Changes in Filament Actin Accompanying IgE-Dependent and -Independent Histamine Release from IL3-Dependent Cultured Human Basophils

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Abstract

When cord blood mononuclear cells were cultured in the presence of rhIL-3 for 5 weeks or more, 40–90% of cultured cells became morphologically mature basophils. We analyzed the kinetics of histamine release, changes in filament actin (F-actin), and movement of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) induced by IgE-dependent (anti-IgE) and -independent (fMLP) stimuli in these cultured basophils. Anti-IgE and fMLP released $24.5 \pm 5.4\%$ and $14.5 \pm 4.5\%$ histamine from the cells, respectively. Anti-IgE caused actin polymerization with a peak response at 15 min, which began much later than the elevation of $[\text{Ca}^{2+}]_i$. In contrast to anti-IgE stimulation, fMLP induced rapid actin polymerization with a peak response at 30 s in correlation with kinetics of histamine release. Our results indicate that cord blood-derived cultured basophils show similar cell functions to mature basophils, and are useful models with which to investigate the mechanisms of degranulation, specifically when a large amount of highly purified cells are required.

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Introduction

Mast cells and basophils play an important role in IgE-mediated hypersensitivity through release of inflammatory mediators [1]. Cytoskeletal movements are involved in many kinds of cell function, such as locomotion [2] and secretion [3]. To clarify cytoskeletal involvements in IgE-dependent and -independent degranulation of basophils, we used rhIL-3-dependent cultured basophils in this experiment.

Measurements of $[\text{Ca}^{2+}]_i$. $[\text{Ca}^{2+}]_i$ was measured using fura-2/AM (Molecular Probes) and a spectrofluorometer as reported [7].

Relative F-Actin Content. Relative F-actin content was determined using the technique of Howard and Meyer [2]. After stimulation with anti-IgE or fMLP, the reaction was stopped by the addition of formaldehyde at the desired time. Cells were permeabilized by the addition of lysophosphatidylcholine (Sigma), and stained with nitrobenzoxiazole (NBD)-phalloidin (Molecular Probes). 50,000 stained cells were analyzed by flow cytometry in each experiment. Relative F-actin content was expressed as the ratio of linear fluorescence intensity between stimulated and unstimulated cells [8].

Materials and Methods

Cell Culture. Cultured basophils were obtained as reported [4]. Briefly, cord blood mononuclear cells were cultured in RPMI 1640 (Gibco) supplement with 10% FCS, 2×10^{-5} M 2-ME, antibiotics and 2.5 U/ml of rhIL-3 (Genzyme) for more than 5 weeks. The purity of cultured basophils used in the analysis of F-actin content and measurements of $[Ca^{2+}]_i$ was more than 80%. Cultured basophils were sensitized with human myeloma-derived IgE in overnight cultures, then used for experiments.

Histamine Release. Histamine release was carried out using standard methodologies [5].

Sensitized cultured basophils were challenged with rabbit serum specific for human IgE (anti-IgE) and f-Met-Leu-Phe (fMLP) at optimal concentrations. Histamine was measured using an automatic histamine analyzer as reported [6].

Results and Discussion

After 5 weeks of culture in the presence of rhIL-3, 40–90% of total cells became mature basophils. We found that basophils could survive longer than the other cell types in the present culture condition. Cultured basophils contained 0.3–0.5 pg/cell of histamine. Flow cytometric analysis revealed the expression of FcεRI on the surface of these cells. Taken together, these cultured basophils seemed to be useful models with which to investigate the mechanisms of basophil degranulation.

We compared the effects of anti-IgE and fMLP on

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30□ a

10 20

Time, min

Fig. 1. Anti-IgE (0.5 μ g/ml) (a) and fMLP (10 $\sim$ 5 M) (b) induced histamine release and cytoskeletal changes in cultured basophils. Histamine release: □ = anti-IgE (mean of 6 separate experiments); (O) = fMLP (mean of 3 separate experiments). F-actin content: ■ = anti-IgE (mean $\pm$ SD, n = 4); · = fMLP (n = 4); Δ = control (n = 3).

Fig. 2. Anti-IgE and fMLP induced intracellular Ca^{2+} movements in cultured basophils. a Anti-IgE (0.5 $\mu\text{g/ml}$). b fMLP (10^{-5} M). Intracellular Ca^{2+} concentration values are derived from the following equation: $[\text{Ca}^{2+}]_i \text{ (nM)} = K_d (R - R_{\text{min}}) / (R_{\text{max}} - R)$, where $K_d = 224$.

kinetics of histamine release, $[\text{Ca}^{2+}]_i$ movements, and changes in F-actin content in these cells. As shown in figure 1, anti-IgE-induced histamine release began 2 min after stimulation and reached a plateau at 30 min ($24.5 \pm 5.4\%$). Relative F-actin content had increased from 2 min after stimulation and reached a maximum value at 15 min (2.205 ± 0.253). By 30 min after stimulation relative F-actin content decreased to 1.646 ± 0.146 . Controls, which received Tyrode solution, did not show significant differences in relative F-actin content from prestimulation levels. Anti-IgE-induced elevation of $[\text{Ca}^{2+}]_i$ consists of two phases. The first phase (over 25 s after stimulation) was a rapid elevation (99 nM) from baseline (49.5 nM). A gradual elevation was then observed following the first phase (fig. 2). $[\text{Ca}^{2+}]_i$ movement associated with IgE-dependent basophil degranulation was proceeded to kinetics of actin polymerization. This cytoskeletal change was in agreement with the observation by Warner et al. [9] using purified peripheral basophils.

In contrast to anti-IgE stimulation, fMLP induced histamine release from cultured basophils within 15 s ($14.5 \pm 4.5\%$). The fMLP-induced change in relative F-actin content was much more rapid than anti-IgE-induced change. The reaction reached a maximum value at 30 s (2.02 ± 0.24) and decreased to close to baseline at 10 min (1.265 ± 0.11). fMLP induced much more rapid elevation in $[\text{Ca}^{2+}]_i$ (within 15 s) than anti-IgE. After the rapid elevation, the reaction reached a plateau (fig. 2). Kinetics of histamine release, relative F-actin content and $[\text{Ca}^{2+}]_i$ movement were much more rapid in fMLP stimulation than those in anti-IgE stimulation. fMLP-induced cytoskeletal change in cultured basophils showed similar kinetics in neutrophils stimulated by fMLP [2] and eosinophils by PAF[10].

These results indicate that the F-actin involved in IgE-dependent and -independent histamine release from cultured basophils is differently regulated.

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