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NERVE GROWTH FACTOR, MAST CELLS AND ARTHRITIS

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SUMMARY

• *Nerve growth factor is a well-characterized neurotrophic protein required for the survival and differentiation of a variety ofneuronal cell types both in the peripheral and central nervous systems. Recent studies indicate that nerve growth factor also plays a role in cells originating in the immune system, since it is synthesized by cells of immune system lineage and its level increases during inflammatory responses. Moreover, it has been shown that cytokines such as interleukin-1J3 and tumor necrosis factor-a are potent inducers of nerve growth factor secretion. These studies were recently confirmed and extended by demonstrating that cells normally present in inflammatory tissues, such as mast cells and lymphocytes, express nerve growth factor receptors and are receptive to the action of nerve growth factor. The aim of the present review is to outline the current understanding of mast cells and nerve growth factor in autoimmune diseases and particularly in arthritis.*

INTRODUCTION

• The term nerve growth factor (NGF) was introduced more than 40 years ago to define a newly discovered protein inducing neurite outgrowth from sensory and sympathetic ganglia *in vivo* and *in vitro* (1,2). NGF is synthesized and released in large amounts by the submaxillary salivary gland (SMG) of adult male mice(l-3). Lesser amounts have been found in snake venom (2), guinea pig prostate gland and seminal fluids of guinea pig and of bull (2,4), while nanogram quantities have been located in a variety ofneuronal and non-neuronal cells, both in the central and peripheral nervous systems (2,5,6). The 2.5S NGF molecule isolated and purified from the SMG is a dimer of two identical subunits linked together by non-covalent bonds and has a molecular weight of about 30 kD (7). The amino acid sequence and the primary structure of NGF from murine as well as from other species have been characterized and indicate highly conserved homology of murine NGF with other species, including humans (2,6,8-10).

The functional significance of NGF in the SMG and the physiological role of such a high concentration of NGF in these glands is largely unknown, although recent studies have shown that NGF released from the mouse SMG into the bloodstream following intraspecific aggressive behavior exerts an effect on at least two NGF target cells, the chromaffm cells and peritoneal mast cells (MC) (11-14).

In recent years, mainly through the use of exogenous NGF and NGF-antibody administration, it has been shown that NGF has specific cell targets not only in the peripheral and central nervous system, but also in the endocrine and immune systems. Accordingly, current knowledge indicates that NGF plays a functional role not only in neuropathological events associated with learning, memory and aging but also in autoimmune diseases, including rheumatoid arthritis (RA).

NGF AMD THE PERIPHERAL NERVOUS SYSTEM

• NGF functions as a retrograde trophic messenger between target tissues and their innervating nerve cells. NGF and NGF mRNA have been detected in peripheral tissues of various mammalian species, whereas NGF receptor mRNA is mostly produced by the corresponding sensory and sympathetic ganglia (2,15,16). NGF exerts its neurotrophic activity in re-

sponse to ligand-receptor binding followed by internalization and retrograde transport of the NGF-receptor complex to the neuronal cell body. Recent studies showed the presence of two different types of NGF receptors: p75 acting as a low-affinity receptor (Kd=10^{9}M) and pWO* high-affinity NGF receptor (Kd=10-"M) (17-19).

Daily injection of purified NGF into neonatal rodents results in an increase in volume of sympathetic ganglia, hypertrophy of sensory ganglia, and in an augmentation of their neurotransrnitiers within NGF target cells (2,15,20-22). The effect of NGF in sympathetic nerve cells is even more dramatically documented by the finding that removal of circulating NGF in living animals, via administration of specific NGF antibodies, results in death of sympathetic neurons, leading to what has been known for years as immunosympathectomy (23).

NGF AMD THE CENTRAL NERVOUS SYSTEM

• The actions of NGF are not limited to the peripheral nervous system. Numerous studies published in the last ten years have shown that NGF is produced in the central nervous system (5,6), that cholinergic basal forebrain neurons (CBFN) bear NGF receptors (17,19,24,25), and that these neurons are highly receptive to the action of NGF. NGF and its receptor are axonally transported retrogradely from cortex and hippocampus to CBFN, where they exert a trophic action. It has also been clearly demonstrated that the degeneration of CBFN following transection of the septo-hippocampal pathway is markedly reduced by exogenous administration of NGF. These studies support the notion mat NGF exerts a functional effect on these brain neurons (26) not only during development but also in adult and aged life. Moreover, the widespread presence of NGF mRNA throughout cells of the central nervous system suggests other functions of this protein within the brain (13,14,27-30).

NGF AMD THE ENDOCRINE SYSTEM

• Apart from its well-known roles in the survival and development of peripheral sympathetic and sensory neurons and in CBFN, NGF is also known to take part in the regulation of specific neuroendocrine functions (13,30-34). In fact, it has been shown that both NGF and its mRNA increase in the hypothalamus following stressful events (11-14). Moreover, the injection of NGF antibodies into rat fetuses results in a marked neuroendocrine deficit in postnatal life, including atrophied peripheral sympatetic and sensory ganglia (31). Deleterious effects of NGF deprivation during fetal life have also been reported in rats and guinea pigs (32,33). Furthermore, maternal exposure to NGF antibodies causes, in newborn pups, loss of body weight, sensory deficits, and high mortality, probably associated to neuroendocrine and immune alterations (30). In this context, it is worth mentioning that exogenous NGF administration acts on the hypothalamic-pituitary-adrenal axis by influencing, most probably, the release of hypothalamic hormones (35).

HGF AND THE IMMUNE SYSTEM

• There is ample evidence that NGF is able to exert a wide variety of effects on cells of the immune system (36-47). These studies demonstrated that *in vivo* administration of NGF to neonatal rats causes a widespread increase in the size and number of MC in several peripheral tissues (36). Exposure of spleen cells to NGF antibodies results in a cytological alteration and a reduction of MC numbers. Recently, it has been shown that MC bear NGF receptors and that NGF induces degranulation and histamine release from isolated peritoneal MC (48-51). That NGF plays a role in the immune system is also suggested by findings which show that administration of NGF in young rats prior to and after immunization with sheep erythrocytes results in an enhancement of T-lymphocyte-dependent antibody synthesis (52).

NGF has also been shown to be involved in inflammatory responses. The observation that inflammation causes an increase in NGF levels was first reported by Levi-Montalcini and Angeletti, who showed that carrageenan-induced granuloma causes a dramatic local increase in NGF levels, far above that found with sarcoma 180 (53). These studies were recently confirmed and extended, demonstrating mat cells normally present in inflammatory tissues, such as MC and lymphocytes, express NGF receptors (46,50,54) and are receptive to the action of NGF (36,37). These findings along with the observation that NGF accumulates at the site of the lesion and inflammation and that cytokines, such as interleukin-lB (IL-1l3) and tumor necrosis factor-a (TNF-a), are potent inducers of NGF secretion (55-57) suggest a functional link between cytokines and NGF in certain inflammatory diseases.

HGF AMD AUTOIMMUNE DISEASES

• The observation that the concentration of NGF is enhanced in inflammatory response, along with the emerging evidence that cells of immune system lineage are able to respond to and/or synthesize NGF (30) led to the hypothesis that this molecule might be involved in autoimmune diseases. Thus, it was assumed that autoimmune diseases characterized by abnormal activation of the immune system and by alteration of numerous biologically active mediators may influence the synthesis and release of NGF. Indeed, recent studies carried out in our laboratory demonstrated elevated NGF levels in a large number of autoimmune diseases (58-66). For example, NGF has been found in the synovial fluid of RA patients and other forms of chronic arthritis, as well as in the

synovium of animals with pharmacologically-induced arthritis (58-60). In the latter instance, destruction of peripheral sympathetic innervation reduced both the inflammation and the increased level of NGF caused by carrageenan injection (59). More recently, it was demonstrated that the synovium of transgenic arthritic mice carrying and expressing the human TNF-oc gene accumulates elevated levels of NGF. Interestingly, subcutaneous injection of NGF antibodies in these transgenic animals attenuated the wasting effect of TNF-oc (61).

Basal levels of NGF were found to be altered in the presence of other autoimmune diseases. For example, the concentration of NGF increases in the cerebrospinal fluid of patients with multiple sclerosis (MS) (62) and in the brain of rats affected by experimental allergic encephalomyelitis (EAE), an animal model of MS (63). A significant increase in NGF levels was observed in the sera of patients with systemic lupus erythematosus (SLE) and in dermis of patients affected by systemic sclerosis (SSc) (64,65). In most of these diseases, the increase of NGF was invariably associated with the accumulation of MC. In view of the fact that MC secrete NGF (67), express NGF mRNA and bear low- and high-affinity NGF receptors (50,54), and since both NGF and MC are up-regulated during inflammation, it is highly possible that either singly or cooperatively they may be functionally involved in these diseases (68-75).

MGF AMD MAST CELLS

• Under normal conditions MC are found in diverse tissues and organs, including nervous tissue, skin, gastrointestinal and respiratory tracts, vascular wall and endocrine glands (68-70,76). MC have been identified in a variety of inflammatory states, such as SLE, mixed connective tissue disease, SSc, atopic dermatitis, MS, and EAE. They respond to and produce a variety of cytokines, such as IL-1,3,4,5,6, TNF-oc, interferon-y, granulocyte-macrophage colony stimulating factor and TNF-p (69,71). In addition, peptides derived from granulocytes, platelets, and mononuclear cells, as well as complement-derived peptides and certain proteases are able to stimulate MC secretion. MC can be activated by nervous and immunological mediators such as neuropeptides (e.g. substance P) or immunoglobulin E (IgE) and are known to be involved in the development of immediate and delayed hypersensibility reactions (75,77-79).

The observation that MC respond to NGF by increasing in numbers and by releasing biological mediators (30,48,67) and that they accumulate in the synovium of patients affected by arthritis and other inflammatory disorders (72-75), suggested that attention be focused on the role of NGF in autoimmune diseases. As a first approach to carry out this studies, we selected human and rodent arthritis. We therefore decided to study the relationship of MC and NGF in rodents and humans affected by this type of disease. Our data clearly indicate that human and experimentally-induced arthritis is characterized by an increase in MC numbers and that MC distribution is correlated to NGF levels (65,66). Since MC produce cytokines (69,71) and cytokines are known to influence the synthesis of NGF (55-57), the results of time-course studies will help to elucidate the functional relationship between NGF, MC and cytokines in autoimmune diseases.

MGF. MAST CELLS AMD ARTHRITIS

• RA is a multisystem, chronic, inflammatory disease of not well-defined origin (72-75). The inflammatory conditions are characterized by synovia! hypertrophy and hyperplasia, and mixed cell infiltration, including numerous MC (78-81). The observation that these cells are associated with development of an inflammatory infiltrate in arthritis suggested that the identification of the mechanism leading to the accumulation of MC might contribute to the development of better therapeutic strategies for arthritis.

The fact that MC are multifunctional cells which store and release numerous biological mediators, including histamine, heparin, cytokines (71) and NGF (67,81), raised the important question of what role NGF plays during the course of inflammatory diseases. Our previous studies showing that injection of NGF into neonatal rats causes an increase of MC led to the hypothesis that also in arthritis, the presence of MC is the result of an overproduction of NGF. During synovial inflammation the increase of NGF precedes the accumulation of MC. However, recent observations that injection of highly purified NGF into the knee joint does not induce inflammation and that MC are able to release NGF (66,67) question this hypothesis and raise the possibility that MC may contribute to up-regulation of synovial NGF. Based on the present findings, one possible explanation is that the presence of high NGF levels at the site of inflammation may serve as a regulatory mechanism for a subpopulation of immunocompetent cells that are necessary for healing processes. The fact that MC can alter lymphocyte function favoring suppression of cytotoxic effects, whilst recruiting phagocytic leukocytes, supports this hypothesis (74). However, this hypothesis is consistent with the observations that IL-4 released by both MC/basophils and T-lymphocytes inhibits the production of proinflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-oc (77). It is therefore also possible that NGF acts through the action of IL-4 which is also a potent inhibitor of synthesis by monocytes of both prostaglandin-E2 and collagenase. Indeed, IL-4 was able to reduce disease activities and progression in various arthritic models (77).

Elevated concentrations of NGF have also been observed after

a number of degenerative events within and outside the nervous system and have been associated to neurite regeneration mechanisms (82-85). It is therefore possible that similarly to the nervous system, the increase of NGF and/or MC occurring in certain tissues during autoimmune diseases is linked to protective and reparative mechanisms (86-88). The observation that the level of NGF in SSc, MS and in EAE is high during the acute phase and low during the remission one (62,63,66) is consistent with this hypothesis. Since NGF is known to act on cells of the nervous, immune and endocrine systems (30,76,85), it is possible that during inflammation its role is to participate in peripheral tissue repair and to reestablish physiological conditions.

In this context is also worth mentioning that MC degranulation through release of heparin may also have antiinflammatory effects. For example, inhibition of T-lymphocyte heparanase by heparin has been shown to prevent T-cell migration, whereas treatment with low doses of heparin has been found to inhibit adoptively transferred encephalomyelitis (89,90). Heparin has also been demonstrated to modulate cell proliferation, wound healing, neurite outgrowth, and inflammation (91). Moreover, the potential role of peripheral MC may be related to connective tissue formation in development or repair and in defence against parasite infection (84,85).

CONCLUSIONS

• Several autoimmune diseases, including arthritis, are associated with the release of numerous biologically active mediators from MC and since MC mediators are known to exert not only deleterious (69,72-74), but also beneficial effects (84-93), a better understanding of the functional correlation between NGF and MC might be of great value for the development of therapeutic strategies for treating joint inflammatory diseases.

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