

Effect of interleukin 2 on basophil histamine release

Y. MORITA, M. GOTO and T. MIYAMOTO

Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Japan

Accumulating evidence suggests a link between immediate hypersensitivity and cellular immunity. In this study, we examined the effect of interleukin 2 (IL-2) on basophil histamine release. Histamine-releasing activity of IL-2 was very weak with % histamine release of 2.9 ± 1.3 (mean $\pm$ SEM, $n = 9$) at 1:12 dilution. IL-2 at 1:1200 dilution slightly inhibited anti-IgE-induced histamine release by $22.4 \pm 18.6\%$ ($P > 0.05$). There was a significant potentiation of release at 1:12 dilution of IL-2 with % enhancement of 78.7 ± 42.2 ($P < 0.05$). IL-2 enhanced the calcium ionophore A23187-induced histamine release in a dose-dependent fashion. IL-2 at 1:12 dilution significantly potentiated release by $28.8 \pm 6.3\%$ ($P < 0.05$). There was a slight suppression of formyl-methionyl-leucyl-phenylalanine (FMLP)-induced histamine release at 1:1200 dilution with % inhibition of 23.4 ± 7.4 ($P > 0.05$). At 1:12 dilution, IL-2 significantly potentiated FMLP-induced release by $73.7 \pm 41.6\%$ ($P < 0.05$). Recombinant IL-2 (RIL-2) augmented anti-IgE-induced histamine release with a significant enhancement at 200 units/ml. Conventional IL-2 was more potent than RIL-2 in enhancing release. These results indicate that IL-2 enhances basophil histamine release and some part of the effect of IL-2 on basophils is derived from other factors contained in conventional IL-2.

Key words: basophil; histamine release; interleukin 2; lymphokine; recombinant interleukin 2.

Accepted for publication 19 September 1986

IgE-mediated chemical mediators released from mast cells and basophils play an important role in the pathogenesis of immediate hypersensitivity reaction. Lymphocytes produce lymphokines which either directly induce histamine release or enhance histamine release induced by IgE-mediation (1, 2, 6, 8, 9). These observations suggest that type I allergy is modulated by lymphocyte-derived lymphokines.

Interleukin 2 (IL-2, T cell growth factor) is a lymphokine produced by T cells, which possesses multiple immunoregulatory properties (4). Furthermore, IL-2 has been reported to affect anti-IgE-induced histamine release from human basophils (5).

In the present study, we examined the effect of IL-2 on histamine release induced by not only anti-IgE, but also the calcium ionophore A23187 and formyl-methionyl-leucyl-phenylalanine (FMLP) and determined whether the effect of recombinant

IL-2(RIL-2) on basophils is identical to that of conventional IL-2 produced by phytohemagglutinin (PHA)-stimulated lymphocytes.

MATERIAL AND METHODS

Materials

Piperazine-N,N'-bis [2-ethanesulfonic acid] (Pipes) and formyl-methionyl-leucyl-phenylalanine (FMLP) were purchased from the Sigma Chemical Co., St. Louis, MO. The calcium ionophore A23187 was obtained from Calbiochem-Behring Corp., La Jolla, CA. FMLP and the calcium ionophore A23187 were dissolved in dimethylsulfoxide (Nakarai Chemicals, Kyoto, Japan) at 5×10^{-2} M and 1 mg/ml, respectively and stored at -20°C . Anti-human IgE was purchased from the Medical Biological Laboratory, Nagoya, Japan.

Interleukin 2 (IL-2, human T cell growth factor) was obtained from Electro-Nucleonics, Inc., Silver Spring, MD. It was a product of PHA-stimulated human peripheral leukocytes, and did not contain either PHA or interferon- γ as determined by indirect enzyme-linked immunosorbent assay and bioassay, respectively. Its potency was 640 units/ml as determined by tritiated thymidine uptake in PHA-stimulated human peripheral blood lymphocytes. Therefore, 1:1200, 1:120 and 1:12 dilutions corresponded to 0.5, 5.3 and 53 units/ml, respectively. *E. coli*-derived recombinant interleukin 2 (RIL-2, purity > 95%) was kindly donated by Shionogi Pharmaceutical Co., Osaka, Japan. Its potency was determined by tritiated thymidine uptake in CTLL-2 cells.

Buffers

A Pipes A buffer contained 119 mM NaCl, 5 mM KCl, 25 mM Pipes and 0.03% human serum albumin (pH 7.4). For histamine release reactions, Pipes A containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+} (Pipes ACM) was used.

Histamine release

Heparinized venous blood was obtained from patients with bronchial asthma, rheumatoid arthritis and healthy donors. Erythrocytes were sedimented at room temperature for 90 min with 2.5 ml of 6% dextran-2.8% glucose solution (6% dextran in saline, Midorijuji Pharmaceutical Co., Osaka, Japan) per 10 ml of blood. Isolated leukocytes were washed with Pipes A containing 4 mM ethylenediamine-tetraacetic acid, washed again with Pipes A and finally suspended in adequate amount of Pipes ACM. To test the effect of IL-2 and RIL-2 on histamine release induced by anti-IgE, ionophore A23187 and FMLP from human basophils, leukocytes were preincubated with various concentrations of IL-2 or RIL-2 for 15 min at 37°C in 12 $\times$ 75 mm polystyrene tubes. RPMI 1640 or Pipes ACM was used as a control. Histamine release reaction was initiated by the addition of anti-IgE, ionophore A23187 or FMLP and incubation

was carried out at 37°C for 45 min. The final cell concentrations of the reaction mixture were 1 to 2 $\times$ 10⁶/ml. Final concentrations of anti-IgE, ionophore A23187 and FMLP were 1:1200 (or 1:12000), 0.2 $\mu\text{g}/\text{ml}$ and 10⁻⁴ M, respectively. The tubes were then centrifuged at 400 $\times$ g for 15 min at 4°C. Supernatants were discarded, and cells were washed with Pipes A and finally destroyed by adding perchloric acid (final concentration; 2%) to measure residual histamine in the cells. Supernatants were stored at 4°C until histamine assay. Experiments were performed in duplicate or triplicate. The concentrations of DMSO used for dissolving ionophore A23187 and FMLP did not affect histamine release.

Histamine assay

Histamine was determined by the Shore's method (7). Histamine release was expressed as % of released histamine on the basis of total histamine in the cells.

Statistical analysis

Statistical analysis was performed by using paired t test.

RESULTS

Histamine-releasing activity of IL-2 for human basophils. IL-2 showed very weak histamine-releasing activity for human basophils. IL-2 directly induced histamine release by 0.9 $\pm$ 0.5%, 1.8 $\pm$ 0.9% and 2.9 $\pm$ 1.3% (mean $\pm$ SEM, n = 9) at 1:1200, 1:120 and 1:12 dilutions, respectively.

Effect of IL-2 on anti-IgE-induced histamine release from human basophils is shown in Fig. 1. Lower concentration of IL-2 (1:1200 dilution) slightly inhibited histamine release by 22.4 $\pm$ 18.6% (mean $\pm$ SEM). This inhibition was not statistically significant. In contrast, IL-2 potentiated release at 1:120, and 1:12 dilutions by 27.7 $\pm$ 6.9% and 78.7 $\pm$ 42.2% respectively. There was a significant enhancement with 1:12 dilution of IL-2 (P < 0.05).

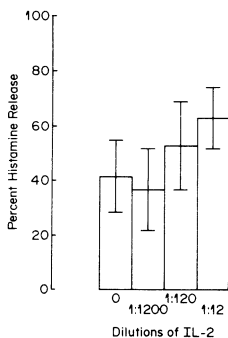


Fig. 1. Effect of IL-2 on anti-IgE-induced histamine release from human basophils: Leukocytes were preincubated with different concentrations of IL-2 for 15 min at 37°C. Anti-IgE (1:1200) was then added, and the incubation continued for another 45 min. Vertical bars represent the mean $\pm$ SEM from three donors. Histamine release in the absence of IL-2 was $40.7 \pm 13.0\%$. There was a significant enhancement of release at 1:12 dilution of IL-2 ($P < 0.05$).

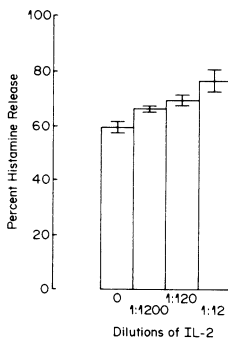


Fig. 2. Effect of IL-2 on the calcium ionophore A23187-induced histamine release from human basophils: For experimental details, see legend to Fig. 1. The calcium ionophore A23187 (0.2 μ g/ml) was added, and the reaction continued for another 45 min. Vertical bars represent the mean $\pm$ SEM from three donors. Histamine release in the absence of IL-2 was $59.1 \pm 1.7\%$. There was a significant enhancement of release at 1:12 dilution of IL-2 ($P < 0.05$).

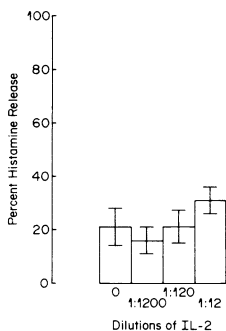


Fig. 3. Effect of IL-2 on FMLP-induced histamine release from human basophils: For experimental details, see legend to Fig. 1. Leukocytes were stimulated by the addition of 10^{-4} FMLP. Vertical bars represent the mean $\pm$ SEM from three donors. Histamine release in the absence of FMLP was $21.2 \pm 7.1\%$. There was a significant enhancement of release at 1:12 dilution of IL-2 ($P < 0.05$).

Effect of IL-2 on ionophore A23187-induced histamine release from human basophils. IL-2 enhanced ionophore A23187-induced histamine release in a dose-dependent fashion (Fig. 2). Percent enhancement was 12.2 ± 3.3 , 17.3 ± 5.5 and 28.8 ± 6.3 at 1:1200, 1:120, and 1:12 dilutions, respectively. Enhancement at 1:12 dilution was statistically significant ($P < 0.05$).

Effect of IL-2 on FMLP-induced histamine release from human basophils. There was a slight inhibition of FMLP-induced histamine release at 1:1200 dilution of IL-2 with % inhibition of 23.4 ± 7.4 ($P > 0.05$, Fig. 3). In contrast, IL-2 at dilution 1:12 enhanced histamine release by $73.7 \pm 41.6\%$ ($P < 0.05$, Fig. 3).

Effect of RIL-2 on anti-IgE-induced histamine release from human basophils. To compare the effect of conventional IL-2 and RIL-2 on histamine release induced by anti-IgE, the effect of RIL-2 was examined. Results were shown in Table 1. RIL-2 at 200 units/ml slightly, but significantly enhanced histamine release (% enhancement;

Table 1
Effect of RIL-2 on histamine release from human basophils

Case	Control release ^a	RIL-2 (u/ml) ^b			
		10	25	100	200
		% Histamine release			
T. T.	19.9	22.0	22.6	20.2	21.3
S. N.	32.6	37.7	37.1	36.5	36.9
M. N.	79.0	86.1	91.2	85.5	80.3
K. N.	65.0	66.1	65.0	67.2	69.5
$\bar{x} \pm \text{SEM}$	49.1 $\pm$ 13.8	53.0 $\pm$ 14.3	54.0 $\pm$ 15.2	52.4 $\pm$ 14.8	52.0 $\pm$ 13.8

^a Control release in the absence of RIL-2; ^b Human leukocytes were preincubated with different concentrations of RIL-2 for 15 min at 37°C. Anti-IgE (1:1200) was then added and the reactions continued for 45 min.

5.7 $\pm$ 2.8, $P < 0.05$). RIL-2 by itself did not activate human basophils.

DISCUSSION

Lymphocytes produce a variety of lymphokines which include migration inhibition factor (MIF), macrophage activating factor (MAF) and lymphocyte mitogenic factor (LMF).

Thueson and co-workers demonstrated that human peripheral mononuclear cells stimulated by antigen or concanavalin A produced a factor with histamine-releasing activity for human basophils (8, 9). Similar factors have been reported by several investigators (1, 2, 6). Enhancing activity of interferon for basophil histamine release has also been demonstrated (3).

IL-2 is a lymphokine produced by T cell (4). It has a multiple immunoregulatory activity such as enhancement of the development of cytotoxic T cells and tumor-specific T cells.

Our interest was to determine whether IL-2 has some effect on basophils. Although IL-2 did not directly induce significant histamine release from human basophils, it caused a substantial enhancement of IgE-mediated release at 1:12 dilution. These results are consistent with those reported by Schulman et al. (5).

A similar potentiating effect of IL-2 was observed when basophils were stimulated by other stimuli such as ionophore A23187 and FMLP.

As a next step, we examined the effect of RIL-2 on basophil histamine release because

conventional IL-2 derived from PHA-stimulated lymphocytes could contain factors influencing histamine release other than IL-2. RIL-2 showed a small, but significant enhancement of IgE-mediated histamine release at 200 units/ml. However, conventional IL-2 was more potent than RIL-2. These results indicate that the enhancing effect of IL-2 was partly due to factors other than IL-2 which could be produced by PHA-stimulated lymphocytes, although the nature of these materials has to be clarified.

In summary, IL-2 potentiated basophil histamine release induced by anti-IgE, ionophore A23187 and FMLP. However, some part of the effect is derived from other factors contained in conventional IL-2.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Manabe Medical Foundation. We thank Miss Mayumi Asakawa for her excellent technical assistance and Miss Yuka Nishina for her expert secretarial assistance. We thank also Dr. Reuben P. Siraganian for useful discussion.

REFERENCES

1. Ezeamuzie, I. C. & Assem, E. S. K.: A study of histamine release from human basophils and lung mast cells by products of lymphocyte stimulation. *Agents Actions* 13, 222-230, 1983.
2. Ida, S., Hooks, J. J., Siraganian, R. P. & Notkins, A. L.: Enhancement of IgE-mediated histamine release from human basophils by immune-specific lymphokines. *Clin. Exp. Immunol.* 41, 380-387, 1980.
3. Ida, S., Hooks, J. J., Siraganian, R. P. & Notkins, A. L.: Enhancement of IgE-mediated histamine release from human basophils by viruses: role of interferon. *J. Exp.*

- Med. 145, 892-906, 1977.
4. Morgan, D. A., Ruscetti, F. W. & Gallo, R.: Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 193, 1007-1008, 1976.
 5. Schulman, E. S., Post, T. J. & Vigderman, R. J.: Effects of T cell growth factor (TCGF) on human basophil and lung mast cell (HLMC) histamine release (HR). *J. Allergy Clin. Immunol.* 73, 45, 1984 (Abst).
 6. Sedgwick, J. D., Holt, P. G. & Turner, K. J.: Production of a histamine-releasing lymphokine by antigen- or mitogen-stimulated human peripheral T cells. *Clin. Exp. Immunol.* 45, 409-418, 1981.
 7. Siraganian, R. P. & Hook, W. A.: Histamine release and assay methods for the study of human allergy. In Rose, N. R. & Friedman, H. (eds.): *Manual of clinical immunology*, pp. 808-821. American Society for Microbiology, Washington, D.C., 1980.
 8. Thueson, D. O., Speck, L. S., Lett-Brown, M. A. & Grant, J. A.: Histamine-releasing activity (HRA). I. Production by mitogen- or antigen-stimulated human mononuclear cells. *J. Immunol.* 123, 626-632, 1979.
 9. Thueson, D. O., Speck, L. S., Lett-Brown, M. A. & Grant, J. A.: Histamine-releasing activity (HRA). II. Interaction with basophils and physicochemical characterization. *J. Immunol.* 123, 633-639, 1979.

Address:

Yutaka Morita, M.D., Ph.D.
Dept. of Medicine and Physical Therapy
Faculty of Medicine
University of Tokyo
7-3-1, Hongo, Bunkyo-ku
Tokyo, 113
Japan

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.