

IL-4 Exacerbates Anaphylaxis¹

Richard T. Strait,* Suzanne C. Morris,^{†¶} Kristi Smiley,* Joseph F. Urban, Jr.,^{||} and Fred D. Finkelman^{2†¶§¶}

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 FcγRIII. 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 potentially 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 TG.UG mice (4) (obtained 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)³2-deficient (22) and RAG2/common γ-chain (γ_c) 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-Ia^d (MKD6; ATCC) (26), anti-IFN-γ (XMG-6; DNAX, Palo Alto, CA) (27), anti-mouse FcγRII/III (24G2; ATCC), anti-mouse IgE (EM-95; Zelig Eshhar, Rehoveth, 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 type 1 (H1) antagonist, triprolidine, and type 2 (H2) antagonist, cimetidine were purchased from Sigma-Aldrich and Tocris (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

Divisions of *Emergency Medicine and [†]Immunology, Children's Hospital Medical Center, Cincinnati, OH 45229; [‡]Division of Immunology, Department of Internal Medicine and [§]Department of Pediatrics, University of Cincinnati, College of Medicine, Cincinnati, OH 45267; ^{||}Veterans Administration Medical Center, Cincinnati, OH 45220; and [¶]Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD 20705

Received for publication September 20, 2002. Accepted for publication January 16, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (merit awards to F.D.F. and S.C.M.), National Institutes of Health Grants RO1 AI35987 (to F.D.F.), RO1 AI45766 (to F.D.F.), and KO8 AI50006 (to R.T.S.), and USDA CRIS 1265-32000-049 (to J.F.U.).

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: ffinkelman@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; γ_c, 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 FcγR 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 FcγRIII or to fix C) (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; Physitry 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 mice after 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-FcγRII/RIII mAb. A total of 20,000 stained cells were analyzed with 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; Moredun Scientific, Penicuik, U.K.). PAF levels in spleen were measured as previously described (19).

Statistics

Spearman correlation (r^2) 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 γ_c-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

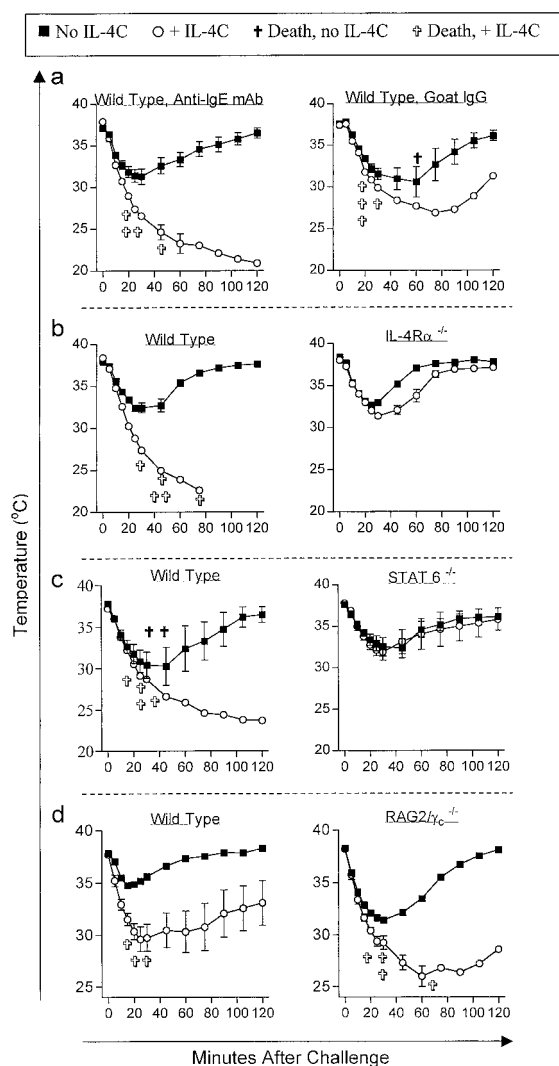


FIGURE 1. IL-4 enhances anaphylaxis by signaling nonimmune cells through an IL-4Rα-dependent, Stat6-dependent, γ_c-independent mechanism. Mice of the strains shown (five per group) were injected i.v. with 0.2 ml of saline or IL-4C (2 μg of IL-4 + 10 μg of 11B11 anti-IL-4 mAb, which saturates each anti-IL-4 mAb molecule with two molecules of IL-4) then challenged the next day by i.v. injection of anti-IgE mAb (a, left panel), or anti-FcγRII/RIII mAb (b–d). Additional mice were immunized i.p. with 0.2 ml of goat anti-mouse IgD antiserum, then challenged i.v. 14 days later with 100 μg of goat IgG (a, right panel). Means and SEM of rectal temperatures are shown for the 2 h following challenge in this and in subsequent figures. Experiments shown in this and in all other figures were repeated at least once, with similar results.

temperature ($r^2 = 0.69$) (19). Pretreatment of mice with IL-4C also exacerbated anaphylaxis in mice that had been primed by injection of goat anti-mouse IgD Ab, which stimulates large IgG and IgE anti-goat IgG responses (32), then challenged with the appropriate Ag (goat IgG; Fig. 1a, right panel).

Although anti-IgE mAb did not induce shock in IL-4R α -deficient, Stat6-deficient, or RAG2/ γ_c -double-deficient mice, all of which lack IgE (data not shown), anti-Fc γ RII/RIII mAb induced shock in all of these strains (Fig. 1, b–d, right panels). Thus, shock induced by Fc γ R cross-linking is B cell-, T cell-, NK cell-, IL-2-, IL-4-, IL-7-, IL-9-, IL-13, and IL-15-independent. Pretreatment of mice with IL-4C considerably increased the severity of anti-IgE mAb-induced shock (greater decreases in rectal temperature and a higher mortality rate) in wild-type mice (Fig. 1a) and the severity of anti-Fc γ RII/RIII mAb-induced shock in wild-type and RAG2/ γ_c double-deficient mice, but not in IL-4R α -deficient or Stat6-deficient mice (Fig. 1, b–d). Thus, IL-4 enhancement of anaphylaxis does not involve the specific immune system or the type 1 IL-4R (IL-4R α/γ_c), but requires IL-4R α and Stat6 signaling. This suggests that IL-4 enhancement of anaphylaxis is mediated through the type 2 IL-4R (IL-4R α /IL-13R α_1), the only receptor other than the type 1 IL-4R that includes the IL-4R α polypeptide and activates Stat6 (33, 34).

IL-13 enhances anaphylaxis through a Stat6-dependent mechanism

Because IL-13 is a ligand for the type 2 IL-4R (33), these observations suggested that IL-13 should also exacerbate anaphylaxis. To test this hypothesis, wild-type and Stat6-deficient mice were pretreated with saline or IL-13, then challenged the next day with anti-Fc γ RII/RIII mAb. IL-13 pretreatment greatly exacerbated anaphylaxis in wild-type mice, but had only a modest effect in Stat6-deficient mice (Fig. 2, upper panels). In contrast, pretreatment of mice with IL-2, IL-5, IL-9, IL-10, or IL-15 had little or no effect on anti-Fc γ RII/RIII mAb-induced shock (data not shown).

IL-4 enhancement of anaphylaxis is suppressed by a combination of IL-12 and IL-18

Because many effects of IL-4 and IL-13 are inhibited by IFN- γ (35, 36), we investigated whether IL-4 enhancement of anaphylaxis could be suppressed by treating mice with a combination of IL-12 and IL-18 that stimulates a large IFN- γ response (37). Treatment of mice daily for 3 days with 10 ng of IL-12 and 500 ng of IL-18 stimulated an ~ 30 -fold increase in serum IFN- γ levels (data not shown). IL-12 plus IL-18 pretreatment, by itself, had no effect on anti-Fc γ RII/RIII mAb-induced anaphylaxis in some experiments and modestly exacerbated anaphylaxis in others, but consistently and considerably suppressed IL-4 enhancement of anaphylaxis (Fig. 2, lower left panel). Inhibition of IL-4 enhancement of anaphylaxis by IL-12 plus IL-18 was IFN- γ -dependent, as inhibition was completely blocked by a neutralizing anti-IFN- γ mAb (Fig. 2, lower right panel).

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-Fc γ RII/RIII mAb was considerably more severe in IL-4 transgenic mice than in wild-type mice (Fig. 3, upper panel). Anti-Fc γ RII/RIII mAb-induced anaphylaxis was also considerably more severe in *T. spiralis*-infected than in uninfected BALB/c mice (40 and 100% mortality in in-

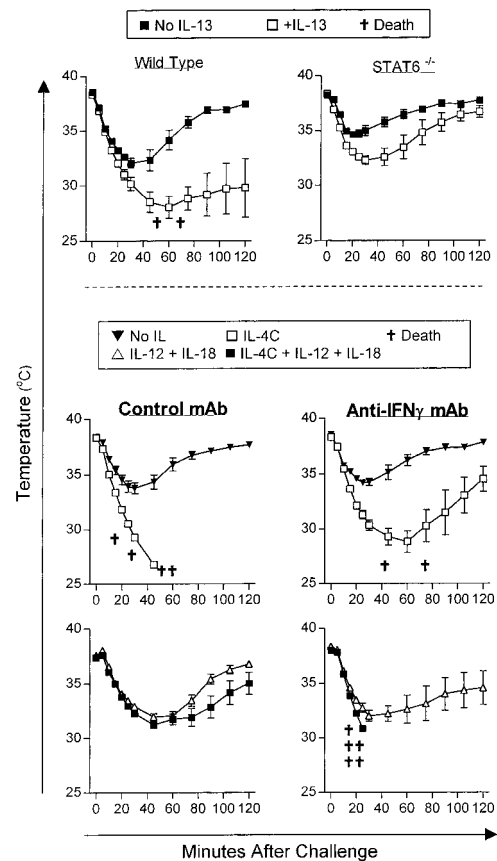


FIGURE 2. IL-13 mimics IL-4 by enhancing anaphylaxis through a Stat6-dependent pathway while IL-12 + IL-18 inhibits IL-4 enhancement of anaphylaxis through an IFN- γ -dependent mechanism. **Upper panels,** BALB/c wild-type and Stat6-deficient mice (five per group) were injected i.v. with saline or 20 μ g of IL-13, then challenged i.v. 1 day later with anti-Fc γ RII/RIII mAb. Deaths and changes in rectal temperature are shown. **Middle and lower panels,** BALB/c mice (five per group) were injected i.p. with a single 1-mg dose of anti-IFN- γ mAb (XMG-6) or an isotype-matched control mAb (GL113) on day 0 and s.c. on days 0, 1, and 2 with 1% autologous serum or 10 ng of IL-12 and 500 ng of IL-18 in 1% autologous serum. In the same experiment, mice were also injected i.v. on day 2 with saline or IL-4C that contained 0.5 μ g of IL-4. Mice were challenged i.v. on day 3 with 0.5 mg of anti-Fc γ RII/RIII mAb. Rectal temperatures and viability were followed for 2 h postchallenge.

fecting 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-4R α -deficient mice infected with *T. spiralis* following anti-Fc γ RII/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-4R α -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

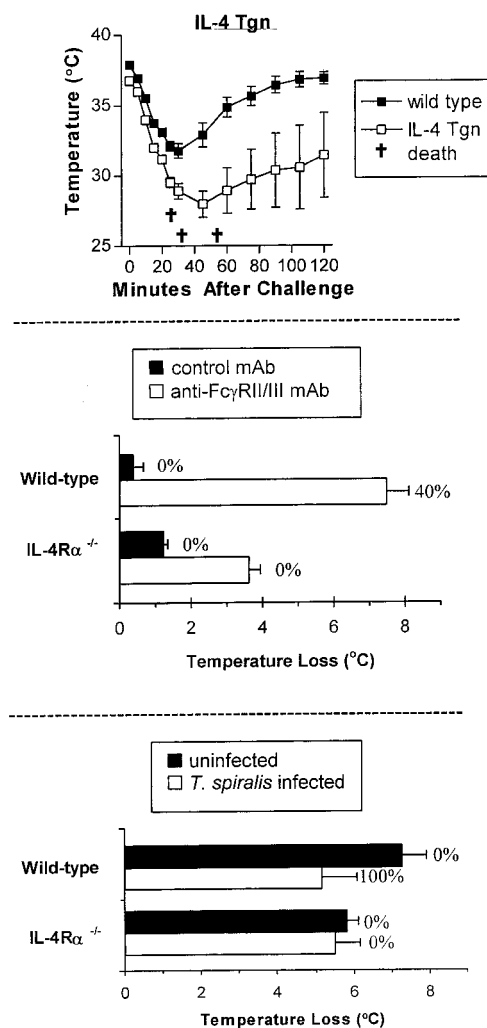


FIGURE 3. Anaphylaxis is enhanced by endogenous IL-4 production. *Top panel*, Wild-type and IL-4 transgenic TG.UG mice (five per group) on the same genetic background were challenged i.v. with anti-Fc γ RII/RIII mAb. Rectal temperatures were followed for the subsequent 2 h. *Middle panel*, BALB/c wild-type and IL-4R α -deficient mice (five per group) were infected with *T. spiralis* and challenged i.v. 8 days later with anti-Fc γ RII/RIII mAb or an isotype-matched control mAb (J1.2). Mortality percentages and maximum decreases in rectal temperature during the 2 h postchallenge are shown. *Bottom panel*, BALB/c mice and IL-4R α -deficient mice (five per group) were left untreated or infected with *T. spiralis*. All mice were challenged i.v. 21 days later with anti-Fc γ RII/RIII mAb. Mortality percentages and maximum decreases in rectal temperature during the 2 h postchallenge are shown.

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-Fc γ RII/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 μ g 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.

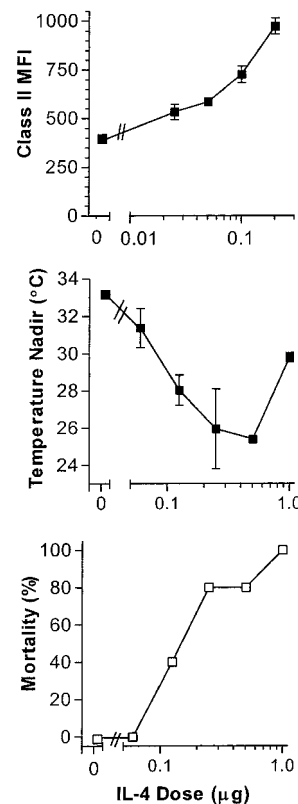


FIGURE 4. Dose-dependence of IL-4 enhancement of anaphylaxis. *Upper panel*, BALB/c mice (three per group) were injected i.v. with IL-4C that contained the quantities of IL-4 shown. Mice were sacrificed the next day and spleen cells were stained with Cy5-anti-B220 mAb (6B2) and FITC-anti-Ia^d mAb (MKD6). Median fluorescence intensity of FITC on Cy5⁺ cells was determined by flow cytometry. Means and SE are shown. *Middle and lower panels*, In a separate experiment with the same lot of IL-4, BALB/c mice (five per group) were injected i.v. with IL-4C that contained the quantities of IL-4 shown and were challenged the next day by i.v. injection of anti-Fc γ RII/RIII mAb. Means and SE of temperature nadirs for mice in each group during the 2 h following challenge are shown in the *middle panel*; 2 h mortality rates are shown in the *lower panel*. Repeat experiments with a different lot of IL-4 had similar results.

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-Fc γ RII/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.

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

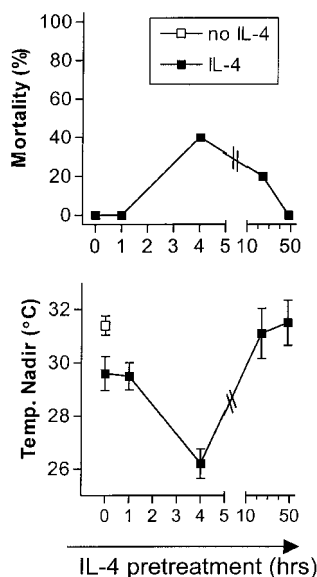


FIGURE 5. IL-4 enhancement of anaphylaxis is rapid and reversible. BALB/c mice (five per group) were injected i.v. with saline or 2 μ g of uncomplexed IL-4 (which has a short in vivo half-life), then challenged i.v. with anti-Fc γ RII/III mAb 0–48 h later. Temperature nadirs and 2 h mortality rates for each group are shown. No mice died in the group receiving no IL-4.

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-Fc γ RII/III 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-Fc γ RIII mAb (data not shown) were inhibited by histamine and PAF antagonists. Additionally, IL-4C did not exacerbate shock induced by the β -blocker, propranolol (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-Fc γ RII/III mAb-induced shock, was enhanced by IL-4 to the same extent in RAG2/ γ_c -deficient 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 effect vasoactive mediator levels^a

Priming	Challenge	IL-4C	Histamine (ng/ml)	PAF (μ g/gm spleen)	MMCP-1 (ng/ml)
—	—	—	<10	nd	10.6 $\pm$ 3.5
—	—	+	<10	nd	5.0 $\pm$ 1.4
—	Anti-Fc γ RII/III mAb	—	nd	nd	7.4 $\pm$ 1.4
—	Anti-Fc γ RII/III mAb	+	nd	nd	9.9 $\pm$ 5.0
—	Anti-IgE mAb	—	2606 $\pm$ 358	nd	2264 $\pm$ 702
—	Anti-IgE mAb	+	2407 $\pm$ 326	nd	2882 $\pm$ 749
GaMD ^b	—	—	nd	3.28 $\pm$ 0.51	139 $\pm$ 4
GaMD	—	+	nd	2.46 $\pm$ 1.46	131 $\pm$ 14
GaMD	Goat IgG	—	nd	7.60 $\pm$ 1.67	265 $\pm$ 90
GaMD	Goat IgG	+	nd	4.34 $\pm$ 0.5	272 $\pm$ 47

^a Mice (five per group) were pretreated with 2 μ g of IL-4C or saline and challenged 24 h later with anti-Fc γ RII/III 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 antiserum. Thirteen days later these mice were injected i.v. with saline or with 2 μ g of IL-4C. Twenty-four hours later these mice were left unchallenged or were challenged i.v. with 100 μ g 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.

^b GaMD, Goat anti-mouse IgD antiserum; nd, not done.

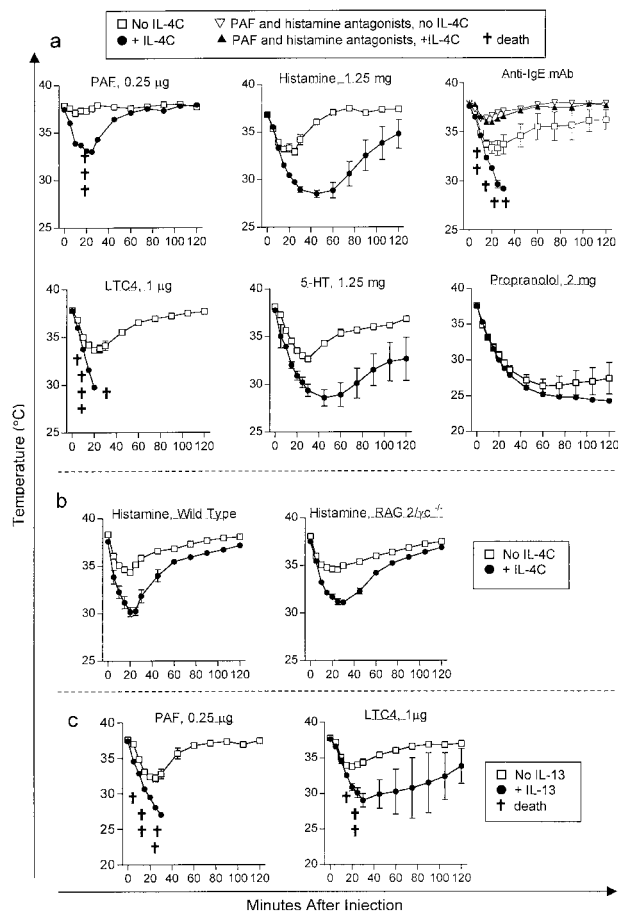


FIGURE 6. IL-4 enhances sensitivity to vasoactive mediators. BALB/c mice (*a* and *c*) or RAG2/ $\gamma_c^{-/-}$ and appropriate wild-type control strains (*b*) (five per group) were injected i.v. with saline or IL-4C that contained 2 μ g of IL-4, then challenged i.v. the next day with 0.25 μ g of PAF, 1.25 mg of histamine, 1 μ g of LTC4, 1.25 mg of 5-HT, 100 μ g of anti-IgE mAb, or 2 mg of propranolol. Some anti-IgE mAb-challenged mice received 66 μ g 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.

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 with a mAb that cross-links Fc γ RIII or Fc ϵ RI, developed severe shock, as measured by hypothermia and decreased activity (activity data not shown), and frequently died, while injection of previously untreated mice with these same mAbs induced only mild, self-limited hypothermia. Although most of our experiments were performed for reasons of convenience and economy with a long-acting complex of IL-4 and anti-IL-4 mAb, IL-4C, anaphylaxis was also enhanced by injection of uncomplexed IL-4 and by endogenously produced transgenic IL-4.

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 ana-

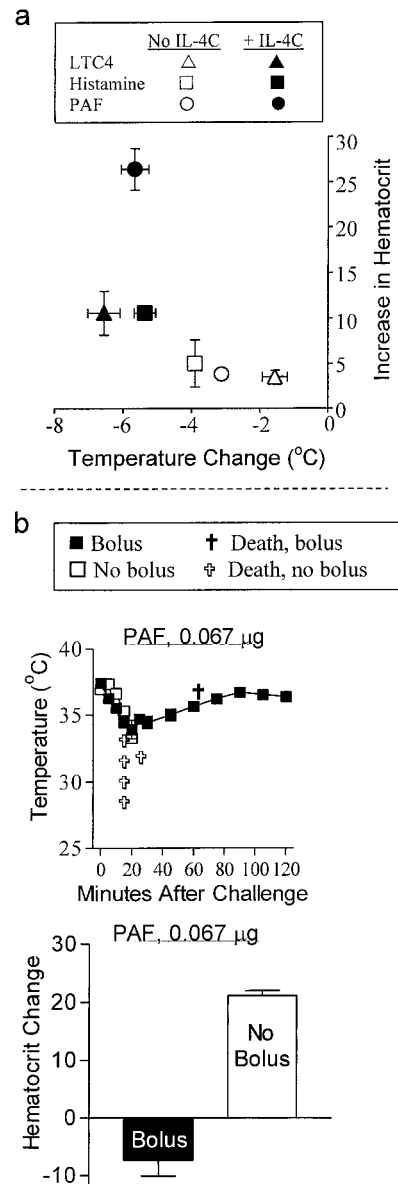


FIGURE 7. IL-4 increases vascular leak in mice injected with vasoactive mediators. *a*, BALB/c mice (five per group) were injected i.v. with saline or IL-4C that contained 2 μ g of IL-4, then injected i.v. the next day with 0.1 μ g of PAF, 5 mg of histamine, or 0.5 μ g of LTC4. Rectal temperatures were 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 μ g of IL-4 and challenged i.v. the next day with 0.067 μ g 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.

phylactic response to anti-Fc γ RII/RIII mAb in wild-type, but not in IL-4R α -deficient mice, even though the latter mice develop a more severe infection and greater morbidity (7).

IL-4 enhanced anaphylaxis rapidly as well as potentially. Exacerbation of anaphylaxis occurred more quickly (within 1 h) than any

previously reported effect of IL-4 on in vivo physiology. This rapidity, and the observation that IL-4 enhances anaphylaxis in RAG2/ γ_c -double-deficient mice (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 μ g of uncomplexed IL-4 while 50 μ g of IL-2, IL-5, IL-9, or IL-10 had no effect (data not shown). In addition, IFN- γ , 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-4R α 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.v. injection of albumin completely prevented hemoconcentration 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 β -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-4R α 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-4R α) 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.

Acknowledgments

We thank Dr. Debra Donaldson for her gift of recombinant mouse IL-13.

References

- Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, A. Zlotnik, and W. E. Paul. 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136:4538.
- Finkelman, F. D., I. M. Katona, J. F. Urban, Jr., J. Holmes, J. Ohara, A. S. Tung, J. V. Sample, and W. E. Paul. 1988. IL-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* 141:2335.
- Madden, K. B., J. F. Urban, Jr., H. J. Ziltener, J. W. Schrader, F. D. Finkelman, and I. M. Katona. 1991. Antibodies to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis. *J. Immunol.* 147:1387.
- Tepper, R. I., D. A. Levinson, B. Z. Stanger, J. Campos-Torres, A. K. Abbas, and P. Leder. 1990. IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. *Cell* 62:457.
- Urban, J. F., Jr., I. M. Katona, W. E. Paul, and F. D. Finkelman. 1991. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proc. Natl. Acad. Sci. USA* 88:5513.
- Urban, J. F., Jr., N. Noben-Trauth, D. D. Donaldson, K. B. Madden, S. C. Morris, M. Collins, and F. D. Finkelman. 1998. IL-13, IL-4R α , and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity* 8:255.
- Urban, J. F., Jr., L. Schopf, S. C. Morris, T. Orekhova, K. B. Madden, C. J. Betts, H. R. Gamble, C. Byrd, D. Donaldson, K. Else, and F. D. Finkelman. 2000. Stat6 signaling promotes protective immunity against *Trichinella spiralis* through a mast cell- and T cell-dependent mechanism. *J. Immunol.* 164:2046.
- Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, and D. D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282:2258.
- Corry, D. B., H. G. Folkesson, M. L. Warnock, D. J. Erle, M. A. Matthay, J. P. Wiener-Kronish, and R. M. Locksley. 1996. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperactivity. *J. Exp. Med.* 183:109.
- Cohn, L., R. J. Homer, H. MacLeod, M. Mohr, F. Brombacher, and K. Bottomly. 1999. Th2-induced airway mucus production is dependent on IL-4R α , but not on eosinophils. *J. Immunol.* 162:6178.
- Thornhill, M. H., S. M. Wellicome, D. L. Mahiouz, J. S. Lanchbury, U. Kyan-Aung, and D. O. Haskard. 1991. Tumor necrosis factor combines with IL-4 or IFN- γ to selectively enhance endothelial cell adhesiveness for T cells: the contribution of vascular cell adhesion molecule-1-dependent and -independent binding mechanisms. *J. Immunol.* 146:592.
- Muchamuel, T., S. Menon, P. Pisacane, M. C. Howard, and D. A. Cockayne. 1997. IL-13 protects mice from lipopolysaccharide-induced lethal endotoxemia:

- correlation with down-modulation of TNF- α , IFN- γ , and IL-12 production. *J. Immunol.* 158:2898.
13. Bischoff, S. C., G. Sellge, A. Lorentz, W. Sebald, R. Raab, and M. P. Manns. 1999. IL-4 enhances proliferation and mediator release in mature human mast cells. *Proc. Natl. Acad. Sci. USA* 96:8080.
 14. Finkelman, F. D., K. B. Madden, S. C. Morris, J. M. Holmes, N. Boiani, I. M. Katona, and C. R. Maliszewski. 1993. Anti-cytokine antibodies as carrier proteins: prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J. Immunol.* 151:1235.
 15. Swain, S. L., A. D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* 145:3796.
 16. Le Gros, G., S. Z. Ben-Sasson, R. Seder, F. D. Finkelman, and W. E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J. Exp. Med.* 172:921.
 17. Fallon, P. G., C. L. Emson, P. Smith, and A. N. McKenzie. 2001. IL-13 overexpression predisposes to anaphylaxis following antigen sensitization. *J. Immunol.* 166:2712.
 18. Dombrowicz, D., V. Flamand, I. Miyajima, J. V. Ravetch, S. J. Galli, and J. P. Kinet. 1997. Absence of Fc ϵ R1 chain results in upregulation of Fc γ RIII-dependent mast cell degranulation and anaphylaxis: evidence of competition between Fc ϵ RI and Fc γ RIII for limiting amounts of FcR β and γ chains. *J. Clin. Invest.* 99:915.
 19. Strait, R. T., S. C. Morris, M. Yang, X. W. Qu, and F. D. Finkelman. 2002. Pathways of anaphylaxis in the mouse. *J. Allergy Clin. Immunol.* 109:658.
 20. Noben-Trauth, N., L. D. Shultz, F. Brombacher, J. F. Urban, Jr., H. Gu, and W. E. Paul. 1997. An interleukin 4 (IL-4)-independent pathway for CD4⁺ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc. Natl. Acad. Sci. USA* 94:10838.
 21. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 4:313.
 22. Spanopoulou, E. 1996. Cellular and molecular analysis of lymphoid development using Rag-deficient mice. *Int. Rev. Immunol.* 13:257.
 23. Colucci, F., C. Soudais, E. Rosmaraki, L. Vanes, V. L. Tybulewicz, and J. P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. *J. Immunol.* 162:2761.
 24. Ohara, J., and W. E. Paul. 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature* 315:333.
 25. Coffman, R. L., and I. L. Weissman. 1981. B220: a B cell-specific member of the T200 glycoprotein family. *Nature* 289:681.
 26. Watts, T. H., A. A. Brian, J. W. Kappler, P. Marrack, and H. M. McConnell. 1984. Antigen presentation by supported planar membranes containing affinity-purified I-A^d. *Proc. Natl. Acad. Sci. USA* 81:7564.
 27. Chervinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann. 1987. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166:1229.
 28. Baniyash, M., and Z. Eshhar. 1984. Inhibition of IgE binding to mast cells and basophils by monoclonal antibodies to murine IgE. *Eur. J. Immunol.* 14:799.
 29. Fattah, D., D. J. Quint, A. Proudfoot, R. O'Malley, E. D. Zanders, and B. R. Champion. 1990. In vitro and in vivo studies with purified recombinant human interleukin 5. *Cytokine* 2:112.
 30. Rosenberg, S. A., E. A. Grimm, M. McGrogan, M. Doyle, E. Kawasaki, K. Koths, and D. F. Mark. 1984. Biological activity of recombinant human interleukin-2 produced in *Escherichia coli*. *Science* 223:1412.
 31. Proding, W., R. Wurzner, A. Erdei, and M. P. Dierich. 1999. Complement. In *Fundamental Immunology*, 4th Ed. W. E. Paul, ed. Lippincott-Raven, Philadelphia, p. 975.
 32. Finkelman, F. D., J. F. Urban, Jr., M. P. Beckmann, K. A. Schooley, J. M. Holmes, and I. M. Katona. 1991. Regulation of murine in vivo IgG and IgE responses by a monoclonal anti-IL-4 receptor antibody. *Int. Immunol.* 3:599.
 33. Finkelman, F. D., T. A. Wynn, D. D. Donaldson, and J. F. Urban, Jr. 1999. The role of IL-13 in helminth-induced inflammation and protective immunity against nematode infections. *Curr. Opin. Immunol.* 11:420.
 34. Jiang, H., M. B. Harris, and P. Rothman. 2000. IL-4/IL-13 signaling beyond JAK/STAT. *J. Allergy Clin. Immunol.* 105:1063.
 35. Mond, J. J., J. Carman, C. Sarma, J. Ohara, and F. D. Finkelman. 1986. Interferon- γ suppresses B cell stimulation factor (BSF-1) induction of class II MHC determinants on B cells. *J. Immunol.* 137:3534.
 36. Urban, J. F., Jr., K. B. Madden, A. W. Cheever, P. P. Trotta, I. M. Katona, and F. D. Finkelman. 1993. IFN inhibits inflammatory responses and protective immunity in mice infected with the nematode parasite. *Nippostrongylus brasiliensis*. *J. Immunol.* 151:7086.
 37. Okamura, H., S. Kashiwamura, H. Tsutsui, T. Yoshimoto, and K. Nakanishi. 1998. Regulation of interferon- γ production by IL-12 and IL-18. *Curr. Opin. Immunol.* 10:259.
 38. Noelle, R., P. H. Krammer, J. Ohara, J. W. Uhr, and E. S. Vitetta. 1984. Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. *Proc. Natl. Acad. Sci. USA* 81:6149.
 39. Urban, J. F., Jr., C. R. Maliszewski, K. B. Madden, I. M. Katona, and F. D. Finkelman. 1995. IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J. Immunol.* 154:4675.
 40. Jancar, S., M. G. Sirois, J. Carrier, P. Braquet, and P. Sirois. 1991. PAF induces rat plasma extravasation and releases eicosanoids during anaphylaxis. *Inflammation* 15:347.
 41. Conrad, D. H., S. Z. Ben-Sasson, G. Le Gros, F. D. Finkelman, and W. E. Paul. 1990. Infection with *Nippostrongylus brasiliensis* or injection of anti-IgD antibodies markedly enhances Fc-receptor-mediated interleukin 4 production by non-B, non-T cells. *J. Exp. Med.* 171:1497.
 42. Ramaswamy, K., D. Negrao-Correa, and R. Bell. 1996. Local intestinal immune responses to infections with *Trichinella spiralis*: real-time, continuous assay of cytokines in the intestinal (afferent) and efferent thoracic duct lymph of rats. *J. Immunol.* 156:4328.
 43. Soudais, C., T. Shiho, L. Sharara, D. Guy-Grand, T. Taniguchi, A. Fischer, and J. Di Santo. 2000. Stable and functional lymphoid reconstitution of common cytokine receptor γ chain deficient mice by retroviral-mediated gene transfer. *Blood* 95:3071.
 44. Finkelman, F. D., I. M. Katona, T. R. Mosmann, and R. L. Coffman. 1988. IFN- γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J. Immunol.* 140:1022.
 45. Shimoda, K., J. van Deursen, M. Y. Sangster, S. R. Sarawar, R. T. Carson, R. A. Tripp, C. Chu, F. W. Quelle, T. Nosaka, D. A. Vignali, et al. 1996. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380:630.
 46. Thivierge, M., J. Stankova, and M. Rola-Pleszczynski. 2001. IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. *J. Immunol.* 167:2855.
 47. Nguer, C. M., O. Pellegrini, P. Galalnaud, J. Benveniste, Y. Thomas, and Y. Richard. 1992. Regulation of PAF-acether receptor expression in human B cells. *J. Immunol.* 149:2742.
 48. Atkins, M. B., G. Vachino, H. J. Tilg, D. D. Karp, N. J. Robert, K. Kappler, and J. W. Mier. 1992. Phase I evaluation of thrice-daily intravenous bolus interleukin-4 in patients with refractory malignancy. *J. Clin. Oncol.* 10:1802.
 49. Dabbagh, K., K. Takeyama, H.-M. Lee, I. F. Ueki, J. A. Lausier, and J. A. Nadel. 1999. IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J. Immunol.* 162:6233.
 50. Venkayya, R., M. Lam, M. Willkom, G. Grünig, D. B. Corry, and D. J. Erle. 2002. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am. J. Respir. Cell Mol. Biol.* 26:202.
 51. Finkelman, F. D., T. Shea-Donohue, J. Goldhill, C. A. Sullivan, S. C. Morris, K. B. Madden, W. C. Gause, and J. F. Urban, Jr. 1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol.* 15:505.
 52. Urban, J. F., Jr., N. Noben-Trauth, L. Schopf, K. B. Madden, and F. D. Finkelman. 2001. Cutting edge: IL-4 receptor expression by non-bone marrow-derived cells is required to expel gastrointestinal nematode parasites. *J. Immunol.* 167:6078.
 53. Laoukili, J., E. Perret, T. Willems, A. Minty, E. Parthoens, O. Houcine, A. Coste, M. Jorissen, F. Marano, D. Caput, and F. Tournier. 2001. IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J. Clin. Invest.* 108:1817.
 54. Doucet, C., D. Brouty-Boyé, C. Pottin-Clémenceau, G. W. Canonica, C. Jasmin, and B. Azzarone. 1998. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts: implication in asthma. *J. Clin. Invest.* 101:2129.
 55. Grunstein, M. M., H. Hakonarson, J. Leiter, M. Chen, R. Whelan, J. S. Grunstein, and S. Chuang. 2002. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 282:L520.
 56. Borish, L. C., H. S. Nelson, M. J. Lanz, L. Claussen, J. B. Whitmore, J. M. Agosti, and L. Garrison. 1999. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med.* 160:1816.