Biomedical Reviews 1998; 9: 93-99.
ISSN 1310-392X

SUBMANDIBULARGLANDSJERVE GROWTH FACTOR ANDNEUROINFLAMMATORY RESPONSES IN RODENTS

LuigiAloe and Marco Fiore
Institute ofNeurobiology, Consiglio Nazionale delle Ricerche (CNR), Rome, Italy

SUMMARY

• *Structural, biochemical, and pharmacological stud ies have provided numerous insights into the role of rodent submandibular glands in a variety of physic/pathological functions. In this review we briefly highlight past and present findings published by our group and others regarding the ro le of rodent submandibular glands and nerve growth factor in inflammatory events. Accordingly, the role of mouse salivary glands and nerve growth factor in neuroinflammatory re sponses, body temperature and parasitic infection are dis cussed, and potential future lines of studies aimed at elucidat ing their physiopathological roles are suggested.* ***(Biomed Rev 1998; 9: 93-99)***

INTRODUCTION

• The submandibular glands (SMG) of rodents are di vided in two different functional and anatomical parts, the acinar and ductal regions. The acinar region contains the secretory cells which produce and release their products in the saliva. The acinar cells are structurally connected to each other by ducts linked to the granulated convoluted tubules, and then to the striated excretory ducts which store the wide variety of biologi cally active polypeptides.

• **The submandibular gland: innervation and growth factors**

SMG are innervated by sympathetic, parasympathetic and peptidergic nerves, and each set of terminal fields contributes to the secretory regulation (1-4). Sympathetic activation has been shown to induce high protein secretion, and parasympathetic activation lead to more liquid saliva production (2-4). The SMG of mice contain small intrinsic cholinergic/peptidergic ganglia whose function is not clearly known. Structurally, mouse SMG are formed by highly differentiated epithelial cells capable of secreting more than 25 different biologically active polypeptides (2,5-8). They contribute, with significant overlapping, to several functional activities involved in the regulation of homeostasis and digestion (4,9). There is considerable evidence indicating that growth factors released by SMG can contribute to reestablishing normal tissue function by promoting cell growth and proliferation, and by regulating inflammatory cell processes (9). Among these proteins, nerve growth factor (NGF) is the first polypeptide identified in salivary glands owing to its property of promoting growth and differentiation of peripheral sympathetic and embryonic sensory neurons (10). In the last fewyears, a number of studies have provided evidence that NGF plays a functional role in forebrain chol inergic neurons, and is also involved in inflammatory events (11-14) inside and outside the nervous system.

The secretion of molecules stored in the SMG cells is regulated by stimuli arising from adrenergic, cholinergic and peptidergic neurons (15,16). Postganglionic sympathetic axons originating in the superior cervical ganglion (SCO) are located in a typical adrenergic ground plexus closely surrounding the SMG acini, while the ducts seem to be completely devoid of adrenergic innervation (17-20). Structural and biochemical studies have shown that adrenergic stimulation results in a depletion of secretion granules containing growth factors, including NGF (17,21). Removal of SCG decreased catecholamine fluorescence and thyroxine hydroxylase immunoreactivity in SCG (22,23), suggesting that, through adrenergic innervation, SCG neurons receive trophic support from the glands. Both a- and (3-adrener-

Received for publication 15 Mayl998 and accepted 29 August 1998.
Corespondence and reprint requests to Dr Luigi Aloe.Institute of Neurobiologv. CNR. viale Marx 15.1-00137 Rome. Italy. Tel: 39 (06) 82 92 592. Fax: 39 (06) 86 0903 70. E-mail: aloe (7jkant.irmkant.rm.cnr.it

gic agents trigger the release of growth factors produced and stored in the glands, although degranulation of cells is more strongly iduced by a- than by (3-adrenergic stimuli. Exogenous administration of cyclocytidine, a drug stimulating oc-adrenergic receptors, produced drastic depletion of NGF from the granular convoluted tubules and increased NGF level in the blood (24). Decentralization of the SCG by surgical lesion of the preganglionic innervation did not significantly affect the SCG neurons (16) and/or the amount of NGF stored in the gland (unpublished observations). Reduction of adrenergic innervation occurs with age, an effect most probably associated with a low availability of NGF in the SMG. Administration of the cholinergic drug pilocarpine to adult rodents induced exocyto-sis of secretion granules in granular convoluted tubule cells (25). Decentralization or superior cervical ganglionectomy did not significantly affect the amount of substance P (SP) present in the SMG, which implies that this neuropeptide is not regulated by preganglionic or postganglionic sympathetic neurons projecting to the SMG (16). This observation suggests that SP is not associated with the SMG sympathetic innervation, but is anatomically and functionally related to the chorda tympani nerve and possibly involved in the parasympathetic innervation of the gland. Accordingly, sectioning of the chorda tympani nerve results in a significant decrease in SP content of the salivary glands.

The SMG also possess rather small intrinsic ganglia located between the lingual nerve, and the submandibular and sublingual gland ducts (26,27). The role of these intrinsic neurons in the activity of the SMG is not fully known; they are supposed to contribute to the secretory activity of the gland.

SALIVARY NGF AMD THE ENDOCRINE SYSTEM

• Although displaying mainly exocrine activity, the SMG also possess an endocrine function. One of the first indications that these glands displayed endocrine activities was reported by Ogata (28) whereas other studies provided evidence that the SMG are able to secrete various proteins (21). They are released by the secretory ducts into the bloodstream, most probably through the fenestrated capillaries underlying the ducts (1). Several studies have shown that NGF is produced, stored and released by the granular convoluted tubules of the SMG, and that its NGF content is higher in male than in female mice (29). The synthesis of growth factors in SMG is hormonally regulated (30). Indeed, testosterone enhances the levels of growth factors, while removal of testis and thyroid gland de creases them (3 1). Moreover, other studies have revealed that removal of the pituitary gland in rats induces atrophy of the SMG and decreases some immunosuppressive capabilities (32), while SMG extirpation is quickly followed by atrophy of the thymus associated with depletion of the thymus-dependent lympho cytes in the spleen and lymph nodes (33). This effect seems to be independent of age and sex.

An involvement of SMG in endocrine mechanisms is also suggested by behavioral studies. Aggressive behavior causes massive degranulation in SMG and release of biologically active compounds such as epidermal growth factor, renin, and NGF (34). As hypothesized some years ago by Levi-Montalcini (10), the mouse salivary NGF seems to be implicated in the regulation of offensive and defensive behaviors. In fact, the increase of blood NGF in fighting mice was found to be associated with the number of agonistic episodes, and is more pronounced in submissive mice than in dominant mice (34-36). Thus the circulating NGF level in submissive mice was increased two-fold compared with dominant animals (3 5). This study also indicates a relationship between the circulatingNGF levels in aggressive mice and the number of fighting episodes, though this relationship is valid only for dominant attacking mice when plasma NGF is analysed in both dominant and submissive animals. The amount of NGF released into the circulation of mice without SMG decreased drastically suggesting that during aggressive behavior, the main source of circulatingNGF is the SMG (3 5). It was also observed that chronic aggressive behavior in mice, which induces a massive release of salivary NGF into the bloodstream, can lead to the production of NGF autoantibodies, thus causing a significant decrease in peripheral sympathetic innervation(37).

Recent studies suggest that adrenergic innervation of SMG participates to some extent in the regulation of NGF release into the circulation. For example, fighting mice, immunosympathectomized with NGF antibody since birth or chemically sympathectomized with 6-hydroxydopamine, still release high levels of NGF into the c irculation (35). Adrenalectomy failed to blockNGF release in fighting mice, and injections of ACTH or crude extract of adrenal gland or hypophysis in isolated adult male mice did not result in NGF release into the bloodstream, suggesting that mediators released by these glands do not exert a primary role in submandibular NGF release (35,38). A correlation between NGF and endocrine functions is suggested by other findings, indicating that NGF is able to stimulate the pituitary-adrenocortical axis releasing ACTH and glucocorticoids (39), and that adrenal gland hormones alter the synthesis of NGF (38,40). Furthermore, administration of NGF antibodies into rat fetuses, which inhibits the availability and activity of endogenous NGF, induced a significant loss in body growth and a marked neuroendocrine deficit in the offspring (41). Likewise, deleterious neuroendocrine effects of NGF have been found in rabbits and guinea pigs after maternal exposure toNGF antibodies (42). Moreover, reproductive organs store and release significant physiological amounts ofNGF, suggestingthatthis molecule is implicated in the functional activity of these tissues (43), while changes in NGF concentration were found during late pregnancy or delivery in both plasma and central nervous system (44,45).

NEUROINFLAMMATORY RESPONSES AMD NGF

• The first evidence showing that NGF is associated with inflammation was reported by Levi-Montalcini who found that experimentally induced granulomas are characterized by altered levels of NGF (46). Subsequent studies demonstrated thatNGFpromotesproliferation and degranulationofmast cells, and it was found that a large number of inflammatory diseases is characterized by high local NGF levels (reviewed in 44,45). Salivary NGF enhances vascular permeability (47), promotes differentiation of granulocytes (48), and induces lymphocyte proliferation (49) and mast cell degranulation (50). The eviden ce that NGF promotes wound healing (51) further suggests that this molecule is involved in the modulation of different inflam matory responses (9). Though the tissues of the immune system are known to be innervated by the sympathetic nervous system (52), the mechanisms regulating the innervation of specific 'tissues are at present not fully understood. Sympathetic nerve tenninals in immune tissues are found to sun'ound blood vessels and T cells areas within lymphoid organs, such as spleen, lymph nodes, and gut-associated lymphoid tissues (53). NGF is also involved in peripheral inflammatory responses (54), playing a crucial role in neuropathologies associated with sensory defi cits (55). Our laboratory studies indicate that NGF increases in several neuroinflammatory diseases (44). A high NGF level has been found in patients with rheumatoid arthritis (56), systemic sclerodemia(57), lupuserythematosus(58,59), Kawasaki dis ease (59a), multiple sclerosis (60) as well as in rodents affected by experimental allergic encephalomyelitis (61,62), an animal in flammatory disorder model considered to closely resemble the human diseases multiple sclerosis and rheumatoid arthritis. We have also shown that pretreatment with NGF antibody reduces or prevents the development of arthritis induced by carrag-eenan, suggesting a functional role of NGF in this type of per-pheral inflammation (63). Similarresults were obtained with arth ritic transgenic mice expressing high levels of TNF-cc in knee joints (64).

It has been reported that certain neuroinflammatory diseases may also be characterized by low levels or absence of circulating NGF and, surprisingly, by a concomitant increase in NGF antibodies (64a). This may due to the generation of autoantibod-ies against NGF by chronic exposure to supranormal NGF amounts in the bloodstream. In fact, evidence supporting this hypothesis was obtained in our laboratory in chronically stressed mice (37). Furthermore, it was recently reported that the NGF level is decreased, whereas the p75 NGF receptor is overexpre-ssed in human atherosclerotic coronary arteries (65).

SIALECTOMY, NGF ANDTHERMOREGULATION

• It was recently demonstrated that salivary NGF is implicated in the regulation of the temperature set point in adult mice (66). Thus, circulatingNGF levels are low in hypothennic and high in hyperthennic mice, whereas injection of purified NGF into the tail vein of normal mice induces an increase in body temperature lasting about 6 hours (66). The observation that body temperature returned to normal values after 6 hours, while the concentration of circulating NGF was still high about 48 hours suggests that NGF may be involved in the early response, most probably *via* the activation of cells of the neuroendocrine axis (39,67). The hypothesis that NGF plays a role in temperature response is also suggested by other observations. For example, supranormal levels of NGF occur in pathophysiological conditions associated with changes in body temperature (reviewed in 68), such as inflammatory responses (69-71). The mechanism leading to NGF increase as a result of body temperature elevation is at present not known. Since NGF synthesis is known to be regulated by cytokines (69.72), the possibility exists that the high levels of NGF are due to the supranormal expression of these molecules during inflammation.

Other studies have shown that SMG are involved in inflammatory responses (9), and that salivary NGF acts on cells of the endocrine and immune systems (52,73). Because the hypothala-mus expresses NGF and NGF receptors (13,35,74-76), another possibility is that hypothalamic NGF-positive neurons might themselves be involved in these events. This hypothesis is supported by evidence demonstrating that a dramatic increase in the expression of NGF occurs in the hypothalamus in response to infection and high body temperature (77). Interestingly, brain ischemia, which alters body temperature (78), is also characterized by an increase in NGF and $mRNA^{NGF}$, while in-tracerebral administration of NGF antibodies during fetal life induces in the offspring physical and behavioral characteristics associated with deficits in thermoregulation (41). Since NGF acts on a variety of cells (10,14,73), the release of NGF during variation of body temperature may well serve to activate NGF responsive cells associated with homeostatic regulation.

SALIVARY GLANDS AMD PARASITIC INFECTION

• To gain further information about the role of salivary NGF in inflammatory responses, we recently earned out studies on mice infected with the trematode *Schistosoma mansoni.* Schistosomiasis is a disease that affects more than two million people in countries of South America, Africa, and Arabia. A key pathogenetic event in this parasitic infection is the formation of granulomas around schistosome eggs trapped in the intestine and liver (79). Immunological. pharmacological, biochemical, and molecular studies have shown that cytokines secreted by macrophages, eosinophils, mast cells, and Th-1 and Th-2 lym phocytes play a crucial role in the formation of these granulomas (79,80). We have shown that NGF occupies a key position in the regulation of parasitic inflammation. Our studies demonstrated that in addition to immunological changes, *Schistosoma manso-*

ni infection induces an increase in liver and brain NGF, the increase of NGF followed by an accumulation of mast cells around the granulomas (77,81). We also found that the exogenous administration of NGF antibodies significantly reduced the presence of these cells and restored the altered thermoregulation induced by schistosomiasis (66). Our findings also indicated a hyperalgesic effect in infected compared with uninfected animals on the hot-plate (82), whereas this nociceptive effect was not observed in infected-sialectomized animals (83). Since NGF administration induces hyperalgesia(54,55), and NGF is known to increase during chronic infection in the paws and in the brain (81), it has been hypothesized that the altered nociceptive expression is related to an increased level of paw NGF in infected mice, while the absence ofhyperalgesia in infected-sialectomized mice is most probably due to a reduced presence of NGF following SMG removal. This latter hypothesis is consistent with the observations that the removal of SMG in mice decreases the plasmaNGF concentration (84), and that infected sialectomized ' mice show similar NGF levels in the plasma compared with uninfected controls (66). However, whetherthese latter effects are directly induced by NGF or mediated through the effect of NGF on inflammatory cells and/or cytokines remains to be verified.

CONCLUSION

• Since its discovery, the presence of large quantities of NGF and other growth factors secreted by the mouse SMG has aroused considerable interest in their pathophysiological func tion. Although salivary glands products are found in the blood stream under conditions of stress, a still unresolved question is whether under normal conditions SMG have to be considered exclusively an exocrine gland or a gland displaying also endo crine activity. As peripheral blood and endocrine cells are recep tive to NGF action, it is highly possible that in appropriate phy siological states, SMG-derived growth factors can reach the bloodstream regulating the activity of these cells. This hypoth esis is supported by findings reported by our group and others showing that mouse SMG release NGF and other growth fac tors during aggressive encounters. Because NGF has been imp licated in autoimmune inflammatory disorders, a potentially important line of research that might be pursued involves the role of SMG and NGF in Sjugren's syndrome (SS), an autoim mune inflammatory disease associated with systemic lupus erythematosus (85), characterized by periductal infiltration of mononuclear cells leading to severe immunological, neurologi cal, and functional deficits of the SMG (86). Significantly, a recent study indicated that the constitutive level of epidermal growth factor, a peptide also produced in the SMG (4), under went significant changes in SS (87). The availability of a mouse strain that spontaneously develops a systemic lupus erythema-tosus-like syndrome renders the possibility of addressing the se questions more feasible. Other areas of research involve studying the hypothesis that SMG and/or growth factors secreted by these glands are involved in tumor growth (88), muscular dystrophy (89,90), pulmonary inflammation (91,92), and aging(93).

1. Penshow JD, Coghlan JP. Secretion of glandular kallikrein and renin from the basolateral pole of mouse subinandibu-lar duct cells: an immunocytochemical study. *JHistochem Cytochem\993-4\:95-\03*.
2. BarkaT, Cubits RM, van derNoen HM. cc-adrenergic stimulation *ofc-fos* expression in the mouse submandibular gland. *Mol CellBiol* 1986; 6:2984-2989.
3. Gutierrez Marin MS, Galera H, Bullon P. Effects of treatment with noradrenaline and isoproterenol on the excretory portion of the submaxillary gland in the rat: an ultrastructural study. *ActaAnat* 1990; 137:324-330.
4. Sabbadini E, Berczi I. The submandibular gland: a key organ in the neuroimmuno-regulatory network. *Neitroimmu-nomodiilation* 1995; 2: 184-202.
5. Hirata Y, Orth DN. Secretion of epidermal growth factor, nerve growth factor, and renin-like enzymes by dispersed male mouse submandibular gland cells *in vitro. Endocri-no/ogy* 1980; 107: 92-97.
6. BarkaT. Biologically active polypeptides in submandibular glands. *J Histochem Cytochem* 1980; 28: 836-859.
7. Murphy RA, Said JD, Blanchard MH, Young M. Nerve growth factor in mouse serum and saliva: role of the submandibular gland. *Proc Nail Acad Sci USA* 1977; 74: 2330-2333.
8. Murphy RA, Watson AY, Metz J, Forssmanri GW. The mouse submandibular gland: an exocrine organ for growth *factors. J Histochem Cytochem* 1980; 28: 890-902.
9. Mathison R, Davison JS, Befus D. Neuroendocrine regulation of inflammation and tissue repair by submandibular gland factors. *Immitnol Today!* \ 994; 15: 527-532.
10. Levi-Montalcini R. The nerve growth factor 35 years later. *Science* 1987; 237: 1154-1162.
11. Thoenen H, Bandtlow C, Heumann R. The physiological function of nerve growth factor in the central nervous system: comparison with the periphery. *Rev Physiol Biochem* 1987; 109:145-178.
12. Thoenen H, Barde YA. Physiology of nerve growth factor. *Physiol Rev* 1980; 60: 1284-1335.
13. Prioro E, Cuello A. Distribution of nerve growth factor receptor-like immunoreactivity in the adult rat central nervous system. Effect of colchicine and correlation with the cholinergic system. *Newoscience* 1990; 34: 57-87.
14. Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A. Nerve growth factor: from neurotrophin to neurokine. *Trends Newosci* 1996; 19:514-520.
15. Garrett JR, Kidd A. The innervation of salivary glands as revealed by morphological methods. *MicroscRes Tech* 1993;

26:75-91. . :• • •• . " ' /
16. Robinson SE, Schwartz JP, Costa E. Substance P in the superior cervical ganglion and the submaxillary gland of the rat. *Brain Res* 1980; 182: 11 -17.
17. Hazen-Martin DJ, Simson JAV. Infrastructure of the secretory response of male mouse submandibular gland granu-lar tubules. *AnatRec* 1986; 214: 253-265.
18. Norberg K-A, Olson E. Adrenergic innervation of the salivary glands in the rats. *ZeitschriftZellforschung* 1965; 68: 183-189.
19. Schneyer CA, Hall HD. Autonomic regulation of changes in rat parotid amylase during postnatal development. ,4/7? *J Physiol* 1972; 223: 172-175.
20. Schneyer EH, Young JA, Schneyer CA. Salivary secretion of electrolytes. *Physiol Rev* 1972; 52: 720-777.
21. Takeda M. Electron microscopy of the adrenergic and cholinergic nerve terminals in the mouse salivary glands. *Archs Oral5*/0/1978; 23: 857-864.
22. Eahtivirta S, Koistinaho J, Hervonen A. A subpopulation of large neurons of the sympathetic superior cervical ganglion innervates the NGF-rich submandibular salivary gland in young adult and aged mice. *JAittonom Nerv Syst* 1995; 50:283-289.
23. Eahtivirta S, Koistinaho J, Hervonen A. Effect of sialectomy on the superior cervical ganglion sympathetic neurons in young adult and aged mice. *Mech Ageing Dev* 1992; 62: 25-33.
24. AloeE. Cozzari C, Eevi-Montalcini R. Cyclocytidine-induced release of nerve growth factor from mouse submandibular glands enhances regeneration of sympathetic fibres in adult mice. *Brain Res* 1985; 332:259-265.
25. Field RB, Hand AR. Secretion of lingual lipase and amylase from rat lingual serous glands. *Am J Physiol* 1987; 253: G217-G225.
26. Snider WD, Johnson Jr EM. Neurotrophic molecules. ,4/7/7 *Neural* 1989; 26:489-506.
27. Snider WD. The dendritic complexity and innervation of submandibular neurons in five species of mammals. *J Newosci* 1987; 7: 1760-1768.
28. OgataT. The internal secretion of salivary glands. *En doer i-noUpn* 1955; 2: 116-128.
29. Eevi-Montalcini R, Aloe E. Synthesis and release of the nerve growth factor from the mouse submaxillary salivary glands: hormonal and neuronal regulatory mechanisms. In: Duinont JE, Nunez J, editors. *Hormones and Cell Regulation. INSERM Ear Symp,E\sev\er.* 1981; 5: 53-72.
30. Walker P. The mouse submandibular gland: a model for the study of hormonally dependent growth factors. *J EndocrinolInvest* 1982; 5: 183-196.
31. Aloe E. Eevi-Montalcini R. Comparative studies on testosterone and L-thyroxine effects on the synthesis of nerve growth factor in mouse submaxillary salivary glands. *Exp Cell Res* 1980; 125:15-22.
32. Nagy E, Berczi I, Sabbadini E. Endocrine control of the immunosuppressive activity of the submandibular gland. *Brain Behav Immitn*992; 6: 418-428.
33. Martinez-Heraandez A, Nakane PK, Pierce GB. Relationship between the submaxillary glands and the thymus. *Lab Invest* 1973; 29:266-271.
34. Alleva E, Aloe E, Bigi S. An update role for nerve growth factor in neurobehavioural regulation of adult vertebrates. *Rev Newosci* 1993; 4:41-62.
35. Aloe E, Alleva E, Buhm A, Eevi-Montalcini R. Aggressive behaviour induces release of nerve growth factor from mouse salivary gland into the bloodstream. *Proc Natl Acad* Sc/LK41986;83:6184-6187.
36. Maestripieri D, De Simone R, Aloe E, Eevi-Montalcini R. Social status and nerve growth factor serum levels after agonistic encounters in mice. *Physiol Behav* 1990; 47: 161-164.
37. Aloe E, Musi B, MiceraA, Santucci D, Tirassa P, Alleva E. NGF antibody production as a result of repeated psychosocial stress in adult mice. *Newosci Res Comm* 1995; 16: 19-28.
38. Aloe E. Adrenalectomy decreases nerve growth factor in young adult rat hippocampus. *Proc Natl Acad Sci USA* 1989; 86:5636-5640.
39. Otten U, Baumann JB, Girard J. Stimulation of the pituitary-adrenocortical axis by nerve growth factor. *Na*-ft//-e 1979; 282:413-414.
40. Dicou E, Eee J, Brachet P. Synthesis of nerve growth factor mRNA and precursor protein in the thyroid and parathyroid glands of the rat. *Proc Natl Acad Sci USA* 1986; 83: 7084-7088.
41. Aloe E, Cozzari C, Calissano P. Eevi-Montalcini R. Somatic and behavioural postnatal effects of fetal injections of nerve growth factor antibodies in the rat. *Nature* 1981; 291: 358-366.
42. Johnson EM Jr, Osborne PA, Rydel RE, Schmidt RE, Pearson J. Characterization of the effects of autoimmune nerve growth factor deprivation in the developing guinea pig. *Neuroscience* 1983; 8: 631-642.
43. Persson H, Ayer-Ee Eievre C, Soder O, Villar MJ, Metsis M, Olson E *el al.* Expression of beta-nerve growth factor receptor mRNA in Sertoli cells downregulated by testosterone. *Science* 1990; 247: 704-707.
44. Aloe E, Skaper SD, Eeon A, Eevi-Montalcini R. Nerve growth factor and autoimmune diseases. *Aiitoimmimity* 1994; 19: 141-150.
45. Aloe E, Bracci-Laudiero E, Bonini S, Manni E. The expanding role of nerve growth factor: from neurotrophic activity to immunologic diseases. *Allergy:* 1997; 52: 883-894.
46. Eevi-Montalcini R, Angeletti PU. In: Kety SS, Elkes J, editors. *Biological Properties of a Nerve Growth Promoting Protein and Its Antisenim. Regional Neitrochemistrv.* The Fourth IntNeurochem Symp, Pergamon Press, New York. 1960.

47. Otten U, Baumann JB, Girard J. Nerve growth factor induces plasma extravasation in rat skin. *Enr J Pharmacol* 1984; 106:199-201.
48. Kannan Y, Ushio H, Koyama H, Okada M, Oikawa M, Yoshihara T *et al.* Nerve growth factor enhances survival, phagocytosis, and superoxide production of murine neutrophils. *Blood* 1991; 77: 1320-1325.
49. Torcia M, Bracci-Laudiero L, Lucibello M, Nencioni L, Labardi D, Rubartelli A *et al.* Nerve growth factor is an autocrine survival factor for memory B lymphocytes. *Cell* 1996; 85:345-356.
50. Aloe L, Levi-Montalcini R. Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Rex* 1977; 133: 358-366.
51. Matsuda H, Koyama H, Sato H, Sawada J, Itakura A, Tanaka A *et al.* Role of nerve growth factor in cutaneous wound healing: accelerating effects in-normal and healing-impaired diabetic mice. *JExp Meet* 1998; 187: 297-306.
52. Levi-Montalcini R, Aloe L, Alleva E. A role for nerve growth factor in nervous, endocrine and immune system. *Prog NeitroEndocrinelmmunol* 1990; 1:1-10.
53. Felten DL. Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Jmmimol Rev* 1987; 100:225-260.
54. Lewin GR, Mendel LM. Nerve growth factor and nociception. *Trends Neurosci* 1993; 16: 353-359.
55. Lewin GR, Hitter AM, Mendell LM. Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. *J Neurosci* 1993; 13:2136-2148.
56. Aloe L, Tuveri MA, Carcassi U, Levi-Montalcini R. Nerve growth factor in the synovial fluid of patients with chronic arthritis. *ArthRheum* 1992; 35: 351-355.
57. Tuveri MA, Passiu G, Mathieu A, Aloe L. Nerve growth factor and mast cell distribution in the skin of patients with systemic sclerosis. *Clin Exp Rheumalol* 1993; 11:319-322.
58. Bracci-Laudiero L, Aloe L, Levi-Montalcini R, Galeazzi M, Schilter D, Scully JL *et al.* Increased levels of NGF in sera of , systemic lupus erythematosus patients. *NenroReport* 1993; 4:563-565.
59. Bracci-Laudiero L, Lundeberg T, Stenfors C, Theodorson E, Tirassa P, Aloe L. Modification of lymphoid and brain •• nerve growth factor levels in systemic lupus erythematosus mice. *Neurosci Lett* 1996; 204: 13-16.
59a. Falcini F, Cerinic MM, Ermini M, Generini S, Lombard) A, Pignone A *et al.* Nerve growth factor circulating levels are increased in Kawasaki disease: Correlation with disease activity and reduced angiotenisin converting enzyme lev-: *e\s.JRheiimatol\996-23:* 1798-1802.
60. Bracci-Laudiero L, Aloe L, Levi-Montalcini R, Buttinelli C, Schilter D, Gillessen S *et al.* Multiple sclerosis patients express increased levels of B-nerve growth factor in cere-brospinal fluid. *Neurosci Lett* 1992; 147: 9-12.
61. Micera A, De Simone R, Aloe L. Elevated levels of nerve growth factor in the thalamus and spinal cord of rats affected by experimental allergic encephalomyelitis. *Arch Ital Biol* 1995; 133: 131-142.
62. De Simone R, Micera A, Tirassa P, Aloe L. mRNAforNGF and p75 in the central nervous system of rats affected by experimental allergic encephalomyelitis. *Newopathol Appl Neurobiol\996; 22:* 54-59.
63. Aloe L, Tuveri MA, Levi-Montalcini R. Studies on carrageenan-induced arthritis in adult rats: presence of nerve growth factor and role of sympathetic innervation. *Rhetimatollnt 1992; 12:213-216.*
64. Aloe L, Probert L, Kollias G, Bracci-Laudiero L, Spillantini MG, Levi-Montalcini R. The synovium of transgenic arthritic mice expressing human tumour necrosis factor contains a high level of nerve growth factor. *Growth Factors* 1993;9:149-155.
64a. Dicou E, Hurez D, Nerdi-ire V. Natural autoantibodies against nerve growth factor in autoimmune diseases. *JNewoimmunol* 1993; 47: 159-168.
65. Chaldakov GN, Properzi F, Ghenev PI, Fiore M, Stankulov IS, Aloe L. Correlative analysis of NGF, p75NGF receptor and mast cells in human coronary atherosclerosis. An artery-to-adipose tissue study, [abstract]. *BiomedRev* 1998; 9: VIII.
66. Aloe L, Moroni R, Angelucci F. Evidence of a role for nerve growth factor in the effect of sialoadenectomy on body temperature of parasite-infected mice. *Archs Oral Biol* 1996; 41:21-26.
67. Taglialatela G, Angelucci L, Schiaccianoce S, Foreman PJ, Perez-Polo JR. Nerve growth factor modulates the activation of the hypothalamo-pituitary-adrenocortical axis during the stress response. *Endocrinology!* 1991; 129: 2212 -2218.
68. Kluger MJ. Fever: role of pyrogens and cryogens. *Physiol Rev* 1991:71:93-127.
69. Lindholm D, Heumann R, Meyer M, Thoenen H. Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* 1987; 330: 658-659.
70. Donnerer J, Schuligoi R, Stein C. Increased content and transport of substance P and calcitonin gene-related peptide in sensor}' nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor *in vivo. Neuroscience* 1992; 49: 693-698.
71. Lindvall O, Kokaia Z, Bengzon J, Elmfir E, Kokaia M. Neurotrophins and brain insults. *Trends Neurosci* 1994; 17: 490493.
72. Hattori A, Tanaka E, Murase K, IshidaN, Chatani Y Tsujimoto M *et al.* Tumor necrosis factor stimulates the synthesis and secretion of biologically active nerve growth factor in non-neuronal cells. *J Biol Chem* 1993; 268: 2577-2582.
73. Levi-Montalcini R, Dal Toso R, della Valle F, Skaper SD, Leon A. Update of the NGF saga. *J N enrol Sci* 1995; 130:

119-127.
74. De Simone R, Alleva E. Aloe L. Changes of NGF level in mouse hypothalamus following intermale aggressive behaviour: biological and immunohistochemical evidence. *Behav Brain Res 1*990; 39: 53-61.
75. Talamini L, Aloe L. Immunohistochemical localization of nerve growth factor (NGF) and NGF-receptor in the hypothalamus of adult rats. *Arch ltd Biol* 1993; 131:255-266.
76. Conner JM. Varon S. Nerve growth factor immuno-reactiv-ity in the anterior pituitary of the rat. *NewoReport* 1993; 4: 395-398.
77. Aloe L, Moroni R, Mollinari C, Tirassa P. *Schistosoma mansoni* infection enhances the levels of NGF in the liver and hypothalamus. *NewoReport* 1994; 5: 1030-1032.
78. Memezawa H, Zhao Q, Smith ML, Siesjo BK. Hyperthermia nullifies the ameliorating effect of dizocilpine maleate (MK 801) in focal cerebral ischemia. *Brain Res* 1995; 670:48-52.
79. Warren KS, editor. *Immunology and Molecular Biology of Parasitic Infection,* 3rd ed. Blackwell Scientific Publication, Boston. 1993.
80. Duvaux-Miret O, Stefano GB, Smith EM, Dissous, Capron A. Immunosuppression in the definitive and intermediate host of the human parasite *Schistosoma mansoni* by release of immunoreactive neuropeptides. *Proc Nail Acad SciUSA* 1993; 89: 778-781.
81. Aloe L, Moroni R, Fiore M, Angelucci F. Chronic parasite infection in mice induces brain granulomas and differentially alters brain nerve growth factor levels and thermal responses in *paws.Acta Neuropathol* 1996; 92: 300-305.
82. Fiore M, Moroni R, Alleva E, Aloe L. *Schistosoma mansoni:* schistosomiasis induces neuro-behavioural deficits in adult mice. *Exp ParasitoJ* 1996; 83: 45-58.
83. Fiore M, Moroni R, Aloe L. Removal of the submaxillary salivary glands and infection with the trematode *Schistosoma mansoni* alters exploratory behaviour and pain thresh-olds in female mice. *Physio/ Beha\>* 1997; 62: 399-406.
84. Hendry I A, Eversen LL. Reduction in the concentration of nerve growth factor in mice after sialectomy and castration. *Nature* 1973; 242: 500-504.
85. Wahren M, Tengner P, Gunnarsson I, Eundberg I, Hedfors E, Ringertz NR *et al.* Ro/SS-A and Ea/SS-B antibody level variation in patients with Sjugren's syndrome and systemic lupus erythematosus. *JAutoimmun* 1998; 11: 29-38.
86. Fox RI, Maruyama T. Pathogenesis and treatment of Sjugren's syndrome. *Curr Opin Rheum at ol* 1997; 9: 393-399.
87. Koshi H, Konttinen YT, Hietanen J, Tervo T, Malmstrom M. Epidennal growth factor, transfonning growth factor-alpha and epidermal growth factor receptor in labial salivary glands in Sjugren's syndrome. *Rheumatolology* 1997; 24: 1930-1935.
88. Arnason BGW, Chelmicka-Szorc E, McCully KS, Oger J, Young M. Tumour growth: suppression in mice by submandibular gland extirpation. *JNat/ Cancer Inst* 1975; 55: 1203-1205.
89. Engvall M, Birkhed D. Oral sugar clearance and other caries-related factors in patients with myotonic dystrophy. *ActaOdontolScand\997;* 55: 111-115.
90. Watson AY, Radie K, McCarthy M, Larsen R, Murphy RA. Thyroxine reverses deficits of nerve growth factor and epidermal growth factor in submandibular glands of mice with muscular dystrophy. *Endocrinology'* 1982: 1392-1397.
91. Mathison R, Hogan A, Helmer D, Bauce E, Woolner J, Davison JS *et al.* Role of the submandibular gland in modulating pulmonary inflammation following induction of systemic anaphylaxis. *Brain Behav Immim* 1992; 6: 117-129.
92. Bissonette EY, Mathison R, Carter E, Davison JS, Befus D. Decentralization of the superior cervical ganglia inhibits mast cell mediated TNFoc-dependent cytotoxicity. 1. Potential role of salivary glands. *Brain Behav Imnnin* 1993; 7: 293-300.
93. Nicolson N,.Storms C, Ponds R, Sulon J. Salivary cortisol levels and stress reactivity in human aging. *J Gerontol A BiolSciMedSci* 1997; 52: M68-M75.