

Absence of interleukin-3 does not affect the severity of local and systemic anaphylaxis but does enhance eosinophil infiltration in a mouse model of allergic peritonitis

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Abstract

Interleukin-3 (IL-3), which is derived from T cells and other sources, can promote the differentiation, proliferation, and migration of mast cells, basophils, and eosinophils. However, little is known about the ability of IL-3 to regulate the function of these cells in IgE-dependent and -independent allergic responses *in vivo*. Therefore, we sought to investigate the extent to which endogenously produced IL-3 can influence mast cell secretory function, the expression of local and systemic anaphylactic responses, and ragweed-induced eosinophilic peritonitis. We found that peritoneal mast cells from IL-3 deficient (IL-3 $-/-$) mice released less serotonin following challenge with low doses of anti-IgE antibody or antigen *ex vivo* than do cells isolated from corresponding wild-type (IL-3 $+/+$) mice. Both IL-3 $-/-$ and $+/+$ mice expressed equivalent IgE-dependent passive cutaneous anaphylaxis responses following challenge with specific antigen and exhibited equivalent active systemic anaphylaxis responses to ovalbumin as assessed by changes in body temperature, death rates, total IgE production, and histamine release. In contrast, ragweed allergen immunization and peritoneal allergen challenge resulted in eosinophil recruitment that was greater in IL-3 $-/-$ mice than in IL-3 $+/+$ mice. Our data demonstrates that IL-3 does not appear to be essential for local or systemic anaphylaxis. However, IL-3 production *in vivo* was found to enhance the mediator release from freshly isolated peritoneal mast cells stimulated *ex vivo*, and, unexpectedly, to inhibit the accumulation of eosinophils associated with a ragweed-induced allergic peritonitis model.

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1. Introduction

The cytokine interleukin-3 (IL-3), which can be derived from T cells and other sources, is well documented at promoting the *in vitro* differentiation and proliferation of murine hematopoietic progenitor cells, leading to the generation of basophils, neutrophils, macrophages, eosinophils, basophils, erythrocytes, megakaryocytes, and dendritic cells [1–4]. Administration of IL-3 to mice [5,6], experimental primates [7], and humans [1] can promote hematopoiesis *in vivo* as well. In both humans and mice, stem cell factor (SCF, also known as KIT ligand) is a crucial mast cell developmental, survival, and proliferation factor [8–10]. Likewise, IL-3 is known to

be a major growth and differentiation factor for rodent mast cells whereas data are less clear for human mast cells [8–10]. In humans, the ability of IL-3 to promote mast cell development appears to be restricted to certain mast cell subpopulations and the extent to which they express receptors for IL-3 [11–14]. In addition to its effects on the development of mast cells, basophils, and other hematopoietic cells, IL-3 can also enhance antigen presentation for T cell-dependent responses, augment macrophage cytotoxicity and adhesion, promote the secretory function of eosinophils and basophils, and participate in inflammation by inducing expression of adhesion molecules on human endothelial cells [15–22].

Despite these numerous activities, mice lacking IL-3 by homologous recombination in embryonic stem cells display intact steady-state hematopoiesis [23–25] and have normal numbers of tissue mast cells [26]. These findings suggest that IL-3 is either not involved in hematopoietic cell development

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under physiological conditions or is a component of a larger network of redundant cytokines. On the other hand, IL-3 deficient (IL-3 $-/-$) mice do show impaired contact hypersensitivity responses to haptens applied epicutaneously [23,24] and mount attenuated mast cell and basophil responses to nematode infection that result in compromised worm expulsion [26]. In fact, a substantial portion of the mast cell hyperplasia, and virtually all the basophil hyperplasia, that occurs in nematode-infected mice is IL-3 dependent.

Although studies with IL-3 $-/-$ mice indicate that IL-3 contributes to mast cell and basophil development and immunity in mice infected with nematodes, the actual importance of IL-3 in modulating other IgE- and mast cell-associated allergic responses remains unclear. A number of *in vitro* studies with human and murine mast cells (and basophils) do suggest that IL-3 might play a role in certain allergic responses by influencing their function, phenotype, or migration *in vivo*. For example, IgE receptor cross-linkage of human mast cells isolated from intestinal mucosa release significantly more histamine and LTC₄ when stimulated with IL-3 [11]. Studies with murine bone marrow-derived mast cells have demonstrated that IL-3 can importantly regulate the expression of certain mast cell granule proteases that may possess important physiological functions *in vivo* [27] and can upregulate mast cell production of LTC₄ [28]. IL-3 can also induce cultured murine mast cells to synthesize IL-10 and IL-13 [29], and increase IgE- and antigen-dependent mast cell serotonin release from peritoneal mast cells [30].

Acute allergic reactions, which develop within seconds or minutes of allergen exposure, evoke a number of pathophysiological changes that can reflect both mast cell-dependent and independent responses, depending on the experimental model system. In the present study, IL-3 $-/-$ and wild-type (IL-3 $+/+$) mice were used to assess the potential role of IL-3 in three different but well-characterized allergic models. First, passive cutaneous anaphylaxis (PCA), which is entirely mast cell-dependent, was used to specifically assess the ability of mast cells in IL-3 $-/-$ mice to undergo activation and mediator release. The second model we utilized, active systemic anaphylaxis, is not entirely dependent on mast cells [31–33] and can occur in the absence of IgE [34]. This model was used to determine if IL-3 might be involved in regulating any cellular function that typically leads to death of the animal due to anaphylactic shock. Finally, we used IL-3 $-/-$ mice to determine the potential role of IL-3 in a model of ragweed-induced allergic inflammation known to be associated with a selective tissue accumulation of eosinophils [35,36]. Our data demonstrates that IL-3 does not appear to be essential for the pathology associated with PCA or active systemic anaphylaxis. However, IL-3 production *in vivo* was found to enhance the secretory function of freshly isolated peritoneal mast cells stimulated *ex vivo*, and, unexpectedly, to suppress eosinophil accumulation in a ragweed-induced allergic peritonitis model.

2. Materials and methods

2.1. Animals

The production of mice deficient in IL-3 (IL-3 $-/-$ mice), and many of the phenotypic characteristics of these mice, has been described in detail [23,24,26]. Unless otherwise indicated, we used female IL-3 $-/-$ mice that were backcrossed for more than 12 generations with BALB/c mice and used BALB/c mice (purchased from The Jackson Laboratory, Bar Harbor, ME) as IL-3 $+/+$ mice. For these studies, mice were 8–16 weeks of age at the beginning of the experiment. In individual experiments, mice of approximately the same age (within 2–3 weeks) and size were used. Mice were housed in microisolator cages in the Department of Biology's animal facility under a 12 h light–dark cycle and were maintained and used in accordance with James Madison University's Institutional Animal Care guidelines.

2.2. Quantification of mast cell serotonin release

For measurements of serotonin (5-hydroxytryptamine, 5HT) release, freshly isolated peritoneal cells or purified peritoneal mast cells were incubated with ascites IgE anti-dinitrophenyl (IgE anti-DNP), at 10 μ g/ml and [³H]5HT (New England Nuclear, Boston, MA) at 1 μ Ci/ml for 2 h at 37°C, washed, and 5HT serotonin release was calculated as the percentage of total incorporated [³H]5HT present in the cell supernatant 15 min after stimulation at 37°C with the indicated concentrations of polyclonal goat anti-mouse IgE (Bethyl Laboratories, TX), the calcium ionophore A23187 (Sigma), the antigen DNP-human serum albumin (DNP-HSA; Sigma), or vehicle. All cell challenges were performed in triplicate using cells pooled from groups of mice. Purified peritoneal mast cells were obtained by resuspending peritoneal cells in 73% isotonic Percoll (Pharmacia, Piscataway, NJ), and centrifuging at 400 $\times g$ for 10 min. Mast cells comprised 84% of the pelleted cells after density gradient fractionation.

2.3. Passive cutaneous anaphylaxis

Mice received a 20 μ l I.D. injection containing 100 ng of mouse monoclonal IgE anti-DNP, clone SPE-7 (Sigma, St. Louis, MO), in PBS into one ear and a 20 μ l I.D. injection of a vehicle (PBS) into the other ear. Twenty-four hours later, the mice received into the lateral tail vein an injection of 200 μ l of PBS containing 100 μ g DNP-HSA (Sigma) with 2% Evans blue. Ear swelling was measured with a digital micrometer (Mitutoyo Corporation, Kangawa, Japan) just prior to challenge, and at 30 min after challenge with DNP-HSA. Evans blue dye extravasation was measured 30 min after challenge with antigen as previously reported [31]. Briefly, the ears were removed and trimmed to equal size, and incubated in formamide (Sigma) for 3 h at 80°C to extract the dye. Following incubation, 200 μ l of each

sample was placed in a 96-well plate and the optical densities of the samples were read at 630 nm using a microplate reader (Dynex Technologies, Chantilly, VA).

2.4. Active systemic anaphylaxis

Mice received a single I.P. injection of 200 μ l of Imject[®] Alum (Pierce, Rockford, IL) containing 100 μ g chicken ovalbumin (OVA; Sigma) and 300 ng Bordetella pertussis toxin (Sigma). Control (sham-immunized) mice were given a single I.P. injection of 200 μ l of the same solution with the omission of OVA. Eighteen days after immunization, the mice were challenged by a rapid intravenous infusion (via the lateral tail vein) of 200 μ l PBS containing 500 μ g OVA. Blood samples were collected from the tail vein for determination of total IgE levels 2 days prior to OVA or sham-immunization and 2 days before antigen challenge (day 16), and serum was stored at -80°C . Total IgE was quantified using a mouse ELISA kit (Bethyl Laboratories, Montgomery, TX) as per the manufacturer's instructions. To monitor changes in body temperature associated with anaphylaxis, rectal temperature was measured at baseline and, after antigen challenge, at 5 min increments for 60 min or until the death of the animal. Body temperature was determined using a mouse rectal probe linked to a digital recorder (Physitemp Instruments, Clifton, NJ). Animals that did not die from anaphylactic shock were killed 60 min after antigen challenge by cervical dislocation. For measurement of histamine, mice were sensitized as above and blood samples taken 2 min after antigen challenge. Plasma histamine levels were determined using an enzyme immunoassay kit (Cayman Chemical; Ann Arbor, MI) according to the manufacturer's directions.

2.5. Ragweed-induced eosinophilic inflammatory responses

Mice received a subcutaneous injection of short ragweed antigen extract (1:5000 dilution; Greer Laboratories, Lenoir, NC) in 0.2 ml of saline containing penicillin/streptomycin (50 U/ml and 5 μ g/ml) on days 0, 7, and 13. Sham-immunized mice received three injections of saline alone. At 4 days after the last immunization (day 17), mice were challenged by I.P. injection of 0.2 ml ragweed antigen extract. At 24 or 48 h after the antigen challenge (day 19), mice were killed and peritoneal lavage was performed with 3 ml of HBSS containing 0.1% BSA. The total number of nucleated cells was determined microscopically using WBC Unopette (Becton Dickinson, Rutherford, NJ). The proportion of eosinophils was determined by counting a minimum of 300 cells present in cytocentrifuged smears stained with May-Grünwald-Giemsa.

2.6. Statistical analysis

Results are expressed as mean $\pm$ S.E.M. for individual mice or, in the case of serotonin release assays, for triplicate

cultures. Data were compared using the paired or unpaired Student's *t*-tests (two-tailed), repeated measures ANOVA, or the Fisher's exact test as indicated in the figure legends, and were considered significant if $P < 0.05$. Data were analyzed on a Macintosh computer using the Statview 4.5 statistical software package (SAS Institute, Cary, NC).

3. Results

3.1. Peritoneal mast cell serotonin release

To investigate whether endogenous IL-3 can regulate peritoneal mast cell mediator release, we passively sensitized unfractionated IL-3 $-/-$ and $+/+$ peritoneal cells with IgE, and then challenged the cells with various concentrations of anti-mouse IgE antibody and measured release of the cytoplasmic granule-associated mediator serotonin (5HT). As shown in Fig. 1, we found that peritoneal mast cells removed

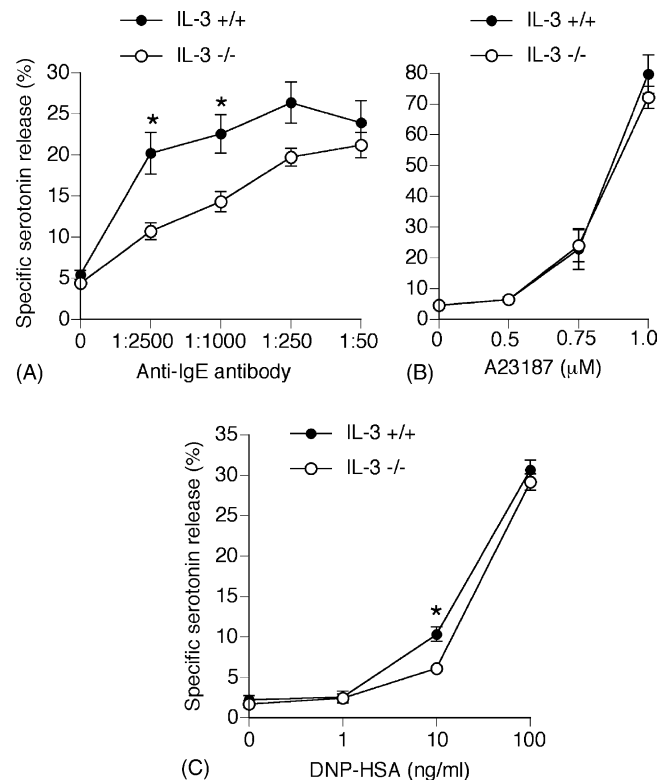


Fig. 1. Release of the preformed mediator serotonin by unfractionated (A, B) or purified (C) IL-3 $-/-$ and $+/+$ peritoneal mast cells at 15 min after challenge with anti-IgE antibody (A), the calcium ionophore A23187 (B), or DNP-HSA (C) as measured by the specific release of [^3H]-hydroxytryptamine creatinine sulfate ([^3H]5HT). One representative experiment out of three is shown using unfractionated peritoneal mast cells and anti-IgE antibody. For the experiment shown in Fig. 1C, purified mast cells from IL-3 $-/-$ and $+/+$ mice released statistically indistinguishable amounts of 5HT in response to stimulation with 1 μ M A23187. Values are mean $\pm$ S.E.M. for triplicate cultures that used cells pooled from groups of 4–10 mice. * $P < 0.05$ vs. corresponding values for mice of the other genotype by the unpaired Student's *t*-test (two-tailed).

from IL-3 $+/+$ mice and tested for their ability to release 5HT upon challenge with relatively low concentrations of anti-IgE antibody in vitro released significantly more 5HT than did cells isolated from IL-3 $-/-$ mice. To use a more biologically realistic means of mast cell challenge, and to address the possibility that non-mast cells (e.g. macrophages and lymphocytes) might have significantly contributed to our results, we used purified mast cells challenged with various concentrations of specific antigen (DNP-HSA). In confirmation of observations using unfractionated peritoneal cells, we found that 5HT release from IL-3 $+/+$ purified mast cells was significantly greater than from IL-3 $-/-$ cells challenged with relatively low concentration of antigen (Fig. 1C).

It is important to note that unfractionated peritoneal cells from IL-3 $-/-$ mice have levels of peritoneal mast cells ($2.9 \pm 0.2\%$, $n = 9$) that are statistically indistinguishable from those in corresponding IL-3 $+/+$ animals ($3.2 \pm 0.2\%$, $n = 12$). Furthermore, the effect of being exposed to endogenous IL-3 levels in vivo on the ability of IL-3 $+/+$ peritoneal mast cells to release 5HT in response to challenge with anti-mouse IgE or specific antigen did not reflect a general enhancement of the secretory responsiveness of IL-3 $+/+$ cells, in that there was no significant parallel increase in the ability of the cells to release 5HT in response to stimulation with the calcium ionophore A23187 (Fig. 1B). Finally, these results do not appear to reflect differences in serum IgE levels or peritoneal mast cell Fc ϵ RI expression as measured by ELISA and flow cytometry, respectively (data not shown).

3.2. Passive cutaneous anaphylaxis

To assess the ability of mast cells in IL-3 $-/-$ mice to undergo activation and mediator release, PCA was induced in IL-3 $+/+$ and $-/-$ mice. PCA was induced by injecting anti-DNP IgE into the ear and 24 h later the mice were challenged intravenously with DNP-HSA in Evans blue. Changes in vascular permeability induced by PCA were assessed by measuring ear swelling and Evans blue dye extravasation. The treated ears of IL-3 $+/+$ and $-/-$ mice exhibited prominent cutaneous extravasation of Evans blue dye that was visually indistinguishable 30 min after antigen challenge. To evaluate Evans blue dye extravasation quantitatively, the ears were removed and the optical density of the extracted dye was measured. Statistically significant increases in vascular permeability were observed in the IgE-treated ears of both the IL-3 $+/+$ and $-/-$ mice as compared to the vehicle-treated ears in both genotypes (Fig. 2A). Importantly, no significant differences were observed between the IgE-treated ears of IL-3 $+/+$ and $-/-$ mice. Measurements of ear swelling were also performed to assess PCA (Fig. 2B). We found the results to be consistent with those obtained by measuring Evans blue extravasation in that both IL-3 $+/+$ and $-/-$ mice exhibited significant ear swelling responses following antigen challenge. Although IL-3 $-/-$ mice exhibited a slightly higher amount

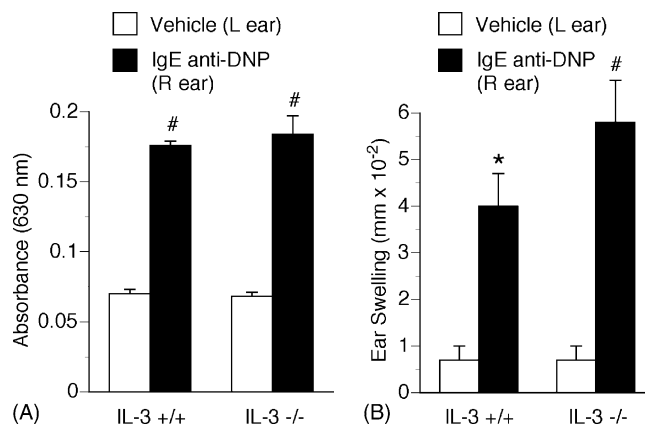


Fig. 2. Passive cutaneous anaphylaxis in IL-3 $+/+$ and $-/-$ mice challenged intravenously with 100 μ g DNP-HSA in 2% Evans blue. Ears were injected intradermally 24 h prior with 100 ng of monoclonal anti-DNP IgE (right ear) or with vehicle (left ear). (A) Extravasation of Evans blue dye (assessed by absorbance at 630 nm) into the ear skin of mice 30 min after challenge. (B) Increase in ear swelling as determined by measurement with a micrometer just prior to and 30 min after challenge. For these studies, both male and female mice were used. All results are mean $\pm$ S.E.M. for individual mice from one experiment ($n = 6$ group) and are representative of at least two experiments. ^{*} $P < 0.05$; [#] $P < 0.001$ vs. corresponding values for vehicle-treated mice of the same genotype by the paired Student's t -test (two-tailed).

of swelling as compared to IL-3 $+/+$ mice, the difference was not statistically significant (Fig. 2B). In a separate experiment, the kinetics of the ear swelling response was evaluated at 30, 60, and 90 min; however, we observed no statistical differences in ear swelling between the two genotypes at any time point (data not shown).

3.3. Active systemic anaphylaxis

Active systemic anaphylaxis was induced by immunizing IL-3 $+/+$ and $-/-$ mice with OVA and challenging them intravenously with OVA 18 days later. Changes in body temperature were used to assess systemic anaphylactic responses in nonanesthetized mice. Body temperatures were taken immediately after intravenous injection with 500 μ g of OVA, and then taken every 5 min for 60 min or until death. Both IL-3 $+/+$ and $-/-$ mice that had been immunized with OVA exhibited significant drops in body temperature upon antigen challenge as compared to mice of the same genotype that were sham-immunized (Fig. 3). Importantly, we observed no statistical differences in the drop in body temperature between the two genotypes at any time point.

The death rates due to active anaphylaxis were statistically indistinguishable in OVA-immunized IL-3 $-/-$ or $+/+$ mice (Table 1). All but one or two of the IL-3 $-/-$ or $+/+$ mice died, most of them within 30 min of antigen challenge, whereas none of the antigen-challenged sham-immunized mice of either genotype died. Both IL-3 $-/-$ and $+/+$ mice exhibited increased serum concentrations of total IgE after OVA immunization (Table 2).

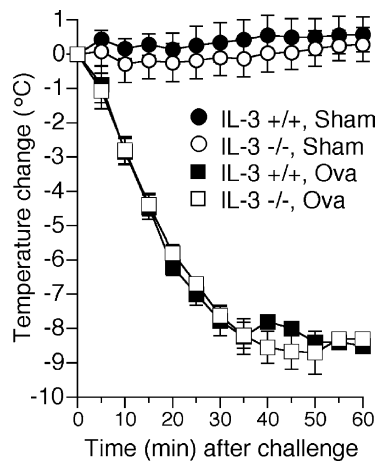


Fig. 3. Changes in body temperature in IL-3 $+/+$ and $-/-$ mice after intravenous challenge with 500 μ g OVA, administered 18 days after immunization with 100 μ g of OVA or vehicle (sham-immunized). All surviving mice were sacrificed 60 min after challenge. Results are shown as the mean $\pm$ S.E.M. for individual mice ($n = 5$ –8/group). In groups in which some mice died before the end of the 60 min period of observation, only mean values are shown for later time periods where $n < 3$. OVA-immunized IL-3 $+/+$ and $-/-$ mice were compared using repeated measures ANOVA over the first 35 min of the response.

However, we observed no statistical differences between total IgE levels in either sham- or OVA-immunized IL-3 $-/-$ and $+/+$ mice challenged intravenously with 500 μ g OVA. Likewise, IL-3 $+/+$ and $-/-$ mice shown in Table 2 had serum concentrations of total IgE 2 days prior to active immunization with OVA that were statistically indistinguishable (data not shown). To confirm if active anaphylaxis in IL-3 $+/+$ and $-/-$ mice was likely associated with mast cell activation, levels of plasma histamine were determined. As shown in Table 2, OVA-immunized IL-3 $+/+$ and $-/-$ mice released significant amounts of histamine immediately upon challenge as compared to corresponding values for sham-immunized mice of the same genotype. However, the postchallenge plasma histamine levels were comparable in similarly treated IL-3 $+/+$ and $-/-$ mice.

3.4. Ragweed-induced allergic peritonitis model

To determine the contribution of IL-3 to peritoneal eosinophilic inflammation, we sensitized mice to ragweed

Table 1

Death rates and times of death in IL-3 $+/+$ and $-/-$ mice, challenged to express active systemic anaphylaxis to OVA

Mice	Treatment	Death rates	Times until death (min)
IL-3 $+/+$	OVA-immunized	6/7*	20, 20, 25, 25, 40, 40
	Sham-immunized	0/5	NA
IL-3 $-/-$	OVA-immunized	6/8*	20, 25, 30, 30, 45, 55
	Sham-immunized	0/6	NA

NA: not applicable. Data are from mice used in the studies reported in Fig. 3.

* $P < 0.05$ by Fisher's exact test vs. values sham-immunized mice of the same genotype.

Table 2

Levels of plasma histamine and total serum IgE antibodies in OVA or sham-immunized IL-3 $+/+$ and $-/-$ mice challenged intravenously with 500 μ g OVA

Mice	Treatment	Histamine (nmol)	Total IgE (ng/ml)
IL-3 $+/+$	OVA-immunized	6306 $\pm$ 191 [‡]	1795 $\pm$ 344*
	Sham-immunized	66 $\pm$ 21	116 $\pm$ 5
IL-3 $-/-$	OVA-immunized	5423 $\pm$ 361 [‡]	1745 $\pm$ 46 [‡]
	Sham-immunized	41 $\pm$ 19	183 $\pm$ 22

Values are mean $\pm$ S.E.M. for individual mice ($n = 4$ –8/group). Values for total IgE are from mice used in the studies reported in Fig. 3 and Table 1. Values for histamine are from separate mice that were immunized and sacrificed 2 min after antigen challenge.

* $P < 0.05$ vs. corresponding values for sham-immunized mice of the same genotype by unpaired Student's t -test (two-tailed).

[‡] $P < 0.0001$ vs. corresponding values for sham-immunized mice of the same genotype by unpaired Student's t -test (two-tailed).

allergen over a 13-day period. On day 17, mice were challenged with an I.P. injection of ragweed allergen and the influx of eosinophils into the peritoneum was examined 24 and 48 h later. Both IL-3 $+/+$ and $-/-$ mice, when immunized and challenged with ragweed allergen, developed a significant peritoneal cavity eosinophilia (Fig. 4) compared with IL-3 $+/+$ and $-/-$ mice that were sham-immunized. Importantly, IL-3 $-/-$ mice immunized with ragweed developed a peritoneal eosinophilia 24 h following challenge with an I.P. injection of ragweed allergen that was statistically greater than that seen in similarly treated IL-3 $+/+$ mice. Similar results were observed 48 h after allergen challenge although the difference between genotypes did

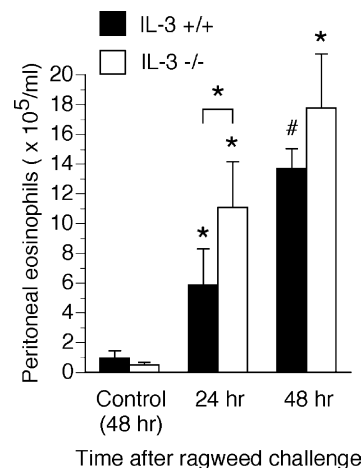


Fig. 4. Eosinophil accumulation in the peritoneal fluid of ragweed-immunized IL-3 $+/+$ and $-/-$ mice 24 h ($n = 5$ mice/group) and 48 h ($n = 6$ –8 mice/group) after I.P. challenge with ragweed allergen. Sham-immunized (control) IL-3 $+/+$ and $-/-$ mice ($n = 3$ /group) were sacrificed 48 h after allergen challenge. All results are mean $\pm$ S.E.M. for individual mice and are representative of the results obtained in two additional experiments in which mice were sacrificed 24 and 48 h after allergen challenge. * $P < 0.05$; # $P < 0.001$ vs. corresponding values for sham-immunized mice or (as indicated by square brackets) vs. corresponding values for mice of the other genotype by the unpaired Student's t -test (two-tailed).

not reach statistical significance (Fig. 4). Mice of either genotype exhibited significant but statistically indistinguishable increases in the total leukocyte count in the peritoneal cavity following ragweed allergen challenge (data not shown).

4. Discussion

Since the discovery of IL-3, most *in vitro* studies have suggested a role for IL-3 in hematopoiesis [1–4]. Despite these studies, relatively little evidence has supported a clear role for IL-3 in hematopoiesis *in vivo*. To date, the most definitive role for IL-3 *in vivo* comes from studies with IL-3 $-/-$ mice. Studies with IL-3 $-/-$ mice have shown that IL-3 plays an important role in modulating contact hypersensitivity, enhancing mast cell and basophil development in response to nematodes, and promoting the rejection of certain parasites that are thought to be susceptible to the influence of mast cell mediators [23,24,26]. However, IL-3 $-/-$ mice have not been used to assess the extent to which IL-3 can regulate mast cell secretory function and the expression of local and systemic allergic responses.

Passive cutaneous anaphylaxis represents one model of acute allergic reactions in which mast cells appear to be essential. In this model, IgE of defined allergen specificity is injected into the skin, and, 24–48 h thereafter, the specific antigen is administered intravenously. The ensuing Fc ϵ RI aggregation of cutaneous mast cells at the site where IgE was injected results in the secretion of mast cell-derived mediators. These products in turn produce multiple local effects including increased cutaneous blood flow, enhanced vascular permeability leading to tissue swelling, and itching due to the stimulation of cutaneous sensory nerves by histamine [37]. Because IL-3 can enhance the release of preformed mediators and upregulate the production of certain newly synthesized mediators from mast cells stimulated *in vitro* [11,28–30], we sought to determine if the physiological responses associated with PCA might be less severe in IL-3 $-/-$ mice.

We found that IL-3 $-/-$ mice exhibited PCA responses that were comparable to that of IL-3 $+/+$ mice when challenged with allergen (Fig. 2). These results clearly indicate that normal PCA responses can occur in the absence of IL-3. However, we cannot rule out the possibility that IL-3 plays no role in mast cell activation and mediator release *in vivo*. For example, PCA responses might be affected during certain immune responses during which IL-3 levels are markedly elevated above physiological levels. Several studies also indicate that IL-3 and other cytokines exhibit functional redundancy. For example, IL-3 $-/-$ or mast cell-deficient Kit^W/Kit^{W-v} mice both exhibit impaired immunity to parasite infection that is even more profoundly reduced in mice that lack the genes for both IL-3 and the stem cell factor receptor, c-kit [26]. Likewise, mice deficient in both IL-3 and GM-CSF develop weaker contact

hypersensitivity reactions to haptens applied epicutaneously than mice deficient in either factor alone [23].

The pathogenesis of active systemic anaphylactic responses is more complex than IgE-dependent passive systemic anaphylaxis. This is perhaps not surprising since immunization for active anaphylaxis is likely to involve many changes that are not associated with passive anaphylaxis. While mast cells undergo degranulation during active systemic anaphylaxis and appear to contribute to some of the features of the response, they are not essential for the associated pulmonary changes and mortality [31–33]. Yet, minimal mast cell involvement does not rule out a role for IL-3 in active anaphylaxis. As pointed out in Section 1, IL-3 may importantly influence the function of other cell types (including basophils) that are thought to be involved with active anaphylaxis.

In the present study, we used a well-characterized experimental model of active systemic anaphylaxis in which IL-3 $-/-$ and $+/+$ mice were immunized with OVA I.P. and challenged intravenously 18 days later with OVA. In mice, systemic anaphylactic responses normally produce a striking diminution in body temperature as well as cardiopulmonary changes. Therefore, we used measurements of body temperature to detect and quantify active systemic anaphylaxis in nonanesthetized IL-3 $+/+$ and $-/-$ mice which were subjected to neither surgical manipulations nor artificial ventilation. Based on changes in body temperature and death rates in IL-3 $+/+$ and $-/-$ mice, our results clearly indicate that active systemic anaphylaxis can occur in the absence of IL-3.

Our experiments also evaluated the ability of IL-3 to regulate IgE production and mast cell activation *in vivo*. Analysis of serum samples indicates that IL-3 $-/-$ mice are capable of producing significant amounts of total IgE antibodies in response to immunization and challenge with OVA. Although serum levels of OVA-specific IgE were not measured, it appears unlikely that IL-3 plays a significant role in regulating IgE synthesis. Histamine is a major vasoactive mediator released primarily by activated mast cells. Analysis of postchallenge histamine levels (Table 2), and morphological assessment of the extent of peritoneal mast cell degranulation (data not shown), in OVA-immunized IL-3 $+/+$ and $-/-$ mice indicate that it is unlikely that differences exist between genotypes in the extent of mast cell activation. In accord with our previous work [26], our experiments with peritoneal mast cells also showed that IL-3 $-/-$ mice have physiological levels of peritoneal mast cells that are statistically indistinguishable from those in the corresponding IL-3 $+/+$ animals.

Yet, our *in vitro* findings indicate that exposure of peritoneal mast cells to endogenous levels of IL-3 *in vivo* alters the secretory potential of these cells when challenged with relatively low concentrations of specific antigen or anti-IgE antibodies (Fig. 1). Although the explanation for this interesting finding is not yet clear, one possible explanation for our results is that low levels of endogenous IL-3 might

only influence anaphylaxis in response to relatively weak biological signals. Alternatively, mast cell populations other than those in the peritoneum may be less affected or not exposed to biologically significant amounts of IL-3 during active anaphylaxis. As a consequence, subtle differences in the reactivity of IL-3 $+/+$ and $-/-$ peritoneal mast cells during active anaphylaxis might not be observed. Whatever the explanation for our *in vitro* findings not being corroborated by our studies of active anaphylaxis, the results clearly illustrate that temperature changes, as well as the death, associated with active systemic anaphylaxis can occur in the complete absence of IL-3.

Tissue eosinophilia is a characteristic abnormality in allergy and asthma, and eosinophil-derived proteins contribute to their specific pathologic features. Although IL-5 plays a central role in eosinophilic inflammation, IL-3 has also been shown to influence eosinophil development and function [22], and adhesion molecules that influence eosinophil migration [15,19]. Although physiological levels of blood and bone marrow eosinophils are normal in IL-3 $-/-$ mice [23,24], we wanted to determine whether IL-3 $-/-$ mice develop typical allergen-induced eosinophilic inflammatory responses. To address this question, we analyzed IL-3 $-/-$ mice in a well-established murine model of allergic inflammation known to be associated with a selective tissue accumulation of eosinophils. Interestingly, the results obtained with the ragweed-induced peritonitis model indicated that 24 h after ragweed challenge, IL-3 actually inhibited the recruitment of eosinophils to the peritoneum (Fig. 4). Although the results did not reach statistical significance, a similar pattern of response was observed 48 h after ragweed challenge, and in the ear swelling response of the PCA reaction (Fig. 2B). While the mechanism(s) responsible for this inhibition is not yet known, these results suggest that in some circumstances IL-3 might have a net suppressive effect on cellular development and/or migration. Indeed, mice deficient in both IL-3 and GM-CSF, but not single knockouts, show increased numbers of circulating eosinophils [23]. Likewise, in certain tissues, mast cell levels after 21, daily subcutaneous injections of stem cell factor are significantly greater in IL-3 $-/-$ mice than in corresponding IL-3 $+/+$ mice [26].

In conclusion, the data presented here strongly argues against a requirement for IL-3 in local and systemic anaphylaxis. However, it cannot be concluded that IL-3 does not contribute at all to this type of allergic response. For example, it is possible that IL-3 and another cytokine(s) mediate redundant functions *in vivo*. It is also important to note that IL-3 levels in blood or tissues are low or undetectable in normal mice using most assays [24,38]. Therefore, we cannot exclude a role for IL-3 in anaphylaxis under circumstances in which there exists other pathological conditions associated with elevated levels of IL-3. Other types of allergic responses might also promote IL-3 production and/or be more sensitive to the effects of IL-3. Indeed, our findings clearly show that IL-3 regulates ragweed-induced allergic inflam-

mation by selectively inhibiting eosinophil accumulation in the peritoneum. Accordingly, we anticipate that the continued analysis of allergic disease in IL-3 $-/-$ mice, and IL-3 $-/-$ mice interbred with other cytokine knockouts, will provide new insights into the pathogenesis of allergic disorders.

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