THE ROLE OF INTERLEUKIN-1ß AND INTERLEUKIN-33 IN ATOPIC DERMATITIS

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Abstract

Introduction: Interleukin-1 super family is a group of cytokines that play a role in the regulation of immune and inflammatory responses. Interleukin-33 is a member of this family and is known to induce expression of the T helper2 cytokines that are important players in atopic dermatitis.

Aim: To evaluate the expression of interleukins-1ß and 33 as T helper2 cytokines inducers in patients with atopic dermatitis.

Materials and Methods: This study included 20 atopic patients and 20 apparently healthy individuals serving as controls. Skin biopsies from all participants will be examined for detection of interleukins-1ß and 33 by ELISA technique.

Results: Both interleukins were statistically higher (P<0.001) in patients than in controls. A statistically significant (P=0.011) highest levels of interleukin-33 was detected in severe cases of atopics when compared to mild and moderate cases. A significant correlation (r=0.632, P=0.003) between both interleukins was detected in atopics.

Conclusions: This is the first study to evaluate both interleukin-1ß and 33 together in atopic patients. Both interleukins could play a role in the recruitment of lymphocytes during the inflammatory reaction in atopic dermatitis and could be targeted in the treatment of resistant cases.

Key words: atopic dermatitis; Interleukin-1ß; Interleukin-33

Introduction

Atopic dermatitis (AD) is a chronic relapsing skin disease, often occurring within the first year of life and affecting up to 20% of children, the majority of whom outgrow the disease within few years. Despite the occurrence of late-onset AD in some adults, the prevalence of AD in the adult population has been estimated to be much lower (2–9%) [1].

Atopic diseases are characterized by IgE sensitization to environmental allergens. The gene-environment interactions leading to the development of AD are only partially understood [2]. Traditionally, two competing hypothesis are presented to explain the pathogenesis of AD. The inside-out hypothesis suggests that an immunological defect predisposes to atopy and that this IgE-mediated sensitization will result in AD, whereas the outside-in hypothesis proposes that disruption of the skin barrier, either resulting from an intrinsic genetic defect in epidermal skin barrier formation or as a result of an environmental alteration, would lead to sensitization and atopic disease [3].

Interleukin (IL) -1 super family is a group of cytokines that play a central role in the regulation of immune and inflammatory responses to infection and tissue injury. IL-1ß is the best-studied cytokine of the 11 members of this family. On exposure to pathological stimuli, IL-1ß is produced by activated leukocytes, leading to induction and enhancement of inflammatory responses [4].

Interleukin-33 is also a member of the IL-1 family of cytokines [5] and is known to induce expression of the helper (Th)2 cytokines IL-5 and IL-13 in vitro, as well, increased blood eosinophils and serum immunoglobulins when delivered in vivo [6]. IL-33 can also induce the activation and maturation of human mast cells [7,8].

Th2 cells are important players in cutaneous allergic inflammation, an integral part of AD [3]. Th2 cytokines can be secreted by basophils, eosinophils and mast cells. Basophils have recently been detected in AD skin lesions [9] and eosinophils recruitment into the skin is characteristic of AD [3].
In humans, IL-33 mRNA levels are induced almost 10-fold in the skin of AD patients compared to healthy skin [8]. However the role of IL-33 in AD remains to be evaluated. Our aim is to evaluate the expression of IL-1ß and IL-33 as Th2 cytokines inducers in the patients with AD.

**Methods**

This study included 20 atopic patients (Group 1) and 20 apparently healthy individuals serving as controls (Group 2). Participants were selected from the outpatient clinic of the Dermatology Department, Faculty of Medicine, Cairo University. A written informed consent was obtained from all participants before initiation of the study.

Atopic patients (Group 1) included twelve males and eight females; each patient was subjected to full history to detect any history of other atopic manifestations like atopic asthma, and the grade of atopic dermatitis severity was evaluated according to Rajka and Langeland [10] as follows; Mild (score:3-4), Moderate (score:4.5-7.5) and Severe (score:8-9). Parameters for scoring the grade of severity included: extent of involvement (as determined by the „rule of 9”, as used for burn area), course of the disease and intensity of disease, as determined by itch (considered by the authors to be the basic trait for AD) (Tabl. I).

**Assessment of IL-1ß and IL-33:**

A portion of the lesional skin tissue was homogenized for testing in IL-33 and IL-1ß ELISA. Homogenization was performed in homogenate buffer [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 1 mM DTT, and 0.5 mM phenylmethanesulfonyl fluoride] using a vertishear tissue homogenizer. Tissue homogenates were centrifuged at 3,000 g for 15 min at 4°C. The supernatants were subsequently stored at –80°C until the ELISA could be performed for IL-33 and IL-1ß.

The ELISA technique was performed by adding 100 µl of each sample to wells in a 96-well plate of a commercially available human ELISA kit (R&D system quantakine USA). The samples were tested in duplicate. The ELISA was performed according to the manufacturer’s instructions and final results were expressed as picograms per milliliter.

**Statistical analysis:**

Data was coded and entered on computer for analysis. A spread sheet on EXCEL was developed for data entry. Data was then transferred to SPSS version 17 for analysis. Simple frequencies were used for data checking. Descriptive statistics (arithmetic mean and standard deviation) were used for summary of quantitative data, while percentages were used for qualitative data. Appropriate statistical tests of significance were used to test the null hypothesis in comparison of the studied groups. A P value < 0.05 was used for detection of statistical significance in all tests.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Finding</th>
<th>Points</th>
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<tbody>
<tr>
<td>extent</td>
<td>&lt; 9% of body area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>skin involved more than score 1 and less than score 3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt; 36% of body area involved</td>
<td>3</td>
</tr>
<tr>
<td>course</td>
<td>&gt; 3 months of remission during the year</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&lt; 3 months remission during the year</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>continuous course</td>
<td>3</td>
</tr>
<tr>
<td>intensity</td>
<td>mild itch, only exceptionally disturbing night's sleep</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>itch, evaluated to be more than score 1, less than score 3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe itch, usually disturbing night's sleep</td>
<td>3</td>
</tr>
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</table>

Table I. Parameters for scoring of atopic dermatitis severity

**Results**

This study included 20 atopic patients; they included 12 males and eight females aged between 10-46 years (mean age 26.75 years). All patients had never received treatment or had stopped therapy six months beforehand. Twenty healthy subjects were included as controls; they included eleven males and nine females aged between 13-45 years (mean age 27.73 years).

Regarding the atopic patients; personal history of other atopic manifestations like asthma were detected in six patients (30%). Family history was positive in six patients’ (30%). Grading of disease severity in patients with AD was performed and accordingly, atopic patients were divided into three groups:

- Group of mild cases (score 3-4) included eight patients (40%), four males and four females.
- Group of moderate cases (score 4.5-7.5) included five patients (25%), all of them were males.
- Group of severe cases (score 8-9) included seven patients (35%), three males and four females.

Interleukin-1ß was detected in all atopic patients in the range of 14.5 to 38.2 pg/ml with a mean of 25.9±6.66 pg/ml. IL-33 was also detected in all atopic patients in the range of 194.5 to 516.3 pg/ml with a mean of 292.5±89.68 pg/ml. Regarding to healthy individuals (Group 2); IL-1ß was detected in the range of 10.2 to 22.3 pg/ml with a mean of 14.75±4.05 pg/ml. IL-33 was also detected in the range of 102.4 to 216.7 pg/ml with a mean of 151.71±38.63 pg/ml.

Statistical analysis revealed a significant difference (P<0.001) between the two studied groups regarding the mean value of both IL-1ß and IL-33 (Tabl. II).
In atopic patients; statistical analysis revealed no significant difference in the mean value of both IL-1ß and IL-33 between the females and males patients (P=0.168 and 0.735 consecutively). Also, there was no statistically significant difference regarding the mean value of both IL1B and IL33 in patients with positive history of asthma and those with negative history (P=0.249 and 0.172 consecutively). When comparing the mean value of IL-1ß with the severity of atopy in patients group, our results revealed no statistically significant difference (P=0.050) between mild, moderate and severe affection. When comparing the mean value of IL-33 with the severity of atopy in patients group, our results revealed statistically significant difference (P=0.011) between mild, moderate and severe affection with the highest value detected in severe cases (Tabl. III).

Our results revealed no statistically significant correlation between the levels of both IL-1ß and IL-33 in atopics with the age of the patients (P=0.219 and 0.157 consecutively). However there was a statistically significant correlation between both IL-1ß and IL-33 in atopics (r=0.632, P=0.003).

**Table II. IL-1ß and IL-33 levels in atopic patients and healthy individuals**

<table>
<thead>
<tr>
<th></th>
<th>IL-1ß (pg/ml)</th>
<th>IL-33 (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Group1 (n*=20)</td>
<td>25.9</td>
<td>6.66</td>
</tr>
<tr>
<td>Group2 (n=20)</td>
<td>14.75</td>
<td>4.05</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001‡</td>
<td>&lt;0.001‡</td>
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</table>

**Table III. IL-1ß and IL-33 levels in different clinical severity of atopic patients**

<table>
<thead>
<tr>
<th>Grade of severity (score)</th>
<th>IL-1ß (pg/ml)</th>
<th>IL-33 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD*</td>
</tr>
<tr>
<td>Mild (n†=8)</td>
<td>23.17</td>
<td>6.577</td>
</tr>
<tr>
<td>Moderate (n=5)</td>
<td>23.5</td>
<td>4.986</td>
</tr>
<tr>
<td>Severe (n=7)</td>
<td>30.73</td>
<td>5.633</td>
</tr>
<tr>
<td>P value</td>
<td>0.050</td>
<td>0.011‡</td>
</tr>
</tbody>
</table>

**Discussion**

In this study, both IL-1ß and IL-33 are significantly highly expressed in atopics. This could explain a role of both of them in the pathogenesis of atopy and in the trafficking of leucocytes during the inflammation caused by AD. In this study both IL-1ß and IL-33 were statistically higher (P<0.001) in patients than in healthy individuals. IL-1ß is responsible of the acute phase responses to inflammation [4] and this can explain its role as initiator of inflammation in AD. IL-33 is one the IL-1 family and has been described to function as an alarmin. It’s expressed in tissues with predominant barrier function, and released upon cell death with activation of several components of the immune system [8].

IL-33 is expressed by human keratinocytes following any insult to the skin causing cell damage of any kind. Dermal immune cells, such as mast cells which express the IL-33 receptor are stimulated by IL-33 and become activated. Mast cells produce cytokines and chemokines which recruit other immune cells, and in the appropriate context inflammation is maintained and lesions develop. IL-33 has been shown to induce the generation of Th2 cytokines producing innate immune cell populations [11]. The IL-33 receptor, consisting of ST2 and IL-1 receptor accessory protein, is also widely expressed, particularly by Th2 cells and mast cells. IL-33 is host-protective against helminth infection by promoting Th2-type immune responses. IL-33 can also promote the pathogenesis of asthma by expanding Th2 cells and mediate joint inflammation, AD and anaphylaxis by mast cell activation. Thus IL-33 could be a new target for therapeutic intervention across a range of diseases [12].

Our results were in accordance with Hakonarson et al. [13] who detected enhanced mRNA expression and release of IL-1beta protein in patients with allergic asthma. Our findings were also in agreement with Pushparaj et al. [8] who showed that IL-33 is upregulated in AD patients. Matsuda et al. [14] also found IL-33 expression in chronic allergic conjunctivitis but not in the control conjunctivae and that IL-1ß stimulation upregulated IL-33 mRNA expression in conjunctival fibroblasts. The authors also confirmed mature IL-33 protein expression in ocular resident cells by Western blot analysis.

In this study, IL-33 was statistically highly detected in severe cases of AD. This could explain its role in the progression of the disease.
A significant correlation between both IL-1ß and IL-33 was detected in atopics in the current study. This could explain the interrelation of both cytokines to initiate the inflammation in AD. To the best of our knowledge, no previous studies examined these two interleukins together in AD. As a conclusion, IL-1ß and IL-33 could play a role in the recruitment of lymphocytes during the inflammatory reaction in AD and could be targeted in the treatment of resistant cases; however this finding should be confirmed by further studies with large scales of patients.

REFERENCES