Specific allergen induces Fas (CD95) and FasL (CD95L) expression on peripheral blood mononuclear cells from allergic subjects

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Abstract

Apoptosis, a programmed cell death results from interaction between surface molecule Fas and its ligand FasL. Fas expression is increased on activated T cells. FasL is upregulated following TCR stimulation.

The aim of our study was to investigate Fas and FasL expression following stimulation of PBMC with specific allergen.

17 allergic subjects, sensitive to grass pollen and 15 healthy controls were included in the study. PBMC were isolated by density gradient centrifugation and incubated with various concentrations (1, 10, 100 ng/ml) of allergen for 18 h at 4°C. Standard immunofluorescence test was performed using rabbit IgG against human Fas and FasL.

The expression of both Fas and FasL after stimulation with allergen was significantly increased in allergic subjects whereas in healthy ones incubation with allergen did not affect the expression of these molecules.

Our results indicate that specific allergen stimulation of lymphocytes from allergic subjects might induce Fas-FasL apoptotic pathway, which might result in deletion of specific cells. This might be a possible mechanism of allergen tolerance achieved naturally or following specific immunotherapy.

Key words: *apoptosis, Fas-FasL pathway, specific allergen, pollinosis, PBMC*

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Introduction

Apoptosis is an essential regulator of the size of population of immunocompetent cells. This term, introduced by Kerr et al. in 1972 [1], designates physiological, programmed, active process of self elimination of cells without induction of an inflammatory reaction. The authors call attention to the role of apoptosis not only in embryogenesis but also in physiology and pathology of adult organisms. Throughout the whole life, apoptosis eliminates an excess of cells as well as obsolete and potentially dangerous cells [2]. It was estimated that in an average adult 2 x 10^{12} cells die every day [3].

Apoptosis is induced by various endo and exogenous factors and in case of T lymphocytes also by a signal from antigen receptor - TCR. Stimulation of this receptor in dependence on concentration, time and frequency of interactions, functional status of a cell, participation of antigen presenting and costimulating molecules may lead to proliferation or apoptosis [4]. Elimination of activated T lymphocytes by apoptosis is designated as activation-induced cell death [5]. Apoptosis may be induced by antigen-specific receptor both in central (thymus, bone marrow) and peripheral lymphoid organs. Elimination of these lymphocytes on periphery enables induction of tolerance and also limiting an immunological response [5, 6].

The main mediator of apoptosis is the system of surface molecules Fas – Fas ligand expressed after activation of cells. Activated lymphocytes may have simultaneous expression of Fas and its ligand FasL or these molecules may be

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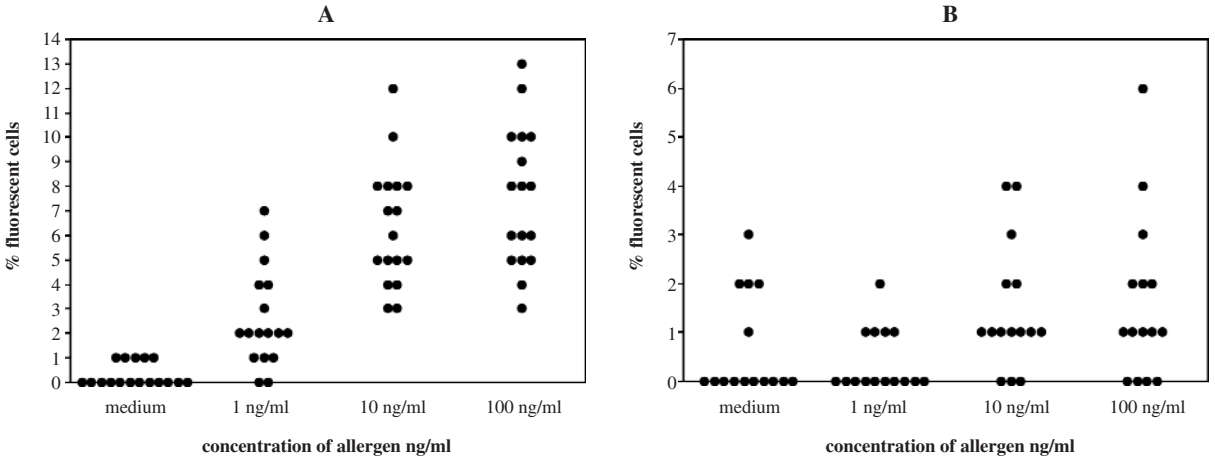

Fig. 1. Expression of Fas on PBMC in response to mixed grass pollen allergen in pollinotic (A) and healthy (B) subjects

expressed seperately. As a result of interaction between them, the cell with the expression of Fas undergoes apoptosis.

The aim of our work was the investigation of influence of specific allergens on the expression of Fas and FasL on cells of patients sensitive to them and healthy subjects.

Material and methods

The study was performed on cells of 15 nonatopic control subjects (7 females, 8 males) aged 25.2±5.5 years and 17 pollinotic patients (7 females, 10 males) aged 24.8±4.6 years. Skin prick test with grass pollen allergen was negative in control and positive in atopic subjects. Skin and cellular tests were performed with mixed grass pollen allergen (Agrostis tenuis, Apera spica venti, Cynosurus cristatus, Festuca pratensis, Holcus lanatus, Lolium perenne, Pheum pratense; Biomed, Cracow, Poland). During investigation, the patients did not take any medication.

Peripheral blood mononuclear cells (PBMC) were isolated by Gradisol L gradient centrifugation [7]. The viability of cells determined by Trypan blue exclusion was >98%. Isolated cells were suspended in PBS containing 1.5% human serum of AB group and gentamicin (40 µg/ml, Polfa, Poland). Suspension of 2 x 10^6 cells was incubated for 18 h at 4°C with mixed grass pollen allergen in concentrations 1, 10, 100 ng/ml or with medium alone as a control. Incubation parameters were selected on basis of preliminary experiments. After incubation, the cells were washed 3 times in PBS and immunofluorescence test was performed according to the protocol of Santa Cruz Biotechnology, USA, the manufacturer of rabbit affinity purified polyclonal IgG against Fas and FasL of human origin. Fluorescein-conjugated swine anti rabbit immunoglobulins (DAKO A/S, Denmark) was used as secondary antibody. The percentage of positive cells was determined under fluorescence microscope Olympus BX-50.

Statistical analysis within the groups was performed using Student t Test. Between-group differences were compared with the 2-tailed Mann-Whitney U Test. P values less than 0.05 were considered significant.

Results

Figure 1 shows the influence of mixed grass pollen allergen on the expression of Fas on PBMC of healthy subjects and pollinotics. Fas was expressed on 0.66±1.0% cells from healthy subjects incubated in medium alone and 0.29±0.4% cells from pollinotics – difference statistically nonsignificant.

Expression of Fas on PBMC of healthy subjects incubated with mixed grass pollen allergens in concentrations 1, 10, 100 ng/ml was respectively 0.38±0.6%, 1.53±1.3%, 1.6±1.62% and did not differ significantly from that in control.

Following incubation of PBMC of atopic subjects with specific allergens, concentration- dependent, significant increase in Fas expression was found, in comparison to medium control. Specific allergen caused the increase in Fas expression to 2.58±1.9% at the concentration 1 ng/ml ($p<0.001$), to 6.35±2.39% at 10 ng/ml ($p<0.001$) and to 7.52±2.76 at 100 ng/ml ($p<0.001$). The differences between groups of pollinotic and healthy subjects (Fig. 1. A, B) were statistically significant in all concentrations of allergen (1 ng – $p<0.001$, 10 ng – $p<0.000004$ and 100 ng – $p<0.000006$).

The expression of ligand FasL on PBMC of both groups is shown on Fig. 2. No significant differences in the expression of FasL on PBMC of healthy subjects after incubation with grass pollen allergen were found, as compared to the control value 0.4±0.6%. At the concentration of allergen 1 ng/ml, FasL was expressed on 0.46±0.7% of cells, at 10 ng/ml on 0.92±1.2% and at 100 ng/ml on 1.2±1.0%.

Specific allergen caused the significant increase in the expression of FasL on PBMC of pollinotics at the

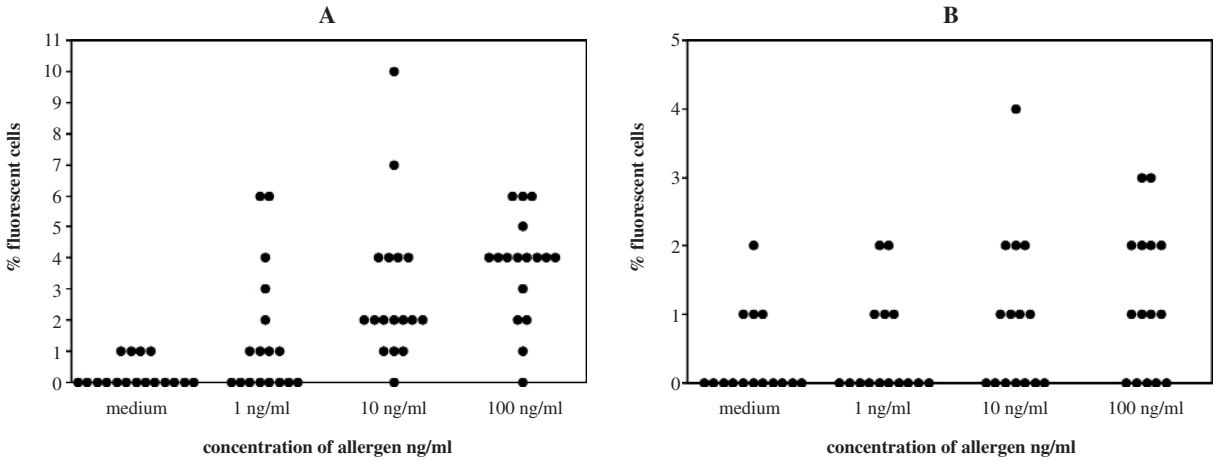

Fig. 2. Expression of FasL on PBMC in response to mixed grass pollen allergen in pollinotic (A) and healthy (B) subjects

concentrations 10 ng/ml – 2.94±2.38% ($p<0.01$) and 100 ng/ml – 3.7±1.63% ($p<0.01$), in comparison to medium control – 0.23±0.42%. Between-group comparison showed, similar to Fas, significant differences: for 1 ng/ml – $p<0.001$, for 10 ng – $p<0.000004$ and for 100 ng – $p<0.000006$.

Discussion

In the investigations presented above, the expression of Fas and FasL was observed on insignificant percentage of PBMC, not different between the groups. This observation corresponds with the data indicating the low expression of Fas on resting lymphocytes and the lack of expression on naive peripheral ones. It is also known that FasL is almost exclusively present on activated T lymphocytes [4].

It was found that the exposure of PBMC to the specific allergen caused concentration-dependent, significantly increased expression of Fas and FasL in atopic subjects only. One may assume that as a result of recognition of specific allergen and subsequent expression of surface proteins Fas and FasL, interaction between them may lead to apoptosis [8] – the phenomenon enabling limiting immunological response to an allergen. It is known from the studies of Zhang et al. that in the induction of peripheral tolerance being a result of activation-induced apoptosis, the essential role play T lymphocytes. However, as a result of the process, simultaneously appeared on periphery the population of antigen-specific cells resistant to apoptosis, characterized by high level of synthesis of cytokines of Th2 type [9].

Elimination of peripheral activated, Fas+ lymphocytes through the pathway of apoptosis can be induced by antigen presenting cells that express FasL [10].

Krug et al. have found in atopic subjects a marked expression of Fas on peripheral T lymphocytes and those from bronchoalveolar lavage fluid (BALF) and negligible expression of FasL. As a result of segmental allergen challenge in these patients, significantly increased the expression of FasL in BALF, remaining unchanged those on peripheral cells [11].

Culturing peripheral blood non-adherent mononuclear cells (MNC) after 24-72 h enhanced spontaneous apoptosis of cells from asymptomatic, pollen-sensitive subjects as compared to the controls and during pollen season [12]. Increased spontaneous apoptosis of peripheral MNC was also found in atopic asthmatic patients. Intensity of the process correlated with serum concentration of apoptotic markers (TNFα, ICE/caspase-1). It was also found that the concentration of soluble Fas in these patients was significantly lower than in control and negatively correlated with the enhancement of apoptosis [13].

The main mediator of apoptosis of lymphocytes is the system Fas – FasL. Dysfunction of the system leads to disturbances of immunological regulation observed in many diseases.

FasL is expressed on activated lymphocytes only. Physiologically, constitutive FasL is expressed on the cells of immune privileged sites (eye, testes or brain). This phenomenon enables elimination through apoptosis Fas+ cells infiltrating these sites. Expression of FasL was also detected in numerous malignancies that leads, in consequence, to apoptosis of cytotoxic lymphocytes infiltrating tumours. Such a situation causes status similar to immunological privilege, preventing an intervention of immunocompetent cells [14, 15].

In conclusion, the observed increase in expression of Fas and FasL on PBMC after exposure to specific allergen may participate in the mechanism defending an organism from excessive immune stimulation under the conditions of natural exposure to an allergen and during specific immunotherapy.

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