Effect of Nerve Growth Factor on the Release of Inflammatory Mediators by Mature Human Basophils

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Nerve growth factor (NGF) is a neurotrophic cytokine known to regulate the survival and function of peripheral and central neuronal cells. Recently, the spectrum of action could be extended to non-neuronal cell types such as rat mast cells and human B lymphocytes. The present study shows that NGF affects the function of mature human basophils isolated from the peripheral blood of healthy donors. Both murine NGF 7S and recombinant human NGF β enhance histamine release and strongly modulate the formation of lipid mediators by basophils in response to various stimuli. This priming effect of NGF on basophils occurs rapidly within 10 to 15 minutes of preincubation, is dose-dependent, and requires similarly low concentrations (1 to 40 pmol/L) of human NGF β as the induction of neurite outgrowth in ganglion cells. Cell fractionation studies indicate that NGF acts directly on human basophils without an involvement of other cell types, suggesting the presence of high-affinity NGF receptors on basophils. NGF by itself (up to 4 nmol/L of human NGF β) does not induce the release of inflammatory mediators directly. The effect of human NGF on basophil mediator release is similar to that of the hematopoietic growth factors interleukin-3, interleukin-5, and granulocyte-macrophage colony-stimulating factor. The present study further demonstrates that NGF acts as a pleiotropic cytokine at the interface between the nervous and the immune system, and that NGF may be involved in inflammatory processes and hypersensitivity reactions.

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For most experiments, leukocytes were separated from erythrocytes by dextran-sedimentation and fractionated by Ficoll Hypaque density centrifugation (400g for 40 minutes at room temperature). The basophil-containing mononuclear cell layer was harvested, washed, and finally suspended at a density of 2.5 to 5 × 10⁶ cells/mL in HACM-buffer (20 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L glucose, 0.025% BSA, 1 mmol/L CaCl₂, and 2 mmol/L MgCl₂). These preparations contained 1% to 6% (mean, 2%) basophils as determined by stained cytospin slides. In some experiments, leukocytes were fractionated on discontinuous Percoll gradients (densities: 1.079, 1.070, and 1.062 containing the leukocytes) and centrifuged (500g for 30 minutes at room temperature). The cell layers at the interface between the 1.070 and 1.070 density (20% to 50% basophils) were harvested and the latter was depleted of contaminating T cells, B cells, NK cells, and neutrophils using immunomagnetic beads (cell/beads ratio = 1:10) coated with mouse anti-CD2 (60%), anti-CD19 (10%), and anti-CD16 monoclonal Ab (MoAb) (30%). The resulting basophil preparation (purity, 85% to 96%) was washed twice in HA-buffer and finally resuspended in HACM-buffer at a density of 1 to 2 × 10⁶ cells/mL.

Cell stimuli. The β-form of rhuNGF, expressed in Chinese hamster ovary cells and purified to greater than 98%, was a generous gift from Genentech, Inc (South San Francisco, CA). The biologic activity of rhuNGF (molecular weight [MW] of the dimer, 25.6 Kd) was determined by neurite formation in P1C12 cells (a rat pheochromocytoma cell line). A half-maximal activity in this assay was produced by 1.5 to 2 pmol/L rhuNGF.²³ Murine NGF 7S complex (muNGF 7S, MW = 140 Kd), prepared from male mouse salivary glands and purified to 99% as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), was from Boehringer Mannheim, Inc. MuNGF 7S activity was determined by fiber outgrowth of chick embryo dorsal root ganglia and became maximal at 400 to 700 pmol/L. The lyophilized preparation was reconstituted according to the manufacturers instructions and further diluted in HA buffer containing 1 mg/mL BSA. Dr Y.A. Barde (Max-Planck-Institute of Psychiatry, Planegg-Martinsried, Germany) kindly provided us with COS-conditioned medium containing recombinant brain-derived neurotrophic factor (BDNF). Final dilutions of 1:100 to 1:300 were maximally active in promoting the survival of spinal sensory neurons isolated from chick embryos.²⁴ rhuIL-3 and rhuGM-CSF were from Sandoz (Basel, Switzerland).²⁵ Supernatants from Escherichia coli cultures expressing the human IL-5 protein were a generous gift from Dr C.J. Sanderson (National Institute for Medical Research, London, UK). These supernatants enhanced basophil mediator release (maximal effect at a dilution of 1:1,000) as the purified preparation.²⁴ The complement cleavage product C5a was purified from plasma by anion-exchange chromatography (-Sepharose CL-4B). Twenty micrograms of C5a were mixed with 0.4 mL of assay buffer and 1 mL of HACM buffer, centrifuged (400g for 10 minutes at 4°C) and divided into two parts: 0.4 mL were directly stored at −70°C for histamine measurement, 0.5 mL were mixed with 0.5 mL of HACM buffer and 1 mL of HClO₄, centrifuged (600g for 15 minutes at 4°C), and these supernatants were also stored at −70°C for histamine measurement using an automated fluorometric method.³

Leukotriene measurement. The sLT including LTC₄/D₄/E₄ were measured by a fluid phase radioimmunoassay (RIA) based on competition between unlabeled LTC₄ (standards or samples) and a fixed quantity of tritium-labeled LTC₄ (tracer) for binding to a limited quantity of MoAb directed against sLT. The LTC₄ tritium tracers, either [14,15-³H]LTC₄ (specific activity, 39.3 Ci/mmol) or the higher labeled [14,15,19,20-³H]LTC₄ (168.4 Ci/mmol), were from NEN research products (Boston, MA). Tracers were diluted in assay buffer (phosphate-buffered saline [PBS] 0.05 mol/L, pH 7.4, containing 10 mg/mL gelatine) to 1.77 and 0.38 pmol/mL, respectively. LTC₄ standards (Amersham Corp, Arlington, IL) were diluted to 0.03 to 8 ng/mL in HACM buffer. The mouse MoAb against sLT (sLT-Ab) was kindly provided by Drs J. Mollenhauer and K. Brune (University of Erlangen-Nürnberg, Germany). This Ab had the same sensitivity for LTC₄, LTD₄, and LTE₄, and showed no cross-reactivity to LTB₄ and its metabolites, to prostaglandins D₂, E₂, F₂α, or to thromboxane B₂.²³ The lyophilized unpurified sLT-Ab from cell culture supernatant was reconstituted in PBS/H₂O 1:1 (1 mg of total protein/mL), and further diluted in assay buffer (1:100 for the less sensitive assay, 1:300 for the high sensitive assay, determined to be optimal for each assay). One hundred microliters of supernatants or of standard LTC₄ solutions + 100 µL of LTC₄ tracer + 100 µL of sLT-MoAb solution + 100 µL of HACM buffer (400 µL in total per tube) were mixed. After overnight incubation at 4°C, 250 µL of charcoal suspension (assay buffer + 800 mg/mL charcoal, 160 mg/mL dextran T70, 1 mg/mL sodium azide) was added for 15 minutes to absorb unbound LTC₄ tracer. These supernatants were transferred into scintillation vials. The amount of LTC₄ tracer bound to the Ab was quantified in the β-counter and was inversely proportional to the concentration of unlabeled sLT. ‘Zero standard’ (without unlabeled LTC₄) was 1,200 to 1,500 cpm (corresponding to 25% to 35% binding of the tracer to the antibody). ‘Nonspecific binding’ (zero standard without antibody addition) was less than 100 cpm. sLT from 12.5 to 800 pg/tube and from 3.125 to 400 pg/tube, respectively, dependent on the assay used for measurement, could be detected (Fig 1). Measurements were identical to those performed with a commercially available LTC₄/D₄/E₄ RIA kit (Amersham Corp) following the manufacturers protocol, despite the fact that the polyclonal antiserum used in this assay recognizes LTD₄ and LTE₄ by only 64% as compared with LTC₄. This is due to the negligible conversion of LTC₄ to LTD₄/E₄ in mononuclear cells under incubation conditions used in this study, as we have shown previously by high performance liquid chromatography (HPLC).²³ Nevertheless, the equal affinity of the sLT-Ab for all sLT is preferable because total sLT are measured, and results are not influenced by possible variations in LTC₄ metabolism.

Presentation of data. All experiments were performed at least in duplicate, and repeated three times or more. Histamine data are presented as mean of percent specific histamine release according to the formula 100 x [release - spontaneous release]/[total histamine content]. Leukotriene data are expressed as mean of picograms of leukotriene per nanogram of total histamine content per tube to correct for different basophil numbers.³ Data derived from several experiments are usually presented as mean ± standard error of the mean (SEM). Mediator release values in response to the same triggering agent within the same cell preparation but with different preincubation conditions (Figs 2 and 3) were analyzed by analysis of variance and the paired t-test according to Sachs.²⁶
preparations also qualitatively changed the release profile to 800 pg/tube, and with the high specific activity tracer Ab. Figure shows the variability of histamine and leukotriene release, respectively, from experiments performed with different donors, as well as mean values from the different stimulation protocols. Both muNGF 7S and rhuNGF enhanced histamine release and sLT generation in response to C5a, anti-IgE Ab, and FMLP. Furthermore, in all experiments, NGF-primed basophils produced large amounts of sLT in response to C5a, while, with the exception of rare cases, no sLT were detected from cells stimulated with C5a alone. It should be noted that in all experiments mediator release in response to the three agonists was enhanced by NGF preincubation, when compared with the corresponding control value (paired t-test, all P < .001). The enhancement of mediator release due to

RESULTS

Figure 2 shows that preincubation of basophils for 10 minutes with either rhuNGF β or muNGF 7S enhanced concentration-dependent histamine release as well as the generation of leukotrienes in response to anti-IgE Ab or FMLP. Apart from these enhancing effects, both NGF preparations also qualitatively changed the release profile in response to C5a, which by itself induced degranulation only. NGF rendered basophils capable of synthesizing lipid mediators in response to C5a, while histamine release was similarly enhanced as the response to FMLP or anti-IgE Ab. Figure 2 shows that this priming effect of rhuNGF β and muNGF 7S on human basophils occurred within a concentration range of 0.03 to 1 ng/mL and 10 to 100 ng/mL, reaching a plateau at 1 to 10 ng/mL and 100 to 1,000 ng/mL, respectively. Thus, basophils are influenced by rhuNGF β (ED50 ≈ 4 pmol/L) and muNGF 7S (ED50 ≈ 140 pmol/L) at concentrations similar to those required for stimulation of neuronal cells. Importantly, neither rhuNGF β nor muNGF 7S did induce any mediator release in basophils by themselves, up to concentrations of 100 ng/mL rhuNGF β and 1,000 ng/mL muNGF 7S, respectively (data not shown). By contrast, BDNF, a neurotrophic protein structurally related to NGF, failed to induce or modulate basophil mediator release at concentrations determined to be optimal for promoting survival of chick embryo sensory neurons (1:1,000 to 1:100 dilutions).

There is a remarkable, well-known donor variability of basophil mediator release in response to anti-IgE Ab or FMLP, and to a lesser extent to C5a. Thus, in a large number of experiments, cells from different donors were preincubated in buffer control or with optimal concentrations of muNGF 7S, rhuNGF β, or rhuIL-3 within the same experiment. After 10 minutes, cells were triggered with maximally effective concentrations of C5a, FMLP, or anti-IgE Ab (Fig 3). IL-3 was included in these experiments, because we found in previous studies that, within the HGF capable of modifying basophil function, IL-3 is the most active cytokine. Figure 3 shows the variability of histamine and leukotriene release, respectively, from experiments performed with different donors, as well as mean values from the different stimulation protocols. Both muNGF 7S and rhuNGF enhanced histamine release and sLT generation in response to C5a, anti-IgE Ab, and FMLP. Furthermore, in all experiments, NGF-primed basophils produced large amounts of sLT in response to C5a, while, with the exception of rare cases, no sLT were detected from cells stimulated with C5a alone. It should be noted that in all experiments mediator release in response to the three agonists was enhanced by NGF preincubation, when compared with the corresponding control value (paired t-test, all P < .001). The enhancement of mediator release due to

![Fig 1](https://www.bloodjournal.org)  
Fig 1. sLT RIA standard curves. The y-axis indicates the percentage of specific binding of a fixed amount of labeled LTC4 to an anti-sLT Ab depending on the concentration of unlabeled LTC4 (x-axis). Specific binding was calculated according to the formula: [Bx - NSB] / [Bo - NSB], whereas Bx = cpm of standards, NSB = cpm of nonspecific binding, and Bo = cpm of maximal binding in the absence of unlabeled LTC4. Using the lower specific activity tracer, sLT could be detected within a concentration range of 12.5 to 800 pg/tube, and with the high specific activity tracer, the range was 3.125 to 400 pg/tube of sLT. Nearly overlapping standard curves were obtained when unlabeled LTC4 was replaced by LTD4 or LTE4 (not shown).

![Fig 2](https://www.bloodjournal.org)  
Fig 2. The concentration-dependent effect of human NGF β and murine NGF 7S on basophil mediator release. Mononuclear cells containing 1% to 6% basophils were preincubated without (0) or with different concentrations of rhuNGF β (left panels) or muNGF 7S (right panels) for 10 minutes, and then stimulated with maximally effective concentrations of C5a (10−9 mol/L), anti-IgE Ab (100 ng/mL), or FMLP (2.5 × 10−5 mol/L) for 20 minutes. Histamine release (C) and leukotriene production (D) were measured in supernatants. Mean ± SEM (n = 3) of three experiments, each performed in duplicate and with different donors, are shown. Neither rhuNGF β nor muNGF 7S induced any mediator release by itself over the whole concentration range (not shown). Statistical analysis: the effect of each NGF concentration was compared to the mediator release in the absence of NGF by the two-sided, paired t-test; *P < .05, **P < .001.
After a preincubation period of 10 minutes in the presence of either muNGF \(7S\) (100 ng/mL), rhuNGF \(7S\) (100 ng/mL), or buffer control (O), basophils were stimulated for 20 minutes with maximally effective concentrations of \(C5a\) (10\(^{-4}\) mol/L), anti-IgE Ab (100 ng/mL), or FMLP (2.5 \(\times\) 10\(^{-6}\) mol/L). Histamine (upper panels) and leukotriene data (lower panels) are shown. Each circle represents the mean of duplicates from one experiment; the data of each experiment are connected by lines; the columns indicate the mean of all data. In all experiments, NGF preincubation without subsequent addition of the IL-3 effect (all \(P > 0.05\)). However, mediator release, in particular in response to anti-IgE stimulation, was less pronounced after NGF preincubation as compared to the IL-3 effect (all \(P > 0.05\)). In previous studies, we defined a set of cytokines (IL-3, GM-CSF, and IL-5) capable of strongly modulating basophil functions.\(^4\) We therefore compared the effect of NGF with that of these HGF within the same experiments (Table 1). The mediator release profile triggered by IgE-independent agonists such as \(C5a\) and FMLP was influenced by all four cytokines in a nearly identical fashion. However, particularly with regards to anti-IgE Ab-induced mediator release, NGF was clearly the least active priming agent. The time interval between addition of NGF and second agonist (\(C5a\), FMLP, anti-IgE Ab) was 10 minutes in all experiments described so far. This preincubation period has been determined to be optimal for basophil priming with HGF, such as IL-3, IL-5, or GM-CSF.\(^4\) To evaluate whether this is transferable to basophil priming with NGF, the time interval between addition of NGF and \(C5a\) was varied from 10 seconds to 40 minutes. An optimal enhancement of \(C5a\)-induced mediator release occurred after a preincubation time of 10 to 15 minutes for both muNGF \(7S\) and rhuNGF (Fig 4A and B). Even 40 minutes after NGF addition, basophils were still able to produce \(sLT\) in response to \(C5a\), albeit slightly less efficiently than after the optimal time of preincubation. The major component of the priming process, in particular with regards to \(sLT\) synthesis, appeared within 2 to 10 minutes after NGF addition for both muNGF \(7S\) and rhuNGF. However, rhuNGF, but not muNGF \(7S\), already enhanced \(C5a\)-induced histamine release significantly 10 seconds after NGF addition, as shown in more detail in Fig 4C. This early effect of rhuNGF was reproducible in three experiments from different donors. Histamine release was raised up to 20% to 30% of total cellular histamine content over the histamine release induced by \(C5a\) alone. The reason for the different effects of muNGF \(7S\) and rhuNGF \(\beta\) at short preincubation times is unclear at present, but may be due to the necessity of dissociation of the NGF \(7S\) complex to become active for basophil priming.\(^8\)

In three experiments, highly purified basophil preparations (85% to 96%) were used to determine the participation of other leukocyte types in the action of NGF on basophils. We also performed experiments with leukocyte preparations fractionated by Percoll gradients containing basophils of different densities and variable proportions of other leukocyte types. Figure 5A shows a representative experiment performed with a cell preparation of 96% basophil purity (contaminating cells were small lymphocytes). The data show that \(C5a\)- and anti-IgE Ab-induced mediator release was enhanced by rhuNGF. NGF-primed, but not unprimed, purified basophils release large amounts of \(sLT\) in response to \(C5a\). The data were not different when, within the same experiment of the same cell fraction, depletion of T cells, B cells, NK cells, and neutrophils was...
omitted (Fig 5B). The pattern of mediator release and the influence of NGF was identical in low density basophils derived from an upper cell layer (between 1.070 and 1.062 density gradients), mainly contaminated with lymphocytes. These cell preparations produced slightly larger amounts of leukotrienes, and in the supernatants of cells triggered by C5a alone some sLT was detected (Fig 5C). We cannot decide at present whether functional differences exist within basophils of different densities, or whether the discretely larger amounts of sLT produced in the cell fractions of lower density are due to contaminating leukocytes, because we have not yet achieved a complete purification of hypodense basophils. However, the data clearly show that the modulating effect of NGF is independent of a contamination with other leukocyte types.

**DISCUSSION**

The present study shows that human NGF at very low concentrations influences the function of one type of mature human effector cells, and shows a first biologic activity of pure rhuNGF β-chain outside the nervous system. This priming effect of NGF, which occurs when basophils have been preincubated for a few minutes in the presence of the neurotrophic cytokine, consists of an enhancement of histamine and sLT release induced by FMLP or anti-IgE Ab, or of a qualitative modification of the basophil response towards C5a, in which case NGF preincubation is required for lipid mediator synthesis.

Previous work with rat peritoneal mast cells showed that purified muNGF directly induces histamine release in a dose-dependent manner. However, degranulation occurred only at high concentrations (5 to 500 nmol/L of NGF), or in the presence of lysophospholipids. Furthermore, mu-NGF 2.5S induced a shape change in rabbit platelets at a concentration of 570 nmol/L. Such concentrations are
orders of magnitude above that required in ganglion or
PC12 cell assays. In vivo, 4 to 40 pmol of muNGF 2.5S
induced plasma extravasation in rat skin, which could be
reduced by capsaicin pretreatment and by histamine antag-
onists, indicating an involvement of both a neurogenic (eg,
via substance P) and a basophil/mast cell-dependent mech-
anism.27 By contrast, in the human system NGF is not a
direct basophil agonist, because it induces no mediator
release by itself, even at concentrations up to 100 ng/mL of
rhuNGF, or up to 1 μg/mL of muNGF 7s. Furthermore,
in the presence of IL-3, in which case several otherwise
inactive basophil triggers (C3a, IL-8, platelet-activating
factor) efficiently induce histamine and sLT release,28,29
NGF is still ineffective (our unpublished results).

The effect of NGF on mature human basophils is
remarkably similar to that of IL-3. In the mouse, IL-3 acts
as a mast cell growth factor, while in human bone marrow
cultures IL-3 induces proliferation and differentiation of
myeloid progenitors into immature histamine-containing
cells, which resemble basophils more than mast cells.30
Furthermore, in contrast to basophils, human mucosal mast
cells do not bear IL-3 receptors, and their function is not
affected by IL-3,31,32 Interestingly, one of the earliest and
most firmly established effects of NGF outside the nervous
system in rats is its capacity to cause an increase in size and
number of mast cells in several peripheral tissues,35 possibly
through an autocrine involvement of mast cell product
release.36 In the human system, however, NGF neither
modulates nor induces granule release in human mucosal
mast cells.32 Therefore, the biologic effects of NGF and
IL-3 seem to be quite similar in the human as well as in the
mouse system, except that the target cells, basophils, and
mast cells, are different.

Among a large number of cytokines examined, only IL-3,
IL-5, and GM-CSF were found to modulate the function of
mature human basophils.24 Thus, NGF is the first basophil
response modifier, which was not primarily described as a
HGF. Of interest, Matsuda et al showed that muNGF also
acts on human myeloid progenitor cells as a growth and
differentiation factor in particular of the eosinophil and
basophil/mast cell lineages.20,23 However, in contrast to the
HGF properties of IL-3, GM-CSF, and IL-5, which affect
the myeloid cells directly, this biologic activity of NGF
appeared to be T-cell–dependent. Because of the similarity
of biologic effects of these HGF and NGF on basophils as
well as on myeloid progenitors, it is tempting to speculate
that NGF stimulates the progenitor cells directly, but in
synergism with a T cell product(s). Indeed, recent data
showed that NGF promotes human granulopoiesis only in
synergy with GM-CSF.22

The NGF effect on basophils is rapid and is independent
of the degree of contamination by other leukocyte types,
suggesting a direct effect of NGF on basophils through
specific receptors. Sutter et al15 described two classes of
NGF receptors (NGFR), a high-affinity receptor (kd = 23
pmol/L) probably mediating nerve cell survival and neurite
outgrowth, and a low-affinity receptor (kd = 1.7 nmol/L),
which seems to be required but not sufficient for functional
expression of high-affinity receptors.36 The low-affinity
NGFR has been cloned in human, rat, and chicken, and
binds NGF and even the related BDNF with equal affinity.12,37
Very recent studies indicate that the trk proto-
ocgene either by itself or in combination with the
low-affinity NGFR forms the high-affinity NGFR in neu-
rnal cells.38,39 There is strong evidence that the biologic
effect of NGF at picomolar concentrations on primary
neurons of animal origin as well as on human basophils, as
shown here, is mediated by a high-affinity NGFR.7,36 It
is less clear whether the higher NGF concentrations required
to affect human B cells and myeloid progenitor cells
(ED50 = 10 to 50 ng/mL = 0.4 to 2 nmol/L)22,23 are due to
the lack of high-affinity binding sites on these target cells or
to a weaker bioactivity of muNGF on human cells. The
low-affinity NGFR belongs to a family of structurally
related membrane proteins including CD40, which has
been regarded as a specific leukocyte marker of B cells.40,41
Recent studies, however, have shown that human baso-
phils, but not mast cells, also express CD40.42 Interestingly,
in humans, B lymphocytes and basophils both respond to
NGF and express CD40, but whether this association is
related to the biologic effects of NGF on these two cell
types is unknown.

NGF belongs to an increasingly large family of neuro-
rophic proteins, which are strongly homologous in primary
structure.24,43 Three family members have been identified,
NGF, BDNF and neurotrophin 3 (NT3), with partially
overlapping, partially distinct biologic activities on neu-
ronal cells. It has been suggested that they could interact
with a common binding protein, the low-affinity NGFR,43
possibly in association with accessory proteins creating
high-affinity states specific for each neurotrophic factor.36,39
Our own preliminary data indicate that BDNF does not
affect basophil function at concentrations promoting sur-
vival of nodose neurons, further supporting the hypothesis
of a high-affinity NGFR on basophils. Clearly, additional
studies are needed to define the functional effects of other
neurotrophic factors on basophils.

muNGF 7s and rhuNGF β prime basophils at a concen-
tration range of 70 to 700 pmol/L and 1 to 40 pmol/L,
respectively. These concentrations of both NGF prepara-
tions correspond well to those required to affect neuronal
cells.7,23 The extremely high biologic activity of rhuNGF β
on basophils indicates that the bioactivity of muNGF 7s is
due to the β-subunit. A definitive assessment of possible
differences of bioactivity between mouse and human NGF
β on human basophils would require a direct comparison of
recombinant purified murine and human material under
identical experimental conditions.

Our results extend and confirm the concept that NGF
affects both the nervous and the immune system. However,
the pathophysiologic significance of this interface between
nervous and immune system, the site of NGF production,
and the regulation of NGF during health and disease is still
unclear.25,18 In particular, informations on human tissue
containing NGF are rare and controversial, in part due to
difficulties and misinterpretations of NGF measurements
by immunoassays or biologic assays.7 Nevertheless, it is
tempting to speculate that NGF is synthesized locally in
many tissues, possibly under the control of other cytokines, and may be involved in different pathologic conditions such as tumor growth, cell regeneration, tissue destruction, and wound healing, or in neurogenic or allergic inflammatory processes.

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REFERENCES

41. Stamenkovic I, Clark EA, Seed B: A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. EMBO J 8:1403, 1989
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