



# Anti-inflammatory activity of IL-37 in asthmatic children: Correlation with inflammatory cytokines TNF- $\alpha$ , IL- $\beta$ , IL-6 and IL-17A



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## ABSTRACT

**Background:** The aim of this study was to assess interleukin (IL)-37 production in asthmatic children in serum and induced sputum and to look to the impact of IL-37 on pro-inflammatory cytokines production (TNF- $\alpha$ , IL-6, IL-1 $\beta$  and IL-17).

**Methods:** Forty children with well-controlled asthma (20 moderate and 20 mild asthmatics) were studied. IL-37 was measured by ELISA in serum and induced sputum (IS) samples, and compared with 22 age- and sex-matched healthy controls. Real-time quantitative PCR was used to determine IL-37 mRNA expression in induced sputum cells. Induced sputum mononuclear cells from 10 moderate asthmatics and 10 healthy controls were stimulated either with lipopolysaccharides (LPS) or LPS plus recombinant IL-37 (rIL-37) comparing pro-inflammatory cytokines production. TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-17 were measured by RT-PCR and ELISA.

**Findings:** The expression of IL-37 mRNA in asthmatic patients was significantly lower than that observed in healthy controls ( $P=0.0001$ ). IL37 mRNA expression depended on asthma severity. Serum and IS IL-37 levels were significantly lower in asthma patients compared to healthy controls. LPS-stimulated sputum cells from asthma patients produced higher levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  than those from HC. Adding rIL-37 suppressed TNF- $\alpha$ , IL-1 $\beta$  and IL-6 production in IS cells. In the same way, stimulating IS CD4<sup>+</sup> T cells in the presence of rIL-37 inhibited IL-17 production both in asthma patients and HC. IL-37 effect on IL-17 was more pronounced in patients than controls.

**Interpretation:** The decrease in IL-37 level observed in IS was found to correlate with disease severity. The increased pro-inflammatory cytokines production from asthma IS cells was abrogated by the addition of rIL-37. IL-37 could be an important cytokine in the control of asthma by suppressing the production of inflammatory cytokines.

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## 1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized mainly by Th2 lymphocyte-mediated immune responses

**Abbreviations:** IL-37, interleukin-37; Asth, asthma; PBMC, peripheral blood mononuclear cell; IS, induced sputum; SMNCs, sputum mononuclear cells; rIL-37, recombinant IL-37; PCR, polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; TNF, tumor necrosis factor.

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and associated with bronchial hyper-responsiveness, airflow obstruction, and airway remodelling. This Th2-biased inflammation is associated with leukocyte recruitment and activation in the airways (mainly lymphocytes, eosinophils, and mast cells) and over production of cytokines including IL-4, IL-5, and IL-13 (Robinson et al., 1992). Recent studies have reported an increased level of TNF- $\alpha$  specifically associated with severe and refractory phenotypes of the disease (Brightling et al., 2008; Bousquet, 2015).

Asthma is characterized mainly by Th1, Th2, Th17 lymphocyte-mediated immune responses. (Raedler et al., 2015; Sharma et al., 2015; Hamzaoui et al., 2014). Interleukin-1 (IL-1) is a central mediator of innate immunity and inflammation. The IL-1 family includes 7 ligands with agonist activity (IL-1 $\alpha$  and  $\beta$ , IL-18, IL-33, IL-36 $\alpha$ ,  $\beta$ ,  $\gamma$ ), three receptor antagonists (IL-1Ra, IL-36Ra, IL-38) and an anti-

**Table 1**  
Patients' characteristics.

Characteristics	Patients	Healthy controls
Number of subjects	40 (20 moderate)	22
Gender (female/male)	12/18	10/12
Age (range), years	8 (4–16)	6.5 (4–12)
Positive skin test results (%)	67.25%	Negative
FVC (%)	80.2 (58–128)	
FEV1 (%)	96.32 (50–127)	
FEV1/FVC ratio	0.9 (0.75–1)	

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity.

inflammatory cytokine (IL-37). Recently, elevated IL-33 expression and its correlation with TNF- $\alpha$  reflected the inflammatory process observed in the lung of young asthmatics (Endo et al., 2015; Savenije et al., 2014; Hamzaoui et al., 2013).

Interleukin 37 (IL-37) an anti-inflammatory orphan members of the IL-1 receptor family, have been shown to down-regulated the expressions of pro-inflammatory cytokines in chronic inflammatory diseases (Ye et al., 2014b; Zhao et al., 2014; Imaeda et al., 2013) suggesting that IL-37 might abrogate pro-inflammatory cytokines productions and reduces inflammatory responses. Recently Lundling et al. (Lundling et al., 2015) demonstrates that IL-37 is able to ablate in mice, a Th2 cell-directed allergic inflammatory response and the hallmarks of experimental asthma in mice, suggesting that IL-37 may be critical for asthma pathogenesis (Imaeda et al., 2013).

In this study, we assessed the serum and induced sputum levels of IL-37 in young asthmatic patients and analysed the relationship between IL-37 and pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-17).

## 2. Materials and methods

### 2.1. Patients

Forty children with well-defined asthma (20 moderate asthma) were recruited from the Department of Paediatrics and Respiratory Disease and Homeostasis and Cell Dysfunction Unit Research, Abderrahman Mami Hospital (Ariana, Tunisia), using the criteria set by the Global Initiative for Asthma guideline (van Weel et al., 2008). Detailed definitions of the inclusion and exclusion criteria for the enrolment of asthmatic subjects were reported previously (Hamzaoui et al., 2013). The protocols for the study were reviewed and approved by the ethics committees of the Abderrahman Mami Hospital, and informed consent was obtained from all participating subjects. Children with mild and moderate asthma were treated with regular inhaled glucocorticoids (ICS), but variable daily doses were required to control the symptoms (at the time of evaluation daily ICS dose ranged 200–800 mg/day). Only patients whose asthma was controlled were retained. Table 1 describes the characteristics of the asthmatic patients in the study. Twenty-two healthy control children (female/male: 10/12) were recruited (aged 4–12 years, means 6.5) with no respiratory no allergic manifestations.

### 2.2. Sputum induction

Sputum was induced as described previously (Hamzaoui et al., 2013). Patients inhaled hypertonic saline solution (3–5% NaCl) briefly after a premedication with 200 mg of inhaled salbutamol. The collected sputum volume was measured, mixed with an equal volume of 0.1% dithiothreitol and then rocked at room temperature for 15 min. The resulting samples were subsequently filtered through a 0.42- $\mu$ m Millipore filter (Billerica, MA) and centrifuged at 1500 g for 10 min. The supernatants were immediately aliquoted and frozen at  $-70^{\circ}\text{C}$  until further analysis for IL-37.

### 2.3. Cell isolation and culture

Sputum mononuclear cells (SMNCs) were purified by Ficoll-Hypaque density gradient centrifugation. SMNCs were cultured in a Roswell Park Memorial Institute 1640 (RPMI1640) medium containing 10% foetal bovine serum and 1% penicillin/streptomycin and seeded into 24-well plates at a concentration of  $1 \times 10^3$  cells/ml. To analyze the effect of IL-37 on cytokine production, SMNCs from healthy controls were plated at  $1 \times 10^3$  cells/ml and stimulated with 100 ng/ml lipopolysaccharides (LPS) (Sigma-Aldrich) in the presence of different concentrations of recombinant IL-37 (rIL-37) (0, 50, 100, and 200 ng/ml); (R&D Systems) for 72 h (Bouali et al., 2015), after which cells and culture supernatants were collected to analyse the mRNA level of cytokines by real-time polymerase chain reaction (RT-PCR) and the protein level by enzyme-linked immunosorbent assay (ELISA). A stock solution of 100 ng/ml of rIL-37 was made in sterile phosphate buffer solution (PBS) and appropriate volumes of this solution were added to the medium with induced sputum mononuclear cells to reach the required final concentration of rIL-37. To analyze the effect of IL-37 on cytokine production by induced sputum mononuclear cells from asthmatic patients and normal controls, SMNCs were plated at  $1 \times 10^3$ /ml and stimulated with 100 ng/ml LPS in the presence or absence of 100 ng/ml rIL-37 for 72 h, and cytokines in the culture supernatants were analyzed for cytokines by ELISA. Control groups were stimulated with the same volume of PBS alone.

CD4<sup>+</sup>T cells were separated from induced sputum cells by magnetic microbeads (purity of CD4<sup>+</sup> cells was  $\geq 94\%$ ; MiltenyiBiotec). CD4<sup>+</sup>T cells were stimulated with anti- CD3/CD28 (2  $\mu$ g/ml; eBioscience) in the presence or absence of 100 ng/ml rIL-37 for 72 h to detect the IL-17 levels in the supernatants.

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

Serum IL-37 levels, IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-17 levels were determined by ELISA following the manufacturer's instructions. IL-37 was quantified using ELISA reagent kits purchased from AdipoGen (San Diego, CA, USA). Detection of the cytokines IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-17 were accomplished using the eBioscience kit (San Diego, CA, USA).

### 2.5. RNA extraction and real-time polymerase chain reaction (RT-PCR)

Total cellular RNA from SMNCs was isolated by using RNeasy micro kit extraction columns (Qiagen, Chatsworth, CA, USA). RNA was reverse-transcribed using oligo(dT) 12–18 primer in the presence of RNAGuard (both from GE Healthcare, Velizy Villacoublay, France) and Superscript II reverse transcriptase (Invitrogen, Life Technologies Products HTDS. 2035 Carthage Airport, Tunis; Tunisia). Quantification of mRNA levels was performed by real-time PCR using the LightCycler PCR (Roche Diagnostics, Indianapolis, Ind.) and QuantitTect SYBR Green PCR master mix (Qiagen, Chatsworth, CA, USA). Relative quantification of the PCR products was achieved using a standard curve, which was obtained by simultaneously amplifying samples with serial dilutions of the amplicon. The results were analyzed with LightCycler software, version 3.5.3 (Roche Diagnostics). Both melting curve analysis and agarose gel electrophoresis were used to assess the specificity of the amplification products as well as primer-dimer formation. Quantification of mRNA encoding endogenous 40S ribosomal protein S9 was performed as a housekeeping gene and used to correct for variations in cDNA content among samples. The ribosomal protein S9 recently ranked in the top 100 best housekeeping genes. Primers were designed to span an intron and the sequences were

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