The cytokine interleukin-26 as a biomarker in pediatric asthma

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Abstract

In this pilot study, we examined associations between local interleukin (IL)-26, disease severity and biomarkers of Th2-mediated inflammation in a well-defined cohort of pediatric patients (14 years median age, 41% females) with controlled (n = 28) or uncontrolled (n = 48) asthma. Sputum IL-26 protein concentrations (ELISA) reflected disease control in patients without local (low exhaled nitric oxide) or systemic (low blood eosinophils) signs of eosinophilic inflammation. Moreover, sputum-IL-26 concentrations correlated with those of blood neutrophils. Our study indicates that IL-26 is a potential biomarker of disease severity in pediatric asthma without signs of Th2-mediated inflammation.

Keywords: Asthma, Children, Eosinophil, FENO, IL-26, Neutrophil, Sputum

Introduction

Dear Editor,

Up to 20% of children with severe asthma have no signs of eosinophilic inflammation [1] and these pediatric patients respond poorly to inhaled corticosteroids and anti-IgE, pharmacotherapy that was originally designed to target severe Th2-mediated inflammation [2]. For these patients, there is a need to increase the understanding of the immunological events underlying the disease, to establish biomarkers for improved diagnosis and monitoring, as well as to identify potential targets for therapy [2].

The presumed Th17 cytokine interleukin (IL)-26 is an intriguing member of the IL-10 family; one that is involved in several chronic inflammatory disorders and can exert both pro- and anti-inflammatory actions, depending upon the setting [3]. However, there is currently no conclusive information on the involvement of IL-26 in the pathogenesis of asthma or any other chronic inflammatory airway disorder [4]. A recent study on healthy human subjects indicates that this cytokine is produced by Th17 cells, as well as by other leukocytes, and that IL-26 contributes to the mobilization of neutrophils in the airways during activation of pulmonary host defense by endotoxin [5]. Given these facts, we hypothesized that local IL-26 is associated with severe disease in pediatric asthma lacking signs of eosinophilic inflammation.

Methods

To address our hypothesis, we utilized data from a previously well-characterized cohort of school-age children (n = 76) with severe uncontrolled (uncontrolled) or persistent controlled (controlled) asthma [6]. Briefly, uncontrolled asthma was diagnosed in a child with reduced asthma control despite treatment with high doses of inhaled corticosteroids (≥800 μg budesonide equivalent per 24 h), whereas controlled asthma was defined as children having an acceptable asthma control with a low to moderate daily dose of inhaled corticosteroids (100–400 μg budesonide equivalent per 24 h). The patients were recruited as previously described [6], after approval by the Regional Ethics Review Committee in Stockholm (Stockholm) and after informed consent from each patient or parent, in full accordance with the Helsinki declaration.

We quantified IL-26 protein concentrations in the airways in relation to asthma severity, blood eosinophils (B-EOS), exhaled nitric oxide (FENO) and blood neutrophils (B-NEUTRO). Specifically, we utilized cell-free samples of induced sputum [7] for the measurement of IL-26 protein concentrations (ng/mL) with ELISA (Cusabio Biotech*) [5].

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Results
Among our included pediatric patients, the median age (range) was 13.7 years (7–19), with 41% being females. The 48 patients with uncontrolled asthma had a reduced score on the asthma control test (17 versus 23, \( p < 0.001 \)), and a lower FEV\(_1\) (82% versus 90% predicted, \( p = 0.04 \)) despite receiving a higher dose of inhaled corticosteroids (800 \( \mu \)g versus 320 \( \mu \)g of budesonide, \( p < 0.001 \)) compared to the 28 children with controlled asthma. In addition, the children with uncontrolled asthma had higher concentrations of B-EOS (0.4 versus 0.2, \( p = 0.02 \)) and B-NEUTRO (3.2 versus 2.6, \( p = 0.03 \)) than those with controlled asthma.

In the entire cohort, the median FENO levels were 18.3 p.p.b and the median B-EOS concentration was 0.3\( \times 10^9 \)/L. These median values were used to categorize children into groups with high and low levels of these biomarkers of Th2-mediated inflammation, independently of the predefined severity classification.

Among children with low concentrations B-EOS (\( \leq 0.3\times 10^9 \)/L), we observed higher concentrations of IL-26 in uncontrolled compared to controlled asthma (Fig. 1a & Table 1). Furthermore, we found a corresponding association between asthma control and IL-26 concentrations among children with low levels of FENO (Fig. 1b & Table 1). A trend towards an association between asthma control and IL-26 concentrations in the entire cohort was observed, but it was not statistically significant (Table 1). Furthermore, when investigating children with high concentrations of B-EOS and high levels of FENO, the difference in distribution of IL-26 between children with uncontrolled and controlled asthma was less pronounced (Table 1).

Finally, we found a weak but statistically significant correlation (the Pearson test: \( r = 0.27, p = 0.018, n = 76 \)) between the concentrations of B-NEUTRO and IL-26 in the entire cohort of pediatric patients with asthma and this correlation tended to be stronger in the patients with low levels of FENO (\( r = 0.35, p = 0.036, n = 36 \)). There was no evident correlation between IL-26 concentrations and age, gender, height or dose of inhaled corticosteroids.

Discussion
Our study is the first to associate local IL-26 protein concentrations in the airways with a certain clinical phenotype of pediatric asthma. The fact that IL-26 protein concentrations are increased in sputum from pediatric patients with uncontrolled asthma but without signs of Th2-mediated inflammation forwards IL-26 as a potential, novel biomarker of disease severity in this

Table 1 Interleukin-26 in the airways of children with uncontrolled versus controlled asthma

|                | IL-26 (ng/ml) | n  | IL-26 (ng/ml) | n  | \( p \)-value* |
|----------------|---------------|----|---------------|----|--------------|
| All patients   | 4.4 (1.9–14)  | 48 | 2.4 (1.3–4.5) | 28 | 0.077        |
| Low B-EOS (<0.3\( \times 10^9 \)/L) | 4.6 (2.3–13) | 27 | 2.3 (1.3–4.5) | 22 | 0.038*       |
| High B-EOS (>0.3\( \times 10^9 \)/L) | 4.1 (1.7–16) | 21 | 2.9 (1–31)   | 5  | 0.61         |
| Low FENO (<183 p.p.b) | 4.1 (2.2–16) | 23 | 2.0 (1.2–3.6) | 13 | 0.055        |
| High FENO (>183 p.p.b) | 5.0 (1.8–13) | 25 | 2.7 (1.4–24) | 15 | 0.54         |

Footnotes: The IL-26 concentrations are presented as medians with inter-quartile ranges. The median values in the entire cohort of the respective biomarker were used to define the cut-off between high and low levels of the various biomarkers. B-EOS blood eosinophils, FENO the fraction of nitric oxide in exhaled air, in parts per billion. *Independent samples, analyzed by Mann–Whitney U-test (SPSS® version 20)
phenotype of asthma. These findings are also supported by a positive correlation between local IL-26 and systemic neutrophils (ie. B-NEUTRO); a correlation in line with a mechanistic link between IL-26 and the mobilization of neutrophils as recently indicated in human airways [5, 8].

One of the effects of treatment with inhaled corticosteroid is an inhibition of the production of Th2 cytokines such as IL-4 and IL-5, which subsequently attenuate eosinophilic recruitment and the release of toxic granule proteins [9]. The observed clinical effects are an improvement of symptoms and pulmonary function and a reduced rate of asthma exacerbations [10]. It is widely accepted that increased concentrations of blood eosinophils and high levels of FENO indicate systemic and local eosinophilic inflammation, respectively [11, 12]. In addition, it is known that low levels of these established biomarkers are associated with a reduced sensitivity to inhaled steroids [13, 14]. Given that our findings are compatible with an increase in local IL-26 being associated with inflammation not mediated by Th2-related mechanisms; this observation forwards the clinically relevant question whether IL-26 is also linked to low sensitivity to inhaled steroids in pediatric patients with non-allergic asthma? New studies are required to address this possibility; a possibility that has implications for the estimated 13 % of children with severe asthma displaying excess neutrophil mobilization [1].

Even though the size of our study material was modest, a particular strength of our current analysis is the standardized and detailed characterization of each individual patient; making it possible to identify various subgroups within this cohort. We think that this facilitated the discovery that IL-26 provides a potentially clinically relevant signal in patients without signs of local or systemic eosinophilic inflammation.

Conclusion
Our pilot study demonstrates that local IL-26 bears potential as a biomarker of disease severity in a clinical phenotype of pediatric asthma that is perpetuated by other than Th2-related mechanisms.

Abbreviations
B-EOS: blood eosinophils; B-NEUTRO: blood neutrophils; FENO: the fraction of nitric oxide in exhaled air; FEV1: forced expiratory volume during 1 s; IL: interleukin; Th: T helper.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
The author contributions were as follows: The conception and design of the study involved JRK, GH and AL; the data analysis and interpretation involved: JRK, BN, BL, GH and ALN; and, finally, the drafting of the manuscript included: JRK, GH and AL. All authors read and approved the final manuscript.

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