Serum levels of Interleukin-33 and its soluble receptor ST2 in asthmatic patients

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KEYWORDS
Interleukin-33; Soluble ST2; Bronchial asthma

Abstract Interleukin-33 is a member of IL-1 family of cytokines and binds to two receptors: ST2 (IL-1-R1) and IL-1 receptor accessory protein (IL-1RAP). There are two isoforms of ST2 proteins: ST2L, a transmembrane form, and a soluble ST2 (sST2), a secreted form, that can serve as a decoy receptor of IL-33. The IL-33/ST2 signaling pathway activates airway eosinophils that exacerbate airway inflammation.

The aim of this study was to analyze the serum level of IL-33 and its receptor sST2 in patients with bronchial asthma to assess if the serum level of IL-33 and/or sST2 may be a marker of the disease severity and potential therapeutic targets.

Patients and methods: This study was carried out at the Microbiology & Immunology and Chest departments, Faculty of Medicine, Zagazig University Hospitals during the period from December 2012 to September 2013. The study included 30 patients diagnosed as bronchial asthma according to GINA 2012. Patients were classified into two groups: Group I: included 15 patients 8 males and 7 females with a mean age 36.2 ± 15.8 during exacerbation of bronchial asthma. Group 2: included 15 patients 8 male and 7 female with mean age 37.3 ± 12.8. They were stable asthmatic patients and the last exacerbation was one month ago. There were 30 normal healthy persons as a control group. All patients were subjected to, full medical history, general and local examination, Plain chest X-ray PA and lateral views, pulmonary function tests, Liver and kidney function tests, intradermal skin test, skin prick test, measurement of serum levels of IL-33 (WEKA MED), IL-33 Receptor (soluble...
ST2) (OmniKine) and total IgE (IMMUNOSPEC) by enzyme linked immunosorbent technique using commercial kits.

Results: There was a highly significant increase in the serum level of IL-33 in both groups of patients (p1 < 0.001) with the highest level 960 ± 336 ng/ml in group 1 followed by 732.2 ± 68.3 ng/ml in group 2 while the normal control group serum level was 174 ± 41 ng/ml.

As regards serum level of sST2, there was a highly significant increase in its level in both groups of patients (p1 < 0.001) with the highest level 96.8 ± 25 µg/ml in group 1 followed by 83.3 ± 5.3 µg/ml in group 2 while the normal control group serum level was 33.9 ± 9.6 µg/ml.

In acute exacerbated patients there was significant –ve correlation between FEV1 and serum level of both total IgE and IL-33 and in stable asthmatic patients there was high significant +ve correlation between PEFR variability and serum level of sST2.

Conclusion: The serum levels of IL-33 and its receptor sST2 were markedly elevated in patients with bronchial asthma and this supports the concept of sST2 and Interleukin-33 as a therapeutic target in asthma.

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Introduction

Bronchial asthma is thought to be T helper 2 (Th2) cell-mediated diseases. Th2 cells produce cytokines, such as interleukin (IL)-33 which is also a chemoattractant for human Th2 cells. IL-33 is produced by mast cells after immunoglobulin (Ig) E-mediated activation and is able to trigger mast cells to release proinflammatory cytokines in vitro [1]. IL-33 is a member of the IL-1 family of cytokines and binds to two receptors: ST2 (IL-1R1) and IL-1 receptor accessory protein (IL-1RAP). There are two isoforms of ST2 proteins: ST2L, a transmembrane form, and soluble ST2 (sST2), a secreted form that can serve as a decoy receptor of IL-33. ST2 is highly expressed on mast cells and selectively on Th2 cells [2].

High levels of sST2 have been found in the sera of adults and children with acute asthma [3].

The IL-33/ST2 signaling pathway activates airway eosinophils that exacerbate airway inflammation. This pathway is critical for the progression of IgE-dependent inflammation. Mutations in the gene for IL1RL1 (ST2) have been linked to atopic dermatitis and asthma [4]. Steroids and combination therapies with long-acting β-agonists are the mainstay of asthma treatment and effectively suppress cytokine expression and acute inflammatory symptoms. However, they do not prevent, reverse or treat the underlying causes of disease. These treatments require constant monitoring and are associated with side-effects and resistance. Therefore, there is an urgent need for new and more effective treatments and cytokines have been extensively investigated as potential therapeutic targets. So the aim of this study was to analyze the serum level of IL-33 and its receptor sST2 in patients with bronchial asthma to assess if the serum level of IL-33 and/or sST2 may be a marker of the disease severity and potential therapeutic targets.

Patients and methods

This study was carried out at the Microbiology & Immunology and Chest Departments, Faculty of Medicine, Zagazig University Hospitals during the period from November 2012 to November 2013. The study included 30 patients with mean age 36.7 ± 14.2 diagnosed as bronchial asthma according to GINA 2012 [5] as follows:

- Recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning.
- Pulmonary function test demonstrating reversible airway obstruction, manifested by post bronchodilator increase in FEV1 > 15%.
- Peak expiratory flow (PEF): variability by 7–20%.

Patients were classified to two groups:

- Group I (asthmatic patients during acute exacerbations):

  This group included 15 patients; 8 males and 7 females with a mean age 36.2 ± 15.8, during exacerbation of bronchial asthma.

  The severity of exacerbations was assessed according to GINA (2012) [5], as mild, moderate, severe and respiratory arrest imminent.

- Group 2 (stable asthmatic patients):

  This group included 15 patients 8 males and 7 females with mean age 37.3 ± 12.8. They were stable asthmatic patients and the last exacerbation was one month ago. They were classified according to GINA 2012 into: controlled, partially controlled and uncontrolled.

- Control group:

  There were 30 normal healthy persons as a control group they were 15 males and 15 females with mean age 34.5 ± 9.

  All patients were subjected to full medical history, general and local examination, Plain chest X-ray PA and latera views, pulmonary function tests, Liver and kidney function tests and eosinophilic count. Measurement of serum levels of IL-33, sST2 and total IgE and commercial enzyme-linked immuno-sorbent assays were used to measure serum levels of IL-33 (WKEA MED), sST2 (OmniKine) and total IgE (IMMUNOSPEC). The assays were performed using the protocols recommended by the manufacturers.

Statistical analysis

Statistical analysis was performed with SPSS version 19 software package (SPSS, Inc. Chicago). Categorical variables were
expressed as proportions, and continuous variables that were or were not normally distributed were expressed as means ± SD or medians (quartiles), respectively. The \( t \)-test or Mann–Whitney test was used to compare means or medians between different groups, for variables that were or were not normally distributed, respectively. For all analyses, \( P \) value <0.05 was considered significant.

Results

Table 1 shows that there is a highly significant difference \((p1 < 0.001)\) in serum levels of IL-33 among the three studied groups with the highest level as 960 ± 336 ng/ml in group 1 (asthmatic patients during exacerbations) followed by 732.2 ± 68.3 ng/ml in group 2 (stable asthmatic patients) while the normal control group serum level is 174 ± 41.2 ng/ml.

There is also a significant difference \((P1 < 0.001)\) between group 1 and group 2 (Fig. 1).

Table 2 shows that there is a highly significant difference \((p1 < 0.001)\) in serum levels of sST2 among the three studied groups with the highest level as 96.8 ± 25 \(\mu\)g/ml in group 1 (asthmatic patients during exacerbations) followed by 83.3 ± 5.3 \(\mu\)g/ml in group 2 (stable asthmatic patients) while the normal control group serum level is 33.9 ± 9.6 \(\mu\)g/ml.

There is also a significant difference \((P1 < 0.001)\) between group 1 and group 2 as regards serum level of sST2 (Fig. 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Serum levels of IL-33 in the studied groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-33 (ng/ml)</td>
<td>Group 1</td>
</tr>
<tr>
<td>X ± SD</td>
<td>960 ± 336</td>
</tr>
<tr>
<td>Range</td>
<td>700–1700</td>
</tr>
</tbody>
</table>

\( X = \) mean.

\( P1: \) means probability of difference among the three groups.

\( P2: \) means probability of difference between group1 and group2.

Table 3 shows that there is a highly significant difference \((p1 < 0.001)\) in serum level of IGE among the three studied groups with the highest level as 324.7 ± 133.4 IU/ml in group 1 (asthmatic patients during exacerbations) followed by 68.2 ± 47.3 IU/ml in group 2 (stable asthmatic patients) while the normal control group serum level is 20.6 ± 15.7 IU/ml. There is also a highly significant difference \((p1 < 0.001)\) between group 1 and group (Fig. 3).

Table 4 shows that there is a highly significant difference \((p1 < 0.001)\) in blood eosinophil percentage among the three studied groups with the highest level as 324.7 ± 133.4 in group 1 (asthmatic patients during exacerbations) followed by 68.2 ± 47.3 in group 2 (stable asthmatic patients) while the normal control group percentage is 20.6 ± 15.7. There is also a highly significant a difference \((p1 < 0.001)\) between group 1 and group (Fig. 4).

Table 5 shows that in acute exacerbated patients there is a significant \(\neg\) correlation between FEV1 and total IgE as \((r) = -0.427\) and \(p = 0.113\). There is also significant \(\neg\) correlation between FEV1 and IL-33s as \((r) = -0.776\) and \(P = 0.001\), while there is no correlation between FEV1 and sST2.

Table 6 shows that there is a high significant \(+\) correlation between PEFR variability and serum level of sST2 as \((r) = 0.524\) and \(p = 0.045\), while there is no correlation between PEFR variability and serum levels of either IgE or IL-33.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Serum levels of sST2 in the studied groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>sST2 ((\mu)g/ml)</td>
<td>Group 1</td>
</tr>
<tr>
<td>X ± SD</td>
<td>96.8 ± 25</td>
</tr>
<tr>
<td>Range</td>
<td>72–95</td>
</tr>
</tbody>
</table>

\( P1: \) means probability of difference among the three groups.

\( P2: \) means probability of difference between group1 and group2.
Discusion

Asthma is a chronic inflammatory disease classically characterized by airway hyper-responsiveness, allergic inflammation, elevated serum IgE levels and increased Th2 cytokine production. Given that IL-33 is a strong inducer of Th2 immune responses its role in asthma has been extensively studied [6]. IL-33, a member of the IL1-cytokine family, is considered to be crucial for the induction of T-helper type 2 cell dominant immune responses such as host defense against nematodes and allergic diseases [7].

IL-33 receptor was first identified as IL-1 receptor-like molecule and termed as ST2. ST2 is an Interleukin-1 receptor family member and exists in both membrane – bound isoform and a soluble isoform (sST2) [8].

IL-33 is the functional ligand for ST2 and ST2/IL-33 signaling regulating inflammation and immunity [9]. IL-33 and its receptor are part of IL-1 family, and their interactions promote a variety of actions from a number of different cell types. The IL-33/ST2 axis is thought to be intimately involved in the promotion and maintenance of allergic inflammation via a number of cell types that include Th2 cells, mast cells and basophils, and structural cells such as airway epithelium and smooth muscle cells [10]. IL-33/ST2 signaling pathway activates air way eosinophils that exacerbate air way inflammation [11].

In our study, there was a significant rise in serum levels of both Interlukin-33 and its receptor sST2 in both exacerbated and stable asthmatic patients and the rise in exacerbated patients was significantly higher than the rise in stable patients Tables 1 and 2.

Cytokines regulate important biological processes such as the immune response or hematopoiesis and are involved in the pathogenesis of many diseases. In the physiological state their concentrations in biological fluids and tissues are undetectable or very low. Therefore, any increase in their concentrations suggests activation of pathways involved in an inflammatory response or disease development. That is why cytokines may serve as potential biomarkers of various diseases, and changes of their concentrations may be used in follow-up. Moreover, the cytokine profile in the acute phase of the disease often differs from the chronic phase. Measurements of cytokine concentrations are sufficient to diagnose a disease and their concentrations correlate with the stage of the disease [12].

In our study, in acute exacerbated patients there was significant +ve correlation between FEV1 and both total IgE and IL-33 while there was no correlation between FEV1 and serum level of sST2 (Table 5). In stable asthmatic patients there was high significant + ve correlation between PEFR variability and serum level of sST2 while there was no correlation between

**Table 3**  Serum levels of IgE level in the studied groups.

<table>
<thead>
<tr>
<th>IgE (IU/ml)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
<th>KW</th>
<th>P1</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>X ± SD</td>
<td>324.7 ± 133.4</td>
<td>68.2 ± 47.3</td>
<td>20.6 ± 15.7</td>
<td>41.825</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>360</td>
<td>50.7</td>
<td>12.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>145–600</td>
<td>6.8–185.7</td>
<td>5.6–50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X = mean.
KW = Kruskal Wallis test (non parametric test).
P1: means probability of difference among the three groups.
P2: means probability of difference between group1 and group2.

**Table 4** Peripheral Blood eosinophil percentage in the studied groups.

<table>
<thead>
<tr>
<th>Eosinophil %</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Control</th>
<th>F</th>
<th>P1</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>X ± SD</td>
<td>16.5 ± 2.6</td>
<td>5.13 ± 2.3</td>
<td>1 ± 0.9</td>
<td>354.129</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Range</td>
<td>(10–21)</td>
<td>(2–10)</td>
<td>(0–3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1: means probability of difference among the three groups.
P2: means probability of difference between group1 and group2.
Serum levels of Interleukin-33 and its soluble receptor ST2

Table 5  Correlation between FEV1 and total IgE, IL-33 and sST2 in group 1 patients.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgE</td>
<td>-0.427</td>
<td>0.113</td>
</tr>
<tr>
<td>IL-33</td>
<td>-0.776</td>
<td>0.001</td>
</tr>
<tr>
<td>sST2</td>
<td>0.256</td>
<td>0.356</td>
</tr>
</tbody>
</table>

Table 6  Correlation between PEFR variability and total IgE, IL-33 and sST2 in group 2 patients.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>0.366</td>
<td>0.179</td>
</tr>
<tr>
<td>IL-33</td>
<td>0.179</td>
<td>0.522</td>
</tr>
<tr>
<td>sST2</td>
<td>0.524</td>
<td>0.045†</td>
</tr>
</tbody>
</table>

† High significant result statistically.

PEFR variability and serum levels of either IgE or IL-33 (Table 6).

IL-33 is increased in Airway smooth muscle and epithelial cells from asthmatics and this increase positively correlates with asthma severity [13]. Soluble ST2 is decoy receptor that is elevated in the serum of asthma patients, soluble ST2 association with IL-33, blocks ST2L-dependent signaling and the immunological effect of IL33 [13]. Previous studies reported that serum ST2 protein levels increased in patients with acute exacerbation of atopic asthma which is a characteristic of Th2-mediated eosinophilic airway inflammation [14].

Expression of IL-33 was found in higher levels in endotracheal biopsies from human asthmatic subjects compared to controls. IL-33 expression was particularly evident in those with severe asthma and the expression was mainly located in bronchial epithelial cells [15].

There are many data suggesting that IL-33 is involved in lung inflammation and support the concept of ST2 as a therapeutic target in asthma. Endobronchial biopsies from adults with mild, moderate, and severe asthma were obtained. Airway smooth muscle cells (ASMC) from asthmatic samples, regardless of severity of disease, expressed increased IL-33 mRNA levels compared with controls. IL-33 protein was predominantly expressed by ASMC and epithelial and endothelial cells in asthmatic lungs but was absent in control samples. Thus, IL-33 is expressed by ASMC in asthmatic lungs and shows promise as a potential inflammatory marker for asthma [16].

Soluble ST2 binds to IL-33 and functions as a decoy receptor of IL-33. Pretreatment with soluble ST2 suppressed IL-33 induced NF-kB activity and IL-4, IL-5 and IL-13 expression [17]. Soluble ST2 has been implicated as an anti-inflammatory mediator in inflammatory responses. Pretreatment with recombinant sST2 protein attenuates expression of TNF, IL-6 and IL-12 in macrophages [18]. ST2/IL-33 interactions on mast cells may serve not only to promote maturation and activation, but also to maintain their localization within the tissue [19].

Conclusion

The serum levels of IL-33 and its receptor sST2 were markedly elevated in patients with bronchial asthma and this supports the concept of sST2 and Interleukin-33 as a therapeutic target in asthma.

References


