ABILITY OF PHAGOCYTES TO GENERATE REACTIVE OXYGEN SPECIES IN CHILDREN WITH POLLINOSIS

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Abstract

The aim of this study was to evaluate the generation of reactive oxygen species (ROS) by neutrophils and monocytes in children with pollinosis treated by specific immunotherapy (SIT). The cells were isolated from peripheral blood of 15 pollinosis patients with and without bronchial asthma (BA). The ROS production by NADPH-oxidase and myeloperoxidase (MPO) of the cells was studied by chemiluminescence (CL) method using luminol and lucigenin as amplifiers. SIT was accompanied by the increase of neutrophil ability to generate the superoxide radicals in children with pollinosis complicated by BA, that contrast with the rise of MPO activity of neutrophils in pollinosis patients without BA. Capability of monocytes to produce ROS was suppressed in patients before SIT in comparison with norm. During SIT the yield of ROS generated by monocytes risen, although monocyte responses to priming agent and stimulation were still abnormal.

KEYWORDS: pollinosis, monocytes, neutrophils, chemiluminescence

1. INTRODUCTION

Allergy is a complex inflammatory response of organism that involves the activation of many leukocytes [1]. The neutrophils and monocytes take part in progression of allergic inflammatory diseases, like rhinitis and asthma [2]. Attracted in inflamed tissues, these cells release the hydrolytic enzymes and reactive oxygen species (ROS) injuring tissues and promoting inflammation [3,4]. Activity of neutrophils and monocytes in patients with pollinosis (seasonal allergic rhinitis) has been slightly investigated. The aim of the present study was to evaluate the ROS generation ability of neutrophils and monocytes in pollinosis patients treated by specific immunotherapy (SIT) before season exacerbation of allergy.

2. PATIENTS AND METHODS

Samples of blood were obtained from 15 children (boys and girls aged 9-17 years) with allergy to pollen of different plants. Four of 15 patients had pollinosis complicated by bronchial asthma (BA). The patients were treated by SIT (exposition to low doses of relative allergens during 16 days) before season exacerbation of allergy. Control group consisted of 10 healthy volunteers aged 15-17 years. Neutrophils and monocytes were isolated from blood by ficoll-verografin gradient centrifugation. Generation of ROS was analysed by chemiluminescence (CL) method [5,6] using chemiluminometer (Model BCL-1, Belarus). In CL assay monocytes (0.5×10^6/mL) or neutrophils (1×10^6/mL) were stimulated during adhesion on glass cuvette bottom and by 1 μmol/L fMLP in the presence of 12.5 μmol/L luminol or lucigenin. To evaluate total intracellular myeloperoxidase (MPO) activity, neutrophils (1×10^6/mL) were destroyed by three freezing (–18 ^{0}C) and defreezing (20 ^{0}C) cycles and MPO activity in supernatants was evaluated as the rate of luminol (12.5 μmol/L) oxidation by hydrogen peroxide (0.1 μmol/L) using CL method. To investigate MPO secretion, neutrophils (1×10^6 cell/mL) were incubated in glass cuvettes at 37 ^{0}C for 30 min, then the cells were removed and MPO activity of supernatants was measured as described above. Results were analysed using Student's statistics. Obtained data were expressed as mean ± confidence interval (P=0.05).

3. RESULTS AND DISCUSSION

As shown early, during adhesion on glass phagocytes generate ROS with participation of NADPH-oxidase and MPO [7]. NADPH-oxidase produces superoxide anion radical ($\bullet O_2^-$), which can be detected sensitively by CL method using lucigenin (Bis-*N*-methylacridinium) at non-redox cycling concentration [5]. As result of $\bullet O_2^-$ enzymatic (with superoxide

dismutase) and spontaneous dismutation, hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are formed. In the presence of H_2O_2, MPO generates hydroxyl radical (OH▪) and hypochlorite. Total ROS production can be detected by measuring of luminol amplified CL, because of all intermediates $•O_2^-$, H_2O_2, OH▪, $^1O_2$ and hypochlorite interact with luminol [6].

We compared the luminol and lucigenin enhanced CL of neutrophils in pollinosis and healthy specimens (fig.1A). The cells were activated during adhesion on glass. It was revealed that the intensity of luminol CL in all patients was higher than in healthy control and significantly increased after treatment with allergens. Lucigenin CL intensity of neutrophils was altered by inverse manner in patients with and without BA symptoms. In patients with BA symptoms, intensity of lucigenin amplified CL did not differ from norm before the therapy, and significantly increased after SIT. In patients without BA symptoms intensity of lucigenin CL decreased during the therapy, but was strongly higher than in norm the both before and after SIT (fig. 1A). Thus, the neutrophils from pollinosis patients in remission generate ROS (particularly, $•O_2^-$) more intensively than in norm and the ROS production is altered after exposure to allergens (*in vivo*). Authors of article [8] also observed the increase of CL, when neutrophils from asthmatic and pollinosis subjects were preincubated *in vitro* with specific allergens. Augmented activity of NADPH-oxidase and/or diminished activity of antioxidant enzymes may cause the rise of $•O_2^-$ yield. The investigation of asthmatic patients has shown a heightened respiratory burst in neutrophils with increased $•O_2^-$ and H_2O_2 generation and suppressed activity of superoxide dismutase [9].

Luminol amplified CL indicates the ROS, which are generated by MPO, particularly [6]. MPO is localised in azurophil granules of neutrophil [4]. The cells secrete MPO in extracellular medium when were stimulated. We measured the total MPO-activity of neutrophils and activity of MPO secreted in medium during adhesion of cells on glass (fig. 1B). Before SIT in pollinosis patients without, but not with BA symptoms, the both types of MPO-activities were decreased in comparison with norm. After exposition of these patients to allergens the meanings of the both parameters increased up to normal values. In contrast, neutrophils had a heightened intracellular MPO-activity and released statistically significantly higher MPO in pollinosis patients with BA not receiving SIT than in control group. Application of SIT led to decrease of the both MPO-activities (fig.1B). Our data allow to conclude that even in period of remission the neutrophils from pollinosis children demonstrate increased ROS production in comparison with norm. It can promote oxygen radical-induced tissue damage. Application of SIT leads to additional rise of the ROS yield that is associated with the increase of $•O_2^-$ production in pollinosis complicated by BA, and the augmentation the MPO activity of neutrophils in pollinosis without BA.

Monocytes can play a role in allergic inflammation because of produce IgE, chemoattractants and other immunomodulators [1,2]. Besides that, monocytes, like neutrophils, can generate ROS [2,3]. We measured luminol amplified CL of monocytes activated during adhesion of the cells on glass and addition of fMLP. Kinetic dependencies of luminol CL for children with pollinosis and healthy subjects are represented in the fig. 2. As shown, the ROS production by monocytes in both types of stimulation was significantly lower in pollinosis children before therapy than in norm. This fact testifies to suppressed functional activity of monocytes in pollinosis patients. Application of SIT was accompanied by the increase of monocyte ability to produce ROS during adhesion on glass and addition of fMLP (fig.2). However, the meanings of these parameters did not reach the values obtained in healthy control subjects.

There are a great number of evidences that ROS, particularly, H_2O_2 at the low concentration modulates signal transduction in different cell types [10]. We studied the influence of H_2O_2 on the ROS generation processes in monocytes from pollinosis children. The cells were pretreated by H_2O_2 (10 μmol/L) at 37 °C for 25 minutes, then luminol CL kinetic dependencies of monocytes during adhesion on glass and stimulation with fMLP were registered (fig. 3). H_2O_2 did not influence on adhesion-associated ROS formation in monocytes from healthy and allergic subjects. However, H_2O_2 induced the significant increase of monocyte response to fMLP in control specimens, but not in pollinosis. In contrast, H_2O_2 led to decrease of fMLP-induced ROS generation in monocytes from pollinosis patients received SIT. So, monocyte ability to generate ROS is depressed in pollinosis patients out of disease exacerbation and increased in result of allergen injections. Monocytes from pollinosis children demonstrate abnormal response to stimulation with H_2O_2 and fMLP.

We conclude that CL method is informative to investigate the mechanisms of neutrophil and monocyte ROS generation in patients with seasonal allergy. Obtained data testify to the stimulative influence of low dose allergens on ability to product ROS of phagocytes in children with pollinosis. Application of SIT leads to particularly restoration of phagocyte functional activity, because of a number of cellular responses remains abnormal.

4. ACKNOWLEDGMENTS

This work has been supported by grant from the Belarus Republican Foundation of Fundamental Researches (project B03M-137).

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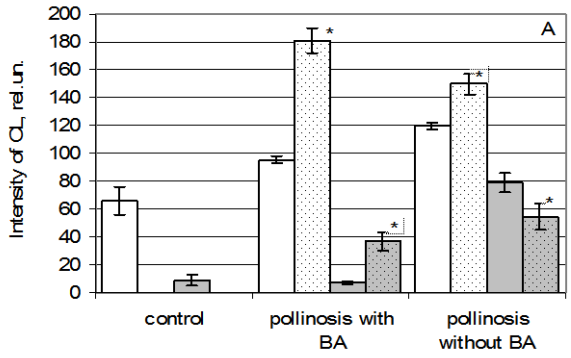

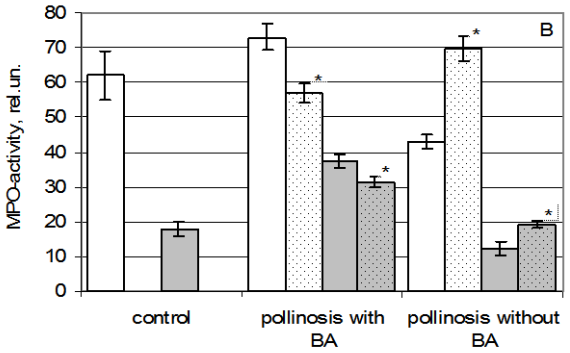

Fig. 1. A. Intensity of luminol (□) and lucigenin (■) induced CL of neutrophils during adhesion on glass. B. Total intracellular MPO activity (□) and secretion of MPO by neutrophils during adhesion on glass (■). Data are represented for healthy people and pollinosis patients with and without BA symptoms before and after (*) SIT.

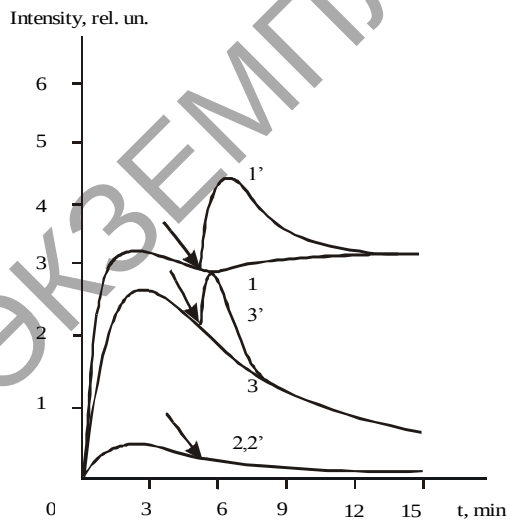

Fig. 2. The kinetics of luminol-induced CL of monocytes from healthy people (1, 1') and pollinosis patients before (2, 2') and after SIT (3, 3'). The cells were stimulated during adhesion on glass (1-3) and with fMLP (1'-3'). Arrows show the moments of fMLP addition.

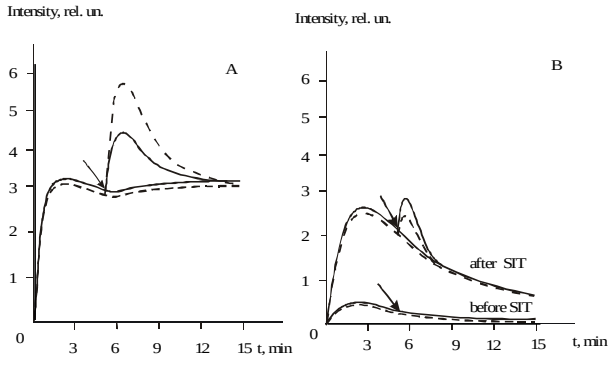

Fig. 3. The kinetics of luminol-induced CL of monocytes from healthy people (A) and children with pollinosis (B) before and after SIT. The cells were stimulated during adhesion on glass and with fMLP in the absence (—) and in the presence (---) of 10 μmol/L H_2O_2. Arrows show the moments of fMLP addition.