

The effect of IL-4 on human nasal mucosal responses

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Interleukin (IL)-4 causes the dose limiting sensation of nasal congestion when administered systemically at doses of 3 µg/kg or higher thrice daily to humans. This side effect was observed in a group of patients treated as part of an immunotherapy protocol for cancer management. To determine the source of this congestion, nasal secretions were collected prospectively in a group of patients at baseline and after provocation with normal saline, methacholine (which stimulates glandular secretion), and histamine (which causes increased vascular permeability). Nasal lavages obtained at baseline and after provocation were analyzed for the presence of these glandular and vascular proteins and inflammatory mediators. Washings and provocations were performed before IL-4 administration, after 24 hours of IL-4 treatment, and after 3 days of treatment, at a time when nasal congestion was maximal. Compared with histamine challenge before IL-4 treatment, the secretion of the plasma proteins albumin and IgG were significantly decreased after 3 days of IL-4 treatment. IL-4 treatment had no apparent effect on methacholine-induced responses. Thus systemically administered IL-4 causes the subjective sensation of nasal congestion, increased histamine in nasal lavages, and the development of vascular unresponsiveness to histamine, without affecting parasympathetic responses to histamine. The relationships among increases in nasal lavage histamine, vascular unresponsiveness to histamine, and the sensation of nasal congestion are unclear. (J ALLERGY CLIN IMMUNOL 1992;90:772-81.)

Key words: Nasal congestion, IL-4, cancer nasal challenge

Human IL-4 stimulates both peripheral blood and thymic T-cell proliferation.^{1,2} Activated T-cell growth (1) and allospecific cytotoxic T-lymphocyte generation are augmented by IL-4 in vitro.^{1,2} IL-4 in conjunction with IL-2 augments the growth of tumor infiltrating lymphocytes, apparently specific for autologous melanoma and induces lymphokine activated

Abbreviations used

NS: Normal saline
IL-4: Interleukin-4
LTC₄: Leukotriene C₄
RGC: Respiratory glucoconjugates
PGD₂: Prostaglandin D₂

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killer activity in cells preactivated by IL-2.^{3,4} These findings suggest that IL-4 might have a role in cancer therapy, particularly if used in conjunction with IL-2 and/or tumor infiltrating lymphocytes.

To assess the antitumor response to IL-4, IL-4 was administered in a clinical protocol in doses up to 30 µg/kg thrice daily to a series of patients with cancer. In addition to generalized edema, occasional gastritis, or gastric ulceration, hypotension, and oliguria, the patients had the subjective sensation of severe nasal congestion with little or no rhinorrhea. These complaints of nasal congestion were volunteered by the patients without prompting from their physicians. No

associated allergic symptoms occurred such as pruritus, urticaria, or sneezing. Subjectively, the dose limiting factor for IL-4 administration was often the discomfort caused by the nasal congestion. To determine the most appropriate therapy for the nasal congestion, we studied the effect of IL-4 on nasal responsiveness to histamine and cholinergic stimulation.

The blood vessels of the nasal mucosa are functionally described as resistance, exchange, and capacitance vessels. The tone of the capacitance vessels, including the cavernous sinusoids, determines nasal patency.⁵ The neurologic control of the capacitance vessels is primarily through sympathetic regulation by constriction,^{5, 6} although recent evidence suggests a role for parasympathetic and nonadrenergic, noncholinergic vasodilatory influences.⁷⁻¹¹ Parasympathetic innervation of the nose regulates glandular secretion because cholinergic fibers are numerous around glands.¹² Recent findings indicate that there are specific M₃ muscarinic receptors both on the gland cells and around the capacitance blood vessels leading to the venous sinusoids, suggesting that parasympathetic input may participate in sinusoidal filling, much as is noted in other organs that have a sinusoidal vascular bed.¹³

Raphael et al.^{14, 15} have demonstrated that nonsecretory IgA, albumin, and IgG in nasal secretions are primarily derived from vascular sources and are disproportionately increased in nasal secretions after topical histamine or antigen challenge. They also demonstrated that secretory IgA,¹⁶ lactoferrin, and lysozyme¹⁷ are glandular products, increasing disproportionately after cholinergic or gustatory stimulation.

We used measurement of baseline secretory products as well as the secretory response to histamine and methacholine nasal provocations before and during IL-4 infusions to determine the possible role of histamine and cholinergic stimulation in IL-4-induced nasal congestion.

METHODS

Patients

Two different groups of patients were studied. The first group of patients received IL-4 as part of a surgery branch, National Cancer Institute Clinical Research Committee approved protocol to treat patients with metastatic malignant melanoma or renal cell carcinoma. Eight selected adult subjects, 28 to 68 years of age, were studied after informed consent was obtained. Three of these patients were only studied at baseline and after 24 hours of IL-4 treatment. These patients discontinued the protocol because of unbearable nasal discomfort. They were, however, able to

tolerate the portion of the protocol needed to assess the levels of the mediators prostaglandin D₂ (PGD₂), leukotriene C₄ (LTC₄), and histamine in nasal lavages. The data on these three patients are included in the mediator section only. The other five patients included three men and two women who received IL-4 for 3 days. These patients received 10 to 20 µg/kg/dose of IL-4 thrice daily over 15 minutes (IL-4 kindly provided by Sterling Pharmaceuticals, Malvern, Pa.). Each patient had the sensation of significant nasal congestion by the second day of treatment. Two of these patients had computerized tomographic scans of their sinuses. Both were noted to have mucosal thickening of their turbinate tissue and sinuses but no air-fluid levels. In addition to IL-4, patients were also given ranitidine (10 mg intravenously per hour) and often acetaminophen (459 mg by mouth every hour), furosemide, promethazine, triethylperazine and/or droperidol were used to control the effects of IL-4. Subjects were not studied within 3 weeks after recovery from an upper respiratory infection.

The second group of patients (*N* = 5) were diagnosed as having Zollinger-Ellison syndrome. These patients were being treated with between 300 to 4600 mg/day of ranitidine, either orally or intravenously. This group of subjects had no nasal complaints and was used to control for the effects of ranitidine.

Nasal provocations

As previously described, subjects were seated comfortably in an upright position.¹⁶ A soft 8F rubber catheter was atraumatically inserted 2 to 4 cm along the floor of the right nostril and was connected to suction to collect nasal secretions and lavage fluid. Catheter insertion causes no subjective irritation and does not induce nasal secretions and has been well tolerated by greater than 900 subjects previously studied. A hand-held nebulizer bottle was used to deliver nasal washes and challenges. In each experiment four sequential washes with normal saline solution (4 ml each) were collected at 1-minute intervals to remove preexisting secretions and to reduce nasal proteins to baseline levels. The first of these washes was reserved for the measurement of inflammatory mediators. After these four washes the nasal challenge protocols formally began. All challenge solutions were delivered to the right nasal cavity in a volume of 0.3 ml. Three consecutive challenges were performed. The first challenge was normal saline solution (0.3 ml). The second challenge was 25 mg/0.3 ml of methacholine, and the last challenge was 1 mg/0.3 ml of histamine. Each challenge was followed by a 10-minute incubation period and then by a 4 ml saline lavage. Lavage samples were kept on ice until the end of the experiment, at which point they were stored at -70° C for subsequent analysis. This challenge scheme was performed before the administration of IL-4, 24 hours after IL-4 therapy was initiated, and after 3 days of IL-4 treatment. The control group with Zollinger-Ellison syndrome was challenged while receiving ranitidine and again when ranitidine levels were felt to be insignificant. Insignificant levels were considered to be present five half-lives or more after the drug had been discontinued.

Assays

All assay results are reported as mean $\pm$ SEM. Assay methods for total protein (Lowry method), albumin (competitive ELISA), total IgA (direct noncompetitive ELISA), secretory IgA (direct noncompetitive ELISA),¹⁴ IgG (direct noncompetitive ELISA),¹⁵ lactoferrin (modified noncompetitive ELISA), and lysozyme (turbidimetric assay)¹⁷ have been described in detail. Nonsecretory IgA was calculated by subtracting secretory IgA from the total IgA. The relative percentages of each specific protein were calculated by dividing the concentration of the specific protein by the total protein and multiplying by 100%.

For PGD₂ analysis, the PGD₂ [³H] assay system (Amersham Co., Arlington Heights, Ill.) was used. [14, 15-³H(N)]-LTC₄ (New England Nuclear, Boston, Mass.), LTC₄ standard, LTC₄/D₄ antibody (Advanced Magnetics Inc., Cambridge, Mass.), and the histamine radioimmunoassay kit (AMAC Inc., Westbrook, Maine) were used for the measurement of LTC₄/D₄ and histamine by competitive radioimmunoassay.^{18, 19} The PGD₂ antibody had 7% cross-reactivity with PGJ₂ and less than 1% cross-reactivity with other major prostaglandins or thromboxane. Because the LTC₄/D₄ antibody has comparable cross-reactivity with LTC₄ and LTD₄ (64% for LTC₄, 100% for LTD₄), the amount of LTC₄/D₄ was expressed as LTC₄ equivalents.

Skin test methods

Patients were skin tested before starting IL-4 and on day 3 or 4 at the end of the infusion. To assess changes in reactivity to histamine skin tests, serial dilutions of histamine diphosphate (2.75, 0.9, 0.3, 0.1, 0.033, and 0.01 mg/ml) were used. The reactivity of the volar surface of the patient's arm was examined with use of epicutaneous testing. After 15 minutes the wheals and flares were measured in the largest vertical and horizontal diameters. These two numbers were added to yield a sum of the crossed diameters at each dilution.

Human nasal mucosal explant culture

Human inferior turbinates were obtained at the time of surgery from a separate group of five patients with chronic nasal obstruction. At the time of surgery, 2% tetracaine hydrochloride and 0.25% phenylephrine hydrochloride were applied topically on nasal packs. The turbinate tissues were injected with 2 to 4 ml of 1% lidocaine with 1:100,000 epinephrine. An incision was made from the lateral wall of the inferior turbinate bone at the level of the infundibulum, through the inferior conchal bone, and then inferiorly along the medial aspect of that bone. The medial flap of turbinate bone that remained was wrapped superiorly and laterally to close the wound. Within 20 minutes of the surgical excision, the nasal mucosa was dissected from the inferior conchal bone. Mucosal specimens for explant culture were placed in L-15 media (Gibco Laboratories, Grand Island, N.Y.) at 4° C supplemented with penicillin (100 µg/ml), streptomycin (100 µg/ml), and amphotericin (0.5 µg/ml) for transport to the laboratory.

To examine the effects of methacholine and recombinant

human IL-4 on nasal glandular secretion, the release of respiratory glycoconjugates (RGCs) (from mucous cells) and lactoferrin (from serous cells) from mucosal fragments were measured. Human nasal mucosa was cut into 3 × 3 mm fragments. Pairs of fragments were placed on gelfoam in petri dishes, and cultured in 2 ml CMRL 1066 containing penicillin (100 µg/ml), streptomycin (100 µg/ml), and amphotericin (0.5 µg/ml) (Grand Island Biologicals, Grand Island, N.Y.). The atmosphere was controlled by purging the culture chamber for 5 minutes with 45% O₂, 50% N₂, and 5% CO₂ before sealing. The explants were incubated at 37° C. The chamber was placed on a rocking platform at 7 cycles per minute.

RGCs were labeled with [³H]-glucosamine (1 µCi/ml) (New England Nuclear) by its incorporation into the culture media. After 48 hours the media was replaced, and nasal mucosal explants were incubated for a 1-hour baseline period (period 1). The culture supernatants were collected at the end of this period, and the cultures were washed with 2 ml of CMRL media. Fresh media alone (control dishes) or media with different doses of recombinant human IL-4 (Genzyme Co., Boston, Mass.) and/or methacholine (Sigma Chemical Co., St. Louis, Mo.) were added for different periods of time (1 and 12 hours, respectively) (period 2). Supernatants from periods 1 and 2 were collected and frozen at -70° C for quantitation of lactoferrin and RGCs.

Measurement of RGCs

Two milliliter aliquots were added to 2 ml of 20% trichloroacetic acid and 2% phosphotungstic acid. After 16 hours at 4° C the samples were centrifuged for 10 minutes at 2000 g and the supernatant decanted. The pellet was washed twice with 1 ml of 10% trichloroacetic acid and 1% phosphotungstic acid. The pellet was suspended in 1 ml of 0.1N NaOH and kept at room temperature. After 16 hours the suspensions were spun in a centrifuge and 0.5 ml aliquots placed in 5 ml of scintillation fluid. Tritium decay was quantitated in a scintillation counter. The secretory index was calculated (disintegrations per minute) for period 2/period 1 for each plate. The results of each treatment and control experiment were determined, and the percent change from control for each treatment was calculated. Each experiment involved quadruplicate plates and was repeated several times.

Statistics

The Wilcoxon signed rank test for paired samples was used in all data analyses. Results are presented as mean $\pm$ SEM.

RESULTS

LTC₄, PGD₂, and histamine

LTC₄, PGD₂, and histamine were measured in the baseline secretions of a group of eight patients before IL-4 infusion and 24 and 72 hours after IL-4 infusion. Only five patients were available for study at 72 hours, and one was studied twice during two different courses

of IL-4. An increase in histamine was seen after 24 hours that did not reach statistical significance ($p = 0.09$; Fig. 1, A). The PGD_2 levels increased significantly at 72 hours ($p < 0.05$, Fig. 1, B). The LTC_4 levels did not change in the three collections (baseline, after 24 hours of treatment, and at 72 hours) (Fig. 1, B). The five patients available for study at 72 hours comprise the group described below.

Secretory proteins

Five patients were studied during six different treatment cycles. (One patient was studied twice during a second IL-4 course.) To determine changes in nasal responses caused by IL-4, a comparison was made for each patient between baseline unstimulated nasal washes (the first prewash) obtained before and after receiving IL-4 for 72 hours. No statistically significant changes were seen in the unstimulated levels of glandular (lysozyme, lactoferrin, secretory IgA) and vascular (albumin, IgG, nonsecretory IgA) proteins either when expressed as absolute amounts or as a percentage of the total protein. Secretory responses to histamine and methacholine after 24 hours of IL-4 treatment also did not differ significantly from pre-IL-4 responses (data not shown).

Histamine challenge

The analyses of the nasal secretory responses to histamine challenges before and after 3 days of receiving IL-4 are shown in Fig. 2. In each provocation, results were compared between histamine and saline challenges. Before receiving IL-4 infusions (Fig. 2, *solid lines, closed squares*), the subjects responded normally to topical histamine challenge. Histamine caused an increase in total protein, albumin, albumin percent, and IgG; each of these was significantly increased compared with baseline values with $p < 0.05$. IgG percent was not increased significantly. These data indicate that at baseline the subjects responded normally to histamine with increased vascular permeability.

After 3 days of IL-4 treatment, histamine no longer produced significant increases in albumin, IgG, albumin percent or IgG percent compared with the saline challenges (Fig. 2, *dashed lines, solid triangles*). Indeed, after 72 hours of IL-4 treatment, the proportion of total protein comprised of albumin (albumin percent) and IgG (IgG percent) actually fell after histamine administration ($p < 0.05$, Fig. 3). Thus the vascular response to histamine is reduced after IL-4 administration.

Histamine reflexively stimulates glandular secretion by interacting with H1 receptors on nerve endings and by initiating a parasympathetic discharge.¹⁴ The pro-

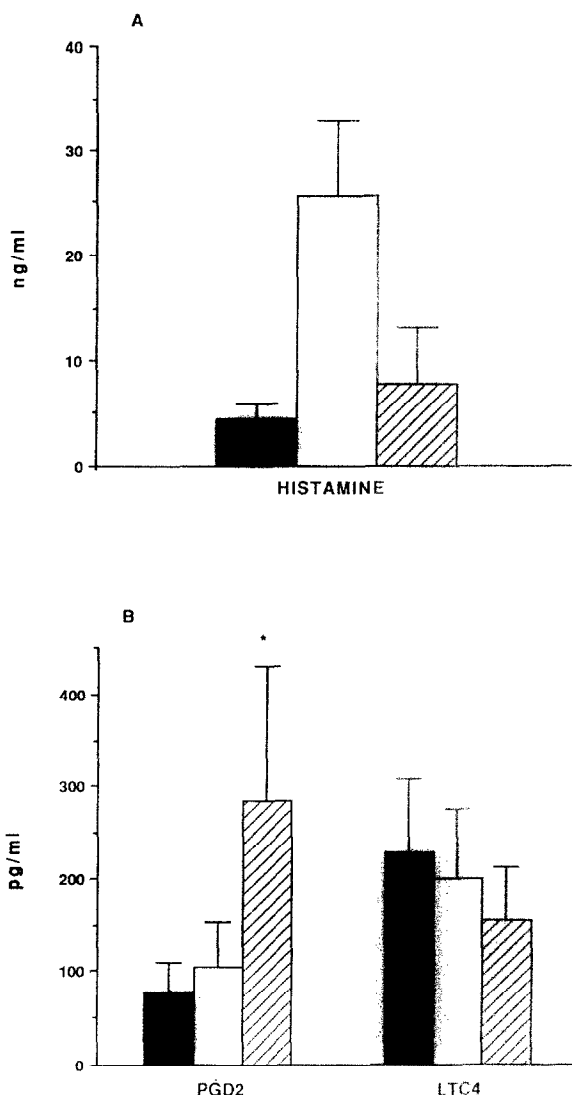


FIG. 1. Effect of IL-4 on histamine, PGD_2 , and LTC_4 content of baseline nasal secretions. Nasal secretions were collected before (filled bar), after 24 hours (open bar), or after 72 hours (striped bar) of IL-4 treatment. * $p < 0.05$ as compared with the baseline collection. $N = 8$ for the baseline and 24-hour treatment data and $N = 5$ for the 72-hour treatment data.

portion of the total nasal lavage protein attributable to the glandular markers lactoferrin and lysozyme (Fig. 3) were significantly increased ($p < 0.05$) in response to histamine after 3 days of IL-4 as compared with samples obtained before IL-4 exposure. In contrast, the absolute concentrations of lactoferrin and lysozyme in histamine-induced secretions were not changed by IL-4 (data not shown), suggesting that proportional changes reflected a reduction in vascular proteins. The relative proportion of IgA and nonsecretory IgA (a plasma protein) in histamine-induced

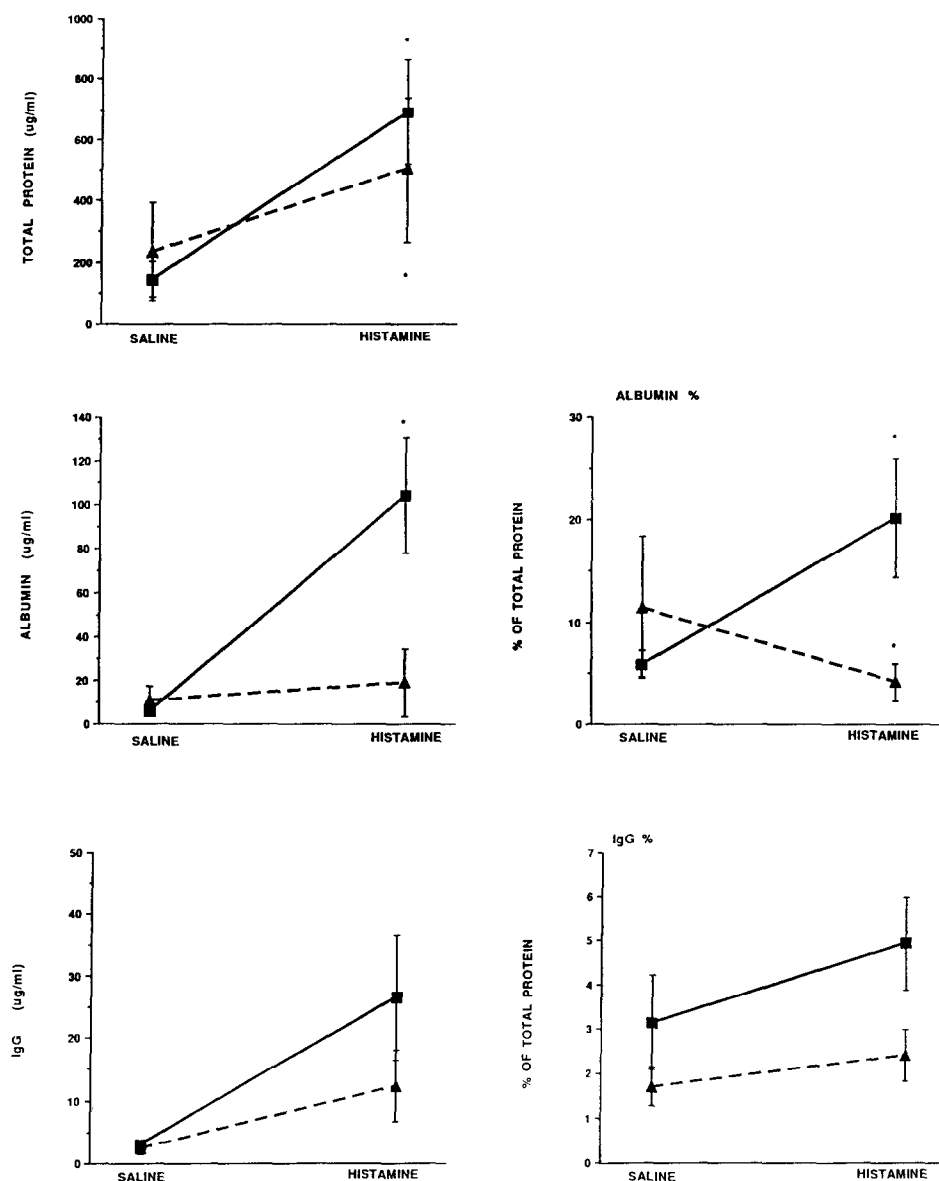


FIG. 2. Effect of 3 days of IL-4 treatment on histamine-induced protein secretion compared with saline challenges. The response to histamine (1 mg/0.3 ml) was compared before (*solid lines, filled squares*) and after 72 hours (*dashed lines, filled triangles*) of IL-4 therapy. Under baseline conditions, histamine caused a significant increase in total protein, albumin, IgG, and albumin percent in nasal washings. *Asterisks* indicate comparisons between increases in these proteins after 72 hours of IL-4 infusion compared with baseline results (* $p < 0.05$, ** $p < 0.01$). Results after IL-4 infusion were significantly reduced compared with baseline responses for total protein, albumin, IgG, and albumin percent. There was no change in the incremental increase in IgG percent comparing pre- and post-IL-4 treatment days (* $p < 0.14$). $N = 5$.

nasal lavage fluids were not affected by IL-4 treatment. It is interesting to note that histamine-induced sneezing, which is also neurally mediated, was not reduced after IL-4 treatment (sneezes induced during the histamine provocations: 5.63 ± 1.19 sneezes before IL-4 infusion versus 3.4 ± 1.89 after IL-4 in-

fusion, $p = 0.46$). Thus IL-4 blunts the vascular response to histamine without affecting the reflex glandular or sneezing responses. Secretory responses to histamine and methacholine after 24 hours of IL-4 treatment also did not differ significantly from pre-IL-4 responses (data not shown).

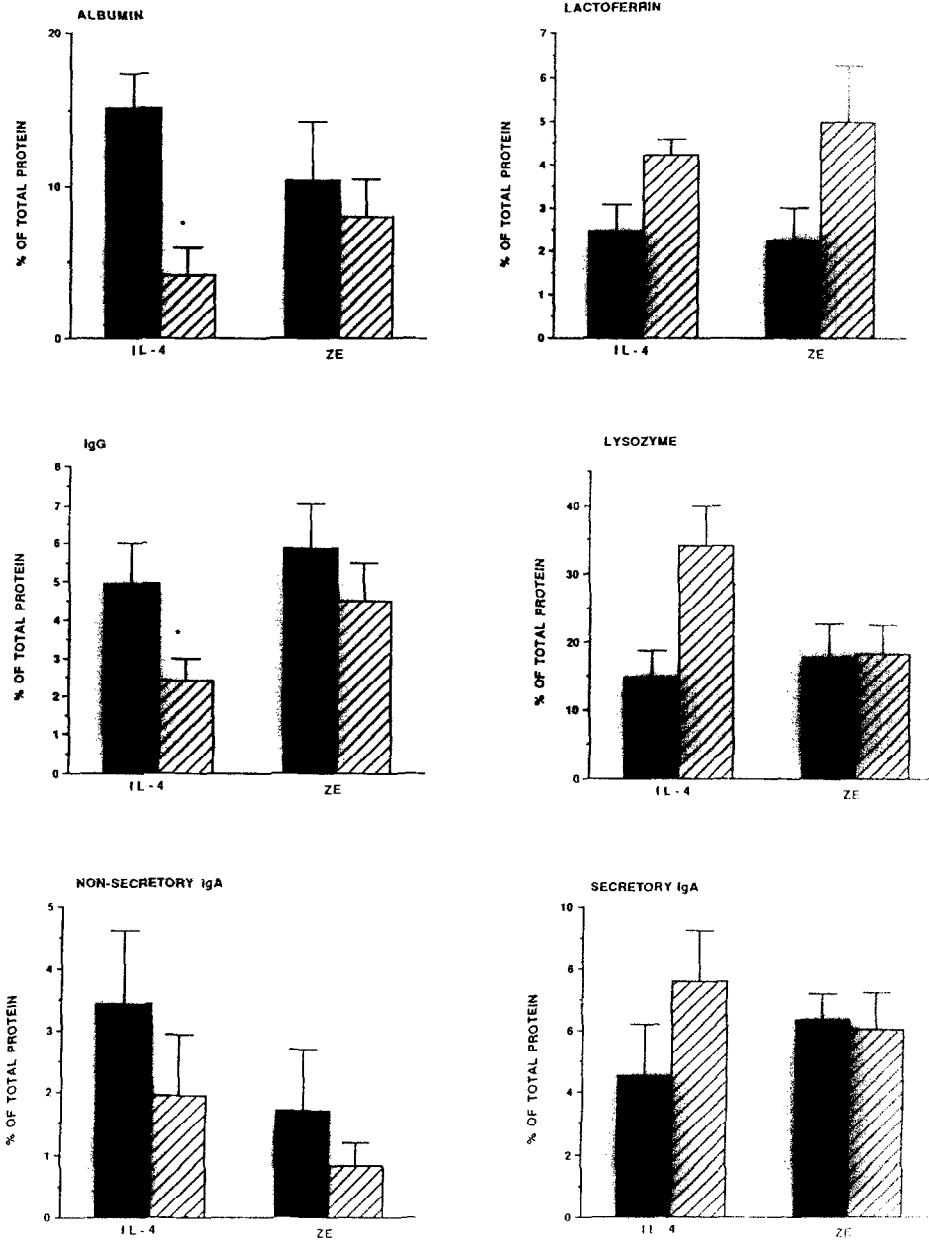


FIG. 3. Effect of 3 days of IL-4 ($N = 5$) or ranitidine (Zollinger-Ellison, $N = 5$) treatment on histamine-induced nasal protein secretions. The response to histamine (1 mg/0.3 ml) is compared before (filled bar) or after 72 hours (striped bar) of IL-4 therapy in patients receiving IL-4 therapy. The patients with Zollinger-Ellison syndrome were studied either after having stopped ranitidine (filled bar) or while receiving ranitidine (striped bar). * $p < 0.05$, *** $p < 0.001$, comparing baseline secretory responses with those on treatments. IL-4 caused a blunted vascular response (significantly reduced albumin and IgG secretion, $p < 0.005$ and 0.05 , respectively), whereas an increase in the secretion of the glandular products lysozyme ($p < 0.05$) and lactoferrin ($p < 0.05$) were seen. Ranitidine had no effect on histamine-induced secretory responses.

Patients with Zollinger-Ellison syndrome

To determine whether the decrease in the response to histamine was due to the effects of IL-4 and not to concomitantly administered ranitidine, which these

patients were receiving in high doses (10 mg/hr), a control group of patients with Zollinger-Ellison syndrome were also studied ($N = 5$, Fig. 3). These patients were also receiving ranitidine at high dose and

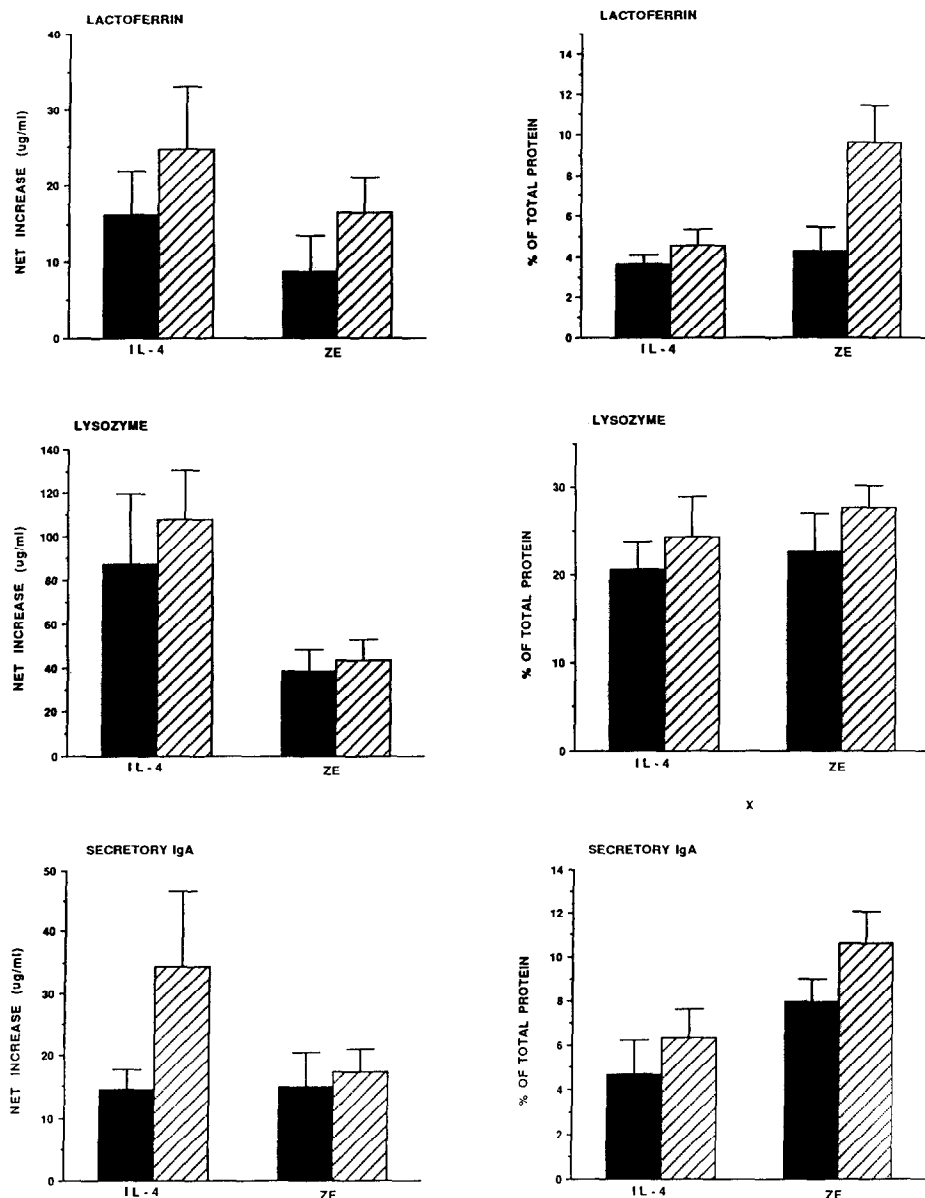


FIG. 4. Comparison of the effects of 3 days of IL-4 ($N = 5$) or ranitidine (Zollinger-Ellison, $N = 5$) treatment on methacholine (25 mg/0.3 ml)-induced nasal glandular secretions. Patients were studied before (filled bar) or after 72 hours (striped bar) of receiving IL-4 (IL-4 patients) or ranitidine (patients with Zollinger-Ellison syndrome) treatment. No IL-4 or ranitidine-induced effects were seen.

were studied while on the agent and after cessation for at least 5 half-lives of the drug. No statistically significant differences occurred in the relative portions of total protein for the vascular markers albumin, IgG, or nonsecretory IgA nor for the glandular markers lactoferrin, lysozyme, or secretory IgA before or after ranitidine treatment. This observation suggests that the decrease in nasal responsiveness to histamine was not due to ranitidine. None of the subjects with Zollinger-Ellison syndrome received IL-4.

Methacholine challenge

Methacholine stimulated secretion of the glandular products lactoferrin, lysozyme, and secretory IgA, and stimulated increases in the percentages of total protein that each of these proteins comprised. These responses were not significantly changed by 24 or 72 hours of IL-4 administration as compared with results obtained before receiving IL-4 (Fig. 4). The glandular responses to methacholine stimulation were largely unchanged by the use of ranitidine in the control group

of patients with Zollinger-Ellison syndrome. However, the recovery of lactoferrin relative to total protein (but not the absolute concentration of lactoferrin) was increased in methacholine-induced nasal lavage fluids in the patients with Zollinger-Ellison syndrome while on ranitidine ($p < 0.05$). Thus neither IL-4 nor ranitidine significantly affected the nasal glandular response to exogenously applied methacholine.

Skin test responses

To assess alterations in reactivity of the skin to histamine after IL-4 treatments, prick skin tests were performed in a dose-response fashion. The wheal and flare reactions were measured in their maximal vertical and horizontal diameters and are expressed as the sum of those measurements in millimeters. Only the data from the highest histamine concentration (2.75 mg/ml) are presented, since concentrations of histamine 0.9 mg/ml or less produced no responses in either group of patients. IL-4 had no effect on either the wheal or the flare response to histamine 2.75 mg/ml and 0.9 mg/ml (Fig. 5). Skin testing was performed on the patients with Zollinger-Ellison syndrome also. The sum of the crossed diameters for the wheal and flare response to either 2.75 mg/ml or 0.9 mg/ml of histamine were not significantly affected by ranitidine. These data demonstrate that cutaneous vascular responses to histamine were not affected by IL-4, whereas nasal vascular responses were.

Human nasal explants

Nasal turbinate tissue responses to IL-4 were studied to examine if IL-4 caused abnormal secretory responses that might provide insights into the phenomena observed in vivo. Recombinant human IL-4 did not significantly affect either [^3H]-RGCs or lactoferrin secretion from human nasal turbinate tissue explants at different incubation times (Table I). Turbinate tissue mucosa incubated for 12 hours with IL-4 responded normally to methacholine (100 $\mu\text{mol/L}$) with secretion of both lactoferrin and RGCs (Table I).

DISCUSSION

Treatment protocols with IL-4 for patients with various tumors including metastatic renal cell carcinoma and malignant melanoma have recently been initiated.²⁰ It was quickly realized that certain side effects from this cytokine were reproducibly observed. IL-4-induced hypotension, oliguria, generalized edema, and occasional gastritis with gastric ulceration.²¹ The side effect of gastritis and gastric ulcerations could be managed with ranitidine.²¹ Patients also had the sensation of severe nasal congestion, which consistently began 24 hours after initiation of IL-4 treatment and progressed until treatment was dis-

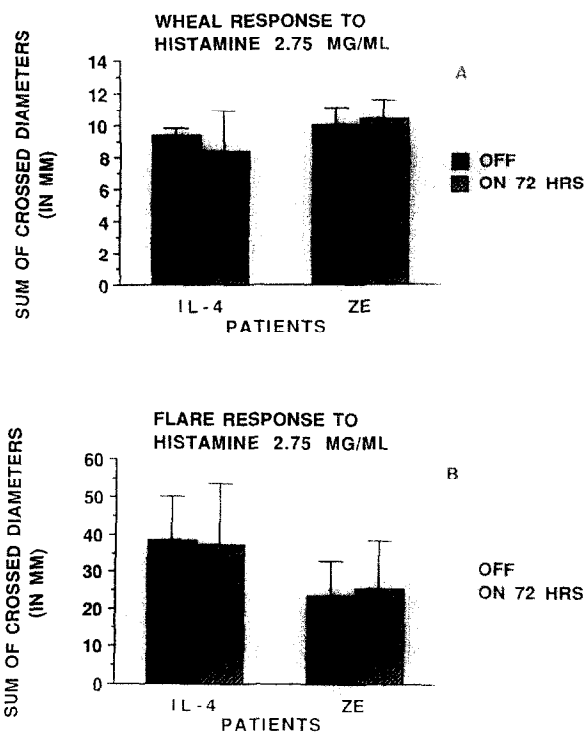


FIG. 5. Effect of 3 days of IL-4 ($N = 5$) or ranitidine (Zollinger-Ellison, $N = 5$) treatment on skin reactivity to two concentrations of histamine. Patients were skin tested with either 2.75 mg/ml or 0.9 mg/ml of histamine either before (filled bars) or after 72 hours (striped bars) of IL-4 or ranitidine treatment. Results are expressed as the sum of the crossed diameters. No effect of IL-4 or ranitidine on skin reactivity to histamine was observed.

continued. This symptom complex was the major subjective complaint for these patients.

To better understand the underlying processes that might contribute to the sensation of nasal congestion, an investigation of IL-4-induced nasal responsiveness was initiated. Before starting IL-4 treatment, the patients responded normally to nasal provocation with histamine and methacholine. However, 3 days after receiving IL-4, a significant decrease in the vascular markers, albumin and IgG, but not the glandular markers, lactoferrin, lysozyme, and secretory IgA, were noted in the nasal lavages after histamine challenge. By contrast, no differences were seen in response to methacholine, which selectively stimulates glandular secretion.¹⁶ These data suggest a selective effect of IL-4 infusions on vascular but not neural responses to histamine, because histamine directly stimulates postcapillary venules to become increasingly permeable but reflexively causes glandular secretion.^{14, 16}

In this study patients received a variety of medications as required to counteract the side effects of their IL-4 chemotherapy. All subjects received ranitidine, and nasal challenge studies performed on a

TABLE I. Effect of recombinant human IL-4 on nasal glandular secretion*

	MC (μ mol/L)	I:L-4 (U/ml)	RGC	No.	Lactoferrin	No.
1 hr	0	1000	-0.3 ± 1.2	2	6.5 ± 3.9	2
	0	2500	5 ± 5.7	2	-18.8 ± 14	2
6 hr	0	1000	16.7 ± 10	3	-16.3 ± 25.6	3
	0	2500	9 ± 8	3	-31.1 ± 9.3	3
12 hr	0	1000	-5 ± 9	4	-21 ± 4	4
	0	2500	8 ± 6	4	-14 ± 9	4
	100	0	20 ± 10	2	569 ± 42	2
	100	1000	17 ± 12	2	549 ± 17	2
	100	2500	4 ± 3	2	845 ± 160	2

N, Number of separate experiments.

*Mucosal explants were exposed to IL-4 (1000 or 2500 U/ml) for 1, 6, or 12 hours, and the secretion of RGC (respiratory glycoconjugates) or lactoferrin was determined. In cultures incubated with or without IL-4 for 12 hours, some replicates were also stimulated with methacholine (100 μ mol/L). Each experiment involved quadruplicate plates containing two mucosal fragments. Results presented compare the supernatants collected during the stimulation period with an equal control period and are expressed as the percent change ($\pm$ SEM).

control population of subjects with Zollinger-Ellison syndrome who were receiving ranitidine demonstrated that the reduced responsiveness to histamine seen in the patients receiving IL-4 was not due to ranitidine. It is possible that prochlorperazine, which was administered to some patients receiving IL-4 might have affected the nasal vascular responsiveness to histamine. We were unable to find studies of prochlorperazine's affect on histamine and cholinergically mediated events in humans. Prochlorperazine has been shown to inhibit histamine-induced contraction of guinea pig intestine.²² However, the same study demonstrated that prochlorperazine was equally effective at inhibiting acetylcholine-induced contraction of rabbit intestine. In the present study the nasal, but not cutaneous, vascular response to histamine was selectively blunted by IL-4 therapy, whereas both the reflex response to histamine as well as the glandular response to methacholine were preserved. Thus it is unlikely that our results can be explained by the use of occasional prochlorperazine.

It is also unlikely that the reduction in the vascular response to histamine observed after IL-4 treatment occurred as a result of congestion interfering with the challenge procedure, since the glandular responses to histamine and to methacholine were unchanged. Histamine was elevated, although not significantly, in nasal washings obtained 24 hours after the onset of IL-4 therapy. PGD₂, another mast cell product that is also produced by epithelial cells, was also significantly elevated after 72 hours of therapy. It is possible that IL-4 might have directly stimulated or facilitated activation of nasal mast cells or basophils, leading to histamine release with resultant congestion. Indeed,

basophils collected from patients receiving IL-4 alone or in combination with IL-2 for cancer immunotherapy were hyperresponsive to histamine-releasing factors found in nasal secretions.²³ An increase in endogenous histamine release in the nasal mucosa after IL-4 therapy could possibly account for the decreased vascular response to histamine that was noted. Tachyphylaxis to histamine challenge has been demonstrated in the airways of anesthetized dogs,^{24, 25} rabbit vascular smooth muscle,²⁶ guinea pig mesenteric artery,²⁷ and in the bronchial smooth muscle of asthmatic patients.²⁸ The mechanism of tachyphylaxis is not clear, although the formation of inhibitory prostaglandins may play a significant role.²⁹ Shore et al.²⁵ demonstrated that the use of the prostaglandin inhibitor indomethacin abrogated the phenomenon of tachyphylaxis in the airways of anesthetized dogs. However, Antol et al.³⁰ observed a persistence of tachyphylaxis despite prostaglandin synthesis inhibition. An argument against this explanation, however, is that tachyphylaxis to histamine was not generalized. IL-4 did not induce a change in the cutaneous response to histamine. Nor was there evidence of histamine release in the lower airways, since bronchoconstriction was not noted in the patients receiving IL-4 except on one occasion in a known asthmatic patient.

An alternative explanation, which has not been tested, might be that IL-4 directly induced vasodilation which led to the sensation of nasal congestion and affected nasal responses to histamine. The occurrence of hypotension in most subjects receiving IL-4 supports this hypothesis. However, the nasal turbinate is a tumescent organ with cavernous sinuses between venules and capillaries similar to those in the

glans penis. Nasal vascular engorgement normally occurs as a result of venous sinusoidal filling, and the lack of priapism in the male subjects receiving IL-4 is evidence against a direct role of IL-4 on venous sinusoids. The nasal vasculature is also unusual in that the vessels feeding the epithelium and glands are fenestrated, similar to those of the renal glomerulus.³¹ Since oliguria was also observed in the patients undergoing IL-4 therapy, it is possible that IL-4 may have an effect on the permeability of fenestrated blood vessels.

The sensation of nasal congestion induced by IL-4 caused significant morbidity in these patients. In addition to the extreme nasal congestion and other side effects, the hesitancy of patients to continue immunotherapy because of nasal congestion may have inhibited the ability of IL-4 to be used as an antitumor agent. If IL-4 is to be used successfully alone, its side effects must be controlled. The data presented herein do not support a major role for either histamine or cholinergic stimulation in the etiology of IL-4-induced nasal congestion. Indeed, oral and topical nasal decongestants as well as oral antihistamine were only minimally effective in controlling congestion. It is interesting to note that in subsequent therapy in more than 40 patients with the combination of IL-2 with IL-4 (at a lower dose) no significant nasal congestion was observed to be associated with the antitumor responses.

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