SHORT COMMUNICATION

Levels of IL-15 in Induced Sputum of Patients with Asthma Disease

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ABSTRACT

The release of various inflammatory mediators into the bronchial lumen of asthmatics is thought to reflect the degree of airway inflammation.Interleukin-15 (IL-15) is a proinflammatory cytokine, involved in the pathogenesis of inflammatory disease. The role of IL-15 in asthma inflammation remains unclear. The goal of the study was to compare IL-15 levels in inducing sputum in patients with severe asthma (20 patients), mild asthma (18 patients) and moderate asthma (10 patients) and 20 healthy controls (HC). The expression of IL-15 was analysed at the protein and mRNA using ELISA and reverse transcription-quantitative polymerase chain reaction. Statistical analysis indicated that sputum IL-15 protein and IL-15 mRNA expression were significantly different between the asthmatic patients and non-asthmatic control group. IL-15 cytokine at the protein and mRNA were elevated according to the disease severity. Severe asthmatics expressed the highest IL-15 levels. No significant differences were found between mild and moderate asthma ($p > 0.05$). Our results suggested that IL-15 might be associated with the pathogenesis of severe asthma. In severe asthmatic significant correlation was found between sputum macrophages and IL-15 levels ($p = 0.0001$). Our results suggested that IL-15 might be associated with the pathogenesis of severe asthma

Key words: Severe asthma, Interleukine-15, Inflammation, macrophages.

INTRODUCTION

Asthma is a chronic, heterogeneous inflammatory disease of the respiratory tract that leads to bronchial hyperreactivity and obturation. [1]Current progress in understanding the pathogenesis of asthma implicates some candidate biomarkers of asthma severity. Considerable candidates are cytokines whose deregulated expression and significance in driving asthma disease are well documented. [2] Importantly, blockade of biological activities of several of them, including TNF-a, IL-6, and IL-1b led to the development of a new class of biological drugs that are today used for the treatment of asthmatic patients. IL-15 could be one of these parameters to study in asthma.

IL-15 is a pleiotropic cytokine that classically acts to support the development, maintenance, and function of killer lymphocytes. [3]As a proinflammatory cytokine, IL-15 is produced by activated monocytes, macrophages and dendritic cells. [4] IL-15 influences cellular adhesion and trans-endothelial inflammation-directed migration of activated T lymphocytes. [5]

Our study focuses on IL-15 production in asthmatic patients, with a particular interest on its expression in the induced sputum.

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MATERIAL AND METHODS

Study Populations

Asthmatic patients and non-asthmatic controls were recruited for this study (Department of Respiratory Diseases, Pavillon B; A. Mami Hospital of lung diseases, Ariana, Tunisia) and investigated for their induced sputum (Figure 1). The diagnosis of asthma was established according to the ATS/ERS criteria. [6]All patients were female as we recently reported. The control group (all females, aged 45–58 years) comprised 20 healthy volunteers with no history of obstructive lung disease. The study protocols were reviewed and approved by the ethics committees of our hospital (A. Mami Hospital, Ariana, Tunisia), and informed written consent was obtained from all participating subjects.

Sputum Induction and Processing

The procedure of sputum induction and processing were the same in control and asthma group and was preceded by inhalation of 400 µg of salbutamol and subsequent pulmonary function testing as we recently reported. [7-8] All patients were pre-treated with two puffs of salbutamol (400 µg) and inhaled a nebulized solution of normal saline followed by increasing concentrations (3%, 4% and 5%) of hypertonic saline using an ultrasonic nebulizer (ULTRA-NEBTM 2000, USA).

IL-15 Cytokine Assay

Sputum fluid concentrations of IL-15 were quantified by enzyme-linked immunosorbent assay (ELISA) (Quantikine; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Undiluted serum and CSF samples were added in duplicate to microtitre wells and assayed according to routine procedures as reported.[9]The lower limit of detection of this assay was 3.9 pg/ml. Interassay variation was <10%.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Sputum cells were collected from the participants in test tubes filled with ethylenediaminetetraacetate (Sarstedt AG, Nümbrecht, and Germany). RNA was then isolated from peripheral blood lymphocytes with the application of TRI ReagentSolution (Ambion; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the standard acid guanidinium-phenol-chloroform procedure (29, 30). The quality of the isolated RNA was evaluated with spectrometryat 260 nm using a Nanodrop ND-1000 analyzer (Thermo Fisher Scientific, Inc.). Following this, 1 µg RNA was reversely transcribed using the ImProm-II™ Reverse Transcription system (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol (annealing at 25˚C for 5 min, first-strand synthesis reaction at 42˚C for 60 min, inactivation of reverse transcriptase at 70˚C for 15 min) and as recently reported.[10] The following gene-specific primers were used: IL-15, forward 5'-GGA ATGTAACAGAATCTGGATG-3' and reverse 5-GTT ATGTCTAAGCAGCAGAG-3' (Sigma-Aldrich; Merck KGaA); and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5'-AGCCACATCGCTGAGACA-3' and reverse 5'-GCCCAATACGACCAAATCC-3'(HTDS, Tunisia).

Statistical Analysis

Data were analysed with the SigmaSTAT software (SPSS, Chicago, IL, USA), and MannWhitney *U*-tests were used for statistical analysis. The level of significance was set at $p < 0.05$.

RESULTS

The study population comprised 20 healthy control subjects, 20 patients with severe asthma, 18 patients with mild asthma and 10 patients with moderate asthma.The clinical characteristics of severe asthma are shown in Table 1.

Table 1. Differential leucocyte profile in the induced sputum

	Severe asthmatics	Non-asthmatic controls
Cells/mL& x 10^6	3.2 ± 0.9*	1.2 ± 0.4
Viability (%)	89.5 ± 2.3	85.8 ± 1.7
Macrophages (%)	63.9 ±6.3*	55.8 ± 2.4
Neutrophil (%)	22.6 ± 3.8	27.6 ± 3.5
Epithelialcells (%)	7.2 + 1.8	11.5 ± 2.3
Lymphocytes (%)	3.4 ± 0.6	4.8 ± 1.5
Eosinophils (%)	0.12 ± 0.1	0.0 ± 0,0

Total and differential cell profiles obtained from induced sputum samples from severe asthma patients and non-asthmatic controls.
Significantly different from controls (*p < 0.05)

IL-15 was correlated with asthma severity

Sputum IL-15 levels were significantly elevated in asthmatic patients (7.73 ± 4.55 pg/ml) compared with non-asthmatic controls (4.35 ± 0.34 pg/ml). No significant differences were observed between moderate (4.46 + 0.52 pg/ml) and mild asthmatics (4.38 ± 0.42). Severe asthma patients exhibited higher levels of IL-15 (12.47 ± 3.24 pg/ml) compared to mild and moderate asthma (Figure 1a).

IL-15 mRNA expression was correlated with asthma severity

Induced sputum cells from asthmatics were tested for the IL-15 mRNA expression. No significant differences were observed between mild and moderate asthma (Figure 1b). Severe asthmatic patients expressed increased IL-15 mRNA comparatively to healthy controls and to mild and moderate asthmatics.

Positive correlation was observed between IL-15 protein and IL-15 mRNA expression (Figure 1c) in severe asthma. No significant correlation was observed between IL-15 protein and the results of the spirometric examination (FEV1, FEV1% and FEV1/FVC) in the group of asthmatic patients. No significant difference of IL-15 levels was observed between severe asthmatic patients with and without allergy (Figure1d).

Significant correlation was observed between the percentage of sputum macrophages and IL-15 protein level in severe asthma (Figure 2a).

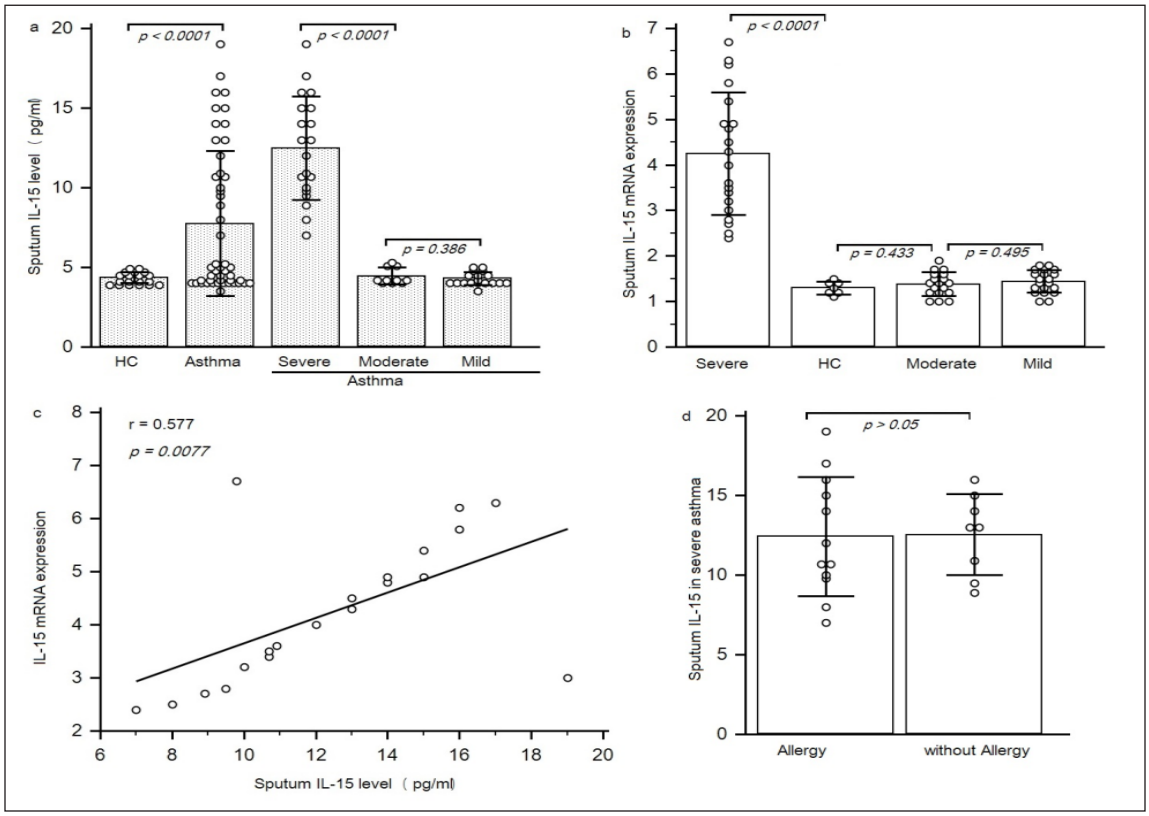

Figure 1. Sputum IL-15 protein levels in asthmatic patients. (a): IL-15 was quantified by ELISA in sputum fluid of asthmatic patients and non-asthmatic control (HC). (b): Expression of IL-15 mRNA in asthmatic patients. (c): Correlation of IL-15 proteinlevel and IL-15 mRNAexpression. (d): IL-15 protein levels between severe asthmatic with and without allergy.

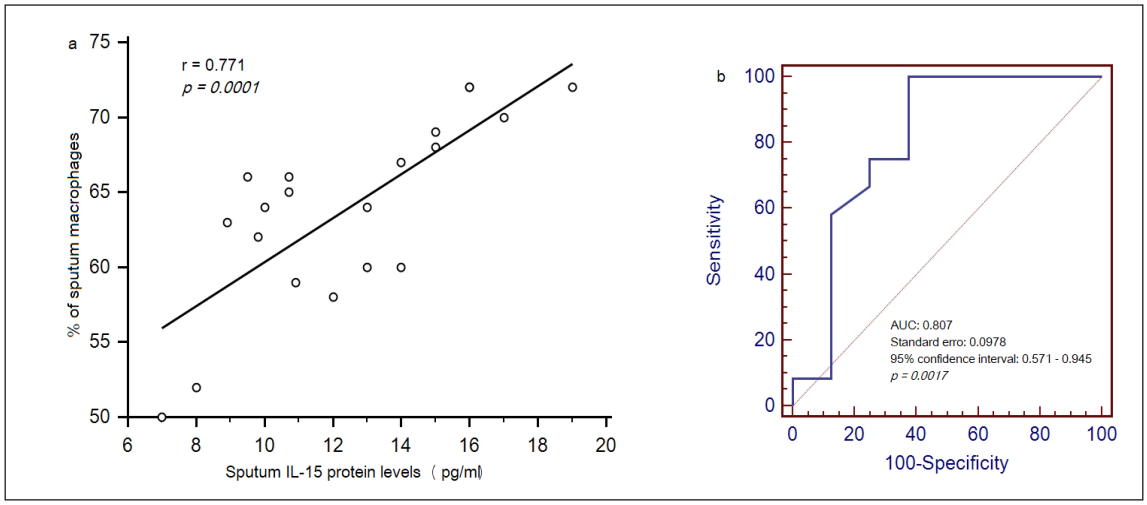

Figure 2. The proportion of alveolarmacrophages was significantly correlated with sputum IL-15 in severe asthma (a). Abbreviationr: Spearman rank correlationcoefficient.(b): Interleukin-15 shows high diagnostic utility in the identification of patients with asthma severity. Receiver operating characteristic curves (ROC) of IL-15 was used.

Interleukin-15 shows high diagnostic utility in the Identification of Patients with low and high IL-15 levels using the Receiver Operating Characteristic Curves (ROC)

Using the area under the curves ROC (AUC) as an index of the overall discriminative abilityof the test, we compared patients with low and high IL-15 levels (Figure 2b). The IL-15 levels differentiated future of severe asthmatic patients with exacerbation than those who did not develop important severity (AUC = 0.807, standard error = 0.0978, 95% CI: 0.571 to 0.945; p = 0.0017).

DISCUSSION

In the present study sputum IL-15 protein and IL-15 mRNA expression levels were elevated in severe asthma compared with moderate-mild asthmatics and healthy controls. In our opinion among many other inflammatory cytokines expressed in severe asthma (IL-1b, IL-6, IL-8, IL-9, and IL-26), IL-15 is one of the mediators that could potentially have a role in this process of exacerbation. The role of IL-15 in asthma has not yet been fully explained.The results of studies conducted so far are equivocal and do not provide clear evidence regarding its role in the etiopathogenesis of asthma.

Attempts to identify the association between major cytokines investigated and IL-15 could provide particularly promising results. Unlike the role of IL-15 and eventually the role of genetic polymorphisms connected with the IL-1b, TNF-a and TGF-β pathway have to be elucidated. The role of IL-15 and TGFbis quite well known. [11] Studies concerning polymorphic forms of the IL-15 gene conducted on a Danish population did not confirm an occurrence of any significant polymorphism of the IL-15 gene in the asthma aetiology. [12]Although one study, conducted using a German population, identified a weak association between IL-15 and childhood asthma, further studies did not confirm any relationship between IL-15 gene polymorphisms and asthma disease. [13] Jonakowki et al reported that IL-15 expression was correlated with TGF-β1 expression among asthmatic patients, and IL- IL-15 activity might be associated with the pathogenesis of asthma. [14]

The results of the present study indicate that IL-15 mRNA expression is elevated in severe asthma. Infections, exacerbations in asthma are an important problem. It has been revealed that deficits of IL-15 in asthmatic patients result from an impaired response to Rhinovirus infection in vitro. These data could explain a possible protective effect of IL-15.[15]Moreover, there is evidence that IL-15 is a significant factor responsible for the production of memory $CD8^+$ T cells in response to viral infection.[16] Our data indicated association between IL-15 protein and IL-15 mRNA expression and severe asthma.

Komai-Komaet al. reported significantly higher levels of IL-15 in the induced sputum of patients taking inhaled glucocorticosteroids compared with patients who had not been administered any therapy.[17]Also, the in vitro culture of sputum mononuclear cells, isolated from asthmatic patients treated with inhaled glucocorticosteroids, showed significantly higher levels of IL-15 synthesis in comparison with the control group.[17]These results highlightthe considerable immunomodulating role of IL-15 in asthma, which involves the induction of changes in the inflammation profile, from a profile connected with Th2 lymphocytes to one connected with Th1 lymphocytes.

IL-15 is a member of g-chain cytokine, is a pro-inflammatory cytokine essential in NK cell proliferation. [18]We reported that the number of NKT ($CD3^+$ $CD56^+$) cells was significantly higher in the sputum of severe asthmatics compared with mild asthmatic and healthy control groups. The increased IL-15 level in severe asthma and not in moderate or mild asthma could probably associated with the increased NK, NKT and TCRgd cells.[19-20]

There are limitations to our study. The evaluation of protein level and genes should be suggested as the next step in evaluating the results of the present study. As the nature of the correlation between IL-15 expression and other inflammatory cytokines expression remains unknown. The regulation and connection of IL-15 with TGF-β1, IL-1b and TNF-a signalling pathway are required for the purpose of identifying their potential implications in the treatment of asthma.

CONCLUSION

Our findings suggest a possible role of IL-15 in the development of severity in asthmatic patients as an actor of the inflammatory process in the lung. We have no information yet about the involvement of this protein in the interaction in blood-brain barrier permeability disturbances between lung and the peripheral circulation. Further studies between IL-15 and other cytokines (IL-8, IL-17, IL-26, IL-13, TNF-a, IL-1b …) are needed to map the inflammatory process in severe asthma.

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