currently underway in the brain, where LIF levels rise after injury (43) and LIF$^-$ mice display abnormalities (44).

Hematopoietic System. LIF overexpression and gene disruption also affects hematopoiesis. Injection of high levels of LIF in mice results in elevated numbers of splenic megakaryocytes and platelets, loss of body fat, an acute phase response, splenomegaly, excess and ectopic bone formation, and gonadal dysgenesis (45–47). Many of these results are consistent with the known distribution of LIF receptors and with the effects of this cytokine on cultured cells. An additional more selective phenotype was obtained by driving overexpression of LIF in T cells. In this transgenic mouse line, the thymus is strikingly deficient in CD4$^+$CD8$^+$ cells, being populated instead by B cells. Lymph nodes, in contrast, contain a vastly expanded population of CD4$^+$CD8$^+$ lymphocytes (48). The profoundly disorganized thymic epithelium in these mice, coupled with the results of bone marrow transplants, suggest that the apparent interconversion of thymic and lymph node phenotypes is due to disruption of stroma–lymphocyte interactions. These results complement those reported earlier for LIF knockout mice. Although the LIF$^-$ mice display apparently normal thymocyte cell populations, there is evidence of reduced myeloid progenitors and defective thymic T-cell activation (49). Here too, bone marrow transplantation studies indicated that the defects in stem cell numbers and T-cell activation are probably due to a requirement for LIF expression in the hematopoietic microenvironment. These two mouse strains present an excellent opportunity to investigate stromal influences in hematopoiesis, an area of study that is relatively underdeveloped.

Interactions Between the Nervous and Immune Systems. It is intriguing that LIF levels have been found to be elevated in various inflammatory conditions, including rheumatoid arthritis, acute kidney rejection, acute bronchoalveolar response, and septic shock (refs. 50–52 and T. Ulich, M.-J. Fann, P.H.P., J. Williams, B. Samal, J. del Castillo, S. Yin, K. Guo, and D. Remick, unpublished data). It is not entirely clear, however, which cell types are responsible for LIF secretion in each of these syndromes. For nerve transection, cited above, glial cells are at least a major contributor to the induction of LIF mRNA (36). An independent body of work has implicated nerve cells in a "neurogenic" component of inflammation. Denervation prior to the experimental induction of inflammation can result in a diminished inflammatory response. Moreover, pharmacological experiments implicate the release of transmitter and neuropeptide from neuronal endings as the active agents in this neurogenic function. In experimental arthritis, for instance, the levels of substance P (SP) and calcitonin gene-related peptide (CGRP) are increased in the sensory neurons that innervate affected joints (53, 54). LIF in synovial fluid from patients with osteoarthritis and rheumatoid arthritis (55) could contribute to this cascade by inducing SP and CGRP in sympathetic neurons that do not normally express them. These neuropeptides enhance monocyte production of cytokines, such as IL-6, tumor necrosis factor α, and IL-1β (56–60). Moreover, SP is chemotactic for monocytes and macrophages (61, 62). Thus, there is a cycle of interdependent reactions such that neuronal gene expression is altered by injury and inflammation, and the induced neuropeptides can actively participate in the inflammation event itself (51, 63, 64). A further interesting complexity is that peripheral blood cells, such as megakaryocytes and T cells, contain high levels of neuropeptides such as neuropeptide Y and enkephalin (65, 66).

Mast cells are also important in this context. They are concentrated in particular locations in the central nervous system (67–69), they can be found in close association with axons in the peripheral nervous system (70), and they contain and release cytokines (including LIF), neurotrophins, neuropeptides, and biogenic amines (71–75). Mast cells release some of these agents when nerves near them are stimulated (76). For instance, SP from sensory nerves stimulates histamine and serotonin release from mast cells, which is one of the mechanisms whereby mast cells participate in neuroinflammatory conditions (77–80). In addition, the release of transmitters, neuropeptides, and cytokines by mast cells could modulate neuronal activity and gene expression in a feedback loop.

While this brief commentary focuses primarily on LIF, other cytokines are also key agents in the interactions between the immune system and nervous system. It seems likely that some of these cytokines are involved in the type of feedback cascades discussed for LIF. Of particular interest in this context are activin, bone morphogenetic protein 2 and 6, transforming growth factor β, IL-1, and γ-interferon, which are cytokines known to influence gene expression and survival of peripheral neurons (5, 38, 81–88).

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