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Monocytes of allergics and non-allergics produce, store and release the neurotrophins NGF, BDNF and NT-3

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Abstract

Introduction: Recent studies have shown that neurotrophins (NTs) are involved in inflammatory processes. Elevated plasma levels of NTs were found allergic diseases with the highest levels in allergic asthma. However, the exact cellular sources involved in the regulation and release of neurotrophins in allergic inflammation are still not well defined.

Objective: The aim of this study was to assess whether monocytes of allergic and non-allergic subjects produce, store and release the neurotrophins NGF, BDNF and NT-3.

Methods: Monocytes of allergic and non-allergic donors were purified by immunomagnetic selection. APAAP-staining for the presence of NTs and their receptors was performed. RT-PCR and Western blot evaluated the production and storage of NTs. Monocytes were incubated and supernatants were collected for measurement of neurotrophic factors after stimulation with lipopolysaccharide (LPS) as inflammatory stimulus. The neurotrophin content in lysates and cell culture supernatants was determined by ELISA.

Results: Human monocytes express the neurotrophins NGF, BDNF and NT-3 but also their specific receptors TrkA, TrkB and TrkC. RT-PCR amplification of isolated mRNA demonstrated expression of the examined neurotrophins. Proteins were detectable by Western blot. NTs were found in the monocyte lysates and supernatants at different levels in allergic and non-allergic donors. Cell stimulation with LPS leads to release of NGF and NT3.

Conclusions: Monocytes, produce, store and release NGF, BDNF and NT-3. They are a possible source of elevated neurotrophin levels found in allergy and asthma.

Keywords: NGF; BDNF; NT-3; Monocytes; Asthma; Allergy; Inflammation

1. Introduction

The neurotrophins (NTs) Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin 3 (NT-3) belong to a family of related polypeptides sharing a high homology and a remarkable range of biological activities modulated through activation of their high affinity specific receptors trkA, trkB, trkC and their low affinity unspecific receptor p75 [1]. NTs play an important and well described role in the development, function and survival of neuronal cells [2].

Recently, several studies have described the effects of NTs in inflammatory and autoimmune diseases [3,4]. In relation to allergic diseases, NTs plasma levels were found to be increased in vernale keratoconjunctivitis [5], allergic rhinitis and at highest levels in allergic asthma [6]. In a previous study, we were able to prove elevated NGF levels

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in allergic asthmatics and also found elevated levels of BDNF and NT-3 in untreated allergic asthmatics. After treatment with inhaled corticosteroids, a decrease in circulating neurotrophin levels was observed [7]. The mechanism of increased NT levels in inflammatory conditions is poorly understood. After segmental allergen provocation, an increase of NTs in the bronchoalveolar lavage (BAL) fluid has been described in the late allergic response [8]. In animal models also the immediate response was influenced while treatment with NGF antibodies reduced airway inflammation [9,10]. In different animal models NGF can influence the asthmatic phenotype by augmenting bronchial hyperresponsiveness (BHR) and allergic inflammation [11]. This effect might be modulated by inducting up-regulation of neuropeptide production and release in sensory neurons [12].

Neurotrophins not only affect nerves but can also activate immune cells. Neurotrophin receptors have been found on various immunological active cells involved in allergic sensitisation and inflammation, e.g., lymphocytes and mast cells [13,14]. Recently also the production of neurotrophins by various immune cells has been proved [15,16]. Documented neurotrophin effects on immune cells include proliferation and activation [17–20] and enhancement of neutrophils and macrophages survival [21–23]. Besides these cells, NGF is produced by residential cells such as epithelial cells [24]. Recently, we were able to demonstrate the production of NGF, BDNF and NT-3 by human peripheral eosinophils [25]. This cell type plays a major role in allergic inflammatory processes of the airways and is found accumulated in the bronchial tissue of asthmatics. While the viability of peripheral eosinophils is not influenced by neurotrophins, an influence of the survival after migration into the lung was lately observed. NGF stimulation leads to an increased production of IL-4 [26,27].

Monocytes are known as main effector cells of the immune system and play a crucial role in host defence mechanisms. They internalize, process and present antigens, tumour cells and parasites, and secrete various cytokines and chemokines.

NGF induces monocytes to generate reactive oxygen metabolites, promotes macrophages chemotaxis and stimulates macrophage phagocytosis and cytokine production [22,23,28]. A low production of NGF in human monocytes/macrophages has been described, increasing after stimulation [29]. Kerschensteiner et al. [30] demonstrated the expression of BDNF only on stimulated human monocytes. NT-3 was not detected on human alveolar macrophages by immunocytochemistry [31]. NT-3 mRNA expression has been shown on mice macrophages after activation by lipopolysaccharide (LPS) [32].

The aim of the presented study was to evaluate if human monocytes are a possible source for the production, storage, and release upon an inflammatory stimulus of the neurotrophins NGF, BDNF and NT-3, particularly in allergic and non-allergic subjects.

2. Materials and methods

2.1. Subjects

Nineteen voluntary blood donors (18 to 56 years), including 10 patients with allergic asthma and nine healthy controls, were examined. Detailed history, skin prick test to common inhalative allergens, with at least one reaction equal to the histamine control, and specific IgE were made to differentiate between atopics and non-atopics. All subjects gave informed consent.

2.2. Isolation of monocytes

Monocytes were isolated from human peripheral blood as previously described [33]. Heparinized venous blood (130 ml) was collected and allowed to sediment in 6% dextran (Sigma, USA) at room temperature for 40 min. The leukocyte rich fraction was aspirated gently, washed and centrifuged on Ficoll-Paque (density 1.077) (Biochrome, Berlin, Germany) for 45 min at $900\times g$. The washed pellet of PBMC was added to micromagnetic beads (Monocyte Isolation Kit; Miltenyi Biotech, Germany) for negative selection. The tagged cells were then passed through a magnetic field (MACS, Miltenyi) and the negative selection was collected. Monocytes were concentrated at high purity of >95% assessed by flow cytometry using anti-CD14 IgG and FITC conjugated antibodies (Jackson Immuno Research, USA). Isolated monocytes demonstrated a viability of more than 95% as evaluated with propidium iodide and trypan blue staining (Sigma).

2.3. Culture and stimulation of isolated monocytes

To asses NTs storage and release after stimulation, isolated cells were cultured in RPMI 1640 medium, containing 10% FCS and 5% penicillin/streptomycin. Approximately 10^5 cells/ well were supplemented with lipopolysaccharide (LPS), (Sigma) of 10, 1 or 0.1 μg/ml, and cultured at 37 °C in humidified atmosphere, 5% CO_2. After 24 and 48 h, the stimulated cells were detached, centrifuged and cell culture supernatant collected.

2.4. Immuncytochemistry

The alkaline phosphatase anti-alkaline phosphatase procedure (APAAP) (Dako, Denmark) was used as previously described. Cytospins of isolated CD14+ cells were air dried, fixed for 20 min in methanol and stained by specific antibodies for NGF, BDNF and NT-3 and their receptors trkA, trkB and trkC (dilution 1:100). For visualisation, a hexazotized new fuchsine was used and counterstaining was performed with Mayer's haematoxylin. For negative controls, the primary antibody was omitted.