Brief Definitive Report

Enhancement of Human Basophil Histamine Release by Interleukin 5

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Summary

Substantial evidence indicates a close relationship between eosinophils and basophils. We examined whether interleukin 5 (IL-5), known to be eosinophilopoietic and capable of selectively regulating eosinophil functions, has an affect on basophil functions. IL-5 enhanced basophil histamine release evoked by anti-IgE, formyl-methionyl-leucyl-phenylalanine, or ionophore A23187 at picomolar concentrations. Direct action of IL-5 on human basophils was confirmed using highly purified basophil populations. These observations reveal the novel fact that IL-5 is able to modulate basophil functions as well as eosinophil functions, and suggest that normal human basophils possess functional receptors for IL-5.

IL-5 is a glycoprotein liberated from activated T cells, and has been shown to possess a variety of biological activities on different cell types (reviewed in reference 1). Besides its action on T and B lymphocytes, IL-5 acts on eosinophil lineages to induce their selective proliferation and differentiation in humans as well as in mice (2, 3). Furthermore, IL-5 stimulates mature eosinophils to enhance their functional activities, such as phagocytosis, motility, and the synthesis of biologically active molecules (4, 5). In as much as substantial evidence strongly indicates a close relationship between basophils and eosinophils, and the fact that we observed that two eosinophilopoietic factors (granulocyte/macrophage [GM]-CSF and IL-3) enhanced basophil histamine release (6), we address the question of whether IL-5 does modulate basophil function. In this paper, we show that IL-5 enhances histamine release from human basophils at picomolar concentrations.

Materials and Methods

Materials. Chemicals used in the experiments were purchased as previously described (6). Purified human (h) (7) and mouse (8) rIL-5, produced by the Suntory Institute of Biomedical Research (Mishima, Osaka, Japan), was kindly donated by Dr. T. Honjo, University of Kyoto (Kyoto, Japan).

Histamine Release. Histamine release experiments were performed as described in the preceding report (6). Briefly, leukocytes were separated from normal peripheral blood by dextran sedimentation, and were incubated in the presence of various concentrations of rIL-5 (400 μl) at 37°C. Incubation was carried out for 30 min, unless otherwise indicated, and histamine release was initiated by the addition of 100 μl of secretagogues. After additional incubation for 45 min at 37°C, supernatants were collected by centrifugation, and released histamine was measured by an automated fluorometric technique. Enhancement of histamine release by IL-5 was calculated by the following formula, as previously described (6): percent enhancement = 100× (histamine released with a stimulus plus IL-5 − histamine released with a stimulus)/total histamine content. Each experiment was performed in duplicate or triplicate.

Purification of Basophils. In some experiments, purified basophils were used instead of crude leukocyte preparations. The details of the purification procedure will be described elsewhere (Yamaguchi, M., K. Hirai, Y. Morita, T. Takaishi, K. Ohta, S. Suzuki, K. Motoyoshi, O. Kawanami, and K. Ito, manuscript submitted for publication). In brief, basophils were separated from normal peripheral blood by discontinuous Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradients with densities of 1.070 and 1.079 g/ml, followed by negative panning selections using anti-Leu-5b (CD2), anti-Leu-12 (CD19), and anti-Leu-M3 (CD14) (Becton Dickinson & Co., Mountain View, CA). Purified basophil suspensions were prepared with a purity of ~90%. Neutrophils and lymphocytes were the most frequent contaminating cells, and preparations contained only small numbers of eosinophils (<0.5%).

Results and Discussion

We first tested whether rhIL-5 itself directly induced histamine release from basophils. However, we observed no significant release in the presence of 3 nM of rhIL-5 (n = 5). We next studied the effect of rhIL-5 on histamine release evoked by various secretagogues. Pre-incubation of leukocytes with rhIL-5 resulted in the dose-dependent augmentation of histamine release initiated by anti-IgE, FMLP, or ionophore

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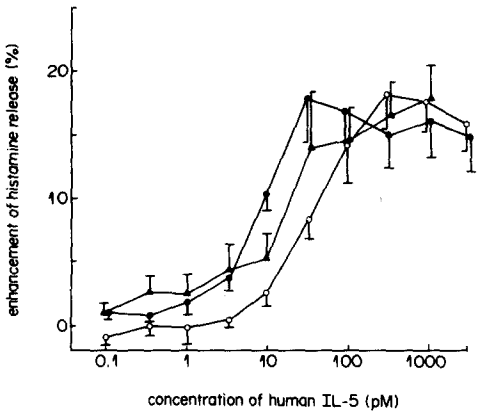

Figure 1. Enhancement of histamine release by rhIL-5 upon stimulation with anti-IgE, FMLP, or ionophore A23187. Leukocytes were pre-incubated for 30 min with serially diluted rhIL-5 and then stimulated with (●) anti-IgE (1:3,000; n = 7), (○) FMLP (10^{-5} M; n = 5), or (▲) ionophore A23187 (0.2 μg/ml; n = 5). Control release in the absence of rhIL-5 was 39.6 ± 5.6% (mean ± SEM) for anti-IgE, 38.0 ± 6.9% for FMLP, and 27.8 ± 10.7% for ionophore A23187. The bar represents SEM.

A23187 (Fig. 1). Maximal enhancement expressed as a percent increase against total histamine content was 19.5 ± 3.3% (mean ± SEM; range, 6.4–32.7%) for anti-IgE; 19.5 ± 2.8% (range, 12.6–27.2%) for FMLP; and 19.6 ± 0.9% (range, 17.1–22.3%) for ionophore A23187. Irrespective of the stimuli used, the enhancement occurred at similar concentrations of rhIL-5; half-maximal enhancement was observed at concentrations ranging from 10 to 30 pM, and the enhancement was observed in a dose-dependent fashion between 0.3 and 300 pM.

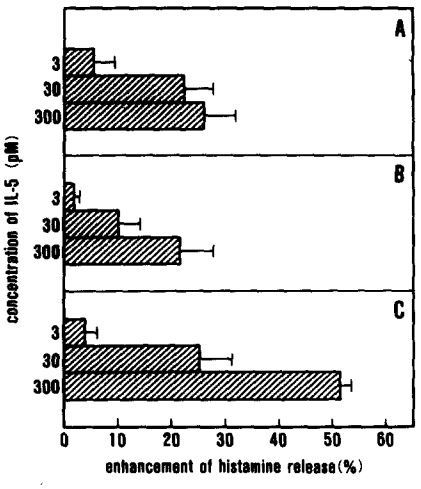

Figure 2. Enhancement of histamine release from purified basophils by rhIL-5. Basophils were purified from normal peripheral blood and tested as described above. (*A*) Anti-IgE (1:3,000; n = 5); (*B*) FMLP (10^{-5} M; n = 5); (*C*) ionophore A23187 (0.2 μg/ml; n = 5). Control release in the absence of rhIL-5 was 27.4 ± 6.2% (mean ± SEM) for anti-IgE, 40.0 ± 4.9% for FMLP, and 27.2 ± 5.2% for ionophore A23187. The bar represents SEM.

Since several lines of evidence have demonstrated that IL-5 activates mature eosinophils and certain eosinophil products such as major basic protein to induce histamine release from human basophils (9), we examined whether rhIL-5 directly acted on basophils rather than indirectly via contaminating eosinophils. We performed similar experiments using purified basophils instead of crude leukocyte preparations, and observed rhIL-5-induced dose-dependent augmentation comparable with that found using crude leukocyte preparations (Fig. 2).

When rhIL-5 was removed by washing before stimulation, we still observed the enhancement, indicating that the presence of rhIL-5 was not required for augmentation to occur during the release process (data not shown). We reported in the preceding paper that the enhancement of histamine release by IL-3 or GM-CSF was time dependent and took place rapidly (6). The enhancement by IL-5 was also a rapid process. Apparent enhancement was observed when the cells were pre-treated with rhIL-5 for 1 min, and the augmentation reached maximum within 30 min of pre-treatment with rhIL-5 (Fig. 3).

Human IL-5 shares high sequence homology with murine IL-5 (~70% at the amino acid level), and hence, murine IL-5 is active on human eosinophils (10). A similar situation was found in basophils; murine IL-5 also enhanced human basophil histamine release (Fig. 4).

Both eosinophils and basophils, constituting elements of circulating white blood cells, play crucial roles in hypersensitivity reactions. Both cells share several properties. First, some eosinophil-specific proteins, such as major basic protein and lysophospholipase (Charcot-Leiden crystal), also exist in basophils (11, 12). Second, both cells generate LTC_4 as a major product of arachidonic acid metabolism. Third, in clonal analyses, the coexistence of eosinophils and basophils has been observed in single colonies derived from human hemopoietic precursors (13, 14). Finally, hybrid cells, which possess both eosinophilic and basophilic granules, have been found in semi-solid cultures (14) as well as in the peripheral blood of patients with chronic myelogenous leukemia (15). These observations collectively indicate the close relationship between

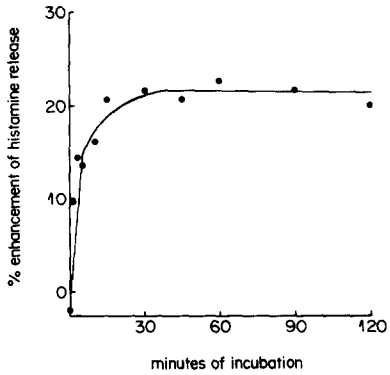

Figure 3. Time dependence of the enhancement of histamine release by rhIL-5. Leukocytes were pre-incubated with 300 pM of rhIL-5 for various periods of time and then stimulated with FMLP (10^{-5} M). Data are the averages of duplicate determinations of an experiment representative of four separate experiments.

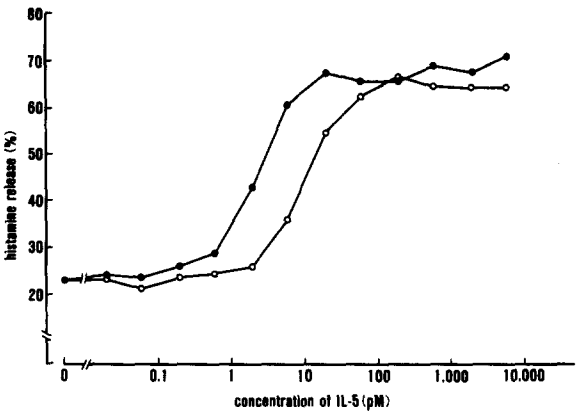

Figure 4. Enhancement of histamine release by murine IL-5. Leukocytes were preincubated for 30 min with serially diluted murine (●) or human (○) rIL-5, and then stimulated with anti-IgE (1:3,000). Data are the averages of duplicate determinations of an experiment representative of three separate experiments.

eosinophils and basophils, and raise the possibility that both cells might potentially derive from a common precursor.

Production of eosinophils is controlled by three eosinophilopoietic factors, including IL-3, GM-CSF, and IL-5. In the preceding paper, we reported that basophil histamine release was amplified by IL-3 and GM-CSF, while G-CSF, M-CSF, and IL-4 had no effect at all (6). In the present paper, we demonstrate that IL-5 also enhances basophil histamine release. It is of interest that all three eosinophilopoietic factors also stimulate basophils to enhance histamine release, and these observations provide additional evidence to support the notion that basophils and eosinophils closely resemble each other; the same hemopoietic growth factors regulate biological functions of both eosinophils and basophils.

The fact that IL-5 modulates basophil functions in vitro indicates a possible mechanism of in vivo regulation by IL-5 in terms of clinical manifestations of hypersensitivity reactions. Migrated basophils at the sites of inflammation are believed to contribute to IgE-mediated late-phase reactions. In concert with IL-3 and GM-CSF, and possibly with IL-8, known to stimulate basophils to trigger histamine release (16) and to induce chemotaxis (17), IL-5 modulates basophil histamine release and could participate in exacerbation and prolongation of hypersensitivity reactions. In fact, IL-5, as well as IL-3 and GM-CSF, induced migration of purified basophils (M. Yamaguchi et al., unpublished observation).

In conclusion, the observation that IL-5 is active on basophils at picomolar concentrations is a novel finding and suggests the presence of functional receptors for IL-5 on normal basophils. These findings add new insight into mechanisms controlling hypersensitivity reactions as well as human basophilopoiesis.

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