Molecular Neurobiology

ISSN0893-7648/98/24(1–3): 183–199/$14.25

Nerve Growth Factor

A Neurokine Orchestrating Neuroimmune-Endocrine Functions

Stephen D. Skaper*

Neurology Centre of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park North, Harlow, Essex, CM19 5AW UK

Abstract

Nerve growth factor (NGF) is widely recognized as a target-derived factor responsible for the survival and maintenance of the phenotype of specific subsets of peripheral neurons and basal forebrain cholinergic nuclei during development and maturation. Other NGF-responsive cells are now known to belong to the hemopoietic-immune system and to populations in the brain involved in neuroendocrine functions. The concentration of NGF is elevated in a number of inflammatory and autoimmune states in conjunction with increased accumulation of mast cells. Mast cells and NGF appear to be involved in neuroimmune interactions and tissue inflammation. Mast cells themselves are capable of producing and responding to NGF, suggesting that alterations in mast cell behavior may trigger maladaptive neuroimmune tissue responses, including those of an autoimmune nature. Moreover, NGF exerts a modulatory role on sensory nociceptive nerve physiology in the adult, and appears to correlate with hyperalgesic phenomena occurring in tissue inflammation. NGF can thus be viewed as a multifactorial modulator of neuroimmune-endocrine functions.

Index Entries: Nerve growth factor; neurotrophin; neuroimmune; mast cells; inflammation; hyperalgesia; pain; autoimmune disease.

Introduction

Neurotrophic polypeptides critically influence developmental events in the nervous system, ranging from naturally occurring cell death to differentiation and process outgrowth. Nerve growth factor (NGF) is clearly the best-characterized neurotrophic protein. It acts on sympathetic and neural crest-derived sensory neurons *(1)*, and is also present in the central nervous system (CNS) where it serves a trophic function in the development and maintenance of basal forebrain cholinergic neurons *(2)*.

* Address to which all correspondence and reprint requests should be sent. E-mail: Stephen_Skaper-1@gsk.com

Considerable evidence has accumulated over the last decade to indicate that the actions of NGF extend far beyond "classical" effects on cells of the nervous system, to encompass a role for this molecule in the interplay between the nervous, immune, and endocrine systems. Additional NGF-responsive cells are now known to include lymphocytes *(3)*, mast cells *(4)*, eosinophils *(5)*, other ectodermal-derived cells such as keratinocytes and melanocytes *(6,7)*, and cellular elements of the endocrine system *(8)*. Expression of the signal-transducing NGF receptor TrkA *(9)* on cells of the nervous, immune, and endocrine systems *(3,9,10)* further strengthens the notion that NGF is a key player in interaction among these three systems. The following article will first briefly summarize NGF and neurotrophin biology, and then review findings that lend credence to the concept of NGF as a mediator of neuroimmune-endocrine functions.

Biology of the Neurotrophins

NGF was discovered almost 50 years ago as a diffusable substance capable of inducing neurite outgrowth in explants from sympathetic and sensory ganglia *(1)*. NGF is the prototypic neurotrophin that defines the properties and functions of this class of growth factors. There are two unique features of the actions of NGF on neurons as opposed to those of growth factors on other types of cells. First, NGF regulates functions of differentiated neurons, i.e., growth as opposed to proliferation. Second, NGF is synthesized at a considerable distance from the cell body by peripheral tissues or other neurons (referred to as targets) that are contacted by axons of the NGF-sensitive neurons. In the periphery, the tissue sources of NGF (and other neurotrophic factors) are typically non-neuronal cells, whereas in the CNS they are synthesized predominantly by neurons under physiological conditions *(11)*. During development, a retrograde flow of NGF is established, transporting NGF from the target into the nerve terminal and up the axon to the cell body *(12)*. Those neurons that establish this flow survive the period of neuronal cell death, while those that do not, degenerate. Once the retrograde flow of NGF is established, it must continue for the lifetime of the neuron to develop and maintain the functional differentiated state of the neuron *(13)*. If the supply of NGF to the target is augmented, some of the neurons that would normally die are rescued *(14)*. Recent studies on the expression and actions of the NGF family indicate that, in addition to target-derived factor acquistion, autocrine and nontarget-derived paracrine modes of factor presentation are likely to be important *(15)*.

The generality of the phenomenon of programmed cell death after target deprivation (axotomy) has suggested that most neurons respond to and are regulated by neurotrophic factors *(16,17)*. This hypothesis was validated by the subsequent isolation of a second neurotrophic factor, designated brain-derived neurotrophic factor (BDNF), capable of supporting the survival of sensory but not sympathetic neurons *(18)*. Molecular cloning of the BDNF gene *(19)* revealed its structural similarity to NGF, leading to the concept of the neurotrophin family. Using a homology cloning approach rather that protein purification, two additional members, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) were later identified *(15,20,21)*. NGF, BDNF, NT-3, and NT-4/5 share approx 50% sequence identity *(22)* and have been found in a wide range of vertebrates, from cartilaginous fish to mammals. The regions of sequence similarity and variation are clustered, indicating probable sections of structural and functional importance *(21)*. A given neurotrophin can address distinct, as well as partially overlapping, neuronal subsets. More recently, two novel neurotrophins from the platyfish and carp have been cloned and designated neurotrophin-6 *(23)* and neurotrophin-7 *(24)*, respectively. In addition to the neurotrophins, a number of other polypeptide growth factors have been shown to possess neurotrophic activities, but will not be discussed here.

Diversity is also apparent in the receptors activated by neurotrophic factors. For example, the neurotrophins interact with two classes of transmembrane glycoproteins on responsive cells: protein tyrosine kinase-type receptors (members of the Trk family) and a smaller binding protein (distantly related to the tumour necrosis factor (TNF) and CD40 receptors) containing a short cytoplasmic tail of unknown function: the p75 low-affinity NGF or neurotrophin receptor, $p75^{LNTR}$ *(9).* The specific biological activities of the four neurotrophins on peripheral and central neurons, to some extent, correlate with their selective interaction with the different members of the Trk family of receptors. Thus TrkA, which is a receptor for NGF, has been found in NGF-responsive neuroal cells, including sympathetic neurons, small spinal sensory neurons of the dorsal root ganglion, and basal forebrain cholingergic neurons. TrkB is a receptor for BDNF and NT-4/5, and it is widely expressed in the peripheral nervous system (PNS) and CNS, including nodose ganglion sensory neurons and spinal-cord motor neurons. Similarly, TrkC expression has been demonstrated in cells that are responsive to NT-3, including large spinal sensory neurons, motor neurons, and the noradrenergic neurons of the locus coeruleus. Nevertheless, some crosstalk may occur; in the case of NT-3, TrkA and TrkB receptors can also be activated, albeit to a lesser extent *(9,25).* Further documenting receptor diversity, a number of isoforms of TrkB and TrkC exist that lack the tyrosine kinase domain or contain inserts in the intracellular domain that influence signaling *(15,25).* In contrast, $p75^{LNTR}$ binds all neurotrophins with similar affinities *(26),* but does not seem to be a functional neurotrophic receptor in the absence of a Trk receptor. It is nonetheless important for the developing nervous system because mice bearing a null mutation in the $p75^{LNTR}$ gene have decreased pain sensitivity and cutaneous innervation *(27),* and sensory neurons cultured from these mice are less sensitive to NGF than are wild-type neurons *(28).* How $p75^{LNTR}$ specifically enhances the neuronal survival response to NGF is not understood, although it may cooperate with Trk receptors to increase the affinity of neurotrophin binding and/or signaling efficiency *(29–32).*

NGF and Immune Cell Function

NGF displays biological activities in a variety of cells outside the nervous system and is produced by a broad range of cell types not normally considered targets for innervation by NGF-dependent neurons, including cells of the immune-hematopoietic lineage. For example, NGF stimulates the proliferation of both B and T lymphocytes *(33,34),* and the production of IgM, IgA *(33),* and IgG4 antibodies *(35).* Exposure to NGF induces high-affinity interleukin-2 (IL-2) receptors on human peripheral blood mononuclear cells *(36,37)* and promotes human hemopoietic cell growth and proliferation *(38).* NGF is also involved in chemotaxis, viability, and functional properties of human polymorphonuclear neutrophils in vitro *(39,40)* and in vivo *(41),* and in the differentiation of thymic stromal nonlymphoid cells *(42).* Additionally, NGF induces shape changes in platelets *(43,44),* accelerates wound healing *(41),* and acts as an autocrine survival factor for memory B lymphocytes *(45).* These effects of NGF are consistent with findings that human monocytes *(46),* activated $CD4^+$ T-cell clones *(47,48),* and lymphocytes *(49,50)* express TrkA. These latter cells also elaborate biologically NGF, suggesting possible autocrine or paracrine actions of this neurotrophic factor in the development and regulation of immune-cell responses.

Mast cells were the first cells of immune lineage to be identified as a target for NGF, both in vivo *(51)* and in vitro *(52).* Neonatal rats given daily injections of NGF showed a massive mast-cell hyperplasia involving connective tissue mast cells in several peripheral tissues *(51),* an effect later extended also to mucosal mast cells *(53,54).* In addition, anti-NGF antibodies reduce rat peritoneal mast-cell numbers *(55).* These

data suggest a specific and direct NGF effect on the differentiation and maturation of mast cells and/or their precursors and are supported by the fact that NGF can induce the development of connective tissue mast cells from mouse bone-marrow cells *(56)* and from spleen cells of newborn rats treated with NGF *(57).* Interestingly, NGF appears to act as a cofactor with IL-3 in the development of basophils or mast cells from human umbilical-cord blood cells *(58),* and can induce mast cell-marker expression in the latter cell cultures *(59).*

NGF is an extremely potent degranulating agent for cultured rat peritoneal mast cells in the presence of phosphatidylserine or its lyso derivative *(60,61).* By itself, NGF at physiological concentrations is a very poor secretagogue for rat peritoneal mast cells, while markedly enhancing antigen or other secretagogue-induced histamine release from such cells *(62).* NGF may thus act as an immunomodulator in the inflammatory response by regulating mediator release from mast cells. Among the neurotrophin family NGF is unique in regulating basophil functions, having a very similar sensitising or priming effect on histamine release in mature human basophils *(63).* In keeping with such findings, subcutaneous injection of NGF produces plasma extravasation *(64),* a response consistent with histamine release caused or facilitated by NGF. Human blood basophils express functional TrkA (but not TrkB and TrkC) receptors that do not require the participation of $p75^{LNTR}$ *(65).* NGF expression by mast cells could thus constitute an important link between mast-cell activation and basophil function in the late-phase allergic reactions.

Rat peritoneal mast cell express TrkA, but not other members of the Trk family or $p75^{LNTR}$ *(4),* and synthesize, store, and release biologically active NGF *(66).* Both the human mast-cell line HMC-1 and cultured human mast cells express functional TrkA and produce active NGF *(67,68);* HMC-1 cells express also TrkB and TrkC full-length proteins, while human lung mast cells express mRNAs for all Trks *(68).* In addition, HMC-1 cells express mRNAs for NGF, BDNF, and neurotrophin-3 (NT-3), whereas NGF and BDNF transcripts were detectable in human umbilical-cord blood mast-cell preparations *(68).* Rat mast cells contain immunoreactive NGF, NT-3, and NT-4/5, but no BDNF, and release active NGF and NT-4/5 (but no NT-3) upon degranulation *(69).* Thus, NGF and other neurotrophins appear capable of affecting mast cell-mediator release, possibly in an autocrine or paracrine fashion. A survival-promoting effect of NGF on rat peritoneal mast cells in vitro may involve prevention of an apoptotic death mechanism *(70–72)* through Trk activation, in analogy to neuronal rescue by NGF. The actions of NGF on immune cell functions are summarized in Table 1.

Table 1
NGF Responsive Cells of the Immune-Hematopoietic Lineage

Cell type	NGF response	References
Mast cells	Maturation, survival, degranulation, chemoattraction (NGF source)	*(51–62, 66–73)*
Basophils	Activation	*(63,65)*
Lymphocytes	Proliferation, antibody production (NGF source)	*(33–35, 47–49)*
Peripheral blood mononuclear cells	II-2 receptor expression	*(36,37)*
Hemopoietic cells	Growth, differentiation	*(38)*
Polymorphonuclear neutrophils	Chemotaxis, viability, phagocytosis	*(39–41)*
Platelets	Shape change	*(43,44)*
Memory B lymphocytes	Autocrine survival factor	*(45)*

Locally produced NGF may play an important role in mast-cell accumulation in allergic and nonallergic inflammatory conditions, given recent evidence that NGF may function as a chemoattractant for mast cells through

both mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase signaling pathways *(73).* NGF modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism *(74),* suggesting a role for prostanoid production in the regulation of local inflammatory responses and neuronal degeneration after tissue injury involving induction of NGF production.

NGF and Endocrine-Nervous System Interaction

NGF appears to take part in the regulation of neuronal and non-neuronal cell populations involved in the control of specific neuroendocrine functions *(75)* and in the acquistion of male and female reproductive capacity *(76,77).* For example, the hypothalamic content of NGF and its gene increase following stressful events *(78),* while the genes encoding NGF and its receptor are expressed in the developing female hypothalamus *(79).* Biologically active NGF is also found in the rat pituitary *(80),* is present in both mammotroph and somatomammotroph cells *(10),* and its release from the pituitary appears to be under neuronal control *(81).* Transgenic mice overexpressing the NGF gene in lactotrophs showed a marked hyperplasia of this cell type and the ability to release NGF *(82).* Thyroid and parathyroid glands of the rat express NGF mRNA and precursor NGF protein *(83).* NGF stimulates the pituitary-adrenocortical axis, enhancing the secretion of adrenocorticotrophic hormone and the concentration of plasma glucocorticoids *(84),* an effect probably mediated through the hypothalamus *(85).* Plasma NGF levels in women during labor and lactation are increased compared with the concentrations found at term or in controls *(86),* a time when plasma levels of the neurohypophyseal hormone oxytocin are high. Administration of oxytocin to female rats significantly raised hypothalamic NGF content *(86).* Upon activation of Trk, NGF directs the differentiation of GH3 cells, a clonal line related to bipotential somatomammotrophs from a prevalent growth hormone-secreting somatotroph phenotype to prolactin producing lactotrophs *(87),* at the same time suppressing the tumorigenicity of human prolactinomas in vivo and in vitro *(88).* NGF may also be present in the pituitary in a stored form and secreted into the circulation in vivo *(80).* This appears to be likely since cell lines of pituitary origin, when transfected with the NGF gene via a replication-defective retroviral vector, are able to store mature β-NGF in secretory granules and to release it upon stimulation *(89).* Together, the data support a physiological role for NGF during pituitary development.

Fetal rats exposed to NGF antibodies are reported to display marked neuroendocrine deficits postnatally *(90).* Such offspring not unexpectedly have severely atrophied sympathetic and sensory ganglia, but also much smaller thyroids among endocrine organs. Deleterious effects of autoimmune NGF deprivation have also been described in rabbits and guinea pigs *(91).* Maternal exposure to NGF antibodies during fetal development causes, in newborn pups, loss of body weight, sensory deficits, and lethality *(92,93).* Exposure to NGF antibodies during the postnatal period perhaps inactivates endogenous NGF in neuroendocrine structures, thereby causing a widespread neuroendocrine immune-deficiency syndrome. The studies cited here, which point to a modulatory role for NGF in the hypothalamo-pituitary-adrenal axis, would support such a notion. Interestingly, natural autoantibodies to NGF have been detected in the sera of some patients with autoimmune diseases *(94).* This topic will be discussed in a later section.

Circulating NGF and Stress

NGF is produced and stored in large amounts in the submaxillary salivary glands of adult male mice *(1),* although the biological significance of this is not fully understood. Snake venom *(95)* and salivary gland of the African rat Mastomys natalensis *(96)* also con-

tain large quantities of NGF. Aggressive interactions with conspecifics is accompanied by a massive release of NGF into the bloodstream *(97)* and, if this type of behavior is prolonged, by hypertrophy of cortical and medullary components of the adrenal gland. Intermale aggressive behavior also induces a large increase in the levels of mRNA for NGF and NGF protein in the hypothalamus *(78).* Serum levels of NGF appeared to correlate positively with the number of fighting episodes in a mouse pair *(97).* The ability of NGF to elicit hypertrophy of the adrenal gland and enhanced functional activity of the medullar and cortical sections of this gland suggests this organ to be the most likely target of NGF *(98).* NGF levels in serum consistently reach higher peaks in the subordinate rather than in the dominant partner *(98)*, suggesting that NGF discharge from salivary gland to bloodstream is a combination of aggressive behavior and anxiety, which would be expected to be more acute in the subordinate mouse. Release of NGF produced by psychosocial stress is not mimicked by physical stresses. Levels of NGF in the blood increase in humans both prior to and after psychologically stressful and anxious situations, while serum levels of II-1, TNF-α, cortisol, and adrenocorticotrophic hormone are not modified *(99).* As mentioned earlier, physiological concentrations of NGF do not appear to activate mast cells and basophils directly but rather modulate their threshold to other triggering stimuli. Thus, circulating NGF could be viewed as a general alert signal used by the brain in settings of stress and anxiety to "prime" the immune system towards noxious inputs.

NGF, Inflammation, and Mast Cells

Mast cells are a heterogeneous immune-effector cell type found in connective tissues throughout the body, occur adjacent to blood and lymphatic vessels, and are concentrated beneath mucosal surfaces *(100).* Morphological and functional mast cell-neuron interactions in vitro and in vivo have been documented *(101)*, where they are found within the PNS and CNS *(102).* For example, mast cells are active in the thalamus in basal conditions and NGF has the potential to elicit long-lasting degranulation of thalamic mast cells in vivo, exerting a direct effect and/or priming these cells to react to endogenous stimuli *(103).* Mast cells represent critical effector cells in allergic diseases and other IgE-dependent responses *(100).* Nervous and immunological mediators such as neuropeptides or IgE can affect the state of mast-cell activation *(104).* Activated mast cells are capable of secreting an enormous array of cytokines and other inflammatory mediators *(100,105).* Mast cells may thus be important messengers between the nervous, endocrine, and immune systems *(106,107).* In addition to being immediate affector cells in immediate hypersensitivity reactions, mast cells appear to be involved in other pathophysiological processes including delayed-type hypersensitivity, wound healing, fibrosis, and disorders of an inflammatory nature *(100,105,108).*

Altered mast-cell numbers or phenotype, as well as mast-cell overactivity, may underlie dysfunctional nervous-immune system interactions. Increased mast-cell numbers have been reported in a variety of immunoinflammatory conditions. Mast-cell numbers are elevated in the derma of patients with early systemic sclerosis *(109)* and in the synovium of rats with rheumatoid synovitis *(110).* NGF was increased in the dermas *(109)* and synovium *(110)* in the latter instances. NGF synthesis and release is dysregulated in the upper airways of patients with allergic rhinitis *(111)*, with increased circulating levels of the protein in humans with allergic diseases and asthma *(112,113).* An increase in NGF content and nerve-fiber sprouting in human allergic contact eczema has been described *(114)*, and this may have a functional impact on skin-associated immune cells, in particular mast cells. Both mast cells and NGF have been implicated also in inflammatory bowel disease *(115)*, Hirschsprung's disease and intestinal neuronal dysplasia *(116)*, human immunodeficiency virus *(117)*, and myeloproliferative pathologies *(118).* A correla-

tion between mast cells and NGF may exist in certain inflammatory diseases, given that NGF is known to cause mast-cell activation and proliferation. IgE receptor-mediated release of NGF by human mast cells further strengthens a link between NGF and mast cells in allergic inflammation *(119).*

Mast cells produce and respond to a variety of cytokines *(105).* Cytokines involved in inflammation and immune responses, such as IL-1β and TNF-α, are strong inducers of NGF synthesis *(120),* and differences in tissue cytokine and NGF expression may provide a positive feedback loop for autocrine/paracrine regulation of mast-cell properties. NGF accumulation in acute inflammatory exudates has widely been attributed to cellular infiltrates or to upregulation of NGF expression. The rapid appearance and level of NGF accumulation in rat skin-blister fluid *(121),* however, seems more in line with NGF being released from resident tissue cells containing stores of the protein. Remodeling of intestinal mucosal nerve fibers during intestinal inflammation follows changes in mast-cell density *(122).* Mast-cells and sympathetic neurons from contacts *(123),* proposing the existence of chemotactic NGF-like gradients between mast cell and nerve ending. The mast cell could thus represent a readily available source of NGF.

Autoimmune Diseases, NGF, and Mast Cells

The etiology and cellular mechanisms underlying autoimmune diseases remain largely to be defined. Presumed autoimmune disorders like multiple sclerosis (MS) *(124),* irritable bowel disease, and interstitial cystisis seem to occur with greater frequency in females *(125),* suggesting a possible hormonal component in the pathophysiology. In MS, plaques in human brain tissue contain mast cells *(104,126).* Mast cells secrete pro-inflammatory cytokines in response to myelin basic protein *(127),* a major suspected immunogen in MS, which also induces peripheral *(128)* and central **(129)** demyelination. Prolonged mast-cell stimulation can damage cultured CNS neurons by the release of mast-cell cytokines, which in turn induce astroglial nitric oxide synthase *(127).* Mast cells, probably originating from the neural crest, have been identified intracranially *(130),* where they show a strict perivascular location *(129)* and where they secrete vasoactive amines in response to mediators *(131).* Interestingly, estradiol and myelin basic protein are reported to act synergistically in triggering mast-cell activation *(132).* The endogenous immune-response system of the brain may also participate in exacerbating the fundamental pathology of Alzheimer's disease, apparently without stimulation by peripheral inflammatory mediators or the peripheral immune system *(133).* A strong inflammatory response might be autotoxic to neurons *(127).* A role for mast cells in Alzheimer's disease, however, remains highly speculative.

MS is accompanied by penetration of blood-borne immune cells within brain parenchyma and subsequent destruction of myelin. T-lymphocytes and monocytes are clearly involved in cellular infiltrates in areas of demyelination *(134,135)* and have a role in experimental allergic encephalomyelitis *(136)* and experimental allergic neuritis. Mast cells also have been reported in MS plaques *(137,138),* and are activated during experimental allergic neuritis *(139).* There is some evidence to suggest that a delayed T-cell response may depend on early release of mast-cell mediators *(140)* and that T-cell products can cause mast-cell activation *(141).* Mast cells themselves appear capable of presenting antigen in a major histocamp-atibility complex class II-restricted manner *(142).* Conceivably, myelin basic protein from demyelinated axons could provoke mast-cell degranulation, leading to a feed-forward reaction. As lymphocytes and monocytes are NGF-responsive, NGF released from brain mast cells could contribute to such a cycle. In this regard, MS patients are reported to express increased levels of NGF in cerebrospinal fluid *(143).*

Mast cells and NGF may also participate in autoimmune diseases of extraneural origin. In

Table 2
Autoimmune and Other Inflammatory Conditions with NGF Elevation

Condition	Species	Tissue site	References
Multiple sclerosis	Human	Cerebrospinal fluid	*(143)*
Chronic arthritis (adult, juvenile)	Human	Synovium, plasma	*(145,146)*
Carrageenan-induced arthritis	Rat	Synovium	*(147)*
Transgenic TNF-α overexpression (arthritis)	Rat	Synovium	*(148)*
Rheumatoid synovitis	Rat	Synovium	*(110)*
Allergic diseases, asthma	Human	Nasal lavage, plasma	*(111–113)*
Allergic contact eczema	Human	Skin	*(114)*
Inflammatory bowel disease	Human	Intestine	*(115)*
Systemic lupus erythematosus	Human	Serum	*(149,150)*
Systemic sclerosis	Human	Dermis	*(109)*
Systemic mastocytosis	Human	Plasma	*(151)*
Carrageenan-induced pleurisy	Rat	Pleural exudate	*(121)*
Noxious thermal stimulus	Rat	Skin blister fluid	*(121)*

the case of rheumatoid arthritis, mast cells and NGF accumulate in the synovial fluid *(110,144).* Increased quantities of NGF are found in several types of chronic arthritis, in systemic lupus erythematosus (SLE), and in mastocytosis *(145–151)* (*see* Table 2). Intrasynovial injection of NGF is reported to induce an increase in the local distribution of mast cells *(110).* Mast cells are frequently found at sites of tissue inflammation, and their abiltiy to release substantial amounts of NGF proposes that these cells may contribute, both acutely and at later times, to NGF expression in inflammatory conditions.

Inflammation, Pain, and NGF

A key feature of inflammation is pain and hyperalgesia. In the periphery, inflammatory mediators increase the sensitivity of high-threshold nociceptors so that a lower stimulus intensity is required to activate them; this is the phenomenon of peripheral sensitisation *(152).* Evidence suggests that, in adult animals, NGF can induce hyperalgesia, and may be an endogenous mediator in some persistent pain states. NGF levels rise markedly in inflamed tissue *(153,154).* This increase is secondary to the increases in levels of cytokines, specifically the interleukins and TNF-α *(155)* that are released from mast cells, phagocytic cells, and antigen-presenting cells, of the immune system. Peripheral administration of NGF in the adult rat rapidly induces a decrease in the threshold of nociceptors to heat and mechanical stimuli *(156–159)* in the absence of $p75^{LNTR}$ *(160),* suggesting that the TrkA receptor is sufficient to mediate the acute noxious action of NGF. Hyperalgesic responses have been observed in transgenic animals overexpressing NGF in the skin *(161),* and increased cutaneous NGF levels selectively affected the survival and functional properties of nociceptors *(162).* In contrast, genetically modified rodents expressing antisense NGF mRNA exhibited hypoalgesia to mechanical stimuli *(161).* Animals treated with anti-NGF antibodies *(90,154)* or TrKA-IgG fusion proteins *(163)* showed substantially reduced sensitivity to noxious mechanical and thermal stimuli, and neutralization of endogenous NGF prevented the sensitization of nociceptors supplying inflamed skin *(164).* Sympathetic postganglionic neurons appear to contribute to thermal hyperalgesia *(159)* but mast cells and sensory neurons are more likely to be important sites for the sustained action of NGF in producing

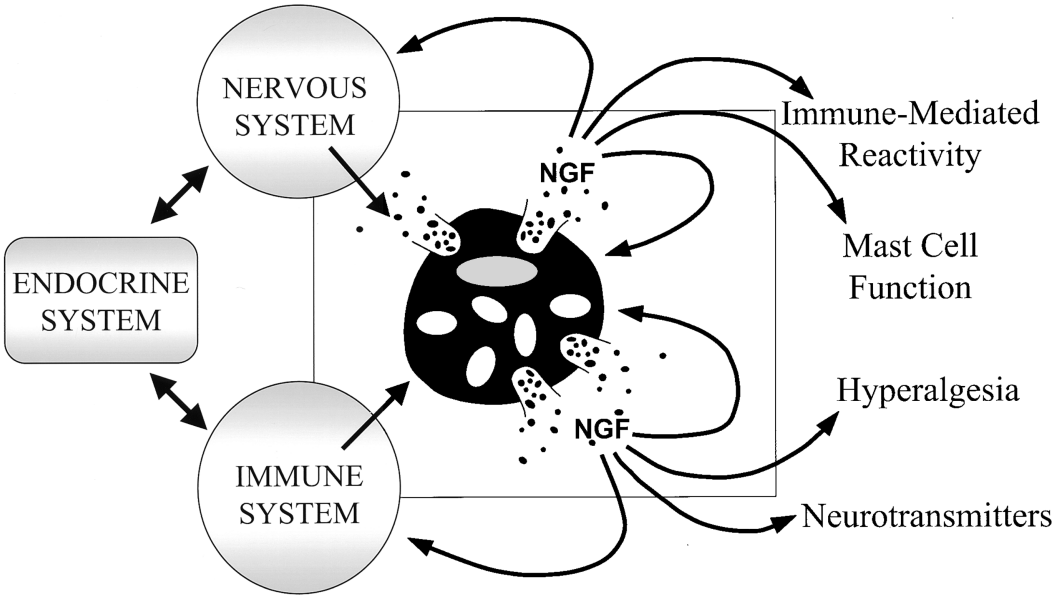

Fig. 1. NGF as a modulator of nervous system-immune-endocrine interactions. Mast cell-released NGF could also function in an autocrine manner. Potential actions of mast cell NGF are shown at right.

increased sensitivity associated with tissue inflammation *(165).*

The precise mechanism of hyperalgesic induction by NGF is not clear. High levels of NGF appear able to strengthen the effects of both orthodromic and antidromic sensory synaptic release by modifying amplitude and/or density of peripheral and central terminal fields, and by increasing peptidergic transmitter content and reducing the activation threshold to noxious heat and mechanical stimuli. The initial, rapid phase of NGF-induced thermal hyperalgesia seems also to be periperally mediated by mast cells and to involve a central glutamatergic receptor-dependent mechanism *(156).* NGF-induced hyperalgesia is qualitatively analogous to that occurring in tissue inflammation.

In addition to upregulation of peptides in sensory somata, NGF also upregulates BDNF in TrKA-positive dorsal-root ganglion neurons *(166).* At peripheral terminals another TrkB agonist, NT-4/5, can sensitize individual sensory afferents to noxious thermal stimulation, as can BDNF *(167).* Neurons lacking BDNF showed a profound and specific reduction in their mechanical sensitivity; postnatal treatment of $BDNF^{+/-}$ mice with BDNF completely rescued the mechanosensitivity deficit *(168).* The sensitizing action of both of these neurotrophins has also been established in behavioral experiments, where NT-4/5 is more potent than BDNF *(167).* Mast-cell depletion prevents NT-4/5 from eliciting behavioral sensitization *(167).* Interestingly, TrkB receptors are known to co-localize with TrkA on some mast cells *(68),* and mast cells themselves are capable of producing and releasing NT-4/5 *(69).*

Concluding Remarks

NGF emerges as a complex pleiotropic agent active on an unexpectedly broad array of cell types and biological functions, something not envisaged on the basis of earlier work establishing its trophic role for sensory and sympathetic neurons during development and adulthood. For example, high levels of NGF have been detected in the inflammatory exu-

dates and biological fluids of patients afflicted with autoimmune diseases. Within this framework, the mast cell can be viewed as a gatekeeper between the immune and nervous systems. Mast cells are involved not only in hypersensitivity but also actively participate in inflammatory phenomena of various types, including those of a neurogenic nature. Mast cells synthesize and release NGF, and NGF itself has both survival and sensitizing actions on mast cells. This raises the possibility of autocrine actions that, if not properly controlled, could have deleterious effects on surrounding tissues and lead to chronic inflammatory processes, e.g., those present in pathologies of an autoimmune nature. Thus, it is now appreciated that neurological dysfunction can occur not only as a result of neurotrophic factor deficiency but also as a consequence of excess. An important goal of future studies will be to more fully understand the involvement of NGF in disorders resulting from dysregulation of tissue homeostasis, which may occur, for example, in chronic inflammatory diseases like MS.

References

1. Levi-Montalcini R. (1987) The nerve growth factor 35 years later. *Science* **237,** 1154–1162.
2. Dreyfus C. F. (1989) Effects of nerve growth factor on cholinergic brain neurons. *Trends Pharmacol. Sci.* **10,** 145–149.
3. Otten U., Scully J. L., Ehrhard P. B., and Gadient R. A. (1994) Neurotrophins: signals between the nervous and immune systems. *Prog. Brain Res.* **103,** 293–305.
4. Horigome K., Pryor E. D., Bullock E. M., and Johnson E. M. Jr. (1993) Mediator release from mast cells by nerve growth factor. Neurotrophin specificity and receptor mediation. *J. Biol. Chem.* **268,** 14,881–14,887.
5. Solomon A., Aloe L., Pe'er J., Frucht-Pery J., Bonini S., and Levi-Schaffer F. (1998) Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J. Allergy Clin. Immunol.* **102,** 454–460.
6. Pincelli C., Sevignani C., Manfredini R., Grande A., Fantini F., Bracci-Laudiero L., et al. (1994) Expression and function of nerve growth factor and nerve growth factor receptor on cultured keratinocytes. *J. Invest. Dermatol.* **103,** 13–18.
7. Yaar M., Eller M. S., DiBenedetto P., Reenstra W. R., Zhai S., McQuaid R., et al. (1994) The trk family of receptors mediates nerve growth factor and neurotrophin-3 effects in melanocytes. *J. Clin. Invest.* **94,** 1550–1562.
8. Polak M., Scharfmann R., Seilheimer B., Eisenbarth G., Dressler D., Verma I. M., and Potter H. (1993) Nerve growth factor induces neuronlike differentiation of a insulin-secreting pancreatic beta cell line. *Proc. Natl. Acad. Sci. USA* **90,** 5781–5785.
9. Meakin S. O. and Shooter E. M. (1992) The nerve growth factor family of receptors. *Trends Neurosci.* **15,** 323–331.
10. Patterson J. C. and Childs G. V. (1994) Nerve growth factor in the anterior pituitary: regulation of secretion. *Endocrinology* **135,** 1697–1704.
11. Thoenen H. (1995) Neurotrophins and neuronal plasticity. *Science 270,* 593–598.
12. Thoenen H. and Barde Y.-A. (1980) Physiology of the nerve growth factor. *Physiol. Rev.* **60,** 1284–1335.
13. Barde Y.-A. (1989) Trophic factors and neuronal survival. *Neuron* **2,** 1525–1534.
14. Hamburger V., Brunso-Bechthold J. K., and Yip J. W. (1981) Neuronal death in the spinal ganglia of the chick embyro and its reduction by nerve growth factor. *J. Neurosci.* **1,** 60–71.
15. Bothwell M. (1995) Functional interactions of neurotrophins and neurotrophin receptors. *Annu. Rev. Neurosci.* **18,** 223–253.
16. Oppenheim R. W. (1991) Cell death during development of the nervous system. *Annu. Rev. Neurosci.* **14,** 451–501.
17. Purves D. (1988) *Body and Brain. A Trophic Theory of Neural Connections.* Harvard University Press, Cambridge, MA.
18. Barde Y.-A., Edgar D., and Thoenen H. (1982) Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* **1,** 549–553.
19. Leibrock J., Lottspeich F., Hohn A., Hofer M., Hengerer B., Masiakowski P., et al. (1989) Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **341,** 149–152.
20. Snider W. D. (1994) Functions of the neurotrophins during development: what the knockouts are teaching us. *Cell* **77,** 627–638.
21. Ibáñez C. F. (1995) Neurotrophic factors: from structure-function studies to designing effective therapeutics. *TIBTECH* **13,** 217–227.

22. Hallböök F., Ibáñez C. F., and Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. *Neuron* **6,** 845–858.
23. Götz R., Köster R., Winkler C., Raulf F., Lottspeich F., Schartl M., and Thoenen H. (1994) Neurotrophin-6 is a new member of the nerve growth factor family. *Nature* **372,** 266–269.
24. Lai K.-O., Fu W.-Y., Ip F. C. F., and Ip N. Y. (1998) Cloning and expression of a novel neurotrophin, NT-7, from carp. *Mol. Cell. Neurosci.* **11,** 64–76.
25. Barbacid M. (1994) The trk family of neurotrophin receptors. *J. Neurobiol.* **25,** 1386–1403.
26. Chao M. V. (1992) Neurotrophin receptors: a window into neuronal differentiation. *Neuron* **9,** 583–593.
27. Lee K.-F., Li E., Huber L. J., Landis S. C., Sharpe A. H., Chao M. V., and Jaenisch R. (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell 69,* 737–749.
28. Davies A. M., Lee K. F., and Jaenisch R. (1993) p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. *Neuron* **11,** 565–575.
29. Barker P. and Shooter E. (1994) Disruption of NGF binding to the low affinity neurotrophin receptor p75LNTR reduces NGF binding to TrkA on PC 12 cells. *Neuron* **13,** 203–215.
30. Jing S., Tapley P., and Barbacid M. (1992) Nerve growth factor mediates signal transduction through trk homodimer receptors. *Neuron* **9,** 1067–1079.
31. Dobrowsky R., Werner M., Castellino A., Chao M., and Hannun Y. (1994) Activation of the sphingomyelin cycle through the low affinity neurotrophin receptor. *Science* **265,** 1596–1599.
32. Casaccia-Bonnefil P., Carter B. D., Dobrowsky R. T., and Chao M. V. (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature* **383,** 716–719.
33. Otten U., Ehrhard P., and Peck R. (1989) Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proc. Natl. Acad. Sci. USA* **86,** 10,059–10,063.
34. Thorpe L. W. and Perez-Polo J. R. (1987) The influence of nerve growth factor on the in vitro proliferative response of rat spleen lymphocytes. *J. Neurosci. Res.* **18,** 134–139.
35. Kimata H., Yoshida A., Ishioka C., Kusunoki T., Hosoi S., and Mikawa H. (1991) Nerve growth factor specifically induces human IgG4 production. *Eur. J. Immunol. 21,* 137–141.
36. Matsuda H., Coughlin M. D., Bienenstock J., and Denburg J. A. (1988) Nerve growth factor promotes human hemopoietic colony growth and differentiation. *Proc. Natl. Acad. Sci. USA* **85,** 6508–6512.
37. Brodie C. and Gelfand E. W. (1992) Functional nerve growth factor receptors on human B lymphocytes: interaction with IL 2. *J. Immunol.* **148,** 171–178.
38. Thorpe L. W., Werrbach-Perez K., and Perez-Polo J. R. (1987) Effects of nerve growth factor expression on interleukin-2 receptors on cultured human lymphocytes. *Ann. NY Acad. Sci. USA* **496,** 310–311.
39. Gee A. P., Boyle M. D. P., Munger K. L., Lawman M. J. P., and Young M. (1983) Nerve growth factor: stimulation of polymorphonuclear leukocyte chemotaxis in vitro. *Proc. Natl. Acad. Sci. USA* **80,** 7215–7218.
40. Kannan Y. H., Ushuo H., Koyama H., Okada M., Oikawa M., Yoshihara T., et al. (1991) 2.5S Nerve growth factor enhances the survival, phagocytosis, and superoxide production of murine neutrophils. *Blood* **77,** 1320–1325.
41. Boyle M. D. P., Lawman M. J. P., Gee A. P., and Young M. (1985) Nerve growth factor: a chemotactic factor for polymorphonuclear leukocytes in vivo. *J. Immunol.* **134,** 564–568.
42. Screpanti I., Meco D., Scarpa S., Morrone S., Frati L., Gulino A., and Modesti A. (1992) Neuromodulatory loop mediated by nerve growth factor and interleukin 6 in thymic stromal cell cultures. *Proc. Natl. Acad. Sci. USA* **89,** 3209–3212.
43. Gudat F., Laubscher A., Otten U., and Pletscher A. (1981) Shape changes induced by biologically active peptides and nerve growth factor in blood platelets of rabbits. *Br. J. Pharmacol.* **74,** 533–538.
44. Pearce F. L. and Thompson H. L. (1986) Some characteristics of histamine secretion from rat peritoneal mast cells stimulated with nerve growth factor. *J. Physiol.* **372,** 379–393.
45. Torcia M., Bracci-Laudiero L., Lucibello M., Rubartelli A., Cozzolino F., Aloe L., and Garaci E. (1996) Nerve growth factor is an autocrine factor for memory B lymphocytes. *Cell* **85,** 1–20.
46. Ehrhard P. B., Ganter U., Bauer J., and Otten U. (1993) Expression of functional *trk* protoonco-

gene in human monocytes. *Proc. Natl. Acad. Sci. USA* **90,** 5423–5427.
47. Ehrhard P. B., Erb P., Graumann U., and Otten U. (1993) Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proc. Natl. Acad. Sci. USA* **90,** 10,984–10,988.
48. Lambiase A., Bracci-Laudiero L., Bonini S., Bonini S., Starace G., D'Elios M. M., et al. (1997) Human CD4+ T cell clones produce and release nerve growth factor and express high-affinity nerve growth factor receptors. *J. Allergy Clin. Immunol.* **100,** 408–414.
49. Santambrogio L., Benedetti M., Chao M. V., Muzaffar R., Kulig K., Gabellini N., and Hochwald G. (1994) Nerve growth factor production by lymphocytes. *J. Immunol.* **153,** 4488–4495.
50. Melamed I., Kelleher C. A., Franklin R. A., Brodie C., Hempstead B., Kaplan D., and Gelfand E. W. (1996) Nerve growth signal transduction in human B lymphocytes is mediated by gp140 trk. *Eur. J. Immunol.* **26,** 1985–1992.
51. Aloe L. and Levi-Montalcini R. (1977) Mast cells increase in tissues of neonatal rats injected with nerve growth factor. *Brain Res.* **133,** 358–366.
52. Böhm A., Aloe L., and Levi-Montalcini R. (1986) Nerve growth factor enhances precocious differentiation and numerical increase in mast cells in cultures of rat splenocytes. *Accad. Naz. Lincei* **80,** 1–6.
53. Stead R. H., Bienenstock J., and Stanisz A. M. (1987) Neuropeptide regulation of mucosal immunity. *Immunol. Rev.* **100,** 333–359.
54. Marshall J. S., Stead R. H., McSharry C., Nielsen L., and Bienenstock J. (1990) The role of mast cell degranulation products in mast cell hyperplasia. I. Mechanism of action of nerve growth factor. *J. Immunol.* **144,** 1886–1892.
55. Aloe L. (1988) The effect of nerve growth factor and its antibody on mast cells in vivo. *J. Neuroimmunol.* **18,** 1–12.
56. Matsuda H., Kannan Y., Ushio H., Kiso Y., Kanemoto T., Suzuki H., and Kitamura Y. (1991) Nerve growth factor induces development of connective tissue-type mast cells in vitro from murine bone marrow cells. *J. Exp. Med.* **174,** 7–14.
57. Aloe L. and De Simone R. (1989) NGF primed spleen cells injected in brain of developing rats differentiate into mast cells. *Int. J. Dev. Neurosci.* **7,** 565–573.
58. Richard A., McColl S. R., and Pelletier G. (1992) Interleukin-4 and nerve growth factor can act as cofactors for interleukin-3-induced histamine production in human umbilical cord blood in serum-free culture. *Br. J. Haematol.* **81,** 6–11.
59. Welker P., Grabbe J., Gibbs B., Zuberbier T., and Henz B. M. (2000) Nerve growth factor-β induces mast-cell marker expression during *in vitro* culture of human umbilical cord blood cells. *Immunology* **99,** 418–426.
60. Bruni A., Bigon E., Boarato E., Mietto L., Leon A., and Toffano G. (1982) Interaction between nerve growth factor and lysophosphatidylserine on rat peritoneal mast cells. *FEBS Lett.* **138,** 190–192.
61. Sugiyama K., Suzuki Y., and Furuta H. (1985) Histamine-release induced by 7S nerve growth factor of mouse submandibular salivary glands. *Arch. Oral Biol.* **30,** 93–95.
62. Tomioka M., Stead R. H., Nielsen L., Coughlin M. D., and Bienenstock J. (1988) Nerve growth factor enhances antigen and other secretagogue-induced histamine release from rat peritoneal mast cells in the absence of phosphatidylserine. *J. Allergy Clin. Immunol.* **82,** 599–607.
63. Bischoff S. C. and Dahinden C. A. (1992) Effect of nerve growth factor on the release of inflammatory mediators by mature human basophils. *Blood* **79,** 2662–2669.
64. Otten U., Baumann J. B., and Girard J. (1984) Nerve growth factor induces plasma extravasation in rat skin. *Eur. J. Pharmacol.* **106,** 199–201.
65. Burgi B., Otten U. H., Ochensberger B., Rihs S., Heese K., Ehrhard P. B., et al. (1996) Basophil priming by neurotrophic factors. Activation through the trk receptor. *J. Immunol.* **157,** 5582–5588.
66. Leon A., Buriani A., Dal Toso R., Fabris M., Romanello S., Aloe L., and Levi-Montalcini R. (1993) Mast cells synthesize, store, and release nerve growth factor. *Proc. Natl. Acad. Sci. USA* **91,** 3739–3743.
67. Nilsson G., Forsberg-Nilsson K., Xiang Z., Hallböök F., Nilsson K., and Metcalfe D. D. (1997) Human mast cells express functional TrkA and are a source of nerve growth factor. *Eur. J. Immunol.* **27,** 2295–2301.
68. Tam S.-Y., Tsai M., Yamaguchi M., Yano K., Butterfield J., and Galli S. J. (1997) Expression

of functional TrkA receptor tyrosine kinase in the HMC-1 human mast cell line and in human mast cells. *Blood* **90,** 1807–1820.
69. Skaper S. D., Pollock M., and Facci L. (2001) Rat peritoneal mast cells differentially express and release active high molecular weight neurotrophins. *J. Neurochem.* **77**(Suppl. 1), 52.
70. Horigome K., Bullock E. D., and Johnson E. M. Jr. (1994) Effects of nerve growth factor on rat peritoneal mast cells. Survival promotion and immediate-early gene induction. *J. Biol. Chem.* **269,** 2695–2702.
71. Kawamoto K., Okada T., Kannan Y., Ushio H., Matsumoto M., and Matsuda H. (1995) Nerve growth factor prevents apoptosis of rat peritoneal mast cells through the trk proto-oncogene receptor. *Blood* **86,** 4638–4644.
72. Bullock E. D. and Johnson E. M. Jr. (1996) Nerve growth factor induces the expression of certain cytokine genes and bcl-2 in mast cells. Potential role in survival promotion. *J. Biol. Chem.* **271,** 27,500–27,508.
73. Sawada J., Itakura A., Tanaka A., Furusaka T., and Matsuda H. (2000) Nerve growth factor functions as a chemoattractant for mast cells through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. *Blood* **95,** 2052–2058.
74. Marshall J. S., Gomi K., Blennerhassett M. G., and Bienenstock J. (1999) Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. *J. Immunol.* **162,** 4271–4276.
75. Aloe L., Skaper S. D., Leon L., and Levi-Montalcini R. (1994) Nerve growth factor and autoimmune diseases. *Autoimmunity* **19,** 141–150.
76. Persson H., Ayer-Le Lievre C., Söder O., Villar M. J., Madis Mrtsis M., Olson L., et al. (1990) Expression of β-nerve growth factor receptor mRNA in Sertoli cells downregulated by testosterone. *Science* **247,** 704–707.
77. Lara H. E., McDonald J. K., and Ojeda S. R. (1990) Involvement of nerve growth factor in female sexual development. *Endocrinology* **126,** 364–375.
78. Spillantini M. G., Aloe L., Alleve E., De Simone R., Goedert M., and Levi-Montalcini R. (1989) Nerve growth factor mRNA and protein increase in hypothalamus in a mouse model of aggression. *Proc. Natl. Acad. Sci. USA* **86,** 8555–8559
79. Ojeda S. R., Hill D. F., and Katz K. H. (1991) The genes encoding nerve growth factor and its receptor are expressed in the developing female rat hypothalamus. *Mol. Brain Res.* **9,** 47–55.
80. Lathinen T., Soinila S., and Lakshmanan J. (1989) Biological demonstration of nerve growth factor in the rat pituitary gland. *Neuroscience* **30,** 165–170.
81. Missale C., Boroni F., Sigala S., Buriani A., Fabris M., Leon A., Dal Toso R., and Spano P. (1996) Nerve growth factor in the anterior pituitary: localization in mammotroph cells and cosecretion with prolactin by a dopamine-regulated mechanism. *Proc. Natl. Acad. Sci. USA* **93,** 4240–4245.
82. Borrelli E., Sawchenko P. E., and Evans R. M. (1992) Pituitary hyperplasia produced by ectopic expression of nerve growth factor. *Proc. Natl. Acad. Sci. USA* **89,** 2764–2768.
83. Dicou E., Lee J., and Brachet P. (1986) Synthesis of nerve growth factor mRNA and precursor protein in the throid and patathyroid glands of the rat. *Proc. Natl. Acad. Sci. USA* **83,** 7084–7088.
84. Otten U., Baumann J. B., and Girard J. (1979) Stimulation of the pituitary-adrenocortical axis by nerve growth factor. *Nature* **282,** 413–414.
85. Scaccianoce S., Cigliana G., Nicolai R., Muscolo L. A., Porcu A., Navarra D., et al. (1993) Hypothalamic involvement in the activation of the pituitary-adrenocortical axis by nerve growth factor. *Neuroendocrinology* **58,** 202–209.
86. Luppi P., Levi-Montalcini R., Bracci-Laudiero L., Bertolini A., Arletti R., Tavernari D., et al. (1993) NGF is released into plasma during human pregnancy: an oxytocin-mediated response? *NeuroReport* **4,** 1063–1065.
87. Missale C., Boroni F., Sigala S., Zanellato A., Dal Toso R., Balsari A., and Spano P. (1994) Nerve growth factor directs differentiation of the bipotential cell line GH-3 into the mammotroph phenotype. *Endocrinology* **135,** 290–298.
88. Missale C., Boroni F., Losa M., Giovanelli M., Zanellato A., Dal Toso R., et al. (1993) Nerve growth factor suppresses the transforming phenotype of human prolactinoma. *Proc. Natl. Acad. Sci. USA* **90,** 7961–7965.
89. Wolf D., Richter-Landsberg C., Short M. P., Cepko C., and Breakefield X. O. (1988) Retrovirus mediated transfer of beta-nerve growth factor into mouse pituitary line AcT-20. *Mol. Biol. Med.* **5,** 43–59.
90. Aloe L., Cozzari C., Calissano P., and Levi-Montalcini R. (1981) Somatic and behavioral

postnatal effects of fetal injections of nerve growth factor antibodies in the rat. *Nature* **291,** 413–415.
91. Johnson E. M. Jr., Osborne P. A., Rydel R. E., Schmidt R. E., and Pearson J. (1983) Characterization of the effects of autoimmune nerve growth factor deprivation in the developing guinea pig. *Neuroscience* **8,** 631–642.
92. Johnson E. M., Gorin P. D., Brandeis L. D., and Pearson J. (1980) Dorsal root ganglion neurons are destroyed by exposure in utero to maternal antibody to nerve growth factor. *Science* **210,** 916–918.
93. Yip H. K., Rich K. M., Lampe P. A., and Johnson E. M. Jr. (1984) The effects of nerve growth factor and its antiserum on the postnatal development and survival after injury of sensory neurons in rat dorsal root ganglia. *J. Neurosci.* **4,** 2986–2992.
94. Dicou E., Hurez D., and Nerrière V. (1993) Natural autoantibodies against nerve growth factor in autoimmune diseases. *J. Neuroimmunol.* **47,** 159–168.
95. Kostiza T. and Meier J. (1996) Nerve growth factors from snake venoms: chemical properties, mode of action and biological significance. *Toxicon* **34,** 787–806.
96. Burcham T. S., Sim I., Bolin L. M., and Shooter E. M. (1991) The NGF complex from the African rat Mastomys natalensis. *Neurochem. Res.* **16,** 603–612.
97. Aloe L., Alleva E., Böhm A., and Levi-Montalcini R. (1986) Aggressive behavior induces release of nerve growth factor from mouse salivary gland into the bloodstream. *Proc. Natl. Acad. Sci. USA* **83,** 6184–6187.
98. Maestripieri D., De Simone R., Aloe L., and Levi-Montalcini R. (1990) Social status and nerve growth factor serum levels after agonistic encounters in mice. *Physiol. Behav.* **47,** 161–164.
99. Aloe L., Bracci-Laudiero L., Alleva E., Lambiase A., Micera A., Tirassa P. (1994) Emotional stress induced by parachute jumping changes blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. *Proc. Natl. Acad. Sci. USA* **91,** 10,440–10,444.
100. Galli S. J. (1993) New concepts about the mast cell. *N. Engl. J. Med.* **328,** 257–265.
101. Roznieckí J. J., Dimitriadou V., Lambracht-Hall M., Pang X., and Theoharides T. C. (1999) Morphological and functional demonstration of rat dura mater mast cell-neuron interactions in vitro and in vivo. *Brain Res.* **849,** 1–15.
102. Johnson D. and Krenger W. (1992) Interactions of mast cells with the nervous system-recent advances. *Neurochem. Res.* **9,** 939–951.
103. Florenzano F. and Bentivoglio M. (2000) Degranulation, density, and distribution of mast cells in the rat thalamus: a light and electron microscopic study in basal conditions and after intracerebroventricular administration of nerve growth factor. *J. Comp. Neurol.* **424,** 651–669.
104. Olsson Y. (1968) Mast cells in the nervous system. *Int. Rev. Cytol.* **24,** 27–70.
105. Gordon J. R., Burd P. R., and Galli S. J. (1990) Mast cells as a source of multifunctional cytokines. *Immunol. Today* **11,** 458–464.
106. Purcell W. M. and Atterwill C. K. (1995) Mast cells in neuroimmune function: neurotoxicological and neuropharmacological perspectives. *Neurochem. Res.* **20,** 521–532.
107. Silver R., Silverman A.-J., Vitkovic L., and Lederhendler I. I. (1996) Mast cells in the brain: evidence and functional significance. *Trends Neurosci.* **19,** 25–31.
108. Hermes B., Welker P., Feldmann-Böddeker I., Krüger-Krasagakis S., Hartmann K., Zuberbier T, and Henz B. M. (2001) Expression of mast cell growth modulating and chemotactic factors and their receptors in human cutaneous scars. *J. Invest. Dermatol.* **116,** 387–393.
109. Tuveri M. A., Passiu G., Mathieu A., and Aloe L. (1993) Nerve growth factor and mast cell distribution in the skin of patients with systemic sclerosis. *Clin. Exp. Rheumatol.* **11,** 319–322.
110. Aloe L., Tuveri M. A., and Levi-Montalcini R. (1992) Nerve growth factor and distribution of mast cells in the synovium of adult rats. *Clin. Exp. Rheumatol.* **10,** 203–204.
111. Sanico A. M., Stanisz A. M., Gleeson T. D., Bora S., Proud D., Bienenstock J., et al. (2000) Nerve growth factor expression and release in allergic inflammatory disease of the upper airways. *Am. J. Respir. Crit. Care Med.* **161,** 1631–1635.
112. Bonini S., Lambiase A., Bonini S., Angelucci F., Magrini L., Manni L., and Aloe L. (1996) Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc. Natl. Acad. Sci. USA* **93,** 10955–10960.
113. Braun A., Lommatzsch M., and Renz H. (2000) The role of neurotrophins in allergic bronchial asthma. *Clin. Exp. Allergy* **30,** 178–186.

114. Kinkelin I., Mötzing S., Koltzenburg, and Bröcker E.-B. (2000) Increase in NGF content and nerve fiber sprouting in human allergic contact eczema. *Cell Tissue Res.* **302,** 31–37.
115. di Mola F. F., Friess H., Zhu Z. W., Koliopanos A., Bley T., Di Sebastiano P., et al. (2000) Nerve growth factor and Trk high affinity receptor (TrkA) gene expression in inflammatory bowel disease. *Gut* **46,** 670–678.
116. Kobayashi H., Yamataka A., Fujimoto T., Lane G. J., and Miyano T. (1999) Mast cells and gut nerve development: implications for Hirschsprung's disease and intestinal neuronal dysplasia. *J. Pediatr. Surg.* **34,** 543–548.
117. Garaci E., Caroleo M. C., Aloe L., Aquaro S., Piacentini M., Costa N., et al. (1999) Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. *Proc. Natl. Acad. Sci. USA* **96,** 14013–14018.
118. Simone M. D., De Santis S., Vigneti E., Papa G., Amadori S., and Aloe L. (1999) Nerve growth factor: a survey of activity on immune and hematopoietic cells. *Hematol. Oncol.* **17,** 1–10.
119. Xiang Z. and Nilsson G. (2000) IgE receptor-mediated release of nerve growth factor by mast cells. *Clin. Exp. Allergy* **30,** 1379–1386.
120. Yoshida K. and Gage F. H. (1992) Cooperative regulation of nerve growth factor synthesis and secretion in fibroblasts and astrocytes by fibroblast growth factor and other cytokines. *Brain Res.* **569,** 14–25.
121. Weskamp G. and Otten U. (1987) An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and peripheral tissues. *J. Neurochem.* **48,** 1779–1786.
122. Stead R. H., Kosecka-Janiszewska U., Oestreicher A. B., Dixon M. F., and Bienenstock J. (1991) Remodelling of B-50 (GAP-43)- and NSE-immunoreactive mucosal nerves in the intestines of rats with *Nippostrongylus brasiliensis. J. Neurosci.* **11,** 3809–3821.
123. Blennerhasset M. G., Tomioka M., and Bienenstock J. (1991) Formation of contacts between mast cells and sympathetic neurons *in vitro. Cell Tissue Res.* **265,** 121–128.
124. Waksman B. H. (1988) Autoimmunity in demyelinating diseases. *Ann. NY Acad. Sci.* **540,** 13–24.
125. Sant G. R. (1989) Interstitial cystitis: pathophysiology, clinical evaluation and treatment. *Urol. Ann.* **3,** 172–179.
126. Theoharides T. C. (1990) Mast cells: the immune gate to the brain *Life Sci.* **46,** 607–617.
127. Skaper S. D., Facci L., Romanello S., and Leon A. (1996) Mast cell activation causes delayed neurodegeneration in mixed hippocampal cultures via the nitric oxide pathway. *J. Neurochem.* **66,** 1157–1166.
128. Johnson D., Seeldrayers P. A., and Weiner H. L. (1988) The role of mast cells in demyelination. I. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P_2 can stimulate mast cell degranulation. *Brain Res.* **444,** 195–198.
129. Theoharides T. C., Baloyannis S. J., and Manolidis L. S. (1991) Activated rat peritoneal mast cells can cause syngeneic brain demyelination *in vitro. Int. J. Immunopath. Pharmacol.* **4,** 137–144.
130. Lambracht-Hall M., Dimitriadou V., and Theoharides T. C. (1990) Migration of mast cells in the developing rat brain. *Dev. Brain Res.* **56,** 151–159.
131. Lambracht-Hall M., Konstantinidou A. D., and Theoharides T. C. (1990) Serotonin release from rat brain mast cells *in vitro. Neuroscience* **30,** 199–207.
132. Theoharides T. C., Dimitriadou V., Letourneau R., Roznieckl J. J., Vliagoftis H., and Boucher W. (1993) Synergistic action of estradiol and myelin basic protein on mast cell secretion and brain myelin changes resembling early stages of demyelination. *Neuroscience* **57,** 861–871.
133. McGeer P. L. and McGeer E. G. (1995) The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res. Rev.* **21,** 195–218.
134. Hauser S. IL., Bhan A. K., Gilles F., Kemp M., Kerr C., and Weiner H. L. (1986) Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol.* **19,** 578–587.
135. Prineas J. W. and Wright R. G. (1978) Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab. Invest.* **38,** 409–421.
136. Sobel R. A., Blanchette B. W., Bhan A. K., and Colvin R. B. (1984) The immunopathology of experimental allergic encephalomyelitis: quantitative analysis of inflammatory cells *in situ. J. Immunol.* **132,** 2392–2401.
137. Olsson Y. (1974) Mast cells in plaques of multiple sclerosis. *Acta Neurol. Scand.* **50,** 611–618.
138. Toms R., Weiner H. L., and Johnson D. (1990) Indentification of IgE-positive cells and mast

cells in frozen sections of multiple sclerosis brains. *J. Neuroimmunol.* **30,** 169–177.
139. Brosnan C. F., Lyman W. D., Tansey F. A., and Carter T. H. (1985) Quantitation of mast cells in experimental allergic neuritis. *J. Neuropath. Exp. Neurol.* **44,** 196–203.
140. Askenase P. W. and Van Loveren H. (1983) Delayed-type hypersensitivity activation of mast cells by antigen-specific T-cell factors initiates the cascade of cellular interactions. *Immunol. Today* **4,** 259–264.
141. Kaplan A. P., Reddigari S., Baeza M., and Kuna P. (1991) Histamine releasing factors and cytokine-dependent activation of basophils and mast cells. *Adv. Immunol.* **50,** 237–260.
142. Frandji P., Oskeritzian C., Cacaraci F., Lapeyre J., Peronet R., David J. G., and Mecheri S. (1993) Antigen-dependent stimulation by bone marrow-derived mast cells of MHC class II-restricted T cell hybridoma. *J. Immunol.* **151,** 6318–6328.
143. Bracci-Laudiero L., Aloe L., Levi-Montalcini R., Buttinelli C., Schilter D., Gillessen S., and Otten U. (1992) Multiple sclerosis patients express increased levels of beta-nerve growth factor in cerebrospinal fluid. *Neurosci. Lett.* **147,** 9–12.
144. Mican J. M. and Metcalfe D. D. (1990) Athritis and mast cell activation. *J. Allergy Appl. Immunol.* **86,** 677–683.
145. Aloe L., Tuveri M. A., Carcassi U., and Levi-Montalcini R. (1992) Nerve growth factor in the synovial fluid of patients with chronic arthritis. *Arthritis Rheum.* **35,** 351–355.
146. Falcini F., Matucci Cerinic M., Lombardi A., Generini S., Pignine A., Tirassa P., et al. (1996) Increased circulating nerve growth factor is directly correlated with disease activity in juvenile chronic arthritis. *Ann. Rheum. Dis.* **55,** 745–748.
147. Aloe L., Tuveri M. A., and Levi-Montalcini R. (1992) Studies on carrageenan-induced arthritis in adut rats: presence of nerve growth factor and role of sympathetic innervation. *Rheumatol. Int.* **12,** 213–216.
148. Aloe L., Probert L., Kollias G., Micera A., and Tirassa P. (1995) Effect of NGF antibodies on mast cell distribution, histamine and substance P levels in the knee joint of TNF-arthritic transgenic mice. *Rheumatol. Int.* **14,** 249–252.
149. Bracci-Laudiero L., Aloe L., Levi-Montalcini R., Galeazzi M., Schilter J. L., and Otten U. (1993) Increased levels of NGF in sera of systemic lupus erythematosus patients. *NeuroReport* **4,** 563–565.
150. Bracci-Laudiero L., Lundeberg T., Stenfors C., Theodorsson E., Tirassa P., and Aloe L. (1996) Modification of lymphoid and brain nerve growth factor levels in systemic lupus erythematosus mice. *Neurosci. Lett.* **204,** 13–16.
151. Kurosawa M., Inamura H., Amano H., Kanbe N., Nagata H., Nagai H., et al. (1999) Nerve growth factor release with mast-cell-derived mediators in a patient with systemic mastocytosis after middle-wave ultraviolet irradiation. *Allergy* **54,** 994–998.
152. Treede R. D., Meyer R. A., Raja S. N., and Campbell J. N. (1992) Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog. Neurobiol.* **38,** 397–421.
153. Donnerer J., Schuligoi R., and Stein C. (1992) Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience* **49,** 693–698.
154. Woolf C. J., Safieh-Garabedian B., Ma Q. P., Crilly P., and Winter J. (1994) Nerve growth factor contributes to the generation of inflammatory sensory hypersensibility. *Neuroscience* **62,** 327–331.
155. Woolf C. J., Allchorne A., Safieh-Garabedian B., and Poole S. (1997) Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. *Br. J. Pharmacol.* **121,** 417–424.
156. Lewin G. R. and Mendell L. M. (1993) Nerve growth factor and nociception. *Trends Neurosci.* **16,** 353–359.
157. Petty B. G., Cornblath D. R., Adornato B. T., Chaudhry V., Flexner C., Wachsman M., et al. (1994) The effect of systematically administered recombinant human nerve growth factor in healthy human subjects. (1994) *Ann. Neurol.* **36,** 244–246.
158. Bennett G., al-Rashad S., Hoult J. R., and Brain S. D. (1998) Nerve growth factor induced hyperalgesia in the rat hind paw is dependent on circulating neutrophils. *Pain* **77,** 315–322.
159. Andreev N. Yu, Dimitrieva N., Koltzenburg M., and McMahon S. B. (1995) Peripheral administration of nerve growth factor produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones. *Pain* **63,** 109–115.

160. Bergmann I., Reiter R., Toyka K. V., and Koltzenburg M. (1998) Nerve growth factor evokes hyperalgesia in mice lacking the low-affinity neurotrophin receptor p75. *Neurosci. Lett.* **255,** 87–90.
161. Davis B. M., Lewin G. R., Mendell L. M., Jones M. E., and Albers K. M. (1993) Altered expression of nerve growth factor in the skin of transgenic mice leads to changes in response to mechanical stimuli. *Neuroscience* **56,** 789–792.
162. Stucky C. L., Koltzenburg M., Schneider M., Engle M. G., Albers K. M., and Davis B. M. (1999) Overexpression of nerve growth factor in skin selectively affects the survival and functional properties of nociceptors.*J. Neurosci.* **19,** 8509–8516.
163. McMahon S. B., Bennett D. L., Priestley J. V., and Shelton D. L. (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. *Nat. Med.* **1,** 774–780.
164. Koltzenburg M., Bennett D. L., Shelton D. L., and McMahon S. B. (1999) Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin. *Eur. J. Neurosci.* **11,** 1698–1704.
165. Woolf C. J., Ma Q. P., Allchorne A., and Poole S. (1996) Peripheral cell types contributing to the hyperalgesic action nerve growth factor in inflamation. *J. Neurosci.* **16,** 2716–2723.
166. Michael G. J., Averill S., Nitkunan A., Rattray M., Bennett D. L., Yan Q., and Priestley J. V. (1997) Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *J. Neurosci.* **17,** 8476–8490.
167. Shu X. Q., Llinas A., and Mendell L. M. (1999) Effects of TrkB and trkC neurotrophin receptor agonists on thermal nociception: a behavioral and electrophysiological study. *Pain* **80,** 463–470.
168. Carroll P., Lewin G. R., Koltzenburg M., Toyka K. V., and Thoenen H. (1998) A role for BDNF in mechanosensation. *Nat. Neurosci.* **1,** 42–46.