Neurotrophins and their receptors in human lingual tonsil: An immunohistochemical analysis

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Abstract. Lymphoid organs are supplied by many nerve endings associated with different kinds of cells and macrophages. The role of this innervation on the release of locally active molecules is still unclear. Lingual tonsils belong to Waldeyer's Ring, in close association with palatine tonsils and nasopharyngeal (adenoids) tonsils, thus constituting part of NALT (nasal-associated lymphoid tissue) together with the tubal tonsils and lateral pharyngeal bands. In this study, we focused our attention on the expression of some neurotrophins (NTs) and their high- and low-affinity receptors in human lingual tonsils. Light immunohistochemistry showed that human tonsillar samples were generally positive for all the NTs investigated (NGF, BDNF, NT-3, NT-4) and their receptors (TrKA, TrKB, TrKC and p75) with some different expression levels. NGF and TrKC were strongly expressed in macrophages, but weakly in lymphocytes. However, BDNF and TrKB was highly expressed in lymphocytes and weaker in macrophages. The low-affinity receptor for NGF, p75, was mainly moderately expressed in the analysed samples. These results suggest the presence of a pattern of neurotrophin innervation in the human lingual tonsil which may play a role in sustaining inflammatory conditions and in modulating a close interaction between the nervous system and the different immune cellular subtypes.

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Introduction

The nasopharyngeal tonsil (adenoids) and palatine and lingual tonsils constitute the major part of Waldeyer's Ring, with the tubal tonsils and lateral pharyngeal bands as less prominent components. All parts of Waldeyer's Ring are located to perform regional immune functions because they are exposed to both airborne and alimentary antigens. Although tonsils and adenoids play an important immune-inductive role as components of MALT (mucosa-associated lymphoid tissue), these structures also present similarities with lymph nodes and may participate as effector organs of local systemic-type adaptive immunity (1). Tonsils and adenoids contain four specialized lymphoid compartments (2), namely the reticular crypt epithelium, the extra-follicular area (T cell-area), the mantle zones of lymphoid follicles and the follicular germinal centres (B cell-inductive compartment).

Both the nervous and immune systems are functionally related and the lymphoid tissues of the human mucosa are considerably innervated (3). As a part of the immune system, tonsils are supposed to closely interact with the nervous system by an extensive network, involving many signalling molecules. In this context, there is still a scarcity of information regarding neurotrophin expression and innervation of the human lingual tonsil. Neurotrophins (NTs), also known as neurotrophic factors, represent a family of dimeric proteins working as polypeptidic growth factors which include NGF, BDNF, NT-3, NT-4/5 and NT-6, the latter being apparently specific for fish (4). Biological actions of NTs are mediated by the binding with two families of membrane receptors, the high-affinity tyrosine kinase (TrK) and the low-affinity p75NTR (5,6). The TrK family includes TrKA, TrKB and TrKC receptors, whereas p75NTR belongs to the transmembrane molecules serving as receptors for tumor necrosis factor and cytokines (6). TrKA is specifically activated by NGF, whereas TrKB and TrKC are primarily receptors for BDNF and NT-3 respectively (7,8). NTs are involved in vertebrate neuronal cell development, differentiation, survival and functional activities (4,9). NTs are also involved in the

modulation of adult central nervous system functions and organization, as well as in the vegetative innervation of several organs (7,9). Moreover, detailed studies have revealed significant actions of neurotrophins in a wide variety of tissues outside the nervous system, especially in the immune system (10-14). In particular, it is thought that immune tissue is capable of concentrating NGF, which in turn may modulate the level of innervation via the sympathetic nervous system (15,16).

In order to gain information on this matter, in the present report we studied the innervation and the expression of NTs and their receptors in different cellular subtypes (lymphocytes, macrophages, endothelium of blood vessels) of human lingual tonsils.

Materials and methods

Patients. Small specimens of human lingual tonsil tissue were surgically removed during a biopsy (after written informed consent of the patients) in the course of operations performed for palatine tonsil removal from ten patients and subjected to immunohistochemical analysis. Experiments were performed in compliance with the Italian laws and guidelines concerning the informed consent of the patients.

Immunohistochemistry. The following molecules were investigated: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), NT-3, NT-4, TrKA, TrKB, TrKC and p75NTR.

For light microscope immunohistochemical analysis, small fragments of tonsil tissue were washed in PBS, fixed in 10% formalin and embedded in paraffin according to a standard procedure. Serial 10 μm thick sections were cut using a rotatory microtome, mounted on gelatine-coated slides and processed for immunohistochemistry. To study the immunolocalization of neurotrophins and their receptors, the antibodies we used were: i) rabbit anti-NGF polyclonal antibody (Santa Cruz Biotechnology, CA, USA), which display <1% cross-reactivity against recombinant human NT-3, NT-4 and BDNF; ii) rabbit anti-BDNF polyclonal antibody (Santa Cruz), which recognizes the amino-terminus of mouse BDNF and do not cross-react with NT-3 or NGF; iii) rabbit anti-NT3 polyclonal antibody (Santa Cruz Biotechnology), raised against the amino-terminus of mouse NT-3 and which does not cross-react with NGF or BDNF; iv) rabbit anti-NT4 polyclonal antibody (Santa Cruz Biotechnology); v) rabbit anti-TrKA polyclonal antibody (Santa Cruz Biotechnology). It recognizes an epitope corresponding to amino acids 763 to 777, mapping adjacent to the carboxy-terminus of human TrKA p140 and is not cross-reactive with TrKB or TrKC; vi) rabbit anti-TrKB polyclonal antibody (Santa Cruz Biotechnology). It recognizes an epitope corresponding to amino acids 794 to 808 of mouse TrKB p145 and is not cross-reactive with TrKA or TrKC; and vii) rabbit anti-TrKC polyclonal antibody (Santa Cruz Biotechnology). It recognizes an epitope corresponding to amino acids 798 to 812 of porcine TrKC p140 and is not cross-reactive with TrKA or TrKB; viii) goat polyclonal antibody to human NGF receptor p75 (Santa Cruz Biotechnology). It recognizes the amino acid sequence mapping the carboxy-terminus of the NGF receptor p75 precursor of human origin and is not cross-reactive with other growth factor receptors. Incubation with primary antibodies was performed overnight at 4˚C at a final concentration of 2-5 μg/ml. Optimal antisera dilutions and incubation times were assessed in a series of preliminary experiments. After exposure to the primary antibodies, slides were rinsed twice in phosphate-buffer and incubated (1 h and 30 min at room temperature) with the appropriate secondary antibody conjugated to horseradish peroxidase (HRP) (final dilution 1:100). The secondary antibody-HRP linked against rabbit immunoglobulins was purchased from Boehringer (Boehringer Mannheim GmbH, Mannheim, Germany), while secondary antibodies-HRP linked against mouse and goat immunoglobulins were from Sigma (Sigma Chemicals Co, St. Louis, MO, USA). After a further wash with phosphate-buffer, slides were treated with 0.05% 3,3-diaminobenzidine and 0.1% H_2O_2. Finally, sections were counterstained with Mayer's hematoxylin and observed by using a light microscope. To block endogenous peroxidase activity, slides were pre-treated with 3% H_2O_2, whereas the non-specific binding of immunoglobulins was prevented by adding 3% fetal calf serum to the incubation medium. Negative control experiments were done: i) by omitting the primary antibody; ii) by substituting the primary antibody with equivalent amount of non-specific immunoglobulins; and iii) by pre-incubating the primary antibody with the specific blocking peptide (antigen/antibody = 5 according to customer's instructions). In preliminary experiments, immunohistochemistry was also performed on frozen sections of human tonsil tissue. We found no differences in the intensity or distribution of immunostaining using the two types of sections, but we preferred paraffin-embedded material because microanatomical details were better preserved. The intensity of immune reaction was assessed microdensitometrically by an IAS 2000 image analyzer (Delta Sistemi, Rome, Italy) connected via a TV camera to the microscope. The system was calibrated taking as zero the background obtained in sections exposed to non-immune serum. Ten 100 μm^2 areas were delineated in each section by a measuring diaphragm. Quantitative data of the intensity of the immune staining were analyzed statistically by analysis of variance (ANOVA) followed by Duncan's multiple range test as a *post hoc* test.

Results

Light microscopy immunohistochemistry. Sections of tonsil samples exposed to the primary/secondary antibodies developed a dark-brown (intense), yellow-brown (slight) or no immunostaining. Immunoreactivity was specific since no immunostaining was obtained in control sections incubated with each primary antibody adsorbed with the specific peptide or with pre-immune serum (data not shown). Immunolabelling was located in macrophages, lymphocytes and blood vessels of the tonsil tissue (Table I).

NGF showed a strong immunoreactivity in germinal centres (GC) and in macrophages (M), while a moderate expression was observed in lymphocytes (L). TrkA showed a moderate expression in germinal centres and macrophages, and a weak immunostaining in lymphocytes. P75 showed a moderate expression in germinal centres and macrophages,

Table I. Immunohistochemistry results.

	Germinal centre	Macrophages	Lymphocytes
NGF	++	++	+
BDNF	+	+	++
NT3	±	+	±
NT4	-	-	-
TrK A	+	+	±
TrK B	+	+	++
TrK C	++	++	±
p75	+	+	±

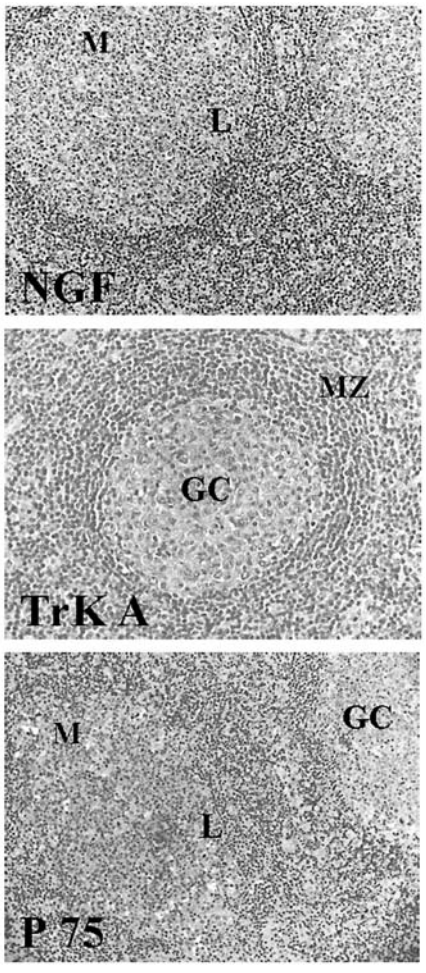

Figure 1. Micrographs of NGF, TrkA and p75 immunostaining in human lingual tonsil tissue, x40. The immunoreactivity of NGF was strongly expressed both in germinal centres (GC) and macrophages (M), and a moderate immunostaining was evident in lymphocytes (L). Immunoreactivity for TrkA and p75 showed a moderate expression both in germinal centres (GC) and macrophages (M), and a weak level in lymphocytes (L).

and a weak expression in lymphocytes. BDNF revealed a moderate expression in germinal centres and in macrophages, and a strong immunoreactivity was observed in lymphocytes. TrkB revealed a moderate immunostaining in germinal centres and macrophages, while a strong expression

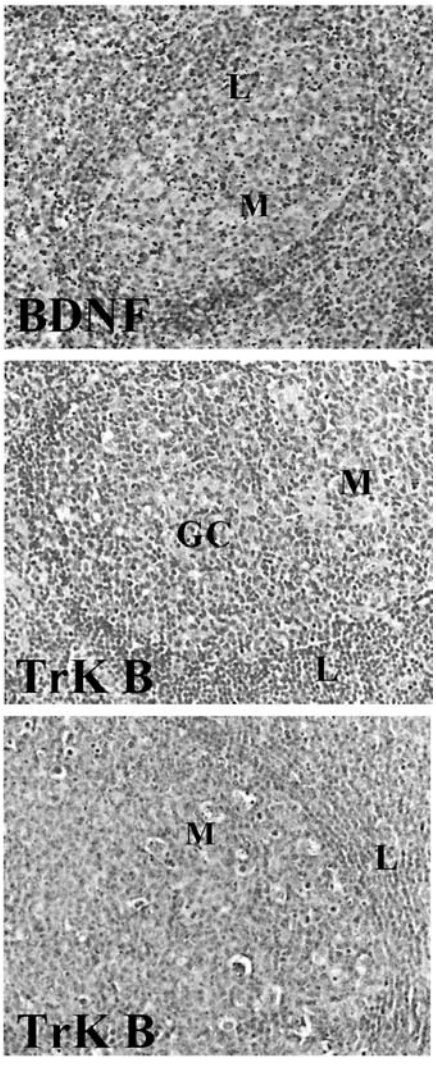

Figure 2. Micrographs of BDNF and TrkB immunostaining in human lingual tonsil tissue, x40. BDNF and its high-affinity receptor TrkB revealed a strong expression in lymphocytes (L), and a moderate immunoreactivity both in germinal centres (GC) and macrophages (M).

was observed in lymphocytes. NT-3 showed a weak immunostaining in germinal centres and lymphocytes, and a moderate expression in macrophages. NT-4 was generally absent in all the specimens analysed. TrkC showed a strong immunoreactivity in germinal centres and macrophages, and a weak immunostaining in lymphocytes (Figs. 1-3).

Discussion

Neurotrophins (NTs) are neurotrophic signalling polypeptides which play physiological roles in the development, maintenance and regeneration of the sympathetic and sensory nervous system (5,9). Moreover, NGF induces differentiation and decreases growth rate in a variety of neoplastic cells with a neurogenic and non-neurogenic origin (17,18). Detailed studies carried out during the last decade regarding the tissue distribution and cellular localization on NT receptors demonstrated their presence in cellular subpopulations of both primary and secondary lymphoid organs (12,19). Therefore, both these tissues and cells are specific targets for NTs.

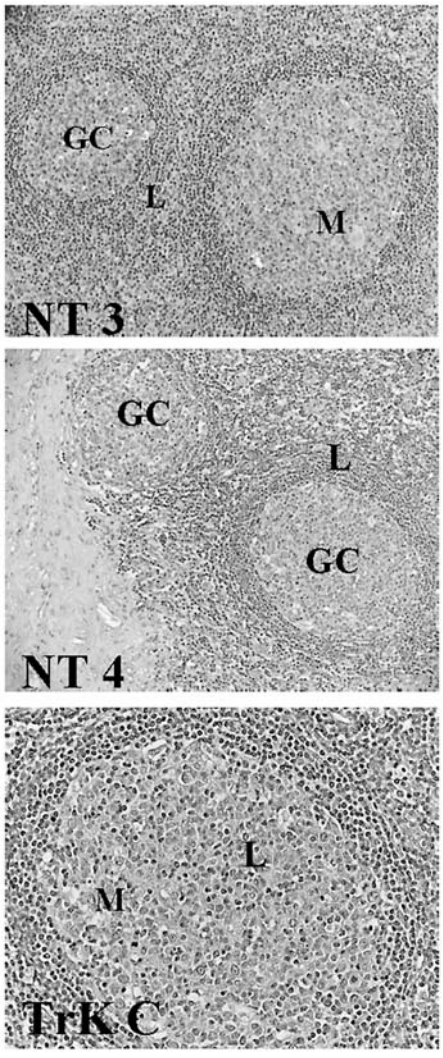

Figure 3. Micrographs of NT-3, NT-4 and TrkC in human lingual tonsil tissue, x40. Immunostaining for NT-3 was generally weak both in germinal centres (GC) and lymphocytes (L), while it was moderate in macrophages (M). NT-4 was generally absent in the analysed specimens. TrkC immunostaining showed a strong expression both in macrophages (M) and germinal centres (GC), and a weak expression in lymphocytes (L).

Interestingly, in all vertebrate species examined, from humans to fish, NTs and their receptors have been detected in lymphoid organs (20-23). Numerous studies provide evidence that NGF acts on a variety of cells of the immune system, including mast cells, eosinophils, and B and T lymphocytes (12). The possibility of neurotrophins acting on lymphocytes was first reported by Dean *et al* (24), who observed that NGF increased the blastogenic response of mouse spleen cells. This finding, which suggested that lymphocytes (and probably other immunocompetent cells) expressed neurotrophin receptors, was followed by the demonstration that these cells also synthesized and released neurotrophins, thus suggesting that there might be an autocrine and paracrine action of NTs on the same cells (19). Interestingly, the expression of both neurotrophins and their receptors by lymphocytes is frequently dependent on cell activation (25,26). In fact, expression of both NGF and TrkA is induced by mitogen activation in $CD4^+$ T cells (27). Moreover, both $CD4^+$ and $CD8^+$ T cells produce NGF, but this is increased after antigenic stimulation in the Th2 subset (28).

Furthermore, other studies (29,30) confirmed that unstimulated human $CD4^+$ Th1 and Th2 cells (but not Th0 cells) express both NGF and TrkA in basal conditions, and Th1 cells express full-length TrkB and low levels of TrkC. In addition, T cells express mRNA and protein specific to BDNF, NT-3 and NT-4/5 (25,26). Otherwise the expression of p75NTR by T cells still remains controversial (31). NGF also induces growth and differentiation in B cells (32) and acts as an autocrine survival factor for memory B cells (33). The expression of TrkA (34,35) and p75NTR (36) in B cells has been reported. According to Schenone *et al* (37) B cells do not express mRNA for either p75NTR or TrkA; moreover, BDNF activates TrkB receptors on B cells. The occurrence of TrkB and TrkC on B cells has been recently confirmed (35,37). Furthermore, it has been shown that B cells produce NGF and NT-3 (33,35,37). It is already known (10,38) that NGF has an inflammatory role and its increase is directly related to inflammation, allergies and diseases of the immune system. NGF levels are also increased in asthma and in other allergic diseases (29,39,40). NGF also causes a significant stimulation of granulocyte differentiation from human peripheral blood and cord blood and murine bone marrow cells; it enhances phagocytosis in macrophages during inflammation and superoxide production of neutrophils (41). Neurotrophins do not modify the antigen-presenting capacities of macrophages or their production of pro-inflammatory cytokines, and NGF acts as a chemotactic factor for these cells (42). Macrophages express TrkB and TrkC but not BDNF, NT-3 or NT-4. The treatment of these cells with NT-3 increases the secretion of nitric oxide in LPS-treated macrophages, suggesting that NT-3 play an important role in the function of macrophages during inflammatory responses (43,44). In our experiments, a moderate to strong immunoreactivity was observed for NTs and NT receptors in human lingual tonsil tissue. In particular, NTs and their own high-affinity receptors were strongly expressed in macrophages and, to a lesser extent, in lymphocytes. The whole tissue showed moderate expression of p75NTR in the analysed patients in conformance with the majority of the reports available in the literature (31). Specific immunoreactivity for NTs and NT receptors was also demonstrated within different layers of large, medium and small-sized arteries and veins, according to previous studies already performed in other mucosa-associated lymphoid tissues (45-47).

Interestingly, we mainly observed a strong expression of NGF and TrkC in macrophages and in germinal centres of the tonsils, suggesting the role of NGF (and NT-3) in the activation and maintenance of the inflammatory condition, as previously demonstrated in the literature (13,41-44,48). On the contrary, we detected a strong expression of BDNF and TrkB in lymphocytes, and a moderate presence in the germinal centres, supporting the major functional role of this factor in the peripheral and differentiated immunocompetent cells.

As revealed by Stanisz *et al* (48) NGF is directly related to the inflammatory response. It induces the proliferation and activation of macrophages and acts as a main factor in the chemotactic response (33,35,37,41,42) and is also a growth

and survival factor for B cells in the germinal centres in the tonsils, as shown by our results. It acts mainly by its own high-affinity receptor TrkA, expressed on the surface of the peripheral blood cells (31,32,42,44) and in close correlation to NT-3 and its own receptor TrkC (43,44). On the contrary, BDNF, with its own receptor TrkB, is mainly involved in the activation of peripheral and differentiated lymphocytes, but not in the maintenance of the memory B cells, supporting the idea that BDNF influences the cytokine expression pattern in antigen-specific T cells and modulate the production of IL-4, TGF-ß, TNF-α, IFN-γ (49). Considering the importance in the Waldeyer's Ring of macrophages and dendritic cells, whose role in the transport of antigens to the extra-follicular T-cell areas and B-cell follicles is well ascertained (38), it is evident that the innervation control of the local micro-environment via the action of neurotrophins on macrophages, germinal centres and lymphocytes may play a relevant role in the modulation of the response to antigens stimulating the Waldeyer's Ring. Due to their localization in the upper aero-digestive tract (38), lingual and palatine tonsils are continuously exposed to antigens. Hence, the generation of antigen-specific immune responses with formation of Ig-producing plasma cells is a very important function that re-evaluates the prominent role of the above-mentioned lymphoid aggregates and emphasizes the significance of the lingual tonsil, that has been considered for a long time almost atrophic or without relevance.

References

1. Brandtzaeg P: Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. International Congress Series 1254: 89-99, 2003.
2. Brandtzaeg P: Immune functions and immunopathology of palatine and nasopharyngeal tonsils. In: Immunology of the Ear. Raven Press, New York, pp63-106, 1987.
3. Felten SY, Felten DL, Bellinger DL and Olschowka JA: Noradrenergic and peptidergic innervation od lymphoid organs. Chem Immunol 52: 25-48, 1992.
4. Lewin GR and Barde YA: Physiology of neurotrophins. Ann Rev Neurosci 9: 217-289, 1996.
5. Barbacid M: The TrK family of neurotrophin receptors. J Neuroimmunol 25: 1386-1403, 1994.
6. Kaplan DR and Miller FD: Signal transduction by the neurotrophin receptors. Curr Opin Cell Biol 9: 213-221, 1997.
7. Lomen HC and Shooter EM: Widespread neurotrophin receptor expression in the immune system and other non-neuronal rat tissue. J Neurochem 64: 1780-1789, 1995.
8. Lommatzsch M, Braun A, Mannsfeldt A, Botchkarev VA, Botchkareva NV, Paus R, Fischer A, Lewin GR and Renz H: Abundant production of brain-derived neurotrophic factor by adult visceral epithelia implications for paracrine and target-derived neurotrophic functions. Am J Pathol 155: 1183-1193, 1999.
9. Snider WD: Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77: 627-638, 1994.
10. Otten U and Gadient RA: Neurotrophins and cytokines-intermediaries between the immune and nervous systems. Int J Dev Neurosci 13: 147-151, 1995.
11. Tessarollo L: Pleiotropic functions of neurotrophins in development. Cytokine Growth Factor Rev 9: 125-137, 1998.
12. Aloe L, Simone MD and Properzi F: Nerve growth factor: a neurotrophin with activity on cells of the immune system. Microsc Res Tech 45: 285-291, 1999.
13. Aloe L: Nerve growth factor and neuroimmune responses: basic and clinical observations. Arch Physiol Biochem 109: 336-354, 2001.
14. Sariola H: The neurotrophic factors in non-neuronal tissues. Cell Mol Life Sci 58: 1061-1066, 2001.
15. Carlson SL, Albers KM, Beiting DJ, Parish M, Conner JM and Davis BM: NGF modulates sympathetic innervation of lymphoid tissues. J Neurosci 15: 5892-5899, 1995.
16. Carlson SL, Johnson S, Parrish ME and Cass WA: Development of immune hyperinnervation in NGF-transgenic mice. Exp Neurol 149: 209-220, 1998.
17. Yaeger MJ, Koestner A, Marushige K and Marushige Y: The reverse transforming effects of nerve growth factor on five human neurogenic tumor cell lines: *in vitro* results. Acta Neuropathol (Berl) 83: 72-80, 1991.
18. Sala R, Viegi A, Rossi FM, Pizzorusso T, Bonanno G, Raiteri M and Maffei L: Nerve growth factor and brain derived neurotrophic factor increase neurotransmitter release in the rat visual cortex. Eur J Neurosci 10: 2185-2191, 1998.
19. Vega JA, Garcia-Suarez O, Hannestad J, Perez-Perez M and Germana A: Neurotrophins and the immune system. J Anat 203: 1-19, 2003.
20. Ciriaco E, Dall'Aglio C, Hannestad J, Huerta JJ, Laura R, Germana G and Vega JA: Localization of TrK neurotrophin receptor-like proteins in avian primary lymphoid organs (thymus and bursa of Fabricius). J Neuroimmunol 69: 73-78, 1996.
21. Hannestad J, Levanti B and Vega JA: Distribution of neurotrophin receptors in human palatine tonsils: an immunohistochemical study. J Neuroimmunol 58: 131-137, 1995.
22. Garcia-Suarez O, Hannestad J, Esteban I, Martinez del Valle M, Naves FJ and Vega JA: Neurotrophin receptor-like protein immunoreactivity in human lymph nodes. Anat Rec 249: 226-232, 1997.
23. Labouyrie E, Parrens M, De Mascarel A, Bloch B and Merlio JP: Distribution of NGF receptors in normal and pathologic human lymphoid tissues. J Neuroimmunol 77: 161-173, 1997.
24. Dean DH, Hiramoto RN and Ghanta VK: Modulation of immune response. A possible role for murine salivary epidermal and nerve growth factors. J Periodontol 58: 498-500, 1987.
25. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H and Hohlfeld R: Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor *in vitro* and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 189: 865-870, 1999.
26. Moalem G, Gdalyahu A, Shani Y, Otten U, Lavarovici P, Cohen IR and Schwartz M: Production of neurotrophins by activated T cells: implications for neuroprotective role autoimmunity. J Autoimmun 15: 331-345, 2000.
27. Ehrhard PB, Erb P, Graumann U and Otten U: 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: 10984-10988, 1993.
28. Santambrogio L, Benedetti M, Chao MV, Muzaffar R, Kulig K, Gabellini N and Hochwald G: Nerve growth factor production by lymphocytes. J Immunol 153: 4488-4495, 1994.
29. Lambiase A, Bracci-Laudiero L, Bonini S, Bonini S, Starace G, D'Elios MM, De Carli M and Aloe L: 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, 1997.
30. Besser M and Wank R: Cuuting edge: clonally restricted production of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3 mRNA by human immune cells and Th1/Th2-polarized expression of their receptors. J Immunol l162: 6303-6306, 1999.
31. Kittur SD, Song L, Endo H and Adler WH: Nerve growth factor receptor gene expression in human peripheral blood lymphocytes in aging. J Neurosci Res 32: 444-448, 1992.
32. Otten U, Ehrhard P and Peck R: Nerve growth factor induces growth and differentiation of human B lymphocytes. Proc Natl Acad Sci USA 86: 10059-10063, 1998.
33. Torcia M, Bracci-Laudierio L, Lucibello M, Nencioni L, Labardi D, Rubartelli A, Cozzolino F, Aloe L and Garaci E: Nerve growth factor is an autocrine survival factor for memory B lymphocytes. Cell 85: 345-356, 1996.
34. Melamed I, Kelleher CA, Franklin RA, Brodie C, Hempstead B, Kaplan D and Gelfand EW: Nerve growth factor signal transduction in human B lymphocytes is mediated by gp140Trk. Eur J Immunol 26: 1985-1992, 1996.
35. D'Onofrio M, De Grazia U, Morrone S, Cuomo L, Spinsanti P, Frati L, Gulino A and Ragona G: Expression of neurotrophin receptors in normal and malignant B lymphocytes. Eur Cytokine Netw 11: 283-291, 2000.

36. Brodie C, Oshiba A, Renz H, Bradley K and Gelfand EW: Nerve growth-factor and anti-CD40 provide opposite signals for the production of IgE in interleukin-4-treated lymphocytes. Eur J Immunol 26: 171-178, 1996.
37. Schenone A, Gill JS, Zacharias DA and Windebank AJ: Expression of high- and low- affinity neurotrophin receptors on human transformed B lymphocytes. J Neuroimmunol 64: 141-149, 1996.
38. Hellings P, Jorissen M and Ceuppens JL: The Waldeyer's Ring. Acta Otorhinolaryngol (Belg) 54: 237-241, 2000.
39. Bonini S, Lambiase A, Angelucci F, Magrini L and Aloe L: Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. Proc Natl Acad Sci USA 93: 10995-10960, 1996.
40. De Vries A, Engels F, Henricks PA, Leusink-Muis T, Fischer A and Nijkamp FP: Antibodies directed against nerve growth factor inhibit the acute bronchoconstriction due to allergen challenge in guinea-pigs. Clin Exp Allergy 32: 325-328, 2002.
41. Susaki Y, Shimizu S, Katakura K and Watanabe N: Functional properties of murine macrophages promoted by nerve growth factor. Blood 88: 4630-4637, 1996.
42. Porcheray F and Gras G: Neurotrophins modulate monocyte chemotaxis without affecting macrophage function. Clin Exp Immunol 151: 476-486, 2008.
43. Barouch R, Appel E, Kazimirsky G and Brodie C: Macrophages express neurotrophins and neurotrophin receptors: regulation of nitric oxide production by NT-3. J Immunol 112: 72-77, 2001.
44. Caroleo MC, Costa N, Bracci-Laudiero L and Aloe L: Human monocytes/macrophages activated by exposure to LPS over-express NGF and NGF receptors. J Immunol 113: 193-201, 2001.
45. Pezzati P, Stanisz AM, Marshall JS, Bienenstock J and Stead RH: Expression of nerve growth factor receptor immunoreactivity on follicular dendritic cells from human mucosa associated lymphoid tissues. Immunology 76: 485-490, 1992.
46. Bronzetti E, Artico M, Lo Vasco VR, Felici LM, Bosco S, Magliulo G, Pompili E and Fumagalli L: Expression of neurotransmitters and neurotrophins in human adenoid tissue. Int J Mol Med 15: 921-928, 2005.
47. Bronzetti E, Artico M, Pompili E, Felici LM, Stringaro A, Bosco S, Magliulo G, Colone M, Arancia G, Vitale M and Fumagalli L: Neurotrophins and neurotransmitters in human palatine tonsils: an immunohistochemical and RT-PCR analysis. Int J Mol Med 18: 49-58, 2006.
48. Stanisz AM and Stanisz JA: Nerve growth factor and neuroimmune interactions in inflammatory diseases. Ann NY Acad Sci 917: 268-272, 2000.
49. Bayas A, Kruse N, Moriabadi NF, Weber F, Hummel V, Wohleben G, Gold R, Toyka KV and Rieckmann P: Modulation of cytokine mRNA expression by brain-derived neurotrophic factor and berve growth factor in human immune cells. Neurosci Lett 335: 155-158, 2003.