STUDY OF SOME ASPECTS OF IL-5 ROLE IN THE ALLERGIC RESPIRATORY PATHOLOGY-ALLERGIC RHINITIS AND ASSOCIATED BRONCHIAL ASTHMA

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ABSTRACT

Background: A lot of evidence exists on the relationship between the upper and lower respiratory airways based on epidemiological, clinical, and pathophysiological levels. However, new aspects of allergic inflammation and released cytokines play a crucial role in this interaction. This study aims to explore the role and involvement of IL-5 in patients with allergic rhinitis (AR) and newly diagnosed allergic bronchial asthma (BA) with a mild course, manifested after many years of previous persistent AR.

Methods: This prospective study included 67 patients with allergic inflammation of the upper respiratory airways, manifested by AR, and involvement of the lower airways, clinically presented as mild atopic BA, recruited between 2020 and 2021.

Results: AR patients were 35 (40.23%), and patients with a combination of AR and newly diagnosed BA - 32 (36.78%). Twenty healthy controls (22.99%) were also included. The most common type of sensitization was to grass pollen 43 (64%) and tree pollen - birch 32 (48%). We did not find significant differences between the serum levels of healthy individuals and patients included in the study. No associations between the IL-5 levels and parameters of functional lung tests or skin prick tests were found. However, we have identified the following dependencies: IL-5 levels were lower in the patients than in the healthy subjects; however, non-significantly. This difference is more prominent when comparing the AR group of patients to the AR with BA group.
Conclusions: Despite the known role and active involvement of IL-5 in allergic diseases, not always IL-5 levels can be found elevated in serum samples of AR and AR with BA patients. Therefore, other mechanisms may play a significant role in airway inflammation, primarily when one allergic disease occurs after another.

Keywords: Allergic Rhinitis, Bronchial Asthma, IL-5, One Way - One Disease, United Airway Disease, Allergic Inflammation, Airway Inflammation.

INTRODUCTION

Allergic rhinitis (AR) affects about 600 million people worldwide (Bousquet et al., 2001). AR is part of a systemic inflammatory process associated with inflammatory mucosal involvement and the development of bronchial asthma (BA), chronic rhinosinusitis and allergic conjunctivitis.

Significant prevalence of BA has been observed in patients with persistent and severe AR (Bousquet et al., 2001). Valero et al. (2009), in a study of 3225 patients, documented the association between skin sensitization, rhinitis and asthma in patients with AR. They concluded that respiratory allergic disease is a systemic disease, and AR and asthma are manifestations of the same disease.

Eosinophils (Eo) are white blood cells, granulocytes, that are rarely found in healthy individuals but increase in blood and tissues during helminthic infections, allergic inflammation, and late-onset eosinophilic BA (Wenzel, 2012). They are thought to be critical regulatory cells involved in the innate immune response. They are found in various tissues and organs, playing an essential role in homeostasis (Rothenberg & Hogan, 2006). In allergic inflammation, Eo levels tend to increase in tissues that generally contain a small number of these cells, as lungs and upper respiratory airways. Additionally, Eo cells store a large number of potent mediators - several cationic proteins (eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin, and major basic protein), different cytokines/chemokines and other mediators (Furuta et al., 2014). These cells and their mediators are found increased in the airways and sputum in patients with AB. IL-5 and eotaxin, when entering the bloodstream, they exert immune modulation. IL-5 stimulates the spread, differentiation, survival, and adhesion of Eo cells (Akdis et al., 2016). In addition to IL-5, tissue Eo cells synthesize and secret other cytokines, chemokines, and growth factors, such as GM-C SF, IL-4, CCL3, MIP-1α, CCL5, RANTES and TGFβ (Dubucquoi et al., 1994). In another study, about 20% of tissue Eos were positive for IL-4 and IL-5 mRNA expression in skin biopsies of allergic individuals, 24 hours after challenge, leading to an increase in 50–60% of protein expression of IL-4 and IL-5 (Barata et al., 1998).

AR and BA are often combined and share common elements in their pathogenesis. Data from epidemiological studies show that nasal symptoms were observed in 78% of patients with asthma, and in 38-40% of patients with AR was observed asthma symptoms (Simons, 1999; Caimmi et al., 2012). Studies have found a temporary link between the onset of rhinitis and asthma, with rhinitis often preceding the development of asthma. It is important to note that patients with AR and no clinical data for asthma often exhibit non-specific bronchial hyperreactivity (Thomas, 2006). Furthermore, it has been observed that the treatment of allergic rhinitis relieves asthma symptoms. This increases interest in the relationship between two diseases (Corrent, 1997; Durham, 1999).

In line with this, we aimed to explore the role and involvement of IL-5 in patients with allergic rhinitis (AR) and newly diagnosed allergic bronchial asthma (BA) with a mild course,
manifested after many years of previous persistent AR..

METHOD

Subjects
In the period from 2020 to 2021, a total of 67 (77.01%) subjects were examined, of which with AR 35 (40.23%), AR patients with newly diagnosed BA 32 (36.78%) and 20 (22.99%) healthy controls.

The patients were included in the following the inclusion criteria: age over 18 years, a diagnose of AR, based on "ARIA - Allergic Rhinitis and Its Impact on Asthma" recommendations (Bousquet et al., 2001) and patients with mild, newly diagnosed atopic BA, diagnosed by the recommendations of the GINA (Global Asthma Initiative) (Bateman et al., 2008).

Clinically manifested by nasal congestion, rhinorrhea, itchy nose and sneezing, AR was classified according to the duration of symptoms and the severity of the course of the ARIA classification as intermittent - in the presence of symptoms less than 4 days a week or for less than 4 consecutive weeks and persistent when symptoms occur more than 4 days a week or more than 4 consecutive weeks. The mild form was considered when there are no disturbances in sleep, daily activities, work and school, while moderate and severe course observed disturbances in sleep or daily activities, sports or problems at work/school.

BA is associated with chronic airway inflammation, multi-cell involvement, and inflammatory mediators. Chronic inflammation is associated with hyperactivity of the airways, leading to recurrent episodes of wheezing, breathlessness, tightness in the chest, and cough. According to the severity of the course, BA was classified based on symptoms, airflow limitation and lung function, such as: mild, moderate or severe.

Methods
We took a detailed history and physical examination of each patient and performed patch testing to establish sensitization, pulmonary functional tests to evaluate them clinically. Rhinoscopy was also performed to assess the mucosal status of the airways - the presence of edema, nasal passages, mucosal color and other pathological changes. X-ray of the sinuses and radiography of the lungs to exclude pathology of the relevant organ.

Skin allergy tests (CAP) - a skin prick test was performed to detect sensitization in the patients. Allergens from Stallergen (France) were used. The tested allergens were instilled on the skin on the front side of the forearm, and the drops were pierced with a lancet on the surface with a depth of 1 mm. An early reaction was sought, and the result was evaluated every 20 minutes. A positive result was considered when the diameter of erythema and papule was ≥ 3 mm. Control tests were also applied: a negative control using saline and a positive control containing histamine hydrochloride (reported with a papule diameter ≥ 3 mm). Venous blood was collected into tubes containing EDTA, centrifuged at 1200 rpm for 15 minutes, divided into six separate tubes and stored at -20-70 • C. Before the investigation, the samples were thawed entirely at room temperature.

To quantify IL-5 in sera in patients, we used a commercial kit, solid-phase sandwich ELISA for in vitro quantification of cytokines in biological materials: Human ELISA kit - Diaclone. We constructed a standard curve according to the calibrators of each kit and determined the amount of cytokine in the test samples. IL-5 serum levels were analyzed in 67 patients (AR, 35; AR and BA, 32; and 20 healthy persons).

Statistical analyses (T-test, descriptive statistics) were performed by SPSS v.19.
RESULTS AND DISCUSSION

Fifty-one of the patients (71.8%) were diagnosed with seasonal AR, 9 (12.7%) of them had year-round AR and 11 (15.5%) - year-round with seasonal exacerbation. The duration of AR in the studied population is mainly lasting more than one year 70 (98.6%). Skin-allergy prick allergy tests were performed to prove atopy in the subjects. Leading sensitization was found to grass pollen 43 (64%) and tree pollen - birch 32 (48%). Newly diagnosed mild atopic BA was detected in 35 (49.3%), while 36 (50.7 %) were with preserved lower airways.

The results of skin prick tests in AR patients are presented in Table 1.

Table 1. Allergens and skin prick tests in patients with AR.

<table>
<thead>
<tr>
<th>Allergens</th>
<th>Number (positive reaction)</th>
<th>Percent</th>
<th>Mean</th>
<th>Net weal size, Median range (max-min) in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. P teronyssinus</td>
<td>23</td>
<td>34.33</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>D. F arinae</td>
<td>18</td>
<td>26.86</td>
<td>5.17</td>
<td>5</td>
</tr>
<tr>
<td>Cockroach</td>
<td>2</td>
<td>2.98</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Down and feathers</td>
<td>4</td>
<td>5.97</td>
<td>6.75</td>
<td>7</td>
</tr>
<tr>
<td>Dog</td>
<td>21</td>
<td>31.34</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cat</td>
<td>22</td>
<td>32.83</td>
<td>7.18</td>
<td>6.5</td>
</tr>
<tr>
<td>Penicillium</td>
<td>5</td>
<td>7</td>
<td>4.6</td>
<td>4</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>4</td>
<td>6</td>
<td>5.75</td>
<td>5.5</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>3</td>
<td>4</td>
<td>4.67</td>
<td>5</td>
</tr>
<tr>
<td>Alternaria</td>
<td>4</td>
<td>6</td>
<td>5.75</td>
<td>5</td>
</tr>
<tr>
<td>Ambrosia</td>
<td>15</td>
<td>22</td>
<td>5.27</td>
<td>5</td>
</tr>
<tr>
<td>Artemisia</td>
<td>26</td>
<td>39</td>
<td>6.38</td>
<td>6.5</td>
</tr>
<tr>
<td>Birch</td>
<td>32</td>
<td>48</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Willow</td>
<td>13</td>
<td>19</td>
<td>3.46</td>
<td>3</td>
</tr>
</tbody>
</table>
The results for IL-5 in the serum samples of the tested individuals are presented in Table 2 and Figure 1A and B.

Table 2. IL-5 (pg/ml) in serum samples of healthy controls, all patients and AR, AR vs. BA. Data are presented as mean ± SD or SE

<table>
<thead>
<tr>
<th></th>
<th>Healthy persons</th>
<th>All patients</th>
<th>Healthy persons vs. patients</th>
<th>AR + BA</th>
<th>AR</th>
<th>AR + BA vs. AR</th>
<th>Healthy persons vs. BA + AR vs. AR (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5, pg/ml</td>
<td>3.58 ± 1.35</td>
<td>3.21 ± 5.31</td>
<td>p = 0.762</td>
<td>2.56 ± 1.67</td>
<td>3.67 ± 1.19</td>
<td>p = 0.391</td>
<td>p = 0.584</td>
</tr>
</tbody>
</table>

We did not find significant differences between the serum levels of healthy individuals and the patients included in the study.

No associations between the IL-5 levels and parameters of functional lung tests or skin prick tests were found. However, we have identified the following dependencies:

1. IL-5 levels were lower in the patients than in the healthy subjects; however, non-significantly. This can be interpreted as IL-5 consumption during recruitment and activation of Eo and the presence of IL-5 mainly in the nasal and bronchial mucosa but not in the circulation.

2. This difference is more prominent when comparing the AR group of patients to the AR with BA group.

3. There is no difference between the IL-5 levels in AR patients and healthy controls. Probably, this can be explained by the initial process of inflammation and united airway disease in the group of AR with BA patients compared to healthy controls.

Additionally, we did not find differences in Eo counts between the study groups, neither correlations between the IL-5 levels and Eo count. It can be considered that AR with BA patients have mild asthma, and a high absolute number of Eo is typical for patients with severe asthma and eosinophilic phenotype.

It is well-known that during the allergic inflammation, activation of Th1 results in the release of IL-2 and INF-γ, while Th2 lymphocytes release IL-2, IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and GM-CSF (Pawankar, 2007). In addition, th2 cells secrete IL-5, stimulate the accumulation of Eo cells and contribute to the induction of hyper reactivity of airways in asthmatic patients. Levels of IL-5, eosinophils and Th2 found in Broncho alveolar lavage correlate with the severity of BA. Th2 cytokines are also involved in the synthesis of IgE antibodies. During the early phase, specific adhesion molecules are expressed on the surface of the endothelium and epithelium, leading to extravasation and...
infiltration of inflammatory cells (Pawankar, 2007).

The role of IL-5 in allergic diseases is well-established. However, in our study, we demonstrated that IL-5 could not correlate with the presence of allergic disease or other clinical signs, and the levels can be as low as in healthy persons. Furthermore, in patients with AR and those with AR with mild newly developed BA, the levels of IL-5 could be indistinguishable.

CONCLUSION
The concept of the "single airway" and the “united airway disease,” introduced recently, has undergone significant development lately with the intensive study of immunological and molecular mechanisms supporting the known pathophysiological and clinical associations.

Our study focuses on some aspects of one of the major proinflammatory cytokines, IL-5, in this complex process. Although we do not find statistically significant differences in serum IL-5 levels, we have identified some dependencies useful in the further diagnostic process.

ACKNOWLEDGMENTS
This study was supported by the scientific project "Young Researcher - 2020" and associated funding D-75/2020 with e№8299/19.11.2019.

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703-708.


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