

INFLUENCE OF HYPEROSMOTIC CONDITIONS ON BASOPHIL CD203C UPREGULATION IN PATIENTS WITH FOOD-DEPENDENT EXERCISE-INDUCED ANAPHYLAXIS

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Abstract: Increased *in vitro* basophil activation in buffers of slightly elevated osmolarity in 2 cases of food-dependent exercise-induced anaphylaxis is presented. These data may suggest the importance of basophil activation degree in pathogenesis of this life threatening syndrome.

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INTRODUCTION

Food-dependent exercise-induced anaphylaxis (FDEIA), first described in 1979 by Maulitz *et al.* [19], is defined as anaphylaxis after food allergen intake followed by vigorous physical exercise. Neither the food allergen nor exercise alone can trigger anaphylactic reaction. The reaction is IgE mediated with mast-cell degranulation [21, 27], but the mechanism by which physical exercise provokes these reactions is not fully understood. Some attempts were made to solve this issue. After food-exercise challenge, the parasympathetic stimulation is increased, so that the sympathetic responsiveness is reduced. Therefore, FDEIA may occur in relation to allergic reactions and decreased activity of the autonomic nervous system [12]. Another important factor predisposing to FDEIA is exercise-induced increased intestinal permeability, which can result in increased allergen absorption [18, 22, 25]. Moreover, the same effects are observed after aspirin intake [1, 13, 14]. In some patients with FDEIA, ingestion of food allergen and aspirin alone

provoked clinical symptoms [1]. These subjects tolerated well aspirin challenge without exercise, therefore the occurrence of clinical symptoms should be recognized as the summarized action of aspirin and allergic reaction to food allergens [1]. Consequently, aspirin is currently regarded as a risk factor for FDEIA. Another suspected factor is the decrease in blood pH during exercise, because bicarbonate pretreatment has an inhibitory effect [16]. In 1986, Banks *et al.* found that hyperosmolar buffers increased the *in vitro* activation of basophils from subjects with food allergy [3]. It is known that physical exercise results FDEIA patients in increased plasma osmolarity [17]. Consequently, in our previous study we demonstrated that in the hyperosmolar buffers histamine release from basophils is significantly increased, comparing to those in food allergy patients and healthy donors. This encouraged the hypothesis that individually increased basophil reactivity might be important in the pathology of FDEIA [4].

Basophil activation might be expressed either as a value of mediators' release (histamine or leukotriene C4) or by

determination of CD63 and CD203c expression on its surface. Many studies have shown the diagnostic usefulness of upregulation of these molecules in diagnosing allergy to a wide range of allergens [5, 6, 8, 9, 24]. These new methods are simpler and less time-consuming than older procedures such as histamine or leukotriene C4 release tests. In this study, the attempt is presented of observation whether basophils obtained from the patients with FDEIA are able to increase CD203c expression in a hyperosmotic condition, in comparison to healthy controls and patients with food allergy urticaria.

CASE DESCRIPTION

The patients were diagnosed as having FDEIA according to widely accepted clinical criteria [7, 23]. Both subjects experienced 2 or more episodes of severe hypotension or syncope and/or upper respiratory tract obstruction with flushing or general urticaria in association with postprandial exercise. The first, a 25-year-old female student with bronchial asthma pollinosis and positive prick tests to mugwort pollens and house dust mites, experienced twice heavy systemic FDEIA incidents one hour after ingesting fresh celery, while vigorous physical exercise. Reevaluation of the patient's allergological status was completed a few months following the last anaphylactic shock. A skin prick test for celery was positive. In contrast, celery-specific IgE test (both Allergopharma, Reinbek, Germany) was negative. An exercise test was performed, but induced neither symptoms of anaphylaxis nor significant findings in spirometric examination. Celery intake not followed with exercise was well tolerated many times before and after these incidents. The patient was informed about FDEIA.

The second patient, a 22-year-old male, experienced many incidents of heavy anaphylaxis after vigorous physical exercises following ingestion of different food allergens – egg, fish and chicken meat. He was diagnosed with bronchial asthma, seasonal rhinitis and food allergy. Initially, food allergy was manifested as atopic dermatitis, and after a few years of remission the patient experienced many incidents of acute recurrent urticaria and angioedema, accompanied by anaphylactic shock on 2 occasions. Ingestion of the mentioned food allergens not accompanied by physical exercise or physical exercise without the allergens ingestion was always well tolerated and never induced skin or systemic reactions. Reevaluation of allergic status showed positive skin tests and the presence of allergen specific IgE to: mites, dog hair, grass pollens, trees pollens, egg, carp meat and chicken meat (Allergopharma, Reinbek, Germany). The patient was informed about FDEIA. Both patients were instructed to avoid all food allergens during the 4 hours preceding physical exercise. Age-matched 4 healthy persons (2 males and 2 females) and 3 persons suffering from allergic urticaria caused by food allergens and well tolerated physical exercise (1 male and 2 females) entered study as the control cases.

MATERIAL AND METHODS

In all tested persons, flow cytometric test for basophil CD203c upregulation was performed. The venous blood specimens were collected on EDTA and centrifuged for 5 minutes at $200 \times g$. The upper leukocyte rich phase was transferred to another polystyrene tube and centrifuged for 10 min. at 500 g. Cell pellet was carefully resuspended in concentration of 0.5×10^6 cells in 150 μ L portions of the stimulation buffers containing IL-3 (Bühlmann Laboratories AG) with increasing osmolarity-280 mOsm, 340 mOsm, and 450 mOsm. Increased osmolarity was achieved by the addition of increased portions (5 and 15% of the final buffer volume) of 20% of aqueous solution of mannitol (Fresenius Kabi, Kutno, Poland) and determined by semimicroosmometer (Knauer, Berlin, Germany). After 15 min. of incubation in an incubator (37°C , humidified atmosphere, 5% CO_2) the activation process was interrupted by the addition of FastImmune EDTA (Becton Dickinson). The samples were centrifuged at 4°C at $500 \times g$ for 5 min. and the supernatants were removed. Basophil CD203c upregulation was determined by the method described by Kahlert *et al.* [15]. Cell pellets were resuspended in 100 μ L of PBS/1% BSA (Institute of Immunology and Experimental Therapy, Wrocław, Poland) and incubated with 20 μ L of anti-CD203c-PE (Coulter Immunotech, Marseille, France) for 45 min at room temperature in the dark. The lysis of red blood cells was performed with a 2 mL of 10 X diluted FACS Lysing Solution (10X) (Becton Dickinson) during 15 min. of incubation. After 2 further washing steps in PBS, the cells were kept in a 1.5% paraformaldehyde solution in PBS until they were analyzed in the FACScan flow cytometer (Becton Dickinson). In each sample, 100,000 cells were analyzed. Basophil population was gated in the plot as CD203c⁺ and SSC^{low} cells. The results were expressed as CD203c^{high} basophils in percentages according to the Kahlert *et al.* method [15]. Coefficient variation of this test is on the level of 4.4%.

RESULTS

At physiological osmolarity (280 mOsm) the percentage of CD203c^{high} basophils was at a low level in all tested persons. After increase of medium osmolarity to 340 mOsm in all the control persons the percentage of CD203c^{high} did not change, but in patients with FDEIA was much higher than in physiological osmolarity. It was almost 3 times higher than in controls ($X=14.7\%$ vs. 5.82% in celery induced urticaria and 5.37% in healthy persons). After increase of medium osmolarity to 450 mOsm, the percentage of CD203c^{high} basophils in FDEIA patients rose to a mean value of 16.9% and was comparable to those in celery induced urticaria ($X=14.96\%$); in healthy controls, however, the mean value was still low (8.05%). Individual results are presented in Figure 1.

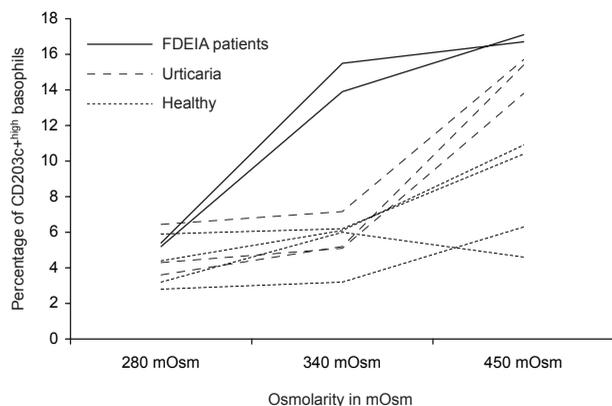


Figure 1. Percentage of CD203c+ high basophils in buffers of increasing osmolarity.

DISCUSSION

The hyperosmolar activation of basophils has been described elsewhere, but its increasing or decreasing effects on the release of histamine may vary according to the cell type tested (mast cell or basophil) and the stimulus used for cell activation [1, 2, 7, 8, 10, 11, 13, 20, 26]. In our first case report we presented augmentation of basophil histamine release in slightly increased medium osmolarity in one patient with FDEIA [4]. This first intriguing observation stimulated subsequent research to clarify the significance of increased medium osmolarity (which partially mimics circumstances of vigorous physical exercise) on basophil activation. As post-exercise plasma osmolarity of 311 ± 2.4 mOsm was reported [17], it was decided to focus on basophil response in a similar hyperosmolar buffer (340 mOsm). Basophil activation in hyperosmotic buffers has been demonstrated by Banks *et al.* [3]. They found that cells from individuals with food allergy were significantly more easily activated comparing to cells from normal volunteers, but not to those from other allergic subjects [3]. Because this study also employed buffers of much higher osmolarity, and maximal response was observed at 770 mOsm, it was decided to include also one additional buffer of the highest osmolarity (450 mOsm); however this very high osmolarity cannot be expected after even extremely intensive physical exercise.

The most important result of our study is the observation of increased basophil activation in slightly elevated medium osmolarity (340 mOsm) only in patients with FDEIA by the means of the percentage of CD203c^{high} basophils (Figure). In all controls, the percentage of CD203c^{high} basophils did not change in this medium osmolarity, when compared to physiologic osmolarity of 280 mOsm. Further increase of medium osmolarity to 450 mOsm resulted in augmentation of patients' basophils activation, but a similar effect was observed in food allergic urticaria controls. The latter finding is consistent with the results reported by Banks *et al.* [3]. Indeed, all food allergic patients, includ-

ing these with FDEIA, exhibited almost the same increased response to the buffer of 450 mOsm, but the response of healthy controls was much lower. In other words, a significantly increased basophil response in slightly elevated osmolarity was observed only in patients with FDEIA. These results support our initial hypothesis that individually increased basophil activity, demonstrated as abnormally increased responsiveness to slightly increased medium osmolarity, might be among the factors predisposing to the occurrence of FDEIA.

The pathogenesis of FDEIA is complex and different predisposing factors might be of different importance in each individual case. In order to make unambiguous statements concerning the significance of unusual basophil activation in a slightly hyperosmolar environment in the pathomechanism of this syndrome requires prospective studies in order to increase the number of observations. However, in our opinion, these initial results deserve description.

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