Original paper

Evaluation of interleukin 33 (IL-33) levels in the course of bronchial asthma (preliminary studies)

Ocena stężeń interleukiny 33 (IL-33) w przebiegu astmy oskrzelowej (badania wstępne)

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Medical Studies/Studia Medyczne 2023; 39 (1): 26–31
DOI: https://doi.org/10.5114/ms.2023.126287

Key words: cytokines, pro-inflammatory cytokines, interleukin-33, prognosis in asthma, allergic diseases.

Słowa kluczowe: cytokiny, cytokiny prozapalne, interleukina 33, rokowanie w astmie, choroby alergiczne.

Abstract

Introduction: Research in the field of immunology has shown a significant role of cytokines in the development of asthma, applying in particular to pro-inflammatory cytokines from Th2 lymphocytes. Interleukin 33 (IL-33) belongs to the interleukin 1 family (IL-1). It intensifies the production of cytokines by these cells. Its action induces the production of very large amounts of such cytokines as IL-4, IL-5, and IL-13, which are important in the development of allergic diseases.
Aim of the research: An assessment of whether IL-33 levels depend on the severity of bronchial asthma in the course of the disease
Material and methods: The study involved 80 people: 20 volunteers and 60 patients with asthma – 20 participants with mild chronic asthma, 20 with moderate, and 20 with severe asthma. All patients were subject to maintained asthma control. From each patient, blood was collected for the evaluation of IL-33 with the ELISA test; all patients had additional spirometry performed (as part of their visits to the clinic).
Results: Elevated levels of IL-33 were found in 17 patients from the examined group of 60 people. Some volunteers, i.e. 10 out of 20, also demonstrated increased concentration of this interleukin.
Conclusions: In our study, no specific answers have been obtained yet. The reason for the increased concentration of this cytokine in the group of volunteers is unclear. According to the medical history, these participants did not suffer from any diseases, including allergy-based. However, taking into account the prevalence of atopy in the general population (45%), it may have been people with this feature.

Streszczenie

Wprowadzenie: Badania z zakresu immunologii wykazały istotną rolę cytokin w rozwoju astmy, ze szczególnym uwzględnieniem cytokin prozapalnych z limfocytów Th2. Interleukina 33 (IL-33) należy do rodziny interleukin 1 (IL-1). Intensyfikuje produkcję cytokin przez te komórki. Jej działanie indukuje produkcję bardzo dużych ilości takich cytokin, jak IL-4, IL-5 i IL-13, które są ważne w rozwoju chorób alergicznych.
Cel pracy: Ocena, czy stężenie IL-33 zależy od ciężkości astmy oskrzelowej w przebiegu choroby.
Materiał i metody: W badaniu wzięło udział 80 osób: 20 ochotników i 60 chorych na astmę – 20 uczestników z astmą przewlekłą łagodną, 20 z astmą umiarkowaną i 20 z astmą ciężką. U wszystkich chorych utrzymywano kontrolę astmy. Od każdego pacjenta pobrano krew do oceny IL-33 testem Elisa; u wszystkich pacjentów wykonano dodatkową spirometrię (w ramach wizyt w poradni).

Wyniki: Podwyższony poziom IL-33 stwierdzono u 17 pacjentów z badanej grupy liczącej 60 osób. U niektórych ochotników, tj. 10 spośród 20, również wystąpiło zwiększone stężenie tej interleukiny.
Wnioski: W naszym badaniu nie uzyskano jeszcze konkretnych odpowiedzi. Przyczyna zwiększonego stężenia tej cytokiny w grupie ochotników wydaje się niejasna. Z wywiadu wynika, że uczestnicy nie cierpieli na żadne choroby, w tym na te o podłożu alergicznym. Ze względu na rozpowszechnienie atopii w populacji ogólnej (ok. 45%) mogły to być osoby z tą cechą.

Introduction

Asthma is a chronic inflammatory disease of the respiratory tract, which affects 1–18% of people, depending on the country, with no correlation between morbidity and mortality in its course [1]. Most therapeutic problems are related to the so-called difficult-to-treat asthma. It is currently estimated that up to 10% of patients with asthma do not react to standard treatment [2]. It is these patients who are exposed to the greatest risk of death, and it is they who should be the focus of innovative diagnostics and treatment [3]. Allergic asthma is a chronic inflammatory disease of the respiratory tract, which involves numerous cells, such as lymphocytes, eosinophils, mast cells, and also neutrophils in severe asthma. With the progress of immunology we know that the substances released by these cells are also extremely significant in the development of asthma, especially cytokines derived from Th2 lymphocytes [1]. Also important are the mediators released by mast cells, which among other things cause bronchospasm. On the other hand, the influence of eosinophilia (acidic granulocytes) on the development of asthma takes place, among others, through the release of eosinophilic cationic protein (ECP), which damages the epithelium of the respiratory tract [2, 3].

The diagnosis and treatment process should therefore focus on these cells and the substances they release. Individual cytokines, which most often have the nature of glycoproteins, have a highly diverse function in the development or suppression of allergic inflammation, but also participate in other vital functions of cells. These include both inflammatory and immunological reactions and the regulation of haematopoietic secretion [4–6]. Most often they show a pleiotropic mechanism of action (the ability to interact with many cells and cause different effects) and redundancy (when different cytokines cause the same effect). They can also act synergistically or antagonistically towards each other and induce positive and negative feedback cascades. Cytokines exert their effects only due to the presence of receptors specific to them on the target cells. They usually convey information between cells at very short distances [5, 6]. Interleukin 33 (IL-33) belongs to the interleukin 1 (IL-1) family. It is subject to expression in many cells and tissues, mainly in epithelium and endothelium cells. It is a cytokine that performs 2 functions: as a traditional cytokine and as a transcription regulator. It intensifies the production of cytokines by Th2 lymphocytes [7]. Its action induces the production of very large amounts of such cytokines as IL-4, IL-5, and IL-13, which are important in the development of allergic diseases, including allergic asthma. However, its importance in the pathogenesis of allergic respiratory tract diseases is not fully explained, as IL-33 may show pro-inflammatory or anti-inflammatory effects, depending on the type of activated cells, the microenvironment, and the presence of co-stimulant factors. It is known that the cytokines it releases stimulate eosinophils and the production of IgE class antibodies associated with the development of allergies. It is also recognised to be one of the cytokines controlling type 2 immune response. Its role seems to be significant in controlling the initiation and severity of the Th2 response, which takes place through the above-mentioned cytokines. And we know that the advantage of Th2 over Th1 is characteristic for allergic diseases [7]. Therefore, it stimulates the immune response mediated by Th2 lymphocytes and thus seems to facilitate the development of inflammatory diseases, such as asthma or other allergic diseases. Knowing the action mechanism of this cytokine may be important in the new therapeutic approach to asthma treatment.

Aim of the research

The aim of the study was to assess whether the concentration level of IL-33 depends of the severity of asthma during the course of the disease.

Material and methods

A sample of venous blood (from the elbow vein) was taken from 80 people: 20 volunteers and 60 patients with asthma. Among the patients with asthma, 20 patients had mild, 20 had moderate, and 20 had severe chronic asthma. Blood samples were placed in test tubes with separating gel – not containing anticoagulant, which was left for 30 min at room temperature. After the coagulation process was completed, the samples were centrifuged for 20 min at 2500 revolutions per minute (1000 g), then the serum was separated into 3 tubes and stored at –70°C until the appropriate number of patients was collected. The concentration of interleukin 33 was determined by ELISA (enzyme linked immunosorbent assay) using the SEB 694Hu reagent kit. The measuring range of the set was from 15.6 to 1000 ng/ml. After preliminary dilution of 10,000 pg/ml, the following standards were obtained: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, and 15.6 pg/ml. The kit uses a plate with 96 openings coated with antibodies specific for IL-33 conjugated with biotin. After adding the standards

Table 1. Classification of asthma severity based on clinical presentation according to GINA

Clinical symptoms	Mild chronic asthma	Moderate chronic asthma	Severe chronic asthma
Day-time symptoms	More than once a week, less than once a day	Occur every day	Occur every day
Night-time symptoms	More than twice a month	More than once a week	Frequent
Escalation	May disrupt sleep and interfere with daily activity	May disrupt sleep and interfere with daily activity	Frequent
FEV_1 or PEF	Above or equal to 80% of the value due (required value)	60–80% of the value due	Below or equal to 60% of the value due
Changeability of FEV_1 or PEF	< 20–30%	> 30%	> 30%
Other	–	Required inhalation of a short-acting β_2-mimetic on a daily basis	Reducing physical activity

FEV_1 – forced expiratory volume in the first second, PEF – peak expiratory flow.

Table 2. Disease control criteria according to GINA

Criterion	Day-time symptoms	Restricted life activity	Night-time symptoms, awakenings	Need for short-term treatment	Pulmonary function	Escalation
Controlled asthma	Do not occur (< or equal to 2 times a week)	Do not occur	Do not occur	Never (< or equal to 2 times a week)	Correct	Do not occur

Table 3. Gender distribution in different levels of asthma severity

Severity level	No. of women	No. of men
Mild chronic asthma	11	9
Moderate chronic asthma	16	4
Severe chronic asthma	12	8

and patients' serum, the plate was incubated for 2 h at 37°C. The contents were extracted and 100 µl of Reagent A was added, then the plate was incubated for 1 h at 37°C. After the openings were rinsed 3 times, 100 µl of Reagent B was added and incubated for another 30 min at 37°C. The contents were extracted and the plate was rinsed 5 times. Then, 90 µl of Substrate Solution (TMB) was added and the plate was incubated for 20 min at 37°C (the plate was protected from the light). Then, 50 µl of sulphuric acid solution (Stop Solution) was added to complete the reaction. The plate was measured in the ELISA reader at 450 nm wavelength with the option to automatically calculate the concentration. The concentration of IL-33 in the samples was determined by comparing the optical density of the tested sample with the standard curve. Out of 80 samples tested, a significant colour reaction was observed in 27 instances. Our study involved 80 people: 20 volunteers and 60 patients with asthma: 20 people with mild chronic asthma, 20 with moderate asthma, and 20 with severe asthma. Clinical symptoms according to GINA (Global Initiative for Asthma – global strategy for diagnosis, treatment, and prevention of asthma) were the criteria for being assigned to a given severity category (Table 1).

All patients were in the maintained disease control (Table 2).

In order for the patient to have controlled asthma, all the following criteria had to be met.

The group of patients consisted of 21 men and 39 women, and the age of patients ranged from 19 to 82 years. In the severe chronic asthma group, there were 8 men and 12 women; 4 men and 16 women with moderate chronic asthma, and 9 men and 11 women with mild asthma (Table 3).

Women prevailed in each asthma severity group. Belonging to a given group was assessed using the severity degree included in the GINA consensus. All study participants were patients of the allergology clinic. In each person, blood was taken for the IL-33 assessment with the Elis test; in addition, all patients received spirometry test (during visits to the outpatient clinic). The accepted normal range for IL-33 in the blood of healthy people is 0.6–180 ng/l. Chronic bronchial asthma was diagnosed during the disease control period. The exclusion criteria were age below 18 and above 82 years , as well as loss of disease control. The volunteers did not suffer from any chronic diseases, including allergic diseases. The laboratory

part of the study was carried out at the Świętokrzyskie Centrum Onkologii (Holy Cross Cancer Centre) in the Department of Tumour Markers. The study was a continuation of the authors' work on the immunological background of bronchial asthma and the possible influence of the concentration of some cytokines on the disease severity.

The study was approved by the Bioethics Committee.

Statistical analysis

The *t*-Student method for independent samples was used for the statistical analysis of the research results. Confidence intervals for IL-33 were determined at a significance level of $\alpha = 0.05$.

Results

Elevated IL-33 levels were found in 17 (28%) out of 60 patients from the examined group, whereas the percentage in the volunteer group was 50% (10 out of 20). Among the patients, 4 (about 7%) suffered from severe chronic asthma, 7 (about 12%) had moderate asthma, and 6 had mild asthma (10%). In all these 27 study participants, a significant colour reaction was observed, sufficient to detect the difference between a blank sample and the lowest standard on the calibration curve (Table 4).

Among the volunteers with a higher level of IL-33, it is noteworthy that, as found in the medical history, everyone was free from allergic diseases and other chronic disorders. Since all patients had controlled asthma, the spirometry results were normal – without obstruction traits.

To compare the measurable feature (parameter) of the 2 study groups, we used Student's *t*-test. We chose the independent samples test. This means that the study groups (samples) were independent of each other, i.e. the measurement results of one group were not dependent on the measurement of the other group. The parameter (measurable feature) that we compared was the value of the level of IL-33 in the group of people with asthma and the group of healthy people. Mean values of IL-33 levels in individual groups of subjects are presented in Table 1. The results of the measurements indicate a lower level of IL-33 in all patients with asthma (regardless of the severity of asthma) compared to healthy subjects. The lowest level of IL-33 was observed in the group of people with severe asthma. The highest level of IL-33 was found in the group of healthy people. At the significance level $\alpha = 0.05$, i.e. with a probability of 95%, it was found that the levels of IL-33 were as follows:

– in healthy people it ranged from 76 to 116, and its average value was 95.7 ng/l,
– people with asthma ranged from 52 to 75, and its average value was 63.9 ng/l;

The adopted null hypothesis assumed no difference in the level of IL-33 between the group of sick people and the group of healthy people. However, the conducted Student's *t*-test showed a statistically significant difference in the level of IL-33 in the group of all patients with asthma compared to the group of healthy people ($0.01 < p < 0.02$, i.e. with a probability between 99% and 98%). A similar statistically significant difference in the level of IL-33 was found between the group of patients with severe asthma and the group of healthy people, but at a lower level of $p < 0.05$, i.e. with a probability of 95%.

However, there was no statistically significant difference in the level of IL-33 between the group of patients with moderate asthma and the group of healthy people. There was also no statistically significant difference in the level of IL-33 between the groups of patients with different degrees of asthma severity (between the group of patients with mild and moderate asthma, severe and moderate asthma, and between moderate and mild asthma) (Table 5).

Table 4. Age distribution of patients

No. of people in total	Age			
	18–29	30–44	45–59	60–82
60	9 (15%)	18 (30%)	17 (28%)	16 (27%)

Discussion

Studies published so far suggest that IL-33 may activate eosinophils during allergic inflammation and be one of the key cytokines involved in Th2 response in asthma [8]. The biological significance of the IL-33

Table 5. Mean value of IL-33 level in groups of examined people

Groups of people	Average age [years]	Average value IL-33 [ng/l]	IL-33 value confidence interval for significance level $\alpha = 0.05$ [ng/l]
All asthma patients	48.6	63.9	52–75
Including: with severe asthma	52.8	53.4	33–73
Moderate	51.0	73.7	52–96
Mild	42.2	64.5	42–86
Healthy people	50.4	95.7	76–116

in the pathogenesis of allergic diseases and asthma has been confirmed using experimental models [8]. Moreover, it has been proven that the *in vitro* stimulation with interleukin 33 of several cell populations (mast cells, basophils, and eosinophils) obtained from allergy sufferers led to activation of these cells and increased secretion of inflammatory cytokines (IL-4, IL-5, IL-13) compared to healthy people's cells stimulated with IL-33 [8]. Interleukin takes part in the induction of inflammation in the respiratory tract, and, as we know, asthma is a chronic inflammatory disease of the airways [8]. Especially important is the action of this cytokine in the initiation control and the severity of the Th2 response; it does so through other cytokines: IL-4, IL-5, IL-13 [9–12], resulting in domination of the Th2 response. And as mentioned before, the dominance of Th2 over Th1 is characteristic of allergic illnesses [1, 11]. The above data suggest that the concentration of this interleukin should increase with the severity of the disease. However, due to pleiotropic effects of most cytokines, including IL-33, numerous studies fail to provide unambiguous results. Some authors also suggest that this cytokine amplifies not only Th2 but also Th1 response and that it is released from damaged cells, acting as alarmin [7, 12–14]. Different effects of this cytokine were observed, especially in the model of animal studies; in some experiments, increased secretion of cytokines associated with Th1 response, e.g. γ-interferon, was observed instead of the expected Th2 response [13]. Also in this study, specific results have not been obtained yet. As regards the number of research participants with asthma, they are as follows: severe asthma – 4 patients, moderate asthma – 7 patients, mild asthma – 6 patients. This group also included 10 volunteers. According to the medical history, the latter did not suffer from any diseases, including allergy-based. However, taking into account the prevalence of atopy in the general population (about 45%), it may have been people with this feature. The results we obtained cannot suggest that the IL-33 concentration is related to the severity of asthma, but for the time being they are only preliminary. Hopefully, conducting further studies will help to achieve more unambiguous data, especially since asthma is also associated with huge socio-economic costs both in Poland and world-wide [1]. The most expensive treatment, multi-drug therapy, applies to severe asthma, especially so-called difficult-to-treat asthma [2]. It is here that the use of a single therapeutic agent (e.g. antibodies) not only may reduce costs, but could also facilitate the treatment of individual patients and even make it possible to obtain and maintain control of this disease in some patients. Therefore, investigating this cytokine seems to be of great importance. Given its mechanism of action, including the release of Th2 cytokines and involvement in reconstruction of the airways in severe asthma, it seems that its concentration should not only correlate with the severity of asthma, but may also change the therapeutic approach to this disease [14–18].

Conclusions

As yet, our study failed to provide an unambiguous outcome. Therefore, it cannot be concluded that the concentration of this cytokine correlates with the asthma severity degree. In particular, the cause of increased concentration of this interleukin in the group of volunteers seems unclear. As found in their medical history, the volunteers did not suffer from any diseases, including allergy-based. However, taking into account the prevalence of atopy in the general population (about 45%), it may have been people with this feature. During the next study, volunteers should also be tested for the presence of possible asymptomatic allergies, using at least such a simple tool as skin prick tests. Taking into account the authors' previous research, it may also be worth using a diagnostic tool with greater specificity than the ELISA test, which is in fact only a screening test. That is why we consider our research as preliminary to further understanding of the cytokine action in people with bronchial asthma.

Conflict of interest

The authors declare no conflict of interest.

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