Biomedical Reviews 1999; 10: 3-14.
Dedicated to Rita Levi-Montalcini

ISSN1310-392X

NERVE GROWTH FACTOR: BASIC FINDINGS AND CLINICAL TRIALS

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The nerve growth factor is the first-discovered and best-characterized member of the family ofneurotrophins. In the introduction of this article we present a briefbiographic view of past andpresent studies of Rita Levi-Montalcini on nerve growth factor: Further, the article focuses onpleiotropic activities of nerve growth factor, exerting on various cell types, including cells of nervous, immune and endocrine system. Implications of these actions of nerve growth factor in the pathogenesis of neurological diseases, autoimmune-inflammatory diseases, allergic diseases, lymphoproliferative diseases, atherosclerotic cardiovascular disease, and neurotrophic corneal ulcers are outlined. ***Biomed Rev 1999; 10: 3-14.***

INTRODUCTION

Rita Levi-Montalcini received her medical degree from the Faculty of Medicine at the University of Turin, Italy, in 1936. In the early postgraduate years, first at the University of Turin with Professor Giuseppe Levi and then at home in spite of severe personal restrictions and difficulties due to racial discrimination during World War II, she carried out studies on the neurogenesis of the cervical and thoracic ganglia of chick that takes place during prenatal development. The results of these studies came to the attention of Viktor Hamburger, a leading scientist at Washington University of St. Louis, MO, USA, who invited her to St Louis to continue these studies and to characterise the effect of wing or limb extirpation on the development of motor and sensory neurons of chick embryos. This invitation marked the beginning of a long and fruitful scientific collaboration between Levi-Montalcini and Hamburger and led to the discovery of the nerve growth factor (NGF). In the following years, she studied the mechanisms of cell migration and neuronal differentiation in the central nervous system (CNS) of developing birds. The results she obtained on transitional morphology and on cell survival or death occurring during nervous system development most probably represent the first and best documented contribution to the knowledge in the field of experimental neuroembryology of what is now more correctly termed apoptosis or programmed cell death.

The NGF story started in 1949 when V. Hamburger showed Levi-Montalcini the results of one of his postgraduate students, Elmer Bueker, who observed that after the implantation of a small fragment of amalignant mouse tumor into the body wall of 3-day chick embryos, sensory fibres invaded the mouse tumor. Bueker hypothesised that this effect was due to the fact that the rapidly expanding tumor offered the possibility to sensory fibres to branch in a much larger field than the embryonic tissues replaced by the neoplastic cells. This hypothesis did not convince Rita Levi-Montalcini, who hypothesized that the mouse sarcoma tissues 180 and 37, when transplanted into a chick embryo, produced and released a diffusible agent that stimulated growth and the differentiation of developing nerve cells. Using *in vitro* methodologies, she demonstrated that these tumor tissues produced and released a molecule that was able to stimulate neurite outgrowth of sensory and sympathetic neurons. In 1952, she gave this substance the name of nerve

Received 15 July 1999 and accepted 22 September 1999.
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growth factor. In the following years, in collaboration with Stanley Cohen, cowinner of the Nobel Prize in 1986, Levi-Montalcini identified in a protein fraction the agent released by the tumor that promoted the effect on embryonic sensory and sympathetic ganglia. These studies led to the discovered that NGF was stored in snake venom and in the mouse submaxillary salivary gland and that this gland produced the largest available source of NGF. This latter finding not only made a better biochemical characterisation of NGF possible, but also that of its biological activities on neonatal and adult mammalians and its purification.

During the early 1950's, Levi-Montalcini, Professor at the Department of Zoology at the Washington University in St Louis, intensified *in vivo* and *in vitro* studies of NGF on small rodents, while first S. Cohen and later P. U. Angeletti investigated the chemical and biochemical properties of NGF and both devised a methodology of purifying large quantities of NGF from both the snake venom and the mouse salivary gland. The availability of large amounts of NGFin the mouse salivary gland provided an additional opportunity to carry out numerous and collaborative *in vivo* studies and to investigate the structural, biochemical and pharmacological action of NGF on sympathetic and sensory nerve cells. Moreover, the large amounts of purified NGF also led to the possibility of producing anti-NGF antibodies and of further characterising the effects of NGF on the development of the peripheral nervous system (PNS) by injecting this antibody into newborn rodents. The immunodepri vation of NGF through exogenous administration of NGF-antibodies to these rodents and the observation that inhibition of circulating NGF levels results in death of NGF-target cells described in the late 1960's, became known as immunosympathectomy. In 1961, Levi-Montalcini established a small neurobiological unit in Rome, first at the Superior Health Institute and in 1970, a larger group at the National Research Council, where she became director of the newly formed Institute of Cell Biology, while in 1986 she founded the Institute of Neurobiology.

The first time one of us (LA) met Rita Levi-Montalcini was in 1968 at the Superior Health Institute during her three months working visit in Rome, when I was working on the structural organisation of the epidermis of the insect *Locusta migratoria* and on *Periplaneta americana.* I spent the first four years of my scientific experience with Levi-Montalcini working on the development of the PNS and CNS of cockroaches and on the identification of molecules endowed with neurite growth-promoting activity on isolated nerve cells of the same or other insects. After two-three years of intense studies on cockroach neurogenesis, we were very close to the identification of the biological mediators present in the foregut of *Periplaneta americana.* Because, the quantity of tissue storing this molecule was very small and recombinant methods were not yet available, Levi-Montalcini decided to give up to idea of isolating and purifying this unknown invertebrate neurotrophic factor.

Looking back, the scientific years spent working on the insect nervous system can be considered an original contribution to the field of invertebrate neurobiology. Surprisingly, these findings passed almost unnoticed by most of the scientific community.

After 1972, Levi-Montalcini returned to NGF studies. The first approach to the renewed interest in this neurotrophin was the study of NGF on chemical and surgical axotomized peripheral neurons. Eugene Gene Johnson, a young pharmacologist working at Washington University Medical School in St. Louis, MO, USA, joined our group and was introduced to the NGF field. These collaborative studies produced the first consistent evidence that NGF can prevent injury or even death of peripheral neurons exposed to sympatholitic drugs. During these same years, numerous other groups working in the USA and in Europe showed great scientific interest in NGF studies. The increased attention to this molecule brought to light original findings regarding its spectrum of action on both the developing and the adult CNS, as well as on cells of the endocrine and immune system. Meanwhile, other investigations led to the identification of NGF receptors (NGFR), protein sequences, gene localisation and to the discovery of a new class of neurotrophins belonging to the NGF families, as well as to a variety of approaches to test the potential clinical use of NGF (see other articles in this volume of *Biomedical Reviews).*

In 1977, Levi-Montalcini published the first indication that NGF acts on cells of nonneuronal origin. Subsequently, other investigators confirmed and extended this observation and provided additional evidence that other cells of the immune system are receptive to the action of NGF. More recent studies carried out by Levi-Montalcini's group demonstrated that NGF is also involved in the regulation of memory B cells and that NGF plays an equally important role in the neuronal cell population of the CNS controlling the activation of the neuroendocrine axis. Other studies carried out in this same Institute showed that NGF is also involved in neuroendocrine responses, as revealed by findings that the circulating and brain NGF levels undergoes significant changes following aggressive behavior and in states of emotional anxiety. These findings lead to the hypothesis that NGF might be implicated in homeostatic interaction and in the pathogenesis of autoimmune inflammatory disorders. In the last ten years murine and recombinant NGF received clinical attention from neurologists who began to utilize NGF and other neurotrophins to induce recovery from neuropathies of the CNS and PNS. Additionally, a collaborative investigation between Institute of Neurobiology, the University of Rome, Tor Vergata, and the division of Ophthalmology, Hospital of Venice also provided a clear clinical evidence that NGF can be used in human neurotrophic corneal ulcers.

THE NGF DISCOVERY

The NGF was discovered in the early 1950's for its properties of stimulating growth and differentiation of peripheral sen-

sory and sympathetic neurons (1,2). It represents the first isolated and best-characterized member of a growing family of neurotrophins, which includes brain-derived neurotrophic factor and neurotrophins 3-5 (3). In recent years, numerous studies have provided evidence that the effects of NGF are not limited to the PNS (4,5) but it also act on cells of the CNS and on cells of the endocrine and immune systems (6). NGF is synthesized and released by a variety of nonneuronal and neuronal cells, though the largest amount of this factor is produced in the salivary gland (SG) of adult male mice which still is considered the best available source of NGF. NGF is also produced by guinea pig prostate and human skin (1,7,8; 9, this volume of *Biomedical Reviews).* The NGF isolated and purified from male mice SG (2.5S) is a dimer of two identical subunits linked together by noncovalent bonds and with a molecular weight of about 26 kD (10). The amino acid sequence and primary structure of this neurotrophin has been characterized (11) and indicates that NGF is a highly conserved molecule that shares a great homology within different species (12). Molecular studies have revealed that the human gene for NGF is located on the proximal short arm of chromosome 1 (13,14).

The levels of NGF not only change with age, but also during neuroendocrine dysregulations (15-18), following neurological insults (19-20) and during autoimmune and allergic diseases (21 - 26). The elevated levels of NGF are often associated with an increase of proinflammatory cytokines, particularly, interleukin-1 beta (IL-1 p^{1}) and tumor necrosis factor-alpha (TNF-cc) (27-30).

MERVE GROWTH FACTOR AMD THE NERVOUS SYSTEM

During development, NGF is known to promote differentiation, growth, and survival of central and PNS neurons (1,5,6). These biological activities are mediated by the NGF binding to two receptors: TrkA, a high-affinity receptor tyrosine kinase, and p75NGFR, a low-affinity receptor which can also bind the other neurotrophins ($p75^{NTR}$) (31-33). In the PNS during postnatal and adult life, one key effect of NGF is to stimulate neurite regeneration of injured nerve cells, regulate synthesis and release of neurotransmitters and neuropeptides (34,35), modulate peripheral pain responses (36,37), and is involved in peripheral inflammatory responses (38-40). In the CNS, the highest amounts of NGF are produced in the cortex and in the hippocampus which are the main target areas for cholinergic neurons localized in the nucleus basalis magnocellularis and medial septum, respectively (5,6,41 -43). Administration of NGF into the brain prevents the degeneration of lesioned cholinergic neurons, augments the activity of nonlesioned cholinergic neurons, and ameliorates spatial impairments in aged rats (44,45). Moreover, intracerebral implantation of NGF-secreting cell lines has also been shown to promote neuronal survival following experimental lesions (46). Clinical studies published in the last ten years seem also to support the hypothesis that NGF, along with other neurotrophins, may play a pivotal role in human forebrain cholinergic deficits, including aging and Alzheimer's disease (AD) (47-51).

Since one of the major limitations in the use of NGF in brain pathologies is its large molecular size and its difficulty in crossing the blood-brain barrier, it was recently suggested for its utilization, that NGF could be conjugated to transferrin receptor antibody (52), or alternatively to use pharmacological agents which act on brain NGF-producing cells (53, also this volume *of Biomedical Reviews).* There is also the possibility that drugs, which stimulate the release of NGF produced by resident cells, including mast cells (MC), may be potential useful to enhance the level of NGF in pathologies associated with the low availability of NGF. This latter aspect is currently under investigation in our laboratory.

Recent studies have also shown that NGF is present in the hypothalamus (54-57) and participate in stress responses (15,17,18) and in neuroendrocrine functions (58,59).

NGF IN NEUROIMMUNE AMD IMMUNE RESPONSES

The first evidence that NGF acts on cells of the immune system lineage was published 1977 by Aloe and Levi-Montalcini who showed that injection of NGF in newborn rats caused an increase in the number and size of MC (60). This observation was confirmed and extended in later years by showing that NGF-primed spleen cells, transplanted into the brain ventricles of donor rats, differentiate into MC (61). MC express NGFR (61-64) and *in vitro* administration of NGF induces MC degranulation and histamine release (61,62,64-66) increasing their survival (63, 66). An important piece of evidence indicating a role of NGF in immune cell survival was published a few years ago. It was shown that NGF prevents programmed cell death (apoptosis) and promotes cell differentiation in developing chick bursal cells (67,68). Recently, it was reported that NGF could support long term growth of human haemopoietic cells (69). NGF has a dose dependent influence on the survival of mature, differentiated cells of myeloid lineage such as eosinophils (70) and neutrophils (71) and in these latter cells, the viability sustaining activity of NGF has been attributed to its effect on apoptosis (72). More recently it has been reported that NGF is a survival factor for memory B cells, both in humans and mice (73). NGF is endogenously produced by B cells and the treatment with neutralizing anti-NGF antibodies induced the disappearance of bcl-2 protein and massive DNA fragmentation, mainly affecting the survival of the IgG or IgA positive cells *in vitro* and *in vivo* (73).

Besides these activities, there is ample evidence that the NGF-NGFR system is able to exert a wide variety of *in vitro* effects on immune cells. NGF acts as a colony-stimulating factor for human and murine myeloid progenitor cells (74,75). It affects specifically basophilic differentiation (76), induces me-

diator release from basophils, eosinophils and neutrophils (71,77,78), acts as a chemoattractant for polymorphonuclear leukocytes (79,80), and enhances neutrophil phagocytosis (71). Studies on mature lymphoid cells demonstrated that NGF has a dose dependent proliferative effect on both B and T cells (81,82) and causes differentiation of B cells into immunoglobulin-secreting plasma cells (82). NGF stimulates B lymphocytes to produce IgM, IgA, and IgG (82-85) and is also able to induce high-affinity interlukin-2 receptors on human peripheral blood mononuclear cells (84,86).

All this *in vitro* data indicates that NGF is involved in the modulation of immune cell development and differentiation. This hypothesis is supported by *in vivo* studies showing NGFR expression on stromal and immune cells within lymphoid organs (87-90). We have demonstrated that the thymus is able to produce and store NGF, that thymic cells express both low- and high- affinity NGFR, and that these cells produce high levels of NGF both in prenatal and early postnatal life (90a).

The effect of NGF on peripheral lymphocytes may be also mediated by centrally produced NGF, since intracerebral injection of this molecule induces proliferation of splenocytes (91). Data from our laboratory and others indicate that several autoimmune inflammatory diseases are characterized by an altered concentration of circulating NGF levels (21,26,92). Thus NGF is involved in the mechanisms related to regulation of immune cell proliferation, differentiation and activation. The fact that cytokines are known to affect NGF synthesis (27-30), differences in tissue cytokines and NGF expression, either alone or in combination, may be functionally correlated in these pathologies. In at least one case, however, anti-NGF antibodies were shown to prevent the wasting induced by TNF-a in transgenic arthritic mice over expressing this cytokine (93). TNF-a is produced and secreted by numerous cells including MC (94). However, whether the increase in NGF is a cause or a result of these immune processes remains to be elucidated. A variety of studies in the last fiftheen years have focused their attention on the possible functional significance of NGF-MC interaction and more recently, MC and TNF-a release. MC are immune cells that are predominantly observed in perivascular locations in mucosal and serosal tissues (94,95). They are also resident within the normal nervous system, and are often observed in close apposition to neurones in a variety of peripheral tissues (94,96). MC are classically associated with the hypersensitivity reactions involving the interaction of allergens with cell-fixed IgE (94,96,97). Although their role in anaphylaxis has been largely elucidated, research on these enigmatic cells has recently gained great interest after the observations that MC respond functionally *(via* specific receptors) to an enormous range of neuroactive compounds including NGF, derived from both the CNS and PNS (60,66,98). Considerable evidence published by numerous investigators indicated that MC and their endogenous products were strongly implicated not only in the classical immune processes, but also in neurophysiological and neuropathological events. MC are implicated in inflammatory and/or autoimmune diseases, such as multiple sclerosis, through the release of biologically active compounds, including NGF (96,97,99). Indirect evidence supporting the hypothesis of apotential functional role of NGF-MC interaction is suggested by recent clinical observations of AD, a neuropathological disorder which seems to be associated not only with severe neurological deficits and loss of cognitive functions (41-43,48,49) but also with altered immunological responses (100-103). The notion that inflammatory mediators are involved in AD was strengthened by observations of patients affected by rheumatoid arthritis who undertook a treatment with a nonsteroidal antiinflammatory drug over long periods of time, and displayed reduced incidence of this disease (101 -103). If one considers that NGF *(i)* exerts powerful activity on basal forebrain cholinergic neurons, *(ii)* prevents or reduces the neurological deficits if administered to AD patients and improves some neuropathological alterations and progressive loss of cognitive function associated with degeneration of brain neurons, *(Hi)* is elevated in inflammatory conditions, and *(iv)* proinflammatory cytokines, such as IL-1 (3 and TNF-a, promote NGF synthesis, the possibility exists that NGF released by proinflammatory cells may be functionally linked to the neuroinflammatory conditions described in AD patients (22,101,102). The possibility also exists that proinflammatory compounds released by MC following exposure to NGF, or NGF itself, may be implicated in promoting or exacerbating the inflammatory responses although, this seems unlikely since exogenous administration of NGF in developing or adult rats does not induce inflammation (21,104). It would be extremely important, therefore, to investigate whether any correlation exists among the distribution and/or activation of brain MC, NGF secretion, and AD.

Recent studies carried out in collaboration with Sergio Bonini of the Department of Allergology and Clinical Immunology, second University of Naples provided the crucial evidence that circulating NGF levels are enhanced in humans with allergic diseases (25). These studies revealed that NGF increases in patients with asthma, rhinoconjunctivitis, and urticaria-angioedema as compared to controls, while patients affected with more than one allergic diseases express higher NGF values than those with a single disease. The fact that NGF levels correlated with total IgE antibody liter further support the hypothesis of a link between NGF and allergic responses. A key question raised by these observations is whether in allergic responses, activated MC or other proinflammatory cells produce the presence of circulating NGF. Eosinophils are the predominant inflammatory cells of the late phase and chronic development of allergic inflammation that along with MC and Th2 cells, play a central role in the pathogenesis of allergic diseases. Collaborative studies with Francesca Levi-Schaffer from the Hebrew University of Jerusalem indicate that NGF can induce circulating human eosinophils to release important proinflammatory

mediators. This release seems not to be necessary linked to a cytotoxic effect of NGF on these cells, since no significant increase in the mortality rate of eosinophils was observed (70). These studies also demonstrated that eosinophils contain variable levels of NGF protein and $mRNA^{NGF}$, suggesting that eosinophils have the ability to produce and store NGF (105). Using an animal model of allergy it has been recently demonstrated that allergic airway inflammation in adult rodents is accompanied by an enhanced local NGF production (106). To gain additional information regarding the role of overespression of circulating NGF and allergic responses and better understand mechanisms of NGF-MC interaction, we have recently investigated the effect of highly purified NGF on the behavior of MC in nasal epithelia and lungs.

There is clinical evidence that overly fearful youngsters are at risk of later emotional distress and allergic disorders, and other clinical observations indicate a close relationship between psychological factors and allergy (107). A significant number of patients displaying anxious, depressive or stress-related behavior seems to suffer more frequently from allergic diseases when compared to healthy subjects (107). Because psychological factors can cause improvement or worsening of the allergic conditions, as well as the release of NGF, one may wonder whether a functional link exists between certain psychosocially stressful events, MC activation and NGF release. As previously reported the levels of circulating NGF, released during stress in male mice are correlated to the number of fighting episodes (15,17). In a subsequent study, it became evident that the increase of NGF in the bloodstream was not simply associated with the expression of aggressive behavior because sera of the mice repeatedly experiencing defeat and submission contained higher NGF levels than those dominating attacking animals (108). Those stimuli of a psychological nature, most likely associated with anxiety, also trigger the synthesis and release of NGF. This hypothesis is suggested by subsequent studies obtained in humans that anxiety induced by alcohol or heroin withdrawal also enhanced blood NGF levels (109). Drugs, which induced sedation, lowered the basal amount of these factors (110). Though the functional significance of elevated levels of NGF in stress-related events remains unclear, it is possible the NGF produced and released by MC is involved in the protection of NGF target cells from potential toxic insults. Thus, MC degranulation and high circulating levels of NGF occurring during allergy could function as a general alerting signal utilized by the organism in settings of stress and anxiety to "prime" the immune system towards external noxious perturbation. Altogether, these observations suggest that beyond its classical role in allergic mechanisms and defense against parasites, MC should be viewed as an important key player in other biological reactions, including neuroimmune regulated physiological processes (96,111).

NGF AND AUTOIMMUNE DISEASES

Several *in vitro* and *in vivo* studies published in the last few years have provided clear evidence that numerous cells of the immune system not only produce NGF (67,82,112,113), but are also receptive to the action of NGF both under normal and pathological conditions (45,114,115). The key *in vivo* 'data suggesting a role of NGF on these cells is that certain autoimmune inflammatory diseases are characterized by an activation of immunocytes and a significant alteration in the basal NGF levels. Alteration of basal NGF levels were found in the synovium of patients affected by rheumatic arthritis (22), knee joints of pharmacological-induced arthritis in rodents (92), cerebrospinal fluid of patients affected by multiple sclerosis (23), plasma of patients affected by lupus erythematosus (24), and the skin of patients with systemic sclerodermia (116). Moreover, studies carried out on animal models have shown that in numerous autoimmune inflammatory diseases, there is a close correlation between the inflammatory conditions and a variation in the constitutive level of NGF (25,26,117). Indeed, in mice affected by lupus erythematosus, the levels of NGF increase in the spleen, kidney and plasma and decrease in the CNS (118). Therefore, a relevant question raised by these observations is whether the changes of NGF levels found in these autoimmune diseases are due to an increase of NGF synthesis, to a low utilization or to an altered turnover of this factor by its target cells. In animal models of inflammatory diseases, the increase of NGF protein is associated with an increased expression of $mRNA^{NGP}$ and NGFR (119), making it highly probable that these events are related to a functional upregulation of NGF.

The elevated levels of NGF in some autoimmune diseases (21), is also associated with an increase in both brain and synovial MC (22,104,120). As indicated above, MC are able to produce NGF (112), are receptive to the action of NGF (60,120) and can release numerous proinflammatory mediators, allowing the possibility that NGF-MC interaction may be associated with inflammatory response dysregulations. The fact that other forms of homeostatic alterations, such as aggressive behavior, stress induced by alcohol or heroin withdrawal, and parasite infection also result in an increase of circulating NGF (15,109, 122), suggests that NGF may be involved in mechanisms correlated with preventing and repairing possible damage to NGF target cells. It has been reported that low levels of or the absence of circulating NGF can also characterize certain autoimmune diseases and, curiously, by a concomitant increase of NGF antibody (123). This apparent contradictory observation may be due to the fact that chronic exposure of supranormal amounts of NGF in the bloodstream can generate production of autoantibodies against NGF. In fact, evidence supporting this hypothesis was recently obtained in our laboratory in chronically-stressed mice (124). It was observed that aggressive behavior in mice, which induces a massive discharge of the endogenous release of NGF in the bloodstream, leads to the

production of NGF autoantibodies and a significant decrease of peripheral sympathetic innervation.

NGF RECEPTORS AS A POTENTIAL MARKER OF IMMUNE DISORDERS

Considerable evidence published in the last ten years indicates that NGF is produced and released by several cells belonging to the immune system and that cells of the immune system lineage are receptive to the action of NGF. Recent studies demonstrated that NGF is produced by bone marrow derived stromal cells (69), and that erythroleukemia cells express NGFR (125) thus indicating that NGF can influence early hematopoiesis. Moreover, detailed immunocytochemical analysis carried out on normal and malignant human tissues indicated that lymphomas, including one classified as a malignant lymphomas of B cell type, as well as in Hodgkin's diseases, overexpress NGFR (126). Surprisingly, no attempt has been made to explore the influence of the pharmacological treatment on NGF circulating levels or on the NGFR expression in peripheral cells or in lymphoid tissues in these diseases. Using peripheral blood cells of patients affected by chronic myeloproliferative disorders, we found that both the basal plasma NGF level and the distribution of NGFR in these cells undergo changes during the course of the disease. For example, we found a significant increase of NGFR expression on mononucleated cells from patients affected by chronic myelogenous leukemia and an altered plasma concentration of NGF (127). This study also showed that drugs, which are routinely used in therapy (such as hydroxyurea), influence both NGF and NGF receptor. These preliminary observations raise the question as to whether either NGF or NGFR may be potentially useful as a marker for some differentiative step of the haemapoietic stem cells or for the analysis of cell deficits. Based on findings obtained with the human neuroblastoma cells indicating that the presence or absence of NGFR a valuable marker for the clinical outcome of this neurological disease (128), the possibility that NGF/receptor expression may be of clinical use for establishing a better prognosis cannot be excluded.

MGF IN CARDIOVASCULAR DISEASE

Because NGF is known to play a crucial role in PNS and in smooth muscle innervation, a question we asked as to whether NGF is involved in the pathogenesis of atherosclerosis. We recently addressed this issue in collaborative studies between George Chaldakov, Medical University, Varna, Bulagaria, and the Institute of Neurobiology, CNR, Rome, Italy. These studies indicate that NGF levels, p75NGFR immunoreacti vity, and MC number undergo significant alteration in human atherosclerotic coronary arteries and surrounding subepicardial adipose tissue, suggesting a role of neuroimmune mediators in the process of pathogenesis of atherosclerotic cardiovascular disease (129, this volume *of Biomedical Reviews).*

NGF AMD HEUROTROPHIC OCULAR ULCER

Corneal healing deficit is the major cause of treatment failure and visual impairment in ocular surface pathology. In these conditions, a persistent epithelial defect may progress into a corneal stromal ulcer, leading to ocular perforation with subsequent visual loss (130). Several noninfectious ocular and systemic diseases may also lead to corneal epithelial damages. Likewise, patients suffering from disorders induced by fifth nerve palsy, postviral infections, chemical burns, corneal surgery, topical anaesthetic abuse, neurotrophic keratitis, diabetes, and multiple sclerosis very often display severe corneal lesions (131). All these diseases are characterized by a deficit of the corneal sensitivity innervation with frequently associated progressive corneal damage. Unfortunately, up to date, no treatment is available which normalizes these pathological changes and thus, the final outcome can frequently be the loss or severe impairment of visual function. We have recently reported that patients affected by corneal ulcer treated for 2-6 weeks with topical application of NGF display corneal healing beginning two days after exposure to this molecule. The effect is not impaired by the severity of corneal ulcer, depth of the stroma lesion, or by the clinical history of the patients, since the treatment with NGF is equally effective. Also, none of the patients with NGF showed systemic or ocular side effects during the NGF treatment or during the follow up period which lasted for over two years (131,132). The restored corneal sensitivity persisted for months after NGF treatment was discontinued, suggesting that this factor may have provided a long-term benefit to sensory innervation. This finding is consistent with the knowledge of the pathophysiological role of sensory innervation in corneal wound healing. The cornea is virtually a vascular tissue, with the most dense innervation in the human body (40 times more than the tooth pulp and 400 times more than skin). Thus, any inflammatory reaction and subsequent healing process undergone is highly controlled by this innervation (5). Experimental observations confirm that corneal nerve damage induce severe alterations in the metabolism and vitality of the epithelium, and clinical evidence shows that surgical (such as in trigeminal manipulation or penetrating keratoplasty) or chemical (such as after local anaesthetic abuse) damage of the corneal innervation induces an impairment of epithelial healing and the development of trophic ulcers (133). The effect of NGF on human neurotrophic corneal ulcer is mediated by high affinity NGF, since the ocular surface express high-affinity NGFR (134). Specifically, NGFR have been found in basal epithelial cells and in stromal cells. These findings suggest therefore that NGF mediated its action through the proliferation and differentiation of corneal epithelial cells. The present data are in line with the current pathogenic

hypothesis for neurotrophic ulcer. It has been thought that corneal nerves release a trophic factor, absent or decreased in neurotrophic ulcer, which stimulates the corneal epithelium to heal. The finding that exogenous NGF restored corneal integrity and sensitivity suggests that the progressive corneal damage observed in some cases of keratitis associated with sensory nerve deficit could originate from a deficit of endogenous NGF in the corneal microenvironment. This NGF deficit hypothesis is in agreement with the effects of this factor observed in other biological systems. Indeed, NGF treatment induces a prompt restoration of corneal ulcer, an absence of relapse of the disease and a persistence of corneal sensitivity without any local or systemic side effects.

CONCLUSION

We have briefly presented past and recent data supporting the hypothesis that in addition to its role as a neurotrophic regulator, NGF displays immunologic and hematological effects, and is involved in inflammatory responses. We have also presented and discussed current knowledge about the key role played by MC in these events. The studies on NGF-MC interaction led to the observation that allergic responses in humans are also characterized by the release into the bloodstream of NGF and that Th-2 cells, which play a pivotal role in allergy, are NGF responsive and NGF-producing cells (25,113). Most, if not all available data on the nervous system indicated that NGF is involved either in preventing or reducing the neuronal damages both in developing and mature nerve cells. There is evidence in animal models, however, that NGF through its effect on neuropetide synthesis and release, enhances hyperalgesia and inflammatory responses. It should be mentioned that to induce hyperalgesia in humans, higher levels of NGF than those observed in allergy patients are required (38). However, injection of highly purified NGF does not induce joint inflammation (92). Nonetheless, the possibility that high levels of NGF can induce undesired effects cannot be excluded. Altogether, the current data favors the hypothesis of a regulatory role of NGF on cells of the immune system. Additional investigations into the different types of allergic responses and changes in basal NGF levels may also provide a better understanding of the NGF role in the mechanisms involved in allergic and/or inflammatory diseases. Table 1 presents a selected list of NGF-related non-neurological diseases.

Table 1. Nonneurological diseases and basal NGF alteration

Humans

Systemic sclerosis*	Kawasaki disease
Juvenile chronic arthritis*	Giant cell arteritis*
Rheumatoid arthritis*	Metabolic syndrome (?)
Lupus erythematosus*	Diabetes mellitus
Vernal keratoconjuctivitis*	Prurigo nodularis*
Rinoconjuctivitis	Psoriasis
Allergic rhinitis	Neuropathic bladder
Allergic urticaria-angioedema	Glomerulophaties
Bronchial asthma*	Breast cancer
Chronic pancreatitis	Lymphoproliferative diseases
Hepatolithiasis	Muscular distrophies*
Systemic mastocytosis	Schizophrenia
Corneal ulcers	
Pressure (skin) ulcers	
Prostate cancer	Depression
	AIDS
	Hirschsprung's disease

Experimental animals

Hypertension	Shistosomiasis
Diabetic, *db/db* mice	Urinary bladder hypertrophy
Obese, *ob/ob* mice	Urinary bladder/cystitis
Skin inflammation	Ocular hypertension
Diabetic skin ulcer	Uveoretinitis
Diabetic myocardium	

: NGF and mast cells are correlatively studied.

ACKNOWLEDGMENTS

This study has been supported by the project "Multiple Sclerosis" from Istituto Superiore di Sanita, Rome, Italy.

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