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Effect of bacterial endotoxin LPS on expression of INF-γ and IL-5 in T-lymphocytes from asthmatics

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Abstract Epidemiological evidence, in vitro studies and animal models suggest that exposure to the bacterial endotoxin lipopolysaccharide (LPS) can influence the development and severity of asthma. Although it is known that signaling through Toll-like receptors (TLR) is required for adaptive T helper cell type 1 and 2 responses, it is unclear whether the LPS ligand TLR 4 is expressed on $CD4^+$ and $CD8^+$ T-lymphocytes and if so, whether LPS could modulate the T_H1 or T_H2 response in this context.

The present authors have, therefore, examined the expression of TLR 4 on peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes using RT-PCR method and FACS analyses. Furthermore, the authors have studied the IL-12-induced expression of the T_H1-associated cytokine INF-γ and the IL-4-induced expression of the T_H2-specific cytokine IL-5 in the presence of LPS using ELISA and compared nine atopic asthmatic subjects and eleven nonatopic normal volunteers.

There was an increased anti-CD3/anti-CD28-induced IL-5 expression in T cells of asthmatics compared with normals ($p<0.01$). In the presence of IL-4 (10 ng/ml), there was an additional increase in IL-5 expression and this additional increase was greater in T cells of normals compared with asthmatics ($p<0.05$). There was an expression of INF-γ in anti-CD3/anti-CD28-induced T-lymphocytes without differences between both groups (NS). In the presence of IL-12 (10 ng/ml), there was an increase in INF-γ release without differences between normals and asthmatics (NS).

In the presence of different concentrations of LPS (10 ng/ml, 1 μg/ml), there was a decrease in IL-4-induced IL-5 expression without differences in both groups, indicating an intact T_H2 response to bacterial endotoxin LPS in asthma. Interestingly, LPS increased the IL-12-induced INF-γ release in a concentration-dependent manner in T-lymphocytes of normals but this could not be found in T cells of asthmatics, indicating an impaired T_H1 response to bacterial endotoxin LPS in asthma.

Abbreviations: IL, interleukin; INF-γ, interferon γ; LPS, lipopolysaccharide; BAL, bronchoalveolar lavage; AHR, airway hyperresponsiveness; TLR, Toll-like receptor.

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In addition, there was a TLR 4 expression on $CD4^+$ T-lymphocytes of normals and to a lesser extent in asthmatics but this TLR 4 expression could not be found on $CD8^+$ T cells of both groups.
In conclusion, there may be an impaired concentration-dependent LPS-induced T_H1 rather than a T_H2 response in allergic adult asthmatics compared with normal volunteers. One reason for this could be a reduced TLR 4 expression on $CD4^+$ T-lymphocytes of asthmatic subjects.

Introduction

Airway infections of viral or bacterial origin are a common cause of respiratory disease and also a well-known reason for asthmatic exacerbations [1]. Expression of specific *Toll-like receptors* (TLRs) on structural cells of the respiratory system is an essential component in the protection against these pathogens [2].

TLRs are involved in innate cell activation by conserved structures expressed by microorganisms and are crucial for recognition of pathogen-derived products and initiate signaling cascades leading to the activation of innate host defenses [3,4]. To date, 10 members of the TLR family that differ in ligand specificities and expression patterns have been described in humans and, among them, TLR 4 is activated by the bacterial endotoxin of Gram-negative bacteria, lipopolysaccharide (LPS) [5]. Binding of pathogen-associated molecular patterns (*PAMPs*) to most of the TLRs initiates a signaling cascade that leads to upregulation of inflammatory cytokines, reactive oxygen intermediates and costimulatory molecule expression.

TLR expression has been reported in several cell types including myeloid cells, neutrophils, airway smooth muscle cells [6] and epithelial cells [3,4]. Interestingly some studies showed that expression of TLRs has been detected also in various upper airway tissues such as tonsils and adenoids. Although some cells of the innate immune system (i.e. macrophages and neutrophils) activated via TLRs differentiate into effector cells that kill infectious agents, others, such as dendritic cells, activate T cells and thereby initiate adaptive immune responses [7–11]. Moreover, human T cells express the mRNA encoding most of Toll-like receptors (TLR) [12].

Among microbial products which might affect the occurrence and severity of allergic asthma, lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria that is ubiquitous in the environment, elicited the greatest interest. The relationship between LPS expose and allergic asthma is poorly understood. Studies in humans have found that exposure to LPS can protect, have no effect or exacerbate allergic asthma. Endotoxin levels in house dust were related inversely to allergen sensitization [13] and development of atopic asthma [14] in children indicating that the exposure seems to depend upon the time of exposure. Tulic et al. reported that endotoxin exposure might exacerbate asthma in patients with established disease and showed in a rat model of asthma that endotoxin was protective when administered before allergen sensitization, whereas it exacerbated lung inflammation when given in sensitized animals [15].

It has been suggested that pathogens or their products elicit T_H1 immune responses, which down-regulate the T_H2 cells, a hallmark of allergic asthma. However, recent studies suggest that a simple change in the ratio of T_H1 and T_H2 cytokines does not account for the ability of pathogens to protect against the progression of asthma [16,17].

The T helper type 1 (T_H1)/T_H2 paradigm was initially proposed as the immunological concept underlying the hygiene hypothesis. This was supported by the evidence that T_H1 responses are compromised in early life because of both an intrinsic T cell defect in interferon (INF) γ production [28] and an impaired capacity of newborn antigen-presenting cells to secrete the bioactive form of interleukin (IL)-12 [19,20]. However, the idea that infections prevent T_H2-driven allergy by promoting T_H1 responses is still a matter of debate [21].

Little is known about the expression of TLR 4 in human peripheral blood $CD4^+$ and $CD8^+$ T-lymphocytes and the direct effect of LPS on T_H1- and T_H2-specific cytokine responses. In the present study, we are first to investigate the expression pattern of TLR 4 in $CD4^+$ and $CD8^+$ peripheral blood T cells. Furthermore we studied the direct effect of LPS on the expression of IL-5 and INF-γ on peripheral blood T-lymphocytes and compared atopic asthmatics and non-atopic normals.

Materials and methods

Subjects

As seen in Table 1 (characteristics of non-atopic normals and atopic asthmatic subjects), the study population consisted of eleven healthy non-atopic non-smoking volunteers (5 male, 6 female; mean age: 30.7 ± 2.4 years), who had no history of atopy and asthma or of any respiratory disease with normal lung function and airway responsiveness to methacholine (PC_{20} >16 mg/ml) and nine mild stable atopic asthmatics (5 male, 4 female; mean age: 28.8 ± 2.8 years) receiving only intermittent treatment with the inhaled β_2-adrenergic agonist aerosol, salbutamol, for relief of wheeze. All asthmatic subjects demonstrated a >15% improvement in forced expiratory volume (FEV_1) following inhalation of 200 μg of salbutamol and with an airway hyperresponsiveness to methacholine (PC_{20} <8 mg/ml), increased IgE (284 ± 13.4 U/l) and increased blood eosinophils. All asthmatic patients were atopic as defined by two or more positive skin prick tests to common allergens. None of the subjects studied had received oral or inhaled glucocorticoids for the preceding 12 months, or any other treatment apart from inhaled β_2 agonists. Current smokers or ex-smokers of more than five pack years and patients with FEV_1 <80% predicted were excluded. The study was approved by the University