Rapid communication

J. JAWIEŃ, S. CHŁOPICKI, R. OLSZANECKI, B. LORKOWSKA, R.J. GRYGŁEWSKI

EOSINOPHIL – EPITHELIAL CELL INTERACTION AUGMENTS CYSTEINYL LEUKOTRIENES SYNTHESIS

Department of Pharmacology, Jagiellonian University School of Medicine, Grzegorzecka Str. 16, 31-531 Krakow, Poland

Eosinophils accumulation in the airways and sustained eosinophil-derived cysteinyl leukotrienes production represent key elements of the inflammatory response seen in asthma. However, it is not known whether activated epithelial cells influence cysteinyl leukotrienes production by eosinophils from healthy volunteers. The aim of the present study was therefore to analyse the effects of interactions between non-atopic eosinophils and epithelial cells on cysteinyl leukotrienes production in vitro. We measured cysteinyl leukotrienes released by phorbol 12-myristate 13-acetate (PMA) –activated human eosinophils or epithelial cells (human bronchial epithelial cell line -BEAS-2B) cultured alone or together. While activated BEAS-2B cells barely formed leukotrienes (1.39 pg/ml ± 0.2) (n=32), activated eosinophils produced considerable amount of them (62.25 pg/ml ± 10.29) (n=32). Interestingly, when activated eosinophils and epithelial cells were co-incubated, production of cysteinyl leukotrienes increased substantially (571.1 pg/ml ± 80.9) (n=32). Thus, eosinophil-epithelial cell interactions, when occur, are associated with increased biosynthesis of cysteinyl leukotrienes.

Key words: BEAS-2B epithelial cell line, eosinophils, cysteinyl leukotrienes, cell-cell interactions

INTRODUCTION

Eosinophils accumulation in the airways, and sustained eosinophil-derived cysteinyl leukotrienes production represent key elements of the inflammatory response seen in asthma (1, 2). Indeed, cysteinyl leukotrienes - LTC₄, LTD₄ and LTE₄ - play pivotal role in various aspects of asthmatic inflammatory response such as bronchoconstriction, increased airway mucus secretion, bronchial wall remodelling, increased recruitment of eosinophils, persistent stimulation of sensory nerves as well as pulmonary epithelial and endothelial injury (3). Interactions between pulmonary
epithelial cells and eosinophils retained within airways are thought to be central in the maintenance of asthmatic inflammatory processes (4). Although the mechanisms involved are not clear, it is well known that epithelial cells support eosinophil inflammatory response by virtue of synthesis and release of potent eosinophil activators such as IL-5 or GM-CSF (5, 6). It was shown that eosinophils from atopic individuals display upregulated LTC4 synthesis, through contact with human epithelial cells (7). However, it is not known whether epithelial cells are capable to modulate cysteinyl leukotrienes production by eosinophils from healthy donors.

Therefore, the aim of our study was to study the influence of interactions between non-atopic eosinophils and epithelial cells on cysteinyl leukotrienes production in vitro. Isolated human eosinophils from healthy donors and human bronchial epithelial cell line (BEAS-2B) were used.

MATERIAL AND METHODS

Isolation of eosinophils from blood

Eosinophils were isolated from the peripheral blood of healthy, nonallergic volunteers. Fifty millilitres of heparinized blood were diluted with an equal volume of phosphate-buffered saline (PBS) containing 5% of foetal calf serum (FCS). Percoll solution (Pharmacia, Sweden) was diluted with Hanks’ buffered saline solution to achieve a density of 1.082 g/ml. The PBS diluted blood was layered above the Percoll at 2:1 ratio and centrifuged at 1,613 g at 20°C for 30 min. Then, plasma and mononuclear cells were discarded, whereas granulocyte / red cell pellet was recovered. The erythrocytes were removed by cold hypotonic lysis. To eliminate neutrophils, anti–CD16 coated magnetic microbeads (Miltenyi Biotech, Germany) were added to the remaining granulocyte mixture (50 µl per 10^7 cells), the mixture was incubated at 4°C for 30 min, and CD16-microbead bound neutrophils were removed using a magnetic separator – VarioMACS (Miltenyi Biotech, Germany) (8). Eosinophils were collected, resuspended in RPMI culture medium (GIBCO, UK) and counted using the Kimura stain method. The whole procedure was conducted in a Ca++-free and Mg++-free medium. Purified eosinophils were suspended in RPMI, supplemented with 1 mM CaCl2 and 1 mM MgCl2.

Culture of bronchial epithelial cells

A human bronchial epithelial cell line BEAS-2B was obtained from the American Type Culture Collection (ATCC; Rockville, USA). The cells were cultured as previously described for endothelial cells (9) in 25-cm² tissue culture flasks in RPMI 1640 Medium, containing 10% FCS, L-glutamine (2 mM), MEM (non-essential aminoacids 2 mM), penicillin (100 IU/ml), streptomycin (100 µg/ml), amphotericin β (0.25 µg/ml) and HEPES (25 mM). All these reagents were purchased from GIBCO, UK.

The cells were cultured at 37°C with 5% CO₂ in humidified air.

Protocol of experiments

Eosinophils were placed in wells of 96-well plate at a density of 10⁴ cells per well and were activated by phorbol 12-myristate 13-acetate (PMA) at a final concentration of 0.25 µM.
BEAS-2B cells passages were seeded in wells of 96-well plate (Nunc, Denmark) at a density of 10^4 cells per well, and were cultured in 300 µL RPMI for 24 hr to allow the cells to reach confluency. Then medium was replaced by the 300 µL of fresh medium containing 0.25 µM PMA. For co-incubation experiments medium from BEAS-2B cells was aspirated and 10^4 eosinophils in 300 µL of the fresh RPMI medium were delivered into each well. Mixture of BEAS-2B cells and eosinophils was activated by 0.25 µM of PMA.

**Measurement of cysteinyl leukotrienes**

After 18 hours of incubation, supernatants (100 µL) were harvested from wells containing separately cultured eosinophils or BEAS-2B cells and from wells containing co-culture of both kinds of cells. The supernatants were spun down at 400 g for 5 minutes, and kept at −80°C until assay. The levels of cysteinyl-leukotrienes (sum of LTC₄, LTD₄ and LTE₄) were measured by enzyme-linked immunoassay method (Cayman, USA).

**Statistical methods**

Results are presented as mean ± SEM (Standard Error of the Means). One-way analysis of variance (ANOVA), followed by post hoc Scheffe test was used for evaluation of differences between the groups. A value of p<0.01 was accepted to be significant.

**RESULTS AND DISCUSSION**

As shown on Fig. 1 cysteinyl leukotrienes released by activated BEAS-2B cells barely reached the level of detection (1.39 pg/ml ± 0.2) (n=32), while activated eosinophils produced substantial amount of them (62.25 pg/ml ± 10.29) (n=32). Interestingly, when activated eosinophils and epithelial cells were co-incubated, production of cysteinyl leukotrienes was nearly 10-fold higher than that of eosinophils alone (571.1 pg/ml ± 80.9) (n=32). Importantly, when unactivated eosinophils and epithelial cells were incubated alone or together, levels of cysteinyl leukotrienes were below the level of detection (<1 pg/ml).

To our knowledge this is the first direct demonstration that interactions between non-atopic eosinophils and epithelial cells result in increased cysteinyl leukotrienes production. It seems that there are at least two plausible mechanisms, which may contribute to the described phenomenon. Increased cysteinyl leukotrienes production could be due to the epithelial-derived paracrine mediator potentiating eosinophil activation or/and due to the transcellular biosynthesis of cysteinyl leukotrienes resulting from the metabolic cooperation of eosinophils and epithelial cells.

Indeed, there are several recent reports supporting reciprocal paracrine interactions between epithelial cells and eosinophils. It was shown that eosinophil peroxidase stimulate the release of granulocyte-macrophage colony-stimulating factor (GM-CSF) from bronchial epithelial cells (6). Activated human epithelial cells in turn, by releasing GM-CSF (10), eotaxin (11) and IL-5 (5), which, stimulates eosinophils degranulation
and support eosinophil survival may contribute to eosinophils retention within airways. Thus, it seems that paracrine action of epithelium-derived mediators may well contribute to the increased generation of cysteinyl leukotrienes, which was observed when eosinophils and epithelial cells were incubated together.

On the other hand, it is tempting to speculate that direct cell-cell interactions are also involved in the phenomenon described here. Indeed, main route of biosynthesis of cysteinyl leukotrienes in the circulation occurs via cell-to-cell transfer of LTA₄ (12). In particular, neutrophil – endothelial cell or neutrophil-platelet interactions lead to outburst of transcellular LTC₄ formation by the intercellular transfer of LTA₄ from neutrophils to endothelial cells or to platelets, while either of these cells alone produce little or no LTC₄ (12). Interestingly, not only LTC₄ but also other eicosanoids may be concomitantly formed by transcellular biosynthesis (13,14). Importantly, intercellular adhesion is mandatory for transcellular biosynthesis to occur (12).

In contrast to neutrophils, eosinophils alone are able to synthetize considerable amount of LTC₄ upon stimulation (15), as also confirmed here. However, when coincubated with epithelial cells, activated eosinophils, similarly to neutrophils may favour their “metabolic” cooperation and transcellular biosynthesis of LTC₄ by the intercellular transfer of LTA₄ from eosinophils to epithelial cells. Interestingly, there is no data as yet concerning activity of LTC₄ synthase in human epithelial cells, however the presence of enzyme converting LTC₄ to LTD₄ in epithelial cells has been recently demonstrated (16). Noteworthy, adhesion molecules involved in eosinophil-epithelial cell tethering are not fully described (17). Thus, it seems that, next to the paracrine

**Fig. 1.** Production of cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) by activated eosinophils (10⁴ cells per well, n=32), activated BEAS-2B epithelial cells (10⁴ cells per well, n=32), and by activated eosinophils and BEAS-2B cells when coincubated (10⁴ and 10⁴cells per well, respectively, n=32). In each case, cysteinyl leukotrienes were measured in supernatant after 18 hours of cell incubation. Each column represents the mean ± SEM. + p<0.01 represents statistically significant difference between eosinophils and BEAS-2B, # p<0.01 between eosinophils and co-culture, * p < 0.01 between co-culture and BEAS-2B.
fashion, adhesion molecules signalling events, which favor transcellular biosynthesis of cysteinyl leukotrienes may contribute to the described phenomenon. However, mechanisms of transcellular synthesis of LTC₄ during eosinophil-epithelial cells interactions require further studies.

It is clear from the data presented above that the epithelial cells are capable of mounting and sustaining an airway inflammatory response by amplifying the production of cysteinyl leukotrienes. This can occur not only in atopic asthma, but also whenever eosinophil-epithelial cell interactions occur. Thus, experimental model of eosinophil epithelial cells interactions proposed here with the use of eosinophil from healthy donors and epithelial cells line give an excellent tool to shed light on the mechanisms involved in the phenomenon of increased cysteinyl production by eosinophil-epithelial cells cooperation, which occurs in disease state. Further studies are on the way.

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Author’s address: Jacek Jawieñ, Department of Pharmacology, Jagiellonian University School of Medicine, Grzegorzecka Str.16, 31-531 Krakow, Poland, e-mail: mmjawien@cyf-kr.edu.pl