Effects of In Vivo Administration of Interleukin-2 (IL-2) and IL-4, Alone and in Combination, on Ex Vivo Human Basophil Histamine Release

By Martha V. White, Yasushi Igarashi, Brian E. Emery, Michael T. Lotze, and Michael A. Kaliner

Human basophils possess receptors for interleukin-2 (IL-2) and IL-4. The effect of 3 days of intravenous administration of IL-2 and/or IL-4 on basophil histamine release was examined in three groups of patients receiving IL-2, IL-4, or the combination of agents as part of a protocol to treat malignant melanoma or renal cell carcinoma. Because all patients received ranitidine for control of side effects, a control group of patients receiving ranitidine for Zollinger-Ellison's syndrome was also studied. IL-4 significantly inhibited IgE-mediated histamine release, while there was a trend for enhancement of IgE-mediated histamine release by IL-2. Administration of the combination of IL-2 and IL-4 did not alter IgE-mediated basophil histamine release. Both IL-2 and IL-4, alone and in combination, enhanced basophil histamine release induced by histamine releasing factors in human nasal washings. The effect of IL-2 alone was significantly greater than that of IL-4 alone or the combination of IL-2 plus IL-4. Taken together, the data suggest that when coadministered, IL-4 may inhibit the effects of IL-2 on basophils. Neither cytokine exerted any effect on basophil histamine release induced by the calcium ionophore A23187, nor did the combination of agents as part of a protocol to treat malignant melanoma or renal cell carcinoma. (1) six patients who received IL-4 (10 to 20 μg/kg/three times daily [tid] dose) alone; (2) seven who received the combination of IL-4 (2 to 6 μg/kg/tid dose) and IL-2 (216,000 to 720,000 IU/kg/tid dose); and (3) three who received IL-2 (216,000 to 720,000 IU/kg/tid dose) alone. The principal side effects of IL-4 consisted of malaise, nausea, vomiting, diarrhea, marked fluid retention, oliguria, and pulmonary congestion. Side effects of IL-4 included generalized edema, gastritis with ulceration, hypotension, oliguria, and severe nasal congestion without rhinorrhea, resistant to H-1 antihistamines and decongestants. All of these subjects received the H-2 antihistamine, ranitidine (10 mg intravenously [IV] per hour), to control the ulcerogenic side effects of IL-4 and/or IL-2. In addition, some subjects received one or more of the following cytokine administration on nasal mucosal and basophil reactivity were studied in these patients. The effects of in vivo administration of IL-4 on nasal reactivity are being published elsewhere. However, in that study, elevated levels of histamine were detected in the nasal secretions of patients who had received IL-4.23 We report here the effects of in vivo administration of IL-2 and/or IL-4 on basophil histamine release induced by anti-IgE, calcium ionophore A23187, and pooled human nasal secretions.

MATERIALS AND METHODS

Subjects. Four different groups of patients, 28 to 68 years of age, were studied. The first three groups consisted of patients participating in various arms of an NIH pilot protocol to treat either metastatic malignant melanoma or renal cell carcinoma: (1) patients who received IL-4 (10 to 20 μg/kg/three times daily [tid] dose) alone; (2) seven who received the combination of IL-4 (2 to 6 μg/kg/tid dose) and IL-2 (216,000 to 720,000 IU/kg/tid dose); and (3) three who received IL-2 (216,000 to 720,000 IU/kg/tid dose) alone. The principal side effects of IL-2 consisted of malaise, nausea, vomiting, diarrhea, marked fluid retention, oliguria, and pulmonary congestion. Side effects of IL-4 included generalized edema, gastritis with ulceration, hypotension, oliguria, and severe nasal congestion without rhinorrhea, resistant to H-1 antihistamines and decongestants. All of these subjects received the H-2 antihistamine, ranitidine (10 mg intravenously [IV] per hour), to control the ulcerogenic side effects of IL-4 and/or IL-2. In addition, some subjects received one or more of the following treatments.

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medications, as indicated: acetaminophen, furosemide, promethazine, triethylperazine, or droperidol. To control for the effects of ranitidine on histamine release, a control group of five patients receiving ranitidine (300 to 4,600 mg/d) for treatment of Zollinger-Ellison syndrome was also studied.

**Basophil histamine release.** Ten to 20 mL of peripheral blood were obtained with consent from each patient before receiving immunomodulator therapy, and at the completion of therapy (2 to 3 days). Blood was obtained from Zollinger-Ellison patients while on long-term ranitidine (300 to 4,600 mg/d) and after discontinuation of ranitidine for at least five half-lives. Basophil histamine release experiments were performed as previously described. Briefly, leukocytes were isolated over Histopaque 1.077 (Sigma, St Louis, MO), washed extensively in HEPES-buffered Hanks’ Balanced Salt Solution without calcium and magnesium (HBSS without C&M; Gibco, Grand Island, NY), and resuspended in 0.75 mL HEPES-buffered HBSS with C&M. The washed leukocytes (10 μL) were incubated with 75 μL HBSS (with or without each of three stimulants [A23187, 0.01 to 0.3 μg/mL [Sigma]; antihuman IgE, 0.03 to 1 μg/mL [Kirkegard & Perry, Gaithersburg, MD]; or pooled nasal washings [1:27-full strength]) for 60 minutes at 37°C. Reactions were terminated by the addition of ice-cold buffer followed by centrifugation and the supernatants removed. Histamine was assayed both in the supernatants and in lysed, unstimulated cells using a commercially available radioimmunoassay (AMAC, Westbrook, ME). Net percent histamine release is defined as histamine in supernatants from stimulated cells minus histamine in supernatants from cells incubated in buffer, divided by total cellular histamine.

**Preparation of human nasal washings.** Nasal washings were obtained and pooled from eight atopic and nonatopic healthy volunteers as previously described. Briefly, an 8 French soft rubber catheter was inserted atraumatically 3 to 4 cm along the floor of each nares and attached to suction. Normal saline sprayed into each nares and the washed secretions collected into a suction trap under constant suction. Secretions were pooled into a single batch that was aliquotted, stored at -80°C, and used throughout the study.

**Statistics.** Differences in stimulant-induced histamine release preimmunomodulator treatment versus postimmunomodulator treatment were analyzed by Wilcoxon signed rank when individual data points were being compared, and by three-way analysis of variance (ANOVA) when entire dose-response curves were being compared. When dose-response curves were found to vary significantly, stimulant dose effects and differences between stimulants were analyzed post hoc by Fisher’s protected least significant difference (LSD) test.

**RESULTS**

**Effects on IgE-mediated histamine release.** To determine whether IL-4 or IL-2 affected IgE-mediated basophil histamine release, basophils were obtained before and after 3 days of IV IL-2 and/or IL-4 treatment, incubated with anti-IgE (0.03 to 1 μg/mL), and histamine release measured. The results are presented in Fig 1. Anti-IgE caused equivalent amounts of dose-related histamine release from all four groups. Neither IL-2 nor IL-4 nor ranitidine had any effect on basophil responsiveness to anti-IgE. There were also no significant differences found in the IL-2 group, although there was a trend towards enhancement of anti-IgE–induced histamine release at the highest dose of anti-IgE (19 ± 6.7 vs. 25.7 ± 12.8, preinduction and postinduction, respectively; P = .1, N = 3). The maximal histamine release induced by anti-IgE (the optimal dose of anti-IgE varied among subjects) decreased after IL-4 (43.6 ± 4.26 ± 11 before and after IL-4, respectively), but the differences did not achieve statistical significance for this or any of the other treatment groups (Table 1). However, analysis of the entire dose response curve by three-way ANOVA indicated that IL-4 significantly (P < .05) reduced anti-IgE–induced histamine release (Fig 1). Thus, IL-4 administered IV for 3 days inhibited ex vivo IgE-mediated basophil histamine release, while there is a trend for enhancement by IL-2.

**Effects on histamine release induced by nasal washings.** One of the dose-limiting side effects of IL-4 is nasal congestion, and nasal washings from patients receiving IL-4 contain increased levels of histamine. The effect of IL-2 and/or IL-4 treatment in vivo on basophil histamine release induced in vitro by unstimulated human nasal washings was therefore examined. Undiluted nasal washings induced low amounts of histamine release from basophils of all four groups before treatment (range, 6.5% to 13.1% net histamine release). Treatment with IL-2, IL-4, and with IL-2 plus IL-4 generally resulted in enhanced basophil histamine release across the dose-response induced by nasal washings (P = .03, .05, and .02, respectively by ANOVA) (Fig 2). The enhancement by IL-2 was significantly greater than that induced by IL-4 alone and by the combination of IL-2 and IL-4 (P < .001, by Fisher’s protected LSD test). Ranitidine had no effect at any dose of nasal washings on nasal washings-induced histamine release. The magnitude of histamine release induced by full-strength nasal washings was also increased after IL-4 (6.5% ± 6.2% ± 15.3% ± 7.6%; P = .04, by Wilcoxon signed rank), but not IL-2 plus IL-4 treatment. There was a trend for the magnitude of histamine release induced by full-strength nasal washings to increase after IL-2 (14.1 ± 10.1 ± 58.3 ± 25.2, preinduction vs. postinduction, respectively; P = .1, by Wilcoxon signed rank, N = 3) but the increase was not statistically significant (Table 1). Thus, both IL-4 and IL-2 caused augmenta-

### Table 1. Effect of Therapy on Maximal Basophil Histamine Release Induced by Anti-IgE, Nasal Washings, and Calcium Ionophore A23187

<table>
<thead>
<tr>
<th>Condition</th>
<th>Anti-IgE (0.03-1 μg/mL)</th>
<th>Nasal Washings (1/27-Nael)</th>
<th>A23187 (0.01-0.3 μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>54.8 ± 13.7</td>
<td>14.4 ± 10.1</td>
<td>66.4 ± 23.1</td>
</tr>
<tr>
<td>Post</td>
<td>42.7 ± 11.2</td>
<td>58.3 ± 25.2</td>
<td>44.2 ± 8.7</td>
</tr>
<tr>
<td>Pre</td>
<td>43.6 ± 14.1</td>
<td>6.8 ± 6.2*</td>
<td>28.9 ± 10.9</td>
</tr>
<tr>
<td>Post</td>
<td>26.0 ± 11.6</td>
<td>15.3 ± 7.6</td>
<td>25.3 ± 9.1</td>
</tr>
<tr>
<td>Pre</td>
<td>36.6 ± 15.7</td>
<td>7.5 ± 2.8</td>
<td>35.7 ± 10.2</td>
</tr>
<tr>
<td>Post</td>
<td>29.6 ± 10.6</td>
<td>14.5 ± 5.3</td>
<td>37.0 ± 7.7</td>
</tr>
<tr>
<td>Pre</td>
<td>32.8 ± 8.9</td>
<td>13.1 ± 4.4</td>
<td>50.9 ± 11.1</td>
</tr>
<tr>
<td>Post</td>
<td>34.7 ± 13.0</td>
<td>18.4 ± 13.0</td>
<td>42.7 ± 11.7</td>
</tr>
</tbody>
</table>

*P < .05 by Wilcoxon ranked sign test.
EFFECT OF IL-2 AND IL-4 ON BASOPHILS

**Fig 1.** Effect of IV IL-2, IL-4, IL-2 plus IL-4, and oral ranitidine on net percentage basophil histamine release induced by various concentrations (0.03 to 1 pg/mL) of anti-IgE. Basophils were obtained before (0) and after (0) 3 days of receiving either (A) IL-2 (N = 3), (B) IL-4 (N = 6), or (C) the combination of IL-2 and IL-4 (N = 7). Basophils were obtained from patients receiving ranitidine at steady-state levels (0) and after stopping ranitidine treatment (0) for at least five half-lives (D) (N = 5). All data are presented as mean ± SEM. Pre- and post-IL-4 histamine release curves were significantly different (P = .05, three-way ANOVA).

**Fig 2.** Effect of IV IL-2, IL-4, IL-2 plus IL-4, and oral ranitidine on net percentage basophil histamine release induced by various concentrations (1/27 to neat) of pooled human nasal washings. Basophils were obtained before (0) and after (0) 3 days of receiving either (A) IL-2 (N = 3), (B) IL-4 (N = 6), or (C) the combination of IL-2 and IL-4 (N = 7). Basophils were obtained from patients receiving ranitidine at steady-state levels (0) and after stopping ranitidine treatment (0) for at least five half-lives (D) (N = 5). All data are presented as mean ± SEM. *P ≤ .05 comparing responses to individual doses of nasal washings pretreatment and posttreatment (Wilcoxon signed rank). The following pretreatment and posttreatment curves were significantly different from each other as compared by three-way ANOVA: IL-2, P = .03; IL-4, P = .05; IL-2 plus IL-4, P = .02.

**DISCUSSION**

This is the first report of the effects of in vivo administration of IL-2 and/or IL-4 on basophil histamine release. IgE-mediated histamine release was inhibited by IL-4; however, this effect was lost when IL-4 was administered in conjunction with IL-2. In contrast, there was a trend towards enhancement by IL-2 of IgE-mediated histamine release, supporting the in vitro observations of Morita et al.29 When IL-2 and IL-4 were administered together, the combined actions resulted in no net change in basophil responsiveness to anti-IgE. These opposing effects of IL-2 and IL-4 are not surprising, and have been observed in other systems. IL-4 has been shown to block the upregulation of IL-2 receptors induced by IL-2 in normal human B cells.25 The effect of IL-4 on basophil IL-2 receptors is not known. The inhibitory effect of IL-4 on IgE-mediated basophil histamine release was unexpected, however, because other basophil growth factors, such as IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), induce and/or enhance IgE-mediated histamine release.12-15

Patients receiving IV IL-4 had elevated levels of histamine in their nasal secretions.24 Pooled crude nasal washings from normal atopic and nonatopic volunteers caused basophil histamine release from all four groups of patients. IV administration of both IL-4 and IL-2 enhanced basophil histamine release induced by subsequent exposure to crude preparations of nasal washings, although the effect of IL-2 was significantly greater than the effect of IL-4. Interestingly, the combination of IL-2 and IL-4 resulted in an effect equivalent to that of IL-4 alone. These data suggest that IL-4 dampened the actions of IL-2, possibly by inhibiting...
were obtained before whereby IL-4 and IL-2 enhance nasal washings-induced secretions (0.01 to 0.3 μg/mL) of calcium ionophore A23187. Basophils were obtained before \( \square \) and after \( \textcircled{O} \) 3 days of receiving either (A) IL-2 \( (N = 3) \), (B) IL-4 \( (N = 6) \), or (C) the combination of IL-2 and IL-4 \( (N = 7) \). Basophils were obtained from patients receiving ranitidine at steady-state levels \( \textcircled{O} \) and after stopping ranitidine treatment \( \square \) for at least five half lives \( \textcircled{D} \) \( (N = 9) \). All data are presented as mean ± SEM. No significant differences were observed.

Fig 3. Effect of IV IL-2, IL-4, IL-2 plus IL-4, and oral ranitidine on net percentage basophil histamine release induced by various concentrations \( (0.01 \text{ to } 0.3 \text{ μg/mL}) \) of calcium ionophore A23187. Basophils were obtained before \( \square \) and after \( \textcircled{O} \) 3 days of receiving either (A) IL-2 \( (N = 3) \), (B) IL-4 \( (N = 6) \), or (C) the combination of IL-2 and IL-4 \( (N = 7) \). Basophils were obtained from patients receiving ranitidine at steady-state levels \( \textcircled{O} \) and after stopping ranitidine treatment \( \square \) for at least five half lives \( \textcircled{D} \) \( (N = 9) \). All data are presented as mean ± SEM. No significant differences were observed.

upregulation of basophil IL-2 receptors. The mechanism whereby IL-4 and IL-2 enhance nasal washings-induced basophil histamine release is not known. One possible explanation is suggested from the observation that IL-4 inhibits the expression of IL-8 from stimulated monocytes.26 There is evidence to suggest that histamine release inhibitory factor, which specifically inhibits cytokine-, but not IgE-induced, basophil histamine release, is IL-8.27 Nasal washings contain several factors of presumed inflammatory cell origin that induce basophil histamine release.28 Thus, it is possible that the production of IL-8 by blood monocytes26 or neutrophils21 might inhibit basophil histamine release when crude leukocytes are used as the basophil source.

The identity of the histamine-releasing factor in nasal lavage fluids that was modulated by IL-2 and IL-4 could not be examined in these patients. A 25 to 50 Kd histamine releasing factor that does not bind to DE52 and is IgE dependent has been described in human nasal secretions.29 However, we did not wish to limit our examination of nasal secretions to this one factor. Subsequent purification of the factor(s) responsible for inducing histamine release would have been desirable; however, there were limited numbers of patients receiving IL-2 and/or IL-4 who were available for study. The patients studied were subjected to multiple procedures and blood draws for clinical as well as various research purposes. In addition, many patients developed anemia from their immunomodulatory therapy. Thus, it was not ethical to obtain enough blood to provide sufficient basophils to screen column fractions for individual histamine-releasing factors to which the patients under study had become hyperresponsive.

Neither IL-2 nor IL-4 had any effect on basophil histamine release stimulated by the calcium ionophore A23187. This is in contrast to a previous report that IL-2 enhances A23187-induced basophil histamine release.29 That study, however, was performed in vitro using a short preincubation with IL-2, while in the current study subjects were treated in vivo for 3 days with IL-2 before their basophils were tested.

To control for the effects of ranitidine, which all subjects received, a separate group of patients receiving high-dose ranitidine for treatment of Zollinger Ellison's syndrome were studied on and off of ranitidine. Ranitidine caused no alterations in basophil reactivity to anti-IgE, nasal washings, or A23187, suggesting that the effects seen in the IL-2, IL-4, and IL-2 plus IL-4 groups were due to the cytokines administered. These results are in contrast with previous observations in which ranitidine, in low doses, inhibited IgE-mediated histamine release, while at doses above \( 10^{-4} \) mol/L ranitidine, augmented histamine release.30 Those results, however, do not necessarily contradict the results presented herein because the referenced study used in vitro administration of ranitidine, while the present study examined the effects of oral ranitidine. The subjects studied were ill and received a variety of other medications, as indicated and outlined above. Controlling for the effects on basophils of these other drugs was not technically feasible, because these other drugs were not received by all subjects and there was no uniformity in the prescription of these drugs amongst those who received them. However, none of these other drugs are known to have the potential to affect basophil function.

In conclusion, IL-2 and IL-4 administered intravenously for 3 days can enhance basophil reactivity to a histamine releasing factor(s) in human nasal washings. This effect may explain the increased histamine levels in nasal lavages of patients receiving IV IL-4. In addition, IL-4 inhibits IgE-mediated basophil histamine release. In general, the effects of IL-2 and IL-4 were opposing. Thus, while IL-4 alone inhibited IgE-mediated histamine release, this effect was negated when IL-2 was administered concomitantly. Likewise, the enhancement by IL-2 of nasal wash-induced histamine release was significantly greater than that of IL-4, but the effect of the combined cytokines was only equal to that of IL-4 alone, suggesting that IL-4 had inhibited the effect of IL-2. Thus, basophils have receptors for IL-2 and IL-4, and in vivo administration of these cytokines can influence basophil reactivity. However, the effect of IL-2 and IL-4 on basophils in health and disease is unclear, and is likely to be dependent on a complex interaction of IL-2 and IL-4 with each other and with other immunomodulators.
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