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IL-4 Exacerbates Anaphylaxis

Richard T. Strait,* Suzanne C. Morris,†Kristi Smiley,* Joseph F. Urban, Jr.,‖and Fred D. Finkelman2‡§¶

We evaluated whether IL-4, a cytokine critical for inducing allergic responses, also contributes to the effector phase of allergy. Pretreatment of mice with IL-4 or the related cytokine, IL-13, rapidly and dramatically increased the severity of anaphylaxis induced by cross-linking FcεRI or FcγRII. This effect was inhibited by endogenously produced IFN-γ, was T cell-, B cell-, and common γ-chain-independent, and required IL-4Rα and Stat6. IL-4Rα signaling also enhanced anaphylaxis in mice infected with a nematode parasite that stimulates IL-4/IL-13 production. IL-4 exacerbated anaphylaxis by acting synergistically with vasoactive mediators to increase vascular permeability. Synergy between IL-4 and vasoactive mediators during the effector phase of allergic inflammation may both contribute to allergic immunopathology and enhance protective immunity against gastrointestinal worms. The Journal of Immunology, 2003, 170: 3835–3842.

Interleukin and IL-13 are critical for the induction of the allergic inflammatory response that protects hosts against nematode parasites and causes immunopathology in mouse models of asthma (1–9). These cytokines induce allergic inflammation through multiple mechanisms: both stimulate mucus production (8, 10), isotype switching to IgE (1, 2), expression of adhesion molecules and chemokines that attract eosinophils and other inflammatory cells to an inflammatory focus (11), and both suppress the production of IL-12, a cytokine that inhibits allergic inflammation (12). In addition, IL-4 stimulates mast cell growth and activation (3, 13, 14) and the differentiation and/or growth of T cells that produce proallergic type 2 cytokines (15, 16). Furthermore, the overexpression of IL-13 during and following Ag sensitization has been shown to enhance the severity of anaphylaxis (17).

To examine the possibility that IL-4/IL-13 promote the effector as well as the induction phase of allergy, we have evaluated the roles of IL-4, IL-13, and related receptors and signaling molecules in well-defined mouse models in which anaphylaxis is induced by the cross-linking of the IgRs FcεRI or FcγRIII (18, 19). Our studies demonstrate that these cytokines rapidly and potentely enhance anaphylaxis through a type 2 IL-4R-, Stat6-dependent mechanism that potentiates the effects of vasoactive mediators.

Materials and Methods

Mice

BALB/c female mice and athymic nude male mice were purchased from the Small Animals Division of the National Cancer Institute (Frederick, MD). IL-4Rα-deficient mice (obtained from N. Noben-Trauth, George Washington University, Washington, DC) (20) and STAT6-deficient mice (obtained from M. Grusby, Harvard University, Cambridge, MA) (21), both on a BALB/c background, were bred in the our animal facility along with the appropriate wild-type controls. IL-4 transgenic T.G.UG mice (4) purchased from P. Leder, Harvard University and wild-type mice on the same mixed genetic background were also bred at the Cincinnati Veterans Administration Medical Center (Cincinnati, OH). C57BL/10 wild-type, recombination-activating gene (RAG)-2-deficient (22) and RAG2/common γ-chain (γc) double-deficient mice (23) were purchased from Taconic Farms (Germantown, NY). All mice were age- and gender-matched within experiments and were used at 7–12 wk of age. All experiments were performed in compliance with the relevant laws and institutional guidelines as approved by the authors’ institutions’ Institutional Animal Care and Use Committee.

Reagents

Purified rat IgG1 anti-mouse IL-4 mAb (11B11) (24) was purchased from Verax (Lebanon, NH). The following hybridomas were grown as ascites in Pristane-primed athymic nude mice and IgG fractions were purified by DE-52 cation exchange chromatography: anti-B220 (6B2; American Type Culture Collection (ATCC), Rockville, MD) (25), anti-IκBα (MKD6; ATCC) (26), anti-IFN-γ (XMG-6; DNAX, Palo Alto, CA) (27), anti-mouse FcγRI/III (24G2; ATCC), anti-mouse IgE (EM-95; Zelig Eshhar, Bethoveth, Israel) (28), control rat IgG1 (GL113; DNAX), and control rat IgG2b (J1.2; DNAX). Some mAbs were labeled with FITC (Calbiochem, La Jolla, CA) or Cy5 (Amersham Life Sciences, Pittsburgh, PA) as suggested by the manufacturer. Platelet-activating factor (PAF), histamine, serotonin (5-HT), propranolol, and 5% bovine albumin were purchased from Sigma-Aldrich (St. Louis, MO). Leukotriene (LT) C4 was purchased from Biomol (Plymouth Meeting, PA). The histamine receptor subtype 1 (H1) antagonist, triprolidine, and type 2 (H2) antagonist, cimetidine were purchased from Sigma-Aldrich and Toxins ((Ellisville, MO), respectively. The PAF antagonist, CV6209, was purchased from Biomol. Recombinant mouse IL-4, IL-9, IL-10, IL-12, and IL-18 were purchased from PeproTech (Rocky Hill, NJ). Recombinant human IL-2 and IL-5 (which activate mouse cells) (29, 30) were obtained from the National Cancer Institute and the Schering-Plough Research Institute (Kenilworth, NJ, respectively. Recombinant mouse IL-13 was a gift of Dr. D. Donaldson (Genetics Institute, Cambridge, MA).

Cytokine administration

In most, but not all experiments, IL-4 was administered as a complex (IL-4C) of two molecules of IL-4 bound by one molecule of a monoclonal

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2 Address correspondence and reprint requests to Dr. Fred D. Finkelman, Research Service (151), Cincinnati Veterans Administration Medical Center, 3200 Vine Street, Cincinnati, OH 45220. E-mail address: finkelman@pol.net
3 Abbreviations used in this paper: RAG, recombination-activating gene; PAF, platelet-activating factor; 5-HT, serotonin; LT, leukotriene; MMCP-1, mouse mast cell protease 1; γc, common γ-chain.
neutralizing anti-IL-4 mAb, 11B11. This complex protects IL-4 from degradation, use, and excretion, and slowly dissociates in vivo, releasing biologically active IL-4 (14). As a result, the in vivo half-life of IL-4 is increased from a few minutes to ~24 h. The biological effects of IL-4C are caused solely by the release of IL-4, rather than by immune complex binding to FcεR or complement receptors. This conclusion is supported by the inability of 11B11 to form complexes with IL-4 that contain more than a single IgG molecule (while at least two IgG molecules must be present in a complex to activate FcγRII or to fix C3) (31), by the inability of IL-4 bound to 11B11 to simultaneously bind to IL-4Rα (14), and by the lack of effect of IL-4C in IL-4Rα-deficient mice.

**Anaphylaxis**

Mice (five per group except where noted otherwise) were challenged i.v. with either 100 μg of rat IgG2a anti-mouse IgE mAb (28) or 500 μg of anti-FcγRII/RIII mAb (18). Additional mice were sensitized i.p. with 200 μl of goat anti-mouse IgD antiserum, then challenged 14 days later by i.v. injection of 100 μg of IgG purified from normal goat serum (19). H1 and H2 were inhibited with 0.2 mg triprolidine and 0.2 mg cimetidine, respectively, given i.p. 30 min before challenge with either anti-IgE mAb or anti-FcγRII/RIII mAb. PAF was inhibited with 66 μg of CV6209 given i.v. 15 min before challenge. Rectal temperatures were measured with a Digital Thermocouple Thermometer (Model BAT-12; Physitery Instruments, Clifton, NJ) just before challenge, then every 5 min for 30 min, then every 15 min for the next 90 min. Activity levels (19) were assessed at the same time the rectal temperatures were obtained. Some mice received 1 ml of 5% bovine albumin i.v. through the tail vein 5 min after i.v. challenge with PAF.

**Hematocrit**

Blood was drawn from incised mouse tail veins into heparinized capillary tubes and centrifuged for 5 min at 10,000 rpm. Hematocrit (percentage of packed RBC volume) was calculated as the length of packed RBCs divided by the total length of serum and red cells in the capillary tube, multiplied by 100%.

**Trichinella spiralis infection**

*T. spiralis* (Beltsville strain) was maintained by serial passage in female Sprague Dawley rats. First-stage larvae (L1) were recovered from infected muscle by pepsin-HCl (1% each) digestion of eviscerated, ground rat carcasses and were washed by settling through several changes of water. Mice were inoculated orally with 50 L1 suspended in 0.2% bacto agar (Difco, Detroit, MI), using an 18-gauge feeding tube. Adult worms were recovered from the intestine was slit lengthwise, rinsed, and placed in HBSS for 4 h at 37°C and were counted with a dissecting microscope.

**Flow cytometry**

Spleen cells from BALB/c mice were ACK-lysed, resuspended in HBSS plus 10% newborn bovine serum and 0.2% NaN₃, and stained with Cy5-anti-B220 mAb and FITC-anti-Ia d mAb in the presence of unlabeled anti-B220 mAb and anti-Ia d mAb. Staining was analyzed using a BD Biosciences FACSCalibur (BD Biosciences, Mountain View, CA) equipped with a red diode laser for median fluorescence intensity of PE (Ia) staining on FITC-positive cells (B lymphocytes).

**Mediator quantitation**

Plasma histamine (drawn on ice) and serum mouse mast cell protease 1 (MMCP-1) levels were measured using commercially available ELISA kits (histamine; IBL, Hamburg, Germany; MMCP-1; Moredu Scientific, Penicuik, U.K.). PAF levels in spleen were measured as previously described (19).

**Statistics**

Spearman correlation (r) for activity and temperature at 30, 60, and 120 min after challenge, mean rectal temperatures, and SEM were calculated with GraphPad Prism 2.0 (GraphPad, San Diego, CA). Fischer’s exact method was used to assess the statistical significance of group differences in death rate and the Mann-Whitney U test was used to compare the mediator concentrations and class II MHC expression between different groups.

**Results**

**IL-4 enhances anaphylaxis by signaling nonimmune cells through an IL-4Rα-, STAT6-dependent, and γc-independent mechanism**

To determine whether IL-4 can enhance anaphylaxis, mice were injected i.v. with saline or with a long-acting formulation of IL-4 (IL-4C) (14), then challenged 24 h later by i.v. injection of either anti-IgE mAb, which induces shock that is FcεRI-, mast cell-, and histamine-dependent, or anti-FcγRII/RIII mAb, which induces shock that is FcγR-, macrophage-, and PAF-dependent (19). Both anti-IgE mAb injection (Fig. 1a, left panel) and anti-FcγRII/RIII mAb injection (Fig. 1b, 1 day) induced shock, as detected by death and by a rapid decline in rectal temperature and activity (activity data not shown) with very good correlation between activity and
Anaphylaxis is exacerbated by endogenous IL-4 production

To address the physiological relevance of IL-4/IL-13 enhancement of anaphylaxis, we examined whether endogenous production of IL-4 and/or IL-13 increases the severity of anaphylaxis in IL-4 transgenic mice (4) or mice infected with a gastrointestinal nematode parasite, *T. spiralis*, which causes a >20-fold increase in IL-4 production (7). Shock induced by anti-FcγRII/RIII mAb was considerably more severe in IL-4 transgenic mice than in wild-type mice (Fig. 3, upper panel). Anti-FcγRII/RIII mAb-induced anaphylaxis was also considerably more severe in *T. spiralis*-infected than in uninfected BALB/c mice (40 and 100% mortality in infected mice in two experiments vs no mortality in uninfected mice, *p* < 0.05; Fig. 3, middle and lower panels). Decreases in rectal temperature were greater in wild-type than IL-4Rα-deficient mice infected with *T. spiralis* following anti-FcγRII/RIII mAb challenge in the first experiment (*p* < 0.05, Fig. 3, middle panel), but not in the second experiment (Fig. 3, lower panel), in which temperature loss was limited by the rapid death of all of the mAb-challenged, wild-type, *T. spiralis*-infected mice. No exacerbation of anaphylaxis was observed in *T. spiralis*-infected IL-4Rα-deficient mice, even though these mice develop a more severe and prolonged infection than wild-type mice (7). Thus, exacerbation of anaphylaxis during *T. spiralis* infection is IL-4- and/or IL-13-dependent.

Dose-dependence of IL-4 enhancement of anaphylaxis

To characterize the potency of the IL-4 effect on anaphylaxis, the sensitivity of the IL-4 effect on anaphylaxis was compared with a particularly sensitive in vivo indicator of IL-4 activity: enhancement of B cell class II MHC (Ia) expression (14, 38). Significant, but suboptimal increases in both the severity of anaphylaxis and
class II MHC expression were observed in mice injected with IL-4C that contained as little as 65 ng of IL-4 (Fig. 4). Larger doses of IL-4 caused further increases in class II MHC expression and severity of anaphylaxis. Anti-FcγRII/RIII mAb challenge caused the death of some mice that were pretreated with IL-4C that contained as little as 125 ng of IL-4, a dose that did not induce a maximal increase in class II MHC, and 100% mortality was observed in mice pretreated with IL-4C that contained 1 μg of IL-4 (Fig. 4, middle and lower panels), a dose substantially less than that required to limit the severity of worm infection (39). These results demonstrate that exacerbation of anaphylaxis is a particularly sensitive effect of IL-4 and provide further evidence that this effect is physiologically relevant.

\[ \text{IL-4 enhances sensitivity to vasoactive mediators} \]

In mice, anaphylaxis is mediated predominantly by vasoactive mediators, such as histamine and PAF (19). IL-4 might exacerbate anaphylaxis by enhancing the effects of these mediators, and this effect might be mediated through the upregulation of class II MHC expression on target cells. However, the precise mechanisms by which IL-4 enhances anaphylaxis are not fully understood and require further investigation.

\[ \text{IL-4 enhancement of anaphylaxis is rapidly inducible and reversible} \]

To gain insight into whether IL-4 enhances anaphylaxis directly or indirectly, we studied the rapidity with which IL-4 enhances anaphylaxis and the speed with which this effect is lost when IL-4 disappears from the circulation. A single dose of uncomplexed IL-4 was used in these experiments, rather than IL-4C, because the short in vivo half-life of uncomplexed IL-4 facilitates study of the resolution of in vivo IL-4 effects. IL-4 slightly exacerbated anaphylaxis when administered simultaneously with anti-FcγRII/RIII mAb challenge, but considerably exacerbated anaphylaxis when administered 4 h before challenge (Fig. 5). A similar effect was observed when IL-13 was administered 4 h before challenge (data not shown). By 24 h after IL-4 administration, most or all of the IL-4 effect had disappeared. Because IL-4 exacerbation of anaphylaxis is even more rapid than IL-4 enhancement of B cell class II MHC expression, which is known to be a direct effect of IL-4 (38), it seems most likely that IL-4 exacerbates anaphylaxis primarily through a direct effect on target cells.

\[ \text{IL-4 enhances sensitivity to vasoactive mediators} \]

In mice, anaphylaxis is mediated predominantly by vasoactive mediators, such as histamine and PAF (19). IL-4 might exacerbate anaphylaxis by enhancing the effects of these mediators, and this effect might be mediated through the upregulation of class II MHC expression on target cells. However, the precise mechanisms by which IL-4 enhances anaphylaxis are not fully understood and require further investigation.
anaphylaxis by increasing production of these mediators or by increasing their effects. Treatment of mice with IL-4C had little or no effect on splenic PAF levels, plasma histamine levels, or serum MMCP-1 levels following challenge with anti-FcγRII/RIII mAb, anti-IgE mAb, or goat IgG (Table I), but dramatically increased the severity of shock induced by injection of histamine, PAF, LTC4, or 5-HT (Fig. 6a). Consistent with these observations, the enhancing effects of IL-4C on anaphylaxis induced by anti-IgE mAb (Fig. 6a) or anti-FcγRIII mAb (data not shown) were inhibited by histamine and PAF antagonists. Additionally, IL-4C did not exacerbate shock induced by the β-blocker, propranalol (Fig. 6a), which decreases cardiac output. Thus, IL-4 appears to enhance the severity of anaphylaxis by increasing the effects of vasoactive mediators that are secreted during an allergic response rather than by increasing mediator production, causing production of different mediators or by exacerbating shock in general. Histamine-induced shock, like anti-FcγRII/RIII mAb-induced shock, was enhanced by IL-4 to the same extent in RAG2−/− mice as in wild-type mice (Fig. 6b) and shock induced by PAF or LTC4 was enhanced by IL-13, which signals through the type 2, but not the type 1 IL-4R (Fig. 6c).

IL-4 increases vascular leak in mice injected with vasoactive mediators

Increased vascular permeability is an important effect of several of the mediators released by activated mast cells and macrophages and contributes to anaphylactic shock by decreasing intravascular volume (40). Because IL-4 exacerbates mediator-induced shock, we examined whether this effect is accompanied by an increase in vascular leak. Because vascular leak causes hemoconcentration by permitting vascular fluid to leak from capillaries and venules, while retaining blood cells, we measured venous hematocrit (packed erythrocyte volume) before challenge and 30 min after unprimed or IL-4-primed mice were injected i.v. with a vasoactive mediator. Priming mice with IL-4C had no direct effect on either rectal temperature or hematocrit (data not shown), while histamine, PAF, 5-HT, or LTC4 injection of unprimed mice caused significant decreases in rectal temperature and increases in hematocrit (Fig. 7a). Both the decreases in rectal temperature and the increases in hematocrit were significantly greater when mice were pretreated with IL-4C, although IL-4 enhanced the effects of different mediators to different extents and IL-4-enhanced increases in hematocrit were not always proportional to IL-4-induced decreases in rectal temperature. To determine whether vascular leak and hemoconcentration contribute to death in IL-4-primed, mediator-challenged mice, we increased intravascular fluid volume in these mice by injecting them i.v. with 1 ml of 5% albumin solution 5 min after PAF challenge. This prevented hemoconcentration, as demonstrated by a decrease, rather than an increase, in hematocrit (Fig. 7b). Only one of five i.v. albumin-treated mice died, as compared with five of five mice that did not receive this treatment (Fig. 7b), and hypothermia, while still present in treated mice, was much less than observed in survivors of lower doses of IL-4C/PAF that did not receive i.v. albumin (data not shown). These observations suggest that enhancement of mediator-induced vascular leak is an important mechanism, but not the only mechanism, by which IL-4 exacerbates anaphylaxis.

### Table I. IL-4 does not affect vasoactive mediator levels

<table>
<thead>
<tr>
<th>Priming</th>
<th>Challenge</th>
<th>IL-4C</th>
<th>Histamine (ng/ml)</th>
<th>PAF (μg/gm spleen)</th>
<th>MMCP-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>&lt;10</td>
<td>nd</td>
<td>10.6 ± 3.5</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>+</td>
<td>&lt;10</td>
<td>nd</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>−</td>
<td>Anti-FcγRII/RIII mAb</td>
<td>−</td>
<td>nd</td>
<td>nd</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>−</td>
<td>Anti-FcγRII/RIII mAb</td>
<td>−</td>
<td>nd</td>
<td>nd</td>
<td>9.9 ± 5.0</td>
</tr>
<tr>
<td>−</td>
<td>Anti-IgE mAb</td>
<td>−</td>
<td>2606 ± 358</td>
<td>nd</td>
<td>2276 ± 700</td>
</tr>
<tr>
<td>−</td>
<td>Anti-IgE mAb</td>
<td>+</td>
<td>2407 ± 326</td>
<td>nd</td>
<td>2882 ± 749</td>
</tr>
<tr>
<td>GaMD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>nd</td>
<td>3.28 ± 0.5</td>
<td>131 ± 4</td>
</tr>
<tr>
<td>GaMD</td>
<td>−</td>
<td>+</td>
<td>nd</td>
<td>2.46 ± 1.46</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>GaMD</td>
<td>Goat IgG</td>
<td>−</td>
<td>nd</td>
<td>7.60 ± 1.67</td>
<td>265 ± 90</td>
</tr>
<tr>
<td>GaMD</td>
<td>Goat IgG</td>
<td>+</td>
<td>nd</td>
<td>4.34 ± 0.5</td>
<td>272 ± 47</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mice (five per group) were pretreated with 2 μg of IL-4C or saline and challenged 24 h later with anti-FcγRII/RIII mAb, anti-IgE mAb, or nothing. Plasma was collected for determination of histamine level 5 min after challenge or serum was collected for determination of MMCP-1 levels 2 h after challenge. Additional mice were immunized i.p. with 0.2 ml of goat anti-mouse IgD antisera. Thirteen days later these mice were injected i.v. with saline or with 2 μg of IL-4C. Twenty-four hours later these mice were left unchallenged or were challenged i.v. with 100 μg of goat IgG. Blood for MMCP-1 analysis was drawn as above. In additional mice, spleens were harvested 15 min after challenge and analyzed for PAF content.

<sup>b</sup> GaMD, Goat anti-mouse IgD antisera; nd, not done.
Discussion

IL-4 and IL-13 have established critical roles in the induction of allergic responses (1–4, 8–10, 13, 17). Our observations demonstrate that these cytokines also contribute to the effector phase of allergy and do so by enhancing the biological effects of allergy-associated vasoactive mediators. Mice pretreated with IL-4 or IL-13, then injected i.v. the next day with 0.25 μg of PAF, 1.25 mg of histamine, 1 μg of LTC4, 1.25 mg of 5-HT, 100 μg of anti-IgE mAb, or 2 mg of propranolol. Some anti-IgE mAb-challenged mice received 66 μg CV6209 (PAF antagonist) i.v. 15 min before challenge and 0.2 mg each of triprolidine and cimetidine (histamine receptor type 1 and 2 antagonist, respectively) i.p. 30 min before challenge (a, upper right panel). Rectal temperatures and deaths are shown for 2 h postchallenge.

FIGURE 6. IL-4 enhances sensitivity to vasoactive mediators. BALB/c mice (a and c) or RAG2/γc−/− and appropriate wild-type control strains (b) (five per group) were injected i.v. with saline or IL-4C that contained 2 μg of IL-4, then challenged i.v. the next day with 0.25 μg of PAF, 1.25 mg of histamine, 1 μg of LTC4, 1.25 mg of 5-HT, 100 μg of anti-IgE mAb, or 2 mg of propranolol. Some anti-IgE mAb-challenged mice received 66 μg CV6209 (PAF antagonist) i.v. 15 min before challenge and 0.2 mg each of triprolidine and cimetidine (histamine receptor type 1 and 2 antagonist, respectively) i.p. 30 min before challenge (a, upper right panel). Rectal temperatures and deaths are shown for 2 h postchallenge.

FIGURE 7. IL-4 increases vascular leak in mice injected with vasoactive mediators. a, BALB/c mice (five per group) were injected i.v. with saline or IL-4C that contained 2 μg of IL-4, then injected i.v. the next day with 0.1 μg of PAF, 5 mg of histamine, or 0.5 μg of LTC4. Rectal temperatures were obtained immediately before and 30 min after challenge. Mice were tail-bled the day before and 30 min after mediator injection and hematocrits were obtained by centrifuging blood in heparinized capillary tubes. Hematocrits were also obtained on untreated mice and on mice treated with IL-4C alone. Means and SEMs of changes in rectal temperature and hematocrit 30 min postmediator injection are shown. IL-4C treatment, by itself, had no effect on rectal temperature or hematocrit. b, BALB/c mice (five per group) were treated with IL-4C that contained 2 μg of IL-4 and challenged i.v. the next day with 0.067 μg PAF. Mice either received no further treatment (no bolus) or were injected with 1 ml of 5% BSA solution i.v. via the tail vein 5 min after PAF injection (bolus). Changes in hematocrits of mice from before to 30 min after PAF challenge are shown in the lower panel; rectal temperatures and mouse deaths for the 2 h following PAF challenge are shown in the upper panel.

IL-4 enhanced anaphylaxis at doses that are physiologically relevant. Anaphylaxis was considerably exacerbated by IL-4 doses lower than those produced in worm-infected mice, as evaluated by the magnitude of IL-4-dependent increases in B cell class II MHC expression (41). Furthermore, *T. spiralis* infection, which stimulates endogenous IL-4/IL-13 production (7, 42), enhanced the anaphylactic response to anti-FcγRII/RIII mAb in wild-type, but not in IL-4Rα-deficient mice, even though the latter mice develop a more severe infection and greater morbidity (7).

IL-4 enhanced anaphylaxis rapidly as well as potently. Exacerbation of anaphylaxis occurred more quickly (within 1 h) than any
previously reported effect of IL-4 on vivo physiology. This rapidity, and the observation that IL-4 enhances anaphylaxis in RAG2\gamma_c-double-deficient mice (7) which lack B cells, T cells, mast cells, eosinophils, and NK cells (Refs. 22 and 43; F. Finkelman, unpublished observations) are consistent with the possibility that IL-4 exacerbates anaphylaxis through a direct effect on an organ or cell type intimately involved in the effector phase of anaphylaxis.

Not all cytokines exacerbate anaphylaxis. Anaphylaxis was significantly exacerbated within 1 h by single doses of 2–6 \mu g of uncomplexed IL-4 while 50 \mu g of IL-2, IL-5, IL-9, or IL-10 had no effect (data not shown). In addition, IFN-\gamma, which inhibits several IL-4-dependent responses, including IgE production (44) and B cell class II MHC expression (35), blocked IL-4 exacerbation of anaphylaxis, although it had little effect itself on anaphylaxis in otherwise untreated mice. However, we cannot rule out the possibility that some cytokines other than IL-4 or IL-13 might enhance anaphylaxis if administered in sufficient quantity for a sufficient period of time.

Our observations also provide information about the cellular and molecular mechanisms by which IL-4 and IL-13 exacerbate anaphylaxis. These cytokines exacerbate anaphylaxis not by increasing vasoactive mediator production, or causing the production of alternative mediators, but by increasing sensitivity to vasoactive mediators, largely through exacerbation of vascular leak. This suggests that IL-4/IL-13 exacerbate anaphylaxis predominantly through an effect on vascular endothelium. Presently, mice that selectively express, or selectively fail to express IL-4 or IL-13 on vascular endothelial cells are being developed and should prove useful in testing this hypothesis.

By demonstrating that IL-4/IL-13 exacerbate anaphylaxis by activating the transcription factor Stat6, our data demonstrate that IL-4/IL-13 affect immunity by modifying gene transcription, rather than through direct activation of signaling pathways that might interact with pathways stimulated by vasoactive mediator ligation of G protein-associated receptors. In this regard, IL-4/IL-13 exacerbation of anaphylaxis resembles IL-4/IL-13 stimulation of IgE production (21, 45), and mucus hypersecretion (8, 10), but differs from IL-4 stimulation of mastocytosis and mast cell degranulation, which are negatively regulated by Stat6 signaling (6). Stat6 activation might exacerbate fluid leak by increasing expression of G protein-associated receptors for vasoactive mediators, as has been shown for a cysteinyl LT and PAF receptors (46, 47), or by changing the expression of proteins involved in a signaling pathway common to vasoactive mediators. Alternatively, Stat6 activation may increase vascular permeability through a structural effect on vascular endothelial cells that is unrelated to signaling mechanisms used by the vasoactive mediators. Because IL-4 acting alone had no effect on hematocrit, such an effect would have to be insufficient to cause significant vascular fluid leak by itself, but be able to act synergistically with separate, mediator-induced structural changes in vascular endothelial cell junctions to exacerbate fluid leak. In this regard, fluid leak has been shown to be a side effect of IL-4 therapy in human cancer patients (48).

These speculations should not obscure the likelihood that allergy-promoting interactions between IL-4/IL-13 and vasoactive mediators are not limited to vascular endothelium: i.e. injection of albumin completely prevented hemococoncentration in IL-4-primed, PAF-challenged mice, but did not completely prevent development of hypothermia (Fig. 7b). Furthermore, treatment of mice with IL-4 and/or IL-13 rapidly induces goblet cell hyperplasia (49) and airway hyperresponsiveness (50) and more slowly increases the ex vivo contractile response of jejunal smooth muscle to LTD4 (51) and the ex vivo secretory response of jejunal epithelium to PGE2 (52). In addition, these cytokines have direct effects on human airway epithelial cells (53), fibroblasts (54), and smooth muscle cells (55) that resemble changes observed in asthmatic individuals. In contrast, it is unlikely that IL-4 has direct mediator-independent effects on cardiac function or vascular tone, because it has little or no effect on shock induced by the \beta-adrenergic blocker propranolol (Fig. 6a), which decreases cardiac output without increasing vascular permeability.

Regardless of the precise mechanism(s) by which IL-4 (and presumably, IL-13) enhance responsiveness to vasoactive mediators, enhanced responsiveness is likely to make a physiologically important contribution to both the protective and the disease-promoting effects of these cytokines. Evidence of a protective effect comes from studies with mice infected with T. spiralis. Both mast cells and IL-4Ra expression by non-bone marrow-derived cells, such as intestinal cells, are required to expel this parasite from the gut (7, 51, 52), consistent with the possibility that the intestinal cells responsible for expulsion must be sensitized with IL-4/IL-13 to respond sufficiently to mast cell-released mediators to expel the worm. A disease-promoting effect is suggested by a phase I clinical trial of an IL-4 antagonist (soluble IL-4Ra) in asthma patients that demonstrated a rapid (<24 h) protective effect in patients withdrawing from inhaled corticosteroids (56). Although it is unlikely that neutralization of IL-4 would suppress IgE or cytokine production during this time frame, our studies demonstrate that enhancement of responsiveness to vasoactive mediators by IL-4 is rapidly reversed once IL-4 is withdrawn (Fig. 4). Thus, inhibiting IL-4/IL-13 enhancement of the effector phase of immediate hypersensitivity may rapidly ameliorate allergic symptoms before inhibitors can suppress IL-4/IL-13 effects on cells responsible for allergy induction.

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References
8. Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schoeldt, T. Y. Neben, C. L. Karp, and F. D. Finkelman. 1998. IL-13, IL-4Ra expression by non-bone marrow-derived cells, such as intestinal cells, are required to expel this parasite from the gut (7, 51, 52), consistent with the possibility that the intestinal cells responsible for expulsion must be sensitized with IL-4/IL-13 to respond sufficiently to mast cell-released mediators to expel the worm. A disease-promoting effect is suggested by a phase I clinical trial of an IL-4 antagonist (soluble IL-4Ra) in asthma patients that demonstrated a rapid (<24 h) protective effect in patients withdrawing from inhaled corticosteroids (56). Although it is unlikely that neutralization of IL-4 would suppress IgE or cytokine production during this time frame, our studies demonstrate that enhancement of responsiveness to vasoactive mediators by IL-4 is rapidly reversed once IL-4 is withdrawn (Fig. 4). Thus, inhibiting IL-4/IL-13 enhancement of the effector phase of immediate hypersensitivity may rapidly ameliorate allergic symptoms before inhibitors can suppress IL-4/IL-13 effects on cells responsible for allergy induction.
34. Jiang, H., M. B. Harris, and P. Rothman. 2000. IL-4/IL-13 signaling beyond
29. Fattah, D., D. J. Quint, A. Proudfoot, R. O'Malley, E. D. Zanders, and
c members of the
17. Fallon, P. G., C. L. Lenson, P. Smith, and A. N. McKenzie. 2001. IL-13 over-
expression predisposes to anaphylaxis following antigen sensitization. J. Immunol.
166:2712.
dependent mast cell degranulation and anaphylaxis: evidence of competition be-
tween FcεRI and FcεRIII for limiting amounts of FcγR β and γ chains. J. Clin.
Invest. 99:915.
15. Swain, S. L., A. D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the
14. Finkelman, F. D., K. B. Madden, S. C. Morris, J. M. Holmes, N. Boiani,