

Serum IL-23 Strongly and Inversely Correlates with FEV₁ in Asthmatic Children

Giorgio Ciprandi^a Caterina Cuppari^c Anna Maria Salpietro^c
Maria Angela Tosca^b Luciana Rigoli^c Luisa Grasso^c Mario La Rosa^d
Gian Luigi Marseglia^e Michele Miraglia del Giudice^f Carmelo Salpietro^c

^aAzienda Ospedaliera Universitaria San Martino, IRCCS, and ^bPediatric Pulmonology and Allergy, Istituto G. Gaslini, IRCCS, Genoa, ^cDepartment of Pediatrics, UOC Genetics and Immunology Paediatrics, University of Messina, Messina, ^dDepartment of Pediatrics, University of Catania, Catania, ^eDepartment of Pediatric Science, Pediatrics Clinic-University of Pavia, Foundation IRCCS San Matteo, Pavia, and ^fF. Fede Department of Pediatrics, Seconda Università di Napoli, Naples, Italy

Key Words

IL-23 · Asthma · Lung function · Children

Abstract

Background: Recently, Th17 cells have been found to participate in the pathogenesis of allergic asthma. IL-23 is a cytokine that may be implicated in modulating Th17 response. This study aimed at evaluating IL-23 and relating it to lung function in asthmatic children. **Methods:** Seventy-eight asthmatic children and 40 healthy children were evaluated. Spirometry and serum IL-23 measurement (ELISA kit) were performed in all asthmatic children. **Results:** IL-23 levels were higher in asthmatic than in healthy children ($p < 0.001$). There was a strong inverse relationship between FEV₁ and IL-23 ($r = -0.787$). **Conclusions:** This preliminary study suggests that serum IL-23 could be a suitable marker of bronchial function impairment in allergic asthmatic children.

Copyright © 2012 S. Karger AG, Basel

Introduction

Asthma is the most common chronic disease of childhood and the leading cause of childhood morbidity as measured by school absences, emergency department visits, and hospitalizations [1]. Besides the paradigmatic Th2/Th1 schema, other T subpopulations, such as regulatory T cells and Th17 cells, may play a pathogenic role in atopic disorders [2]. Several studies suggest that Th17 cells are involved in allergy and asthma [3–5]. The mechanisms of enhanced Th17 immunity in allergic asthma are still unknown even though there are some reports showing a pathogenic activity in allergic rhinitis [6, 7]. Th17 cells are regulated by IL-23, a novel member of the IL-12 heterodimeric cytokine family [8]. IL-23 is a heterodimer comprising the p19 and p40 subunits shared with IL-12p70, and is secreted by dendritic cells in response to immune danger [9]. Moreover, IL-23 may contribute to the differentiation of macrophages. It has been shown that increased expression of IL-23 in the lungs enhances not only antigen-induced IL-17A production and neutrophil recruitment in the airways but also antigen-induced Th2 cytokine production and eosinophil recruitment in the airways [10].

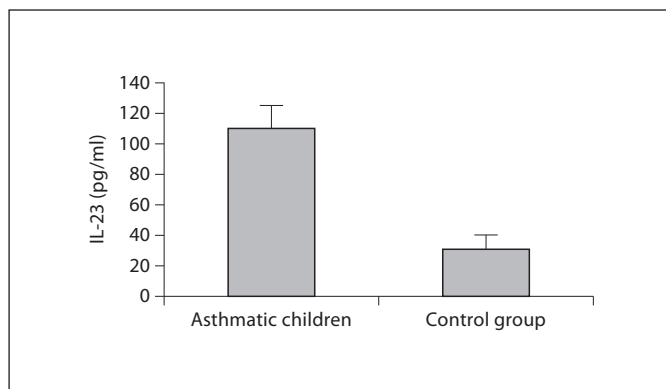


Fig. 1. Serum IL-23 levels in children with asthma and in healthy controls.

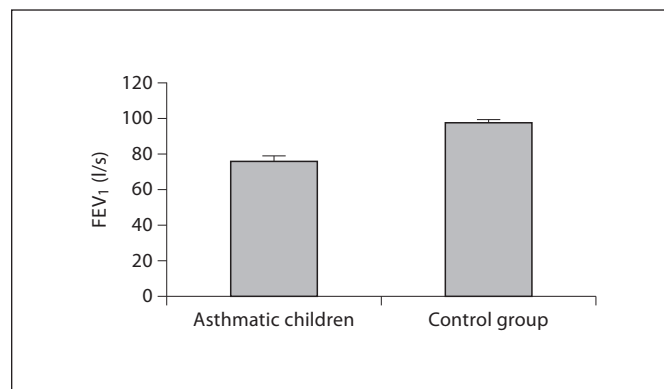


Fig. 2. FEV₁ values in children with asthma and in healthy controls.

These findings suggest a substantial role of IL-23 in causing eosinophilic inflammation in the airways. Therefore, IL-23 may play a pivotal role in bridging innate and adaptive immune responses, but comparatively little is known regarding its regulation.

On the other hand, studies concerning the circulating levels of IL-23 and its functional consequence in asthmatic children are still lacking. Therefore, the aims of the present study were to evaluate serum IL-23 levels in allergic children with asthma compared with healthy controls, and to correlate them to lung function.

Materials and Methods

This study included asthmatic as well as healthy children. Children were recruited in 4 Italian pediatric departments (Messina, Catania, Naples and Pavia). All the participating subjects were of Italian origin. The inclusion criterion was a diagnosis of mild to moderate persistent asthma according to validated criteria (GINA guidelines) [11] and documented reversibility. Exclusion criteria were: previous use of inhaled corticosteroids, pulmonary or cardiac congenital malformations; chronic pulmonary disease (bronchopulmonary dysplasia, cystic fibrosis, bronchiolitis obliterans following a viral infection), prematurity, small for gestational age, concurrent stridor, foreign body aspiration, neurological alteration, contact with tuberculosis and laryngomalacia. In addition, the healthy age-matched control subjects had to have no history of lung disease or allergy, no airway hyperresponsiveness and no upper and lower respiratory infections, and none of the patients should have sustained respiratory infections in the last 3 months before the beginning of the study and none of the children should have received oral corticosteroids in the last 3 months before inclusion into the study.

The study was explained in detail to the parents/guardians of the children who participated in this study, and informed consent was obtained from each parent/guardian. Study approval was re-

quested from the 'Human Ethics Committee' of the University Polyclinic of Messina.

The study was carried out from September 2010 to October 2010, and included 78 asthmatic subjects (40 males and 38 females, median age 9.1 ± 3.5 years) and 40 (22 males and 18 females) healthy age-matched children as controls (median age 9.26 ± 3.9 years; with a negative history of allergic disease, normal levels of total serum IgE and negative skin prick tests to a panel of aeroallergens, such as dust mite, mixed grass and tree pollen, cat and dog).

The blood samples were collected by venipuncture under appropriate conditions. Serum was acquired after blood coagulation for 1–2 h at room temperature. The supernatant was centrifuged for 10 min at 2,000 g. The serum thus obtained was then stored at -70°C .

IL-23 was measured using an ELISA kit (Human interleukin-12/int.-23P40 ELISA© Biovendor) according to the manufacturer's instructions.

Pulmonary function was measured spirometrically (Lungtest 1000; MES Ltd. 30–390, Krakow, Poland). FEV₁ values were expressed as percentage of predicted values, based on the child's age, gender, ethnicity, weight and height.

The data collected were analyzed by SPSS, version 15.0, statistical computer software. A p value <0.05 was considered statistically significant. Means and standard deviations were calculated for the variables and a t test was employed for the comparisons. Correlations between interleukin levels and FEV₁ were established using Spearman's correlation coefficient.

Results

Serum IL-23 levels were significantly higher in asthmatic children than in healthy control subjects (111.38 ± 14.77 vs. 30.63 ± 10.73 pg/ml; $p < 0.001$) as shown in figure 1. The mean FEV₁ values were significantly higher in healthy control subjects compared with asthmatic chil-

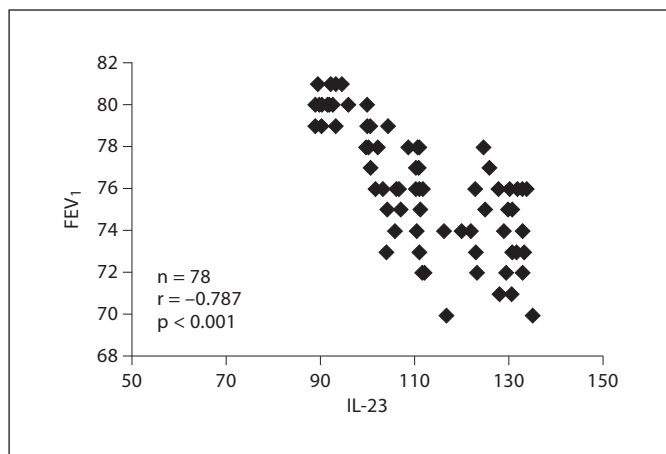


Fig. 3. Relationship between serum IL-23 levels and FEV₁ values in asthmatic children.

dren (96.2 ± 3.05 vs. 75.2 ± 5.7 ; $p < 0.001$) as shown in figure 2. Moreover, there was a strong and inverse significant relationship between serum IL-23 levels and FEV₁ values ($r = -0.787$) in asthmatic children (fig. 3).

Discussion

Asthma is characterized by airway inflammation that is orchestrated by a complex cytokine network. Allergic inflammation sustains the airflow limitation that is the main pathophysiologic feature of asthma. Recently, attention has focused on IL-23 [12–15]. Therefore, this study was designed to be conducted in children with atopic asthma, compared with healthy controls to measure serum levels of IL-23 and to correlate them to lung function.

The findings revealed that asthmatic children had higher serum IL-23 levels than healthy controls. There was a strong inverse relationship between IL-23 and lung function. This finding underlines the possible negative influence of IL-23 on bronchial airflow.

In asthma, IL-23 probably amplifies airway inflammation, recruits neutrophils and potentiates airway hyperresponsiveness. On the other hand, neutrophils can also produce a wide range of cytokines including IL-23 [16], which may be linked to the inflammatory status in asthma. Indeed, IL-23 stimulates survival and proliferation of Th17 cells, a key cell that regulates the production of other proinflammatory cytokines (IL-17, IL-22, IL-21, IL-6, TNF- α). Moreover, it has been shown that there was

an overexpression of IL-23 in the lung during asthma exacerbations that correlated with Th2-dependent cytokine production and eosinophilic infiltrates [17]. These findings could suggest a substantial role of IL-23 in causing eosinophilic airway inflammation.

In fact, recent studies on mice [18] have shown that IL-23 and Th17 cells enhance Th2 cell-mediated eosinophilic airway inflammation, suggesting that IL-23 and Th17 cells upregulate Th2 cell-mediated eosinophilic airway inflammation in two phases: IL-23 enhances antigen-induced activation of Th2 cells in the airways and antigen-specific Th17 cells cooperate to enhance Th2 cell-mediated eosinophil recruitment into the airways. This could thus suggest that IL-23 may be implicated in Th2 cell differentiation and this machinery could be involved in part in the IL-23-mediated enhancement of allergic airway inflammation.

Our data support the hypothesis that the altered pulmonary function in allergic asthmatic subjects might be associated with increased production of IL-23. Although additional studies are required to address the molecular mechanisms causing IL-23- and Th17-cell-mediated enhancement of allergic airway inflammation, our results raise the possibility that IL-23 could be a novel marker of allergic status.

On the other hand, this study had some limitations: IL-17A was not measured, nor did we assess IL-6 and TGF- β as both cytokines are involved in IL-17A production.

In conclusion, this preliminary study shows that serum IL-23 levels are increased in allergic children with asthma not treated with corticosteroids, and IL-23 levels are strongly and inversely correlated to lung function. Therefore, IL-23 might be considered a putative marker of reduced lung function in allergic asthmatics.

References

- 1 Masoli M, Fabian D, Holt S, Beasley R: The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004;59:469–478.
- 2 Schmidt-Weber CB, Akdis M, Akdis CA: TH17 cells in the big picture of immunology. *J Allergy Clin Immunol* 2007;120:247–254.
- 3 Hashimoto T, Akiyama K, Kobayashi N, Mori A: Comparison of IL-17 production by helper T cells among atopic and nonatopic asthmatics and control subjects. *Int Arch Allergy Immunol* 2005;137(suppl 1):51–54.
- 4 Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al: IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108:430–438.

- 5 Matsunaga K, Yanagisawa S, Ichikawa T, Ueshima K, Akamatsu K, Hirano T, et al: Airway cytokine expression measured by means of protein array in exhaled breath condensate: correlation with physiologic properties in asthmatic patients. *J Allergy Clin Immunol* 2006;118:84–90.
- 6 Ciprandi G, Fenoglio D, De Amici M, Quaglini S, Negrini S, Filaci G: Serum IL-17 in allergic rhinitis. *J Allergy Clin Immunol* 2008;122:650–651.
- 7 Ciprandi G, De Amici M, Murdaca G, Fenoglio D, Ricciardolo F, Marseglia GL Tosca MA: Serum IL-17 levels are related to clinical severity in allergic rhinitis. *Allergy* 2009;64:1375–1378.
- 8 Hao JS, Shan BE: Immune enhancement and anti-tumour activity of IL-23. *Cancer Immunol Immunother* 2006;55:1426–1431.
- 9 Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA: Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000;13:715–725.
- 10 Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, Puccetti P, Iwamoto I, Nakajima H: IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008;178:1023–1032.
- 11 National Institutes of Health NHLBI: Burden of asthma; in *Global Strategy for Asthma Management and Prevention*. NIH Publication. National Institutes of Health NHLBI, 2002;11–27 Available from: <http://www.gin-asthma.com/workshop.pdf>.
- 12 Durrant DM, Metzger DW: IL-12 can alleviate Th17-mediated allergic lung inflammation through induction of pulmonary IL-10 expression. *Mucosal Immunol* 2010;3:301–311.
- 13 Nakajima H, Hirose K: Role of IL-23 and Th17 cells in airway inflammation in asthma. *Immune Netw* 2010;10:1–4.
- 14 Peng J, Yang XO, Chang SH, Yang J, Dong C: IL-23 signaling enhances Th2 polarization and regulates allergic airway inflammation. *Cell Res* 2010;20:62–71.
- 15 Zhao Y, Yang J, Gao YD, Guo W: Th17 immunity in patients with allergic asthma. *Int Arch Allergy Immunol* 2010;151:297–307.
- 16 Sampson AP: The role of eosinophils and neutrophils in inflammation. *Clin Exp Allergy* 2000;30(suppl 1):22–27.
- 17 Charoenying Y, Kamchaisatian W, Atamasirikul K, Direkwattanachai C, Manuyakorn W, Benjaponpitak S: Cytokine responses during exacerbation compared with stable phase in asthmatic children. *Asian Pac J Allergy Immunol* 2010;28:35–40.
- 18 Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, Puccetti P, Iwamoto I, Nakajima H: IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008;178:1023–1032.