Immunology Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Increased expression of thymic stromal lymphopoietin in induced sputum from asthmatic children

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ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 6 June 2016
Accepted 8 August 2016
Available online xxx

Keywords:
TSLP
TNF-α
Interleukin-37
Asthma
Children
Induced sputum
Bronchial epithelial cells

ABSTRACT

Objective: Thymic stromal lymphopoietin (TSLP) plays a role in amplifying the inflammatory response in asthmatics. TSLP is also a critical factor in airway remodeling airways. The aim of this study was to assess the expression of TSLP in induced sputum from asthmatic children and to look to the impact of TNF-α and IL-37 on TSLP production in induced sputum from asthmatic children.
Methods: Forty children with well-controlled asthma (20 moderate and 20 mild asthmatics) were studied. TSLP was measured by enzyme-linked immunosorbent assay (ELISA) in induced sputum (IS) samples, and compared with 22 age- and sex-matched healthy controls. Real-time quantitative PCR was used to determine TSLP mRNA expression in induced sputum cells. Sputum cells (ISCs) from 5 moderate asthmatics and 5 healthy controls (HC) were stimulated either with TNF-α or TNF-α plus recombinant IL-37 (rIL-37) comparing the suppression on TSLP production.
Results: The expression of TSLP mRNA in asthmatic patients was significantly higher than that observed in healthy controls [$P = 0.0001$]. Induced sputum fluid TSLP and TNF-α levels were significantly higher in asthmatic patients compared to healthy controls and their levels depend on asthma severity. Sputum cells produced high TSLP levels upon stimulation with TNF-α (10 pg/ml) in asthmatics. TSLP is merely produced by bronchial epithelial cells. Addition of recombinant IL-37 suppressed partially TSLP production in sputum-cultured cells and in bronchial epithelial cultured cells.
Conclusions: The increase in TSLP and TNF-α level observed in IS fluid was found to correlate with disease severity. The increased TSLP production from asthma sputum cells was abrogated by the addition of rIL-37. Regulation of TSLP pathway may be a therapeutic approach for asthma.

1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized mainly by T helper (Th)2 lymphocyte-mediated immune responses and associated with bronchial hyper-responsiveness, airflow obstruction, and airway remodeling. Th2-biased inflammation is associated with leukocytes recruitment and Th2 cytokines production [1]. However, other inflammatory pathways have been identified in asthmatic patients, depending on the clinical phenotype. Th1, Th17, IL-33 and IL-37 have been incriminated in asthma manifestation [2–6]. TSLP has recently emerged as a key cytokine in the development of adaptive immune responses and has wide-ranging impacts on both hematopoietic and non-hematopoietic cell lineages, including dendritic cells (DCs), $CD4^+$, $CD8^+$, natural killer T (NKT) cells, B cells and epithelial cells [7].

TSLP is an IL-7-related cytokine reported to be critical to the pathogenesis of airway inflammation [8], discovered as a growth factor for lymphocyte progenitors and is now recognized as a protein released primarily from epithelial cells in response to irritating stimuli. Previous findings have implicated the involvement of TSLP in asthma [9]. TSLP has been shown to play an important role in the initiation and maintenance of the allergic immune response. TSLP polymorphisms in humans have been linked to increased disease risk for atopic dermatitis, asthma, and allergic rhinitis [9,10].

In the current study, we aimed to investigate the level of TSLP and TNF-α in induced sputum and whether recombinant IL-37 also

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http://dx.doi.org/10.1016/j.imlet.2016.08.004
0165-2478/

Table 1
Characteristics of the study participants. Data are presented as mean (range).

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 (%)	75.52 (50–96)	108. 31 (100–120)
FEV1/FVC ratio	0.9 (0.75–1)	

Sputum analysis (cytospin slides)	Mild Asthma	Moderate Asthma	
Total cell counts (10^6)	1.9 (1.5–2.8)*	3.2 (2.5–3.8)*	1.2 (0.7–1.6)
Alveolar macrophages (%)	71 (65.2–74.2)	73 (68.2–76.8)	71 (67.9–75.9)
Lymphocytes (%)	2.4 (2.1–2.2)	2.6 (2.4–2.9)	2.1 (1.8–2.4)
Neutrophils (%)	12.5 (4.9–35.7)	14.5 (5.8–32.7)	16.2 (7.2–40.5)
Eosinophils (%)	0.9 (0–2.2)*	1.1 (0–2.7)*	0 (0–0.3)
Epithelial cells (%)	4.2 (0.5–7.6)**	5.7 (1.5–8.9)**	1.3 (0.2–2.9)

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity. Significantly different from healthy controls [**]: $P < 0.0001$; [*]: $P = 0.001$.

plays a role in suppressing TSLP production by sputum cells in young asthmatics children.

2. Materials and methods

2.1. Patients

Forty children with well-defined asthma (20 with moderate asthma and 20 with mild asthma) were recruited from the Department of Paediatrics and Respiratory Disease, Homeostasis and Cell Dysfunction Unit Research Abderrahman Mami Hospital (Ariana, Tunisia), using the criteria set by the Global Initiative for Asthma guidelines [11]. Detailed definitions of the inclusion and exclusion criteria for the enrolment of asthmatic subjects were reported previously [6]. The protocols for the study were reviewed and approved by the ethics committees of our 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 μg/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 nor allergic manifestations. All patients were nonsmokers. Spirometry was carried out in the patient and the control group. Using a spirolab II assessed FEV1 of the children.

2.2. (RPMI1640) medium

Complete Roswell Park Memorial Institute (RPMI) 1640 medium was prepared for cell culture containing 10% foetal bovine serum, 1% penicillin/streptomycin and 10 pg/ml of TNF-α (Sigma–Aldrich).

2.3. Sputum induction

Sputum induction was performed according to standard methods and as described previously [12,13]. Patients inhaled hypertonic saline solution (3–5% NaCl) briefly after a premedication with 200 μg of inhaled salbutamol. The collected sputum volume was measured, mixed with an equal volume of 0.1% dithiotreitol and then rocked at room temperature for 15 min.

The sample were filtered through a 0.42-μm Millipore filter (Billerica, MA) to remove mucus and debris and centrifuged at 1500*g* for 10 min and the pellet was resuspended in cell dissociation buffer with 10% FCS. A total cell count was performed (hematocytometer), and cell viability was determined (trypan blue). Specimens with greater than 90% cell viability were used, and differential cell counts were performed on cytospin slides. Cell counts from sputum samples from asthmatic and healthy subjects for the study sample are shown (Table 1). There was a slight increase in the percentage of sputum eosinophils and bronchial epithelial cells in asthmatic patients compared with healthy controls ($P < 0.05$). For the expression of TSLP mRNA, whole sputum samples were used.

The supernatants were immediately aliquoted and frozen at −70 °C until further for TSLP expression by ELISA. Great part of the sputum cells (ISCs) was transferred to RNA extraction buffer for the expression of the mRNA. The other part was diluted in Roswell Park Memorial Institute 1640 (RPMI1640) medium containing 10 pg/ml of TNF-α (Sigma–Aldrich), 10% foetal bovine serum and 1% penicillin/streptomycin.

2.4. Magnetic cell sorting (MACS) of bronchial epithelial cells sorting

We obtained bronchial epithelial cells from sputum samples using magnetic-assisted cell sorting (MACS) with anti-CD14 and anti-CD16 antibody beads as reported by Qui et al. [14]. We used anti-CD14 microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) to deplete the macrophages and anti-CD16 microbeads (Miltenyi Biotech) to remove the neutrophils from sputum samples. Sputum cell suspension (1×10^3 cells) was incubated with the antibody microbeads on ice for 20 min, with gentle agitation. An enrichment column (Miltenyi Biotech) with a diameter of 1.5 cm was washed with 60 mL of ddH2O three times according to the manufacturer's instructions and then placed in a VarioMACS magnetic cell separator (Miltenyi Biotech). The incubated cells were fed into the column and the flow-through (eluate) was collected in a 10 mL sterile tube on ice. The eluate was centrifuged at 300*g* for 10 min. Cell pellet was then suspended in complete RPMI 1640 medium as sorted samples, which contained the enriched bronchial epithelial cells. Bronchial epithelial cells were tested for their positivity using anti-cytokeratin antibody AE1/AE3 (Roche Diagnostics, Indianapolis, IN; custom conjugate Intergen Co., Purchase, NY) as reported by Kramer et al. [15].

Lymphocytes were separated from the sputum using Ficoll–Hypaque density gradient™ PLUS (GE Healthcare) centrifugation. Lymphocytes were piped at the interface without disturbing the cells on the bottom of flask, washed twice with cold PBS, and suspended in complete RPMI 1640 medium.

Macrophages and ganulocytes were harvested from the pellets, washed three times with cold PBS by centrifugation at 300 × *g* and then suspended in complete RPMI 1640 medium. To distinguish sputum macrophages from neutrophils, we performed a

Please cite this article in press as: A. Berraïes, et al., Increased expression of thymic stromal lymphopoietin in induced sputum from asthmatic children, Immunol Lett (2016), http://dx.doi.org/10.1016/j.imlet.2016.08.004