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# LTB4 increases nasal neutrophil activity and conditions neutrophils to exert antiviral effects

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Neutrophils;
Innate defense;
Rhinitis;
Rhinovirus

**Summary**
*Background:* Leukotriene B4 (LTB4) recruits and activates neutrophils. Accordingly, this leukotriene is involved in innate defense actions.
*Objective:* To examine if nasal LTB4 can produce neutrophil activity and to explore whether or not LTB4 can condition neutrophils to exert virucidal effects *in vitro* and *in vivo*.
*Methods:* 1. Twenty-three healthy subjects received nasal LTB4 in a randomized and sham-controlled design. Symptoms were scored and nasal lavages carried out. Myeloperoxidase (MPO) and $\alpha$-defensins were monitored as indices of neutrophil activity. IL-8, eosinophil cationic protein (ECP) and $\alpha_2$-macroglobulin were measured as indices of pro-inflammatory cytokine production, eosinophil activity, and plasma exudation. 2. Supernatants from neutrophils activated by LTB4 *in vitro* were assayed for virucidal activity against respiratory viruses. 3. In 38 healthy individuals, nasal inoculation with human rhinovirus-16 (HRV-16) was performed. In a preliminary study, intervention with LTB4 was given in a randomized and controlled design. Symptoms, virus replication, and antibody-titres were monitored.
*Results:* 1. LTB4 produced statistically significant increases in MPO and $\alpha$-defensins, whereas IL-8, ECP, and $\alpha_2$-macroglobulin were unaffected. 2. The supernatants efficiently killed human coronavirus, respiratory syncytial virus, and influenza B virus. 3. HRV-16 replication was lower in subjects receiving LTB4, but this difference failed to reach statistical significance. Common cold symptoms and incidence of seroconversion were unaffected.
*Conclusion:* Nasal LTB4 induces a selective recruitment/activation of neutrophils. LTB4 can condition neutrophils to exert virucidal effects *in vitro* and may reduce virus replication *in vivo*. We suggest that the condition induced by LTB4 reflects an enhanced state of innate defense.


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## Introduction

Leukotriene B4 (LTB4) is a metabolite of the 5-lipoxygenase pathway. Original findings on the biology of this leukotriene comprise demonstrations of its biosynthesis by, and its potent chemotactic and activating effects on, polymorphonuclear leukocytes .[1–3] Accordingly, and substantiated by a series of subsequent observations,[4–11] LTB4 is regarded as a key mediator of the innate immune system.

Information on the role of LTB4 in human airways can be obtained through experiments involving airway challenges with this leukotriene. Administration of LTB4 to the bronchial airways has been shown to increase neutrophil recruitment,[12,13] without producing exudative inflammation[12] or bronchial hyperresponsiveness.[14] In contrast, there are no reports on effects of LTB4 administered to the human nasal airway. If LTB4 selectively increases human nasal neutrophil activity, topical administration of this leukotriene may be employed to enhance mucosal innate immune defense against infection.

Defensins are cationic antimicrobial peptides grouped into $\alpha$- and $\beta$-defensin subfamilies.[15] $\alpha$-Defensins 1-4 are major components of neutrophil granules, whereas $\beta$-defensins 1-4 are found in epithelial cells.[16] Flamand et al.[10] recently demonstrated that intravenous administration of LTB4 to monkeys produced increased plasma levels of $\alpha$-defensins and that these levels could exert antimicrobial effects *ex vivo*. Whether or not topical administration of LTB4 affects the nasal mucosal output of $\alpha$-defensins is unknown.

In this study, we examined dose- and time-dependent effects of nasal administration of LTB4 in healthy subjects on symptoms, nasal peak inspiratory flow (PIF), and select nasal lavage fluid indices. Accordingly, we monitored the neutrophil granule protein myeloperoxidase (MPO) and $\alpha$-defensins as indices of neutrophil activity. Interleukin-8 (IL-8) was analyzed in order to explore whether or not any neutrophil active effect of LTB4 involved this pro-inflammatory cytokine. Eosinophil cationic protein (ECP) and $\alpha_2$-macroglobulin were monitored as indices of eosinophil activity and plasma exudation, in order to explore whether or not LTB4, or any LTB4-produced neutrophil activity, had any consequence to the nasal mucosa in terms of producing eosinophil, exudative inflammation. In this study, we also studied whether or not exposure of neutrophils to LTB4 *in vitro* induced virucidal effects against respiratory viruses: human coronavirus, human respiratory syncytial virus (RSV), and human influenza B virus. Finally, in a preliminary experiment involving healthy subjects, we examined effects of LTB4 on human rhinovirus-16 (HRV-16) induced virus replication, seroconversion, and symptoms.

## Methods

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board of Lund University (Reference numbers 522/06 and 198/09). All patients provided written informed consent for the collection of samples and subsequent analysis.

### Study design

1. In healthy subjects, nasal challenges with LTB4 were carried out in a double-blinded, randomized, sham-controlled, and crossover design. Nasal lavages were carried out and IL-8, $\alpha$-defensins, MPO, ECP, and $\alpha_2$-macroglobulin were measured.
2. In experiments *in vitro*, possible virucidal effects of supernatants of neutrophils conditioned by LTB4 were examined on human coronavirus, RSV, and influenza B virus.
3. In healthy individuals, nasal inoculation with HRV-16 was carried out. Intervention with LTB4 was administered in a double-blinded, randomized, controlled, and parallel group design. Nasal symptoms, virus replication, and neutralizing antibody-titres were monitored.

### Effects of LTB4 on the human nasal mucosa *in vivo*

#### Subjects

Twenty-three subjects (11 female, 12 male, aged 21–29 years) were recruited. Inclusion criteria were a negative skin-prick test to relevant aeroallergens. Exclusion criteria were allergic rhinitis, other nasal disease (structural abnormalities, rhinosinusitis, and polyposis), chronic disease and/or on-going drug treatment, and pregnancy or lactation. The subjects were without medication and had been so for at least four weeks prior to the study. No medication except the study drug and occasional use over-the-counter pain relievers were allowed during the course of the study.

#### Challenges

All challenges (and all lavages) were given to the right hand side of the nasal cavity. Sham solution (isotonic saline containing 10 mM glycine/sodium hydroxide buffer, pH 10.5), used as control challenge, and two doses of LTB4 (2.0 and 20 $\mu$g) dissolved in sham solution were given in a randomized order. The challenges were given as single actuations using a spray-device delivering 100 $\mu$l per actuation. The washout time between the administrations was at least one week.

#### Clinical measurements

Symptoms, nasal peak inspiratory flow (PIF), and nasal lavages were carried out before each challenge as well as 1 and 4 h thereafter. The symptoms blocked nose, runny nose, and irritation were scored by the subjects on a four-graded scale: 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms. The number of sneezes were counted and transformed into a score: 0 = 0 sneezes, 1 = 1–4 sneezes, 2 = 5–8 sneezes, 3 = more than 9 sneezes.[17] The four scores of blocked nose, runny nose, irritation, and sneezes were then added to a total nasal symptom score (TNSS).[17] Nasal PIF were measured using a PIF-meter (Clement-Clarke, Harlow, U.K.). On each occasion, the best of three PIF-recordings were used in the analysis.

#### Nasal lavages

A pool-device was used for isotonic saline lavage of the nasal mucosa as described previously.[18] The volume of the pool-fluid was 14 ml and the dwell time 5 min (This high-volume lavage