

CD4⁺ T Cell-Specific Deletion of IL-4 Receptor α Prevents Ovalbumin-Induced Anaphylaxis by an IFN- γ -Dependent Mechanism¹

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IL-4R α -mediated STAT6 activation serves an essential role in various animal models of allergy and asthma at both the sensitization and effector phases. IL-4 and IL-13 signaling via the IL-4R α chain exacerbates murine anaphylaxis, but the cell-specific requirements for IL-4R α expression are unclear. The purpose of this study was to elucidate the mechanisms of systemic anaphylaxis to OVA in gene-targeted mice with a deletion of the IL-4R α chain in the macrophage/neutrophil or CD4⁺ T lymphocyte population. Results demonstrated that anaphylaxis in this model was entirely dependent upon the Fc γ RII/III and was associated with mast cell degranulation. Expression of the IL-4R α on CD4⁺ T cells, but not macrophages or neutrophils, was critical for severe anaphylaxis, characterized by diarrhea, hypothermia, and death. Ab depletion experiments demonstrated that IFN- γ protected against mortality and severe intestinal pathology despite the presence of Ag and specific Ab. This protection was associated with reduced levels of mast cell protease, a marker of mast cell degranulation, suggesting that IFN- γ may inhibit mast cell degranulation in vivo. These data suggest that it may be possible to limit the severity of anaphylaxis using rational therapies designed to increase numbers of IFN- γ -producing cells by targeting IL-4R α signaling in CD4⁺ T lymphocytes. *The Journal of Immunology*, 2007, 179: 2758–2765.

Anaphylaxis is a severe, systemic immediate type I hypersensitivity reaction affecting multiple organ systems, and is potentially fatal (1, 2). Common causes of anaphylaxis include insect venom, food, and drugs. Severe life-threatening anaphylaxis affects ~1–3 in every 10,000 people, with manifestations including (but not limited to) hypotension, angioedema, cutaneous reactions, gastrointestinal distress, respiratory failure, and cardiac arrest (1–4). Anaphylactic reactions are immunologically mediated, with Ag-triggered release of vasoactive and inflammatory mediators causing vascular permeability, vasodilation, edema, and smooth muscle contraction, which culminates in life-threatening shock (2, 3). Experimental models of anaphylaxis have demonstrated that two pathways of anaphylaxis exist in mice: IgE cross-linking of Fc ϵ RI in mast cells, and IgG cross-linking of Fc γ RIII on macrophages (5–7).

IgE-mediated anaphylaxis is dependent on Ag cross-linking of IgE bound to the Fc ϵ RI α on mast cells and basophils, which

causes the release of histamine, platelet-activating factor (PAF),⁶ serotonin, cytokines, and leukotrienes. Systemic shock is thought to be mediated predominantly by histamine with minor involvement of PAF (6–8), although in an experimental model penicillin V-induced anaphylaxis was blocked by a PAF inhibitor (9). In contrast, IgG-dependent anaphylaxis can occur independently of mast cells, with macrophages, Fc γ RIII, and PAF serving essential effector roles (5–7, 10). It is not known whether the Fc γ RIII/macrophage pathway exists in humans, but IgE-independent anaphylactic reactions have been reported following drug administration (10–13). Whether IgE- or IgG-mediated anaphylaxis occurs may be dependent upon the concentration of the challenge Ag as well as the quantity of Ag-specific Ab in circulation (7, 14). It has been proposed that most cases of human anaphylaxis are primarily IgE dependent due to the relatively low concentrations of Ab and Ag involved (7, 14).

In mice, Ag-specific IgG can inhibit IgE-mediated anaphylaxis when both IgG and IgE are present (14). Despite mechanistic differences between the IgE- and IgG-mediated pathways, the endpoints of systemic anaphylaxis involve a highly conserved set of cytokines and effector molecules that result in cardiovascular, pulmonary, and gastrointestinal involvement (6).

IL-4 and IL-13 serve important roles in both IgE- and IgG-mediated anaphylaxis via signaling through their shared receptor component IL-4R α (7, 15). IL-4R α forms a functional IL-4R when complexed with a common γ -chain, or a functional IL-13R when complexed with an IL-13R α 1 subunit (16). Produced by Th2 cells, NK cells, eosinophils, basophils, and mast cells, IL-4 and IL-13 have fundamental roles in both the sensitization and effector phases of the anaphylactic response, promoting IgE and IgG1 class

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⁶ Abbreviations used in this paper: PAF, platelet-activating factor; MMCP, mouse mast cell protease.

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switching, mastocytosis, and vascular permeability (17). Enhancement of anaphylaxis through IL-4R α -driven STAT6 activation could involve multiple tissues or cell types that express a functional type 2 receptor (IL-4R α /IL-13R α 1). In the present work, it was asked whether CD4⁺ T cell-specific or macrophage/neutrophil-specific expression of the IL-4R α chain is essential for systemic Ag-induced anaphylaxis. IL-4 signaling through IL-4R α expressed on naive CD4⁺ T cells is critical for the induction of Th2 differentiation (18). Similarly, IL-4 and IL-13 signaling through IL-4R α on myeloid cells induces differentiation into a particular macrophage phenotype termed alternatively activated (19, 20). Alternatively activated macrophages are thought to be important in chronic allergic diseases through their role in fibrosis (21), but the role of alternatively activated macrophages in acute allergic diseases such as anaphylaxis is unknown.

The data presented in this study show that IL-4R α expression by macrophages and/or neutrophils did not significantly affect anaphylactic responses in a model of OVA-induced anaphylaxis. In contrast, mice bearing a CD4⁺ T cell-specific IL-4R α gene deletion were resistant to OVA-induced anaphylaxis, indicating a critical role for CD4⁺ T cells in regulating susceptibility to anaphylaxis. The mechanism of protection was IFN- γ dependent: IFN- γ neutralization inducing susceptibility to fatal responses. Protection in CD4⁺ T cell-specific IL-4R α knockout mice occurred despite the concomitant production of type 2-specific Ab and Th2 cytokines, suggesting regulation of IFN- γ levels as a primary determinant for overall susceptibility to systemic anaphylaxis.

Materials and Methods

Mice

BALB/c mice (8–12 wk old) and IL-4R α ^{-/-} (22), macrophage/neutrophil-specific (LysM^{Cre}IL-4R α ^{-lox}) (23), and CD4⁺ T cell-specific (Lck^{Cre}IL-4R α ^{-lox}) mice (24), all on a BALB/c background, were housed under specific pathogen-free barrier conditions using individual ventilated cages. All experiments complied with the South African Code of Practice, and were approved by the University of Cape Town's Animal Ethics Committee.

Sensitization and challenge protocol

Mice were injected i.p. with 50 μ g of grade V OVA (Sigma-Aldrich) adsorbed to 0.6 mg/ml aluminum hydroxide gel (Sigma-Aldrich) at day 0, and boosted on day 14 with the same preparation. On day 21, mice were challenged i.v. with 750 μ g of OVA in 200 μ l of PBS. Mice were observed for 50–70 min postchallenge, and signs of anaphylaxis were scored using a modification of a previously described scoring system (25), as follows: 0, no symptoms; 1, hypersensitivity to touch, irritability/aggression; 2, diarrhea, puffiness around the eyes, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, cyanosis around mouth and tail, labored breathing, lying flat; 4, loss of consciousness, no activity upon prodding, tremor or convulsions; 5, death. Mice experiencing convulsions invariably die at a later stage, and were thus euthanized for humane reasons and scored as 5. Temperature readings were taken every 10 min using a mouse rectal thermometer (VetTech) lubricated with Vaseline petroleum jelly (Unilever). Mice that survived challenge were anesthetized with ketamine (Anakete-V; Centaur Laboratories) and xylazine (Rompun; Bayer) and killed by cervical dislocation.

Ab depletion

The following Abs were purified by affinity chromatography using protein G-Sepharose and used in this study: rat anti-mouse Fc γ R2/III (2.4G2), rat anti-mouse CD4 (GK1.5), rat anti-mouse IL-4 (11B11), rat anti-mouse CD3 (145-2C11), rat anti-mouse IgE EM95.3, rat anti-mouse IFN- γ (AN18KL6), and anti-c-kit (ACK-2), a gift from R. Grencis (University of Manchester, Manchester, U.K.). All Abs were dialyzed against 1 $\times$ PBS and injected i.p. at days 18 and 20, apart from anti-c-kit, which was injected at days -2, 0, 12, 14, and 20.

ELISPOT

Multiscreen plates (Millipore) were coated with capture Ab for IFN- γ (R46A2; Sigma-Aldrich), IL-4 (11B11; Sigma-Aldrich), or IL-5 (TRFK-5; Sigma-Aldrich) at 10 μ g/ml in borate buffer (pH 8.5), overnight at 4°C. Plates were then washed and blocked with 5% BSA for 2 h at room temperature. Cells were plated in triplicate wells in IMDM supplemented with 10% FCS. Following 72 h of culture at 37°C, cells were removed by washing with PBS/Tween 20 and incubated with the appropriately matched biotinylated anti-mouse secondary Abs diluted in PBS/1% BSA (pH 7.3). Plates were washed before incubation with alkaline phosphatase-labeled streptavidin (BD Pharmingen) for 2 h at room temperature. Finally, plates were washed with PBS/Tween 20 before developing the colorimetric assay by the addition of Sigma Fast 5-bromo-4-chloro-3-indolyl phosphate/NBT tablets (Sigma-Aldrich) for 30 min. The plates were then washed with distilled water and air dried. Spots were quantified by examination under a dissecting microscope.

Ab ELISA

OVA-specific Abs were measured by indirect ELISA using 5 μ g/ml grade V OVA (Sigma-Aldrich) for coating and anti-mouse isotype-specific Abs (Southern Biotechnology Associates) for detection. Absorbance was measured at 405 nm with 492 nm as a reference wavelength. Total IgE was measured by sandwich ELISA using clone 84.1C as a coat and anti-mouse IgE (Southern Biotechnology Associates) for detection.

Mouse mast cell protease (MMCP)-1 detection

MMCP-1 levels were measured in serum and tissue homogenates by ELISA, according to the manufacturer's protocol (Moredun Scientific). Blood was collected and centrifuged for 15 min at 4000 rpm to obtain serum. Intestinal tissue homogenates were prepared, as previously described (9).

Statistical analysis

Values are given as mean $\pm$ SEM, and significant differences were determined using unpaired two-tailed Student's *t* test or ANOVA using computer software (GraphPad Prism). Values of *p* < 0.05 were considered significant.

Results

OVA-induced anaphylaxis in BALB/c mice

A model of Ag-driven anaphylaxis was established in BALB/c mice. Mice were sensitized with two i.p. injections of OVA/alum, followed by i.v. challenge with OVA in PBS. Control mice were sensitized with PBS/alum (sham) and challenged with OVA. OVA-sensitized mice experienced a sharp decline in rectal temperature following i.v. challenge and remained hypothermic up to 1 h postchallenge (Fig. 1A). In contrast, sham-sensitized mice maintained relatively constant temperatures. Temperature loss was accompanied by decreased activity, but this did not always correlate with severity of anaphylaxis (our unpublished observations). A scoring system was used to evaluate a range of reactions, including diarrhea, reduced activity, cyanosis, loss of consciousness, and death (see *Materials and Methods*). Severe systemic anaphylaxis was accompanied by intestinal pathology characterized by venous congestion, hyperemia, and edematous swelling of the small intestine from the gastric fundus to the ileocecal junction (Fig. 1B). Diarrhea was the most common symptom of intestinal anaphylaxis, and severe intestinal pathology correlated nearly 100% with fatality.

OVA-induced anaphylaxis is dependent upon the Fc γ R2/III

Ab-mediated inhibition studies were performed to determine whether anaphylaxis in this model was dependent upon the IgE-Fc ϵ RI and/or IgG-Fc γ R2/III pathway. Blocking was performed by injection of 1 mg of anti-mouse IgE (clone EM95.3) or anti-mouse Fc γ R2/III (2.4G2), respectively, on days 18 and 20 (6). Anti-IgE-treated mice developed severe systemic anaphylaxis with a sharp decline in rectal temperature following i.v. challenge with

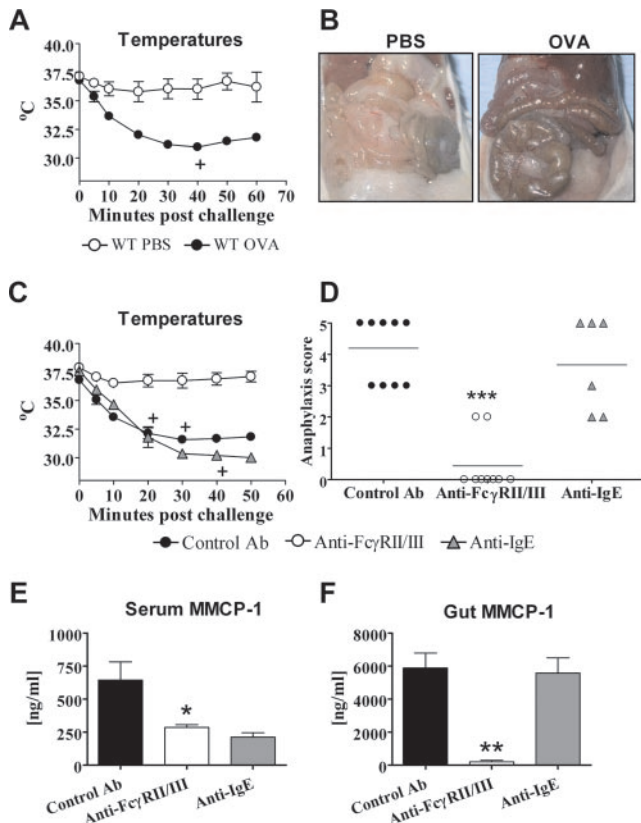


FIGURE 1. Ag-driven anaphylaxis is dependent upon Fc γ RII/III. *A*, BALB/c wild-type mice ($n = 5$) were sensitized i.p. with alum-adsorbed OVA on days 0 and 14 and challenged i.v. with OVA in PBS on day 21. Temperatures were measured with a rectal thermometer. *B*, OVA challenge of sensitized mice led to systemic anaphylaxis accompanied by intestinal pathology. *C*, Sensitized mice were treated with anti-Fc γ RII/III, anti-IgE, or control rat IgG on days 18 and 20, and rectal temperatures were measured for 50 min postchallenge. Results are representative of two experiments. *D*, Signs of anaphylaxis were scored as described in *Materials and Methods* ($n = 6-9$). Data are the combined results of two experiments. *E* and *F*, MMCP-1 was measured in serum (*E*) and gut homogenates (*F*) as a measure of mast cell degranulation. +, Death; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

OVA, similar to control Ab-treated mice (GL113) (Fig. 1*C*). However, anti-Fc γ RII/III-treated mice experienced a significant inhibition in temperature loss, with only two of nine mice having mild reactions (Fig. 1*D*). In addition, levels of MMCP-1, an indication of mast cell degranulation, were significantly reduced in the serum (Fig. 1*E*) and intestine (Fig. 1*F*). Intestinal pathology and diarrhea in susceptible mice were therefore associated with mast cell degranulation. Together, this demonstrates that this model of OVA-induced anaphylaxis is mediated by IgG/Fc γ RIII signaling.

IL-4R α expression on CD4 $^{+}$ T cells is required for anaphylaxis

IL-4 and IL-13 exacerbate murine anaphylaxis by signaling via the IL-4R α (7, 15), but the specific cells in which IL-4R α signaling mediates crucial anaphylactic responses are unidentified. In this model, the complete absence of IL-4R α resulted in a significant reduction in anaphylactic shock and abrogated mortality (Fig. 2*A*). However, the absence of IL-4R α on macrophages/neutrophils (and consequently the absence of alternatively activated macrophages) had no effect on OVA-induced anaphylaxis, with mice developing severe shock and hypothermia similar to their wild-type littermates (Fig. 2*B*). In contrast, when CD4 $^{+}$ T cell-specific IL-4R α -deficient mice were i.v.

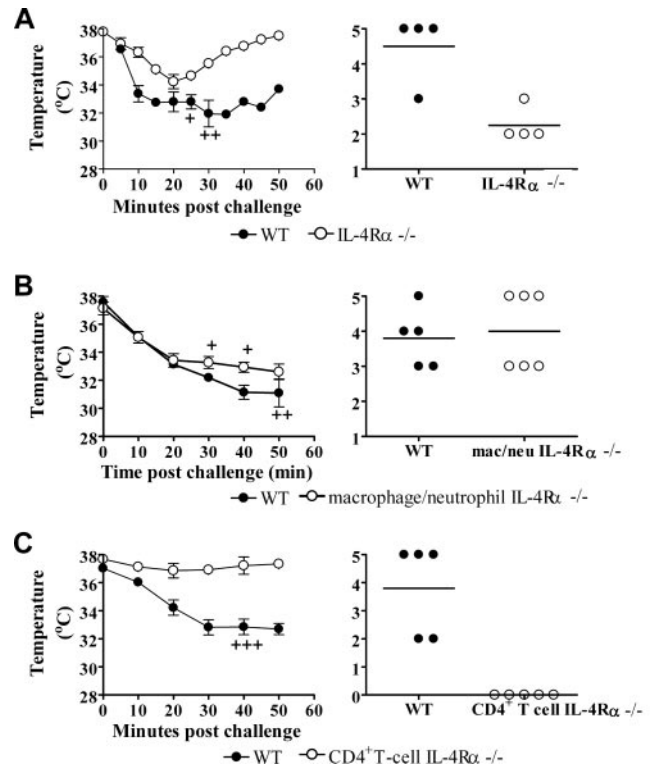


FIGURE 2. Deletion of IL-4R α on CD4 $^{+}$ T cells abrogates systemic anaphylaxis. BALB/c wild-type (*A*), IL-4R $\alpha^{-/-}$ (*A*), macrophage-specific IL-4R $\alpha^{-/-}$ (*B*), and T cell-specific IL-4R $\alpha^{-/-}$ (*C*) mice were sensitized i.p. with alum-adsorbed OVA on days 0 and 14 and challenged i.v. with OVA in PBS on day 21. Rectal temperatures were measured for 50–80 min postchallenge. Results are representative of two to four experiments. +, Death.

challenged, marked abrogation of anaphylaxis occurred (Fig. 2*C*). Mice that lacked IL-4R α specifically in CD4 $^{+}$ T cells failed to develop anaphylactic shock or intestinal pathology, in contrast to wild-type littermates. At equal challenge doses, CD4 $^{+}$ T cell IL-4R α -deficient mice were more protected than complete IL-4R α -deficient mice, although reactions equivalent to those seen in IL-4R α -deficient mice could be elicited by higher challenge doses (data not shown). These results show that IL-4R α responsiveness by CD4 $^{+}$ T cells, but not macrophages/neutrophils, is crucial in mediating susceptibility to anaphylaxis, and indicate that IL-4R α responsiveness on cell populations other than CD4 $^{+}$ T cells may contribute to protection.

CD4 $^{+}$ T cell-specific IL-4R α -deficient mice exhibit mixed type 1/type 2 responses

ELISPOT analysis was performed to determine whether protection from anaphylaxis in CD4 $^{+}$ T cell-specific IL-4R α -deficient mice was associated with a change in the phenotype of cytokine production. Spontaneous release of IFN- γ vs IL-4 from splenocytes was used to indicate Th1 vs Th2 frequency, respectively. The results demonstrated that wild-type mice had high numbers of IL-4-secreting cells, but few IFN- γ effectors, suggesting that these mice were strictly Th2 polarized. In contrast, IL-4R α -deficient and CD4 $^{+}$ T cell-specific IL-4R α -deficient mice had significant numbers of both IFN- γ - and IL-4-secreting cells (Fig. 3, *A* and *B*). IFN- γ -secreting cells were most likely CD4 $^{+}$ T cells, because in vivo depletion with GK1.5 (anti-mouse CD4) eliminated IFN- γ -positive cells as determined by ELISPOT (data not shown). In

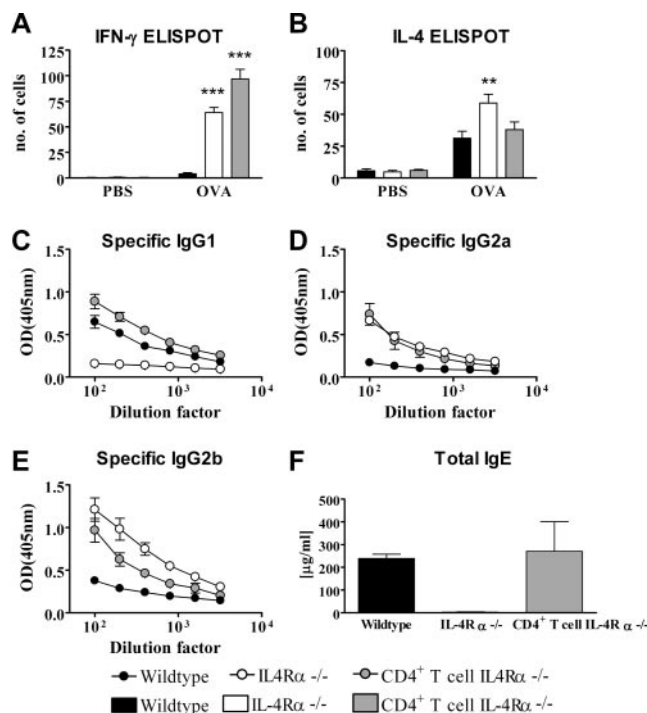


FIGURE 3. CD4⁺ T cell-specific IL-4R α -/- mice exhibit mixed type 1/type 2 responses. Mice were sensitized with alum-adsorbed OVA at days 0 and 14 and challenged i.v. with OVA at day 21. *A* and *B*, Splenocytes were harvested after challenge ($n = 4$ –5 per group) and ELISPOTS were performed to detect the number of IFN- γ - and IL-4-producing cells. Nine wells were counted per group, and results are representative of two experiments. *C*–*F*, OVA-specific IgG1, IgG2a, and IgG2b, and total IgE were measured in serum taken after challenge.

accordance, ELISA measurement of Ag-specific IgG isotypes confirmed ELISPOTS, with raised levels of both type 2 (IgG1/total IgE) (Fig. 3, *C* and *F*) and type 1 (IgG2a/IgG2b) isotypes (Fig. 3,

D and *E*) in CD4⁺ T cell-specific IL-4R α -deficient mice. As expected, wild-type mice had predominantly IgG1/IgE responses, and IL-4R α -deficient mice mounted a predominant IgG2a/IgG2b response. These results indicated that a shift toward Th1 responses is associated with protection from severe anaphylaxis.

IFN- γ depletion abrogates protection against anaphylaxis in CD4⁺ T cell-specific IL-4R α knockout mice

To address whether the Th1 cytokine IFN- γ served a protective role against OVA-induced anaphylaxis, mice were sensitized with OVA/alum at days 0 and 14 and injected i.p. with 1 mg of anti-IFN- γ Ab at days 18 and 20. This protocol aimed to allow sensitization to occur before mAb-mediated cytokine neutralization. Results showed that IFN- γ depletion of CD4⁺ T cell-specific IL-4R α -deficient mice dramatically increased the severity of shock when compared with mice of the same genotype that were administered IgG control Ab (Fig. 4). This was reflected by the rapid decline in rectal temperature and significant increase in mortality in CD4⁺ T cell-specific IL-4R α -deficient mice that were given the anti-IFN- γ mAb. Neutralization of IFN- γ also caused a slight increase in mortality in wild-type mice (Fig. 4). In addition, IL-4R α -deficient mice treated with anti-IFN- γ had a slight decrease in temperatures postchallenge as compared with control Ab-treated mice, but did not show significantly different anaphylactic reactions and did not experience mortality. Overall, these data demonstrate that IFN- γ protects against severe shock and mortality in a model of OVA-induced anaphylaxis.

The effects of IFN- γ depletion on Ab levels, Th1/Th2 cytokine production, MMCP-1, and intestinal pathology

We sought to determine whether anti-IFN- γ administration influenced the type of immune response generated in sensitized animals. Analysis showed that anti-IFN- γ treatment at days 18 and 20 did not significantly influence the generation of type 1/type 2 Abs (Fig. 5*A*) nor the secretion of IL-4, IL-5, IL-13,

FIGURE 4. Protection against anaphylaxis in CD4⁺ T cell-specific IL-4R α KO mice is abrogated by IFN- γ depletion. Mice were sensitized with alum-adsorbed OVA at days 0 and 14, treated with anti-IFN- γ mAb or control Ab at days 18 and 20, and challenged i.v. with OVA at day 21. Results are representative of two experiments. Rectal temperatures were followed for 1 h after challenge, and anaphylactic reactions were scored as described in *Materials and Methods*. Survival was plotted against time. +, Death.

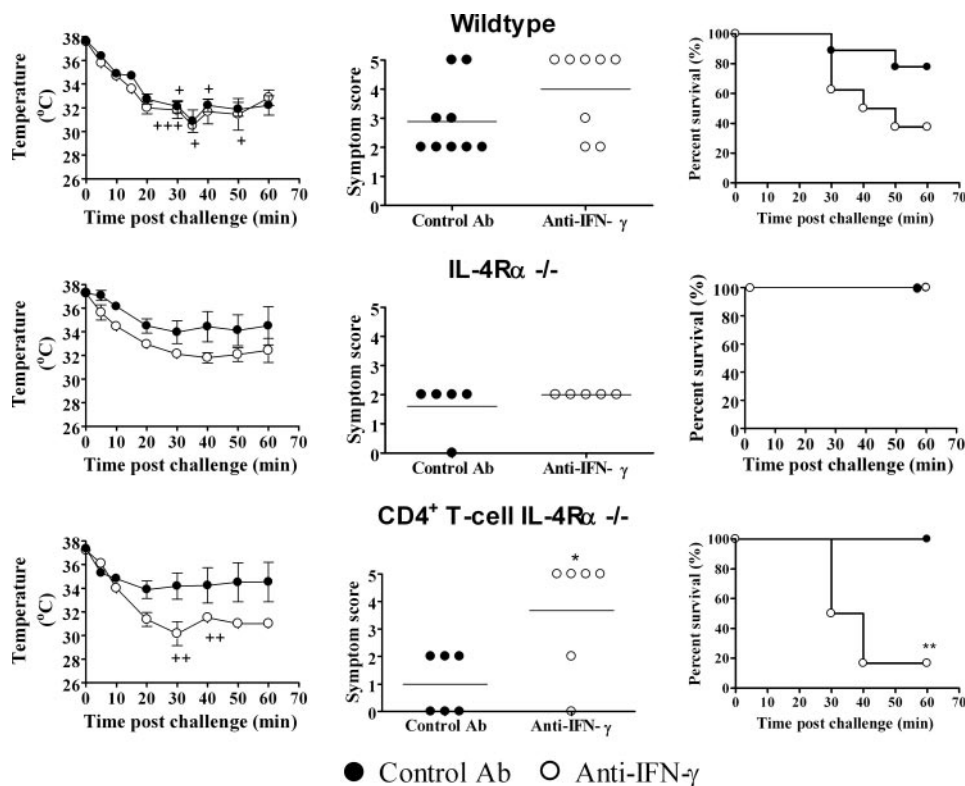
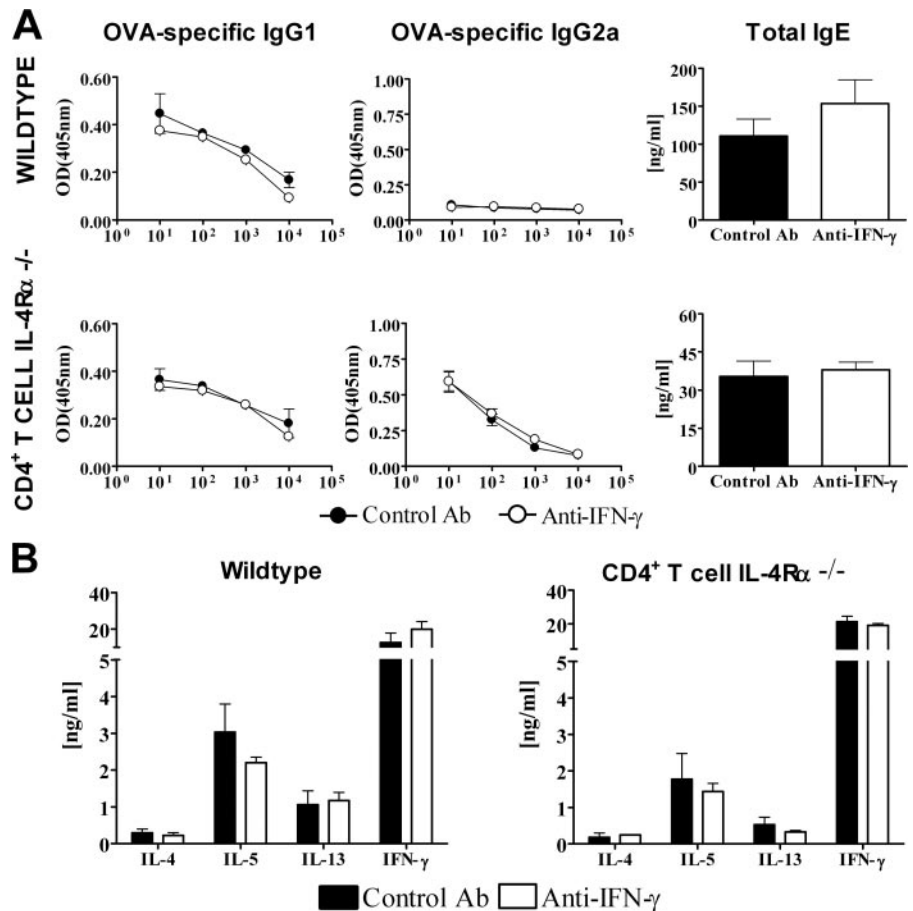


FIGURE 5. Anti-IFN- γ treatment does not alter serum Abs or splenocyte Th1/Th2-type cytokine production. Mice were sensitized with alum-adsorbed OVA at days 0 and 14, treated with anti-IFN- γ mAb or control Ab at days 18 and 20, and challenged i.v. with OVA at day 21. **A**, Specific IgG1 and IgG2a Abs and total IgE in control-treated and anti-IFN- γ -treated mice (day 21). **B**, Splenocyte cytokine production after restimulation with anti-CD3. Splenocytes were harvested at day 21.



and IFN- γ from restimulated splenocytes (Fig. 5B) in wild-type or CD4⁺ T cell IL-4R α -deficient mice. However, susceptibility to severe anaphylaxis correlated with alterations in the mast cell degranulation product MMCP-1. Serum MMCP-1 levels were

highly elevated in wild-type mice and did not increase further following administration of anti-IFN- γ Ab. In contrast, the low levels of serum MMCP-1 observed in control Ab-treated CD4⁺ T cell IL-4R α -deficient mice were greatly elevated following

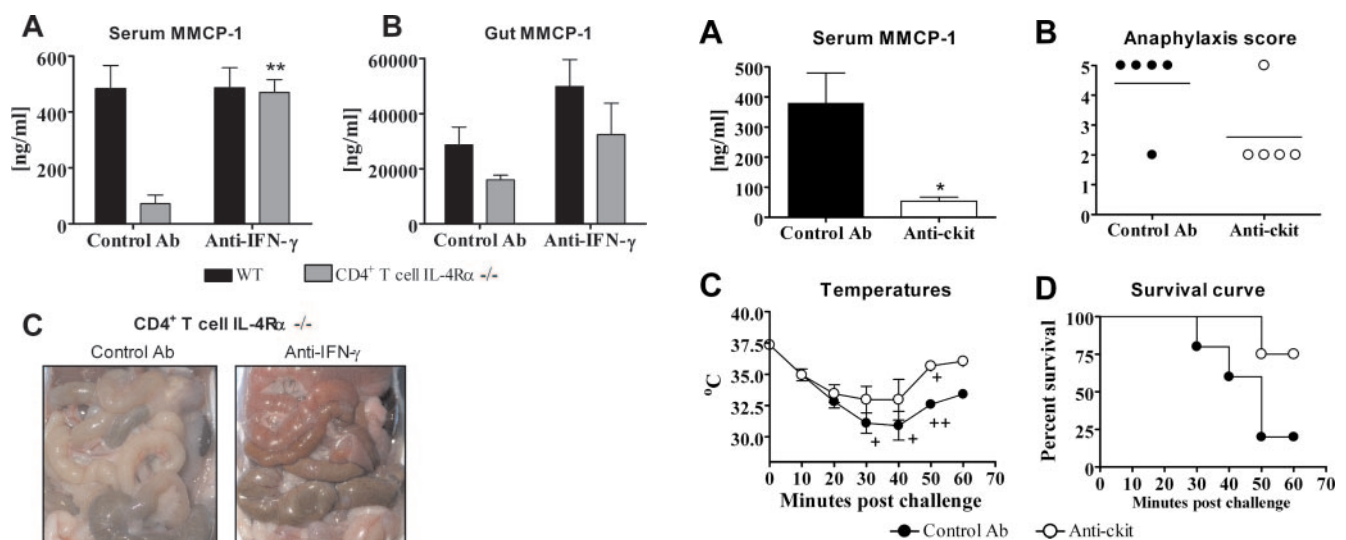


FIGURE 6. Anti-IFN- γ treatment increases MMCP-1 levels and intestinal pathology in CD4⁺ T cell-specific IL-4R α -deficient mice. **A** and **B**, MMCP-1 levels were determined in serum (**A**) and gut homogenates (**B**) of control Ab- and anti-IFN- γ -treated mice after i.v. OVA challenge as an indication of mast cell degranulation. **C**, Anti-IFN- γ treatment increased intestinal pathology in CD4⁺ T cell-specific IL-4R α -deficient mice.

FIGURE 7. Decreased MMCP-1 levels are associated with decreased severity of anaphylaxis. Results are representative of two experiments, $n = 4-5$. **A**, Serum MMCP-1 levels after i.v. OVA challenge in control Ab and anti-ckit-treated OVA/alum-sensitized mice. **B**, Anaphylactic reactions were scored as described in *Materials and Methods*. **C**, Rectal temperatures were measured for 1 h postchallenge. +, Death. **D**, Survival was plotted against time.

anti-IFN- γ mAb treatment (Fig. 6A). Similarly, IFN- γ depletion raised gut levels of MMCP-1 in both wild-type and CD4⁺ T cell-specific IL-4R α -deficient mice (Fig. 6B), indicating a role for IFN- γ in inhibiting mast cell degranulation and/or accumulation. After IFN- γ depletion, CD4⁺ T cell IL-4R α -deficient mice showed intestinal edema and hyperemia to the same degree as wild-type mice, whereas control Ab-treated mice failed to develop severe pathology (Fig. 6C).

Mast cells exacerbate anaphylaxis in wild-type mice

Mast cell depletion experiments were performed to directly address the role of mast cells in our model of anaphylaxis. Administration of rat anti-mouse *c-kit* mAb was performed before sensitization and challenge (days -2, 0, 12, 14, and 20) in an attempt to completely remove circulating mast cells present at the time of i.v. challenge. The treatment significantly reduced numbers of mast cells, illustrated by significantly decreased levels of serum MMCP-1 after challenge (Fig. 7A). In comparison with control Ab-treated animals, anti-*c-kit*-treated mice showed a trend toward less severe reactions, decreased hypothermia, and increased survival (Fig. 7, B-D). These data indicate that mast cell degranulation contributes to the severity of anaphylaxis in this model.

Discussion

Understanding the immunological mechanisms surrounding anaphylaxis is essential for the design of better preventative and therapeutic strategies. To date, various murine models of anaphylaxis have been used to elucidate pathways of anaphylaxis. In this study, we use cell-specific IL-4R α -deficient mice to show that IL-4R α expressed on CD4⁺ T cells, but not macrophages and neutrophils, is critical in mediating responses that lead to Ag-induced anaphylactic shock, and that IFN- γ plays a key role in protection against anaphylaxis.

Anaphylaxis in this model is Fc γ RII/III dependent and independent of IgE. Murine models of anaphylactic shock have shown that both Fc ϵ RI/IgE (8, 9)- and Fc γ RIII/IgG-dependent pathways exist (5, 6, 26). Studies by Strait et al. (6) have demonstrated that Fc ϵ RI- and Fc γ RIII-mediated anaphylaxis are dependent on mast cells and macrophages, respectively. Their studies and ours show that Ag-induced anaphylaxis in mice is primarily Fc γ RIII dependent. However, in contrast to their model, in which Ag-induced anaphylaxis was virtually independent of mast cells, in our study anti-*c-kit* treatment reduced the severity of anaphylactic reactions. Fc γ RII/III-dependent anaphylaxis was associated with significantly raised levels of MMCP-1, and anti-IgE treatment did not abrogate MMCP-1 levels. This, together with the presence of Fc γ RIII on mast cells (27), suggests that IgG/Fc γ RIII-dependent mast cell degranulation occurred in our model and could represent an additional pathway for anaphylaxis in mice. This is supported by evidence from Miyajima et al. (5) showing that mice lacking a functional Fc ϵ RI maintain mast cell degranulation, whereas mice deficient in the Fc ϵ R γ subunit do not. Together these data indicate a more important role for mast cells in Ag-induced anaphylaxis in this study than in the model used by Strait et al.

IgG-mediated anaphylactic reactions have been reported in humans (10, 12, 28), but the mechanisms of IgG-dependent anaphylaxis are uncertain. In vitro, Ag-specific IgG can cause human basophil/mast degranulation and release of mediators (29, 30). The same mediators are released by both IgG- and IgE-mediated degranulation (30). Clinically, IgE-mediated and IgG-mediated reactions are virtually identical, and so whereas IgE-independent reactions used to be called anaphylactoid, both forms of the disease are now known as anaphylaxis (31). Similarly, in mice, the outcome of IgE-mediated and IgG-mediated pathways is much the

same, with involvement of the same target organs (6). Therapies relevant to mice may therefore have possible application in humans.

Anaphylaxis is IL-4R α dependent. In the present model, as in others, the IL-4R α was found to play an essential role in anaphylaxis, with IL-4R α null mutant mice maintaining resistance to death and intestinal pathology (6). The IL-4R α is essential for various forms of allergic inflammation and hyperresponsiveness through signal transduction following IL-4 and/or IL-13 binding (32-35). These cytokines cause B cell IgE and IgG1 secretion, Th2 differentiation, and IL-4R α -dependent alternative macrophage activation. IL-4/IL-13 also exerts effects upon epithelial, endothelial, and smooth muscle cell lineages by promoting chemokine production, vasodilation, and contractility, respectively. Both IL-4 and IL-13 influence intestinal and systemic anaphylaxis by increasing cellular responsiveness to vasoactive mediators and shifting the balance of ion and fluid flow (7, 15, 36, 37). It has been shown that IL-4- and IL-13-mediated exacerbation of murine anaphylaxis can occur even in the absence of T cells, B cells, and the common γ -chain, but requires the IL-4R α and STAT6 (7, 15). The IL-4R α subunit is expressed on a wide range of cells (17). Whether IL-4/IL-13-driven anaphylaxis could be inhibited by targeting individual components of an intact immune system was not known. Our study is the first to examine the effects of in vivo dissociation of IL-4/IL-13 responsiveness on individual cell types during systemic Ag-driven anaphylaxis. Macrophage/Neutrophil-specific and CD4⁺ T cell-specific IL-4R α knockout mice were generated to determine the role of IL-4R α signaling in these cells independently of other cell populations. Studies have shown that macrophages can be important mediators of IgG-dependent anaphylaxis through PAF release (5, 6), whereas CD4⁺ Th cells play an important role in shaping adaptive immune responses by the secretion of a wide variety of cytokines. Our observations show that macrophage and neutrophil activation through the IL-4R α is not required for severe pathology and mortality in anaphylaxis. This demonstrates that alternatively activated macrophages, which are essential during certain Th2-driven pathologies (21, 23), have a minimal role during this type of allergic response. In contrast, deletion of IL-4R α from CD4⁺ T cells abrogated anaphylaxis almost completely. Murine T cells do not express the IL-13R α 1 component and cannot respond to IL-13. Therefore, CD4⁺ T cell IL-4R α -deficient mice enable specific investigation into the importance of IL-4 signaling through CD4⁺ T cells in anaphylaxis. Unexpectedly, abrogation of IL-4 signaling through IL-4R α on CD4⁺ T cells alone was enough to protect mice against anaphylaxis, despite maintained signaling of IL-4 and IL-13 through other cell types. This novel finding illustrates a critical role for CD4⁺ T cells in controlling susceptibility to anaphylaxis.

Subsequently, we aimed to determine the mechanism of protection in CD4⁺ T cell IL-4R α -deficient mice. The data showed that this strain was protected against anaphylaxis despite maintenance of Th2-type responses and production of IgG1 and IgE. However, a notable feature of this strain, seen both in our anaphylaxis experiments and in other disease models used in our laboratory (24, 38), was a tendency to produce high numbers of IFN- γ -producing cells and raised levels of IFN- γ . The number of IFN- γ -producing cells in CD4⁺ T cell-specific IL-4R α -deficient mice was significantly increased at the time of antigenic challenge. This indicated a potential role for IFN- γ in resistance to anaphylaxis. IFN- γ neutralization in CD4⁺ T cell IL-4R α knockout mice led to a rapid subversion to severe anaphylaxis, comparable to that of wild-type littermates (Fig. 4), demonstrating the importance of IFN- γ in protection. Because IFN- γ was only neutralized after sensitization

was complete, we concluded that protection occurred at the effector phase of the allergic response. Levels of IgG1 and IgE as well as Th1/Th2-type cytokine production by splenocytes were unaffected by IFN- γ neutralization (Fig. 5).

In a previous study, Strait et al. (15) found no effect of raised serum IFN- γ levels on anti-Fc γ R2/3 mAb-induced anaphylaxis, although IFN- γ reversed IL-4 exacerbation of anaphylaxis. However, this model lacked a sensitization phase, effectively eliminating possible influences from activated/sensitized bystander cells such as T cells and mast cells to the anaphylactic phenotype. Indeed, in this model, anaphylaxis was independent of mast cells, and MMCP-1 was not raised after anti-Fc γ R2/3 challenge (6, 15). This is in direct contrast to our model, in which anaphylaxis was Fc γ R2/3 dependent, but associated with raised levels of MMCP-1 (Fig. 1, E and F), and anti-c-kit treatment was associated with reduced severity of anaphylaxis (Fig. 7). The loss of resistance to anaphylaxis in anti-IFN- γ -treated CD4⁺ T cell-specific IL-4R α knockout mice was associated with a 6-fold increase in serum MMCP-1, a marker of mast cell degranulation (Fig. 6A).

Several studies have shown in vitro inhibition of mast cell degranulation by IFN- γ , mediated by NO (39, 40). The NO was thought to be produced by accessory cells such as macrophages. IFN- γ binds to glycosaminoglycan dermatan sulfate on mast cells and is then presented to the IFN- γ receptor on macrophages to induce NO (41). Therefore, an IFN- γ -dependent mechanism exists that may inhibit the release of mediators such as histamine, PAF, and MMCP-1, a protease known to increase intestinal permeability (42). For the first time, we show data indicative of IFN- γ -mediated inhibition of mast cell degranulation in vivo. It is also possible that IFN- γ has effector roles that extend beyond the inhibition of mast cell degranulation. It has already been shown that IFN- γ reverses IL-4-induced sensitivity to vasoactive mediators such as histamine, PAF, serotonin, and cysteinyl leukotrienes in a STAT-6-dependent fashion (15). The release of vasoactive mediators and subsequent binding of mediators to cell receptors results in the vascular permeability and vasodilation that are primarily responsible for the symptoms of anaphylactic shock. An in vitro study has shown that IFN- γ mediates suppression of PAF-induced activities by down-regulation of PAF receptor number and binding affinity (43). Up- or down-regulation of mediator receptors by IL-4/IL-13 and IFN- γ , respectively, offers an intriguing possibility by which the IL-4/IFN- γ balance could control cellular responsiveness to PAF and other anaphylactic mediators.

In summary, the data show that IL-4R α expression on CD4⁺ T cells plays a crucial role in mediating anaphylaxis and that IFN- γ may play a direct role in preventing life-threatening anaphylactic reactions by inhibiting the degranulation of mast cells. Interestingly, successful allergen-specific immunotherapy in humans is often associated with increased IFN- γ and IL-10, rather than a decrease in Th2 cytokines (44, 45), and tolerance to food allergens has been associated with high levels of IFN- γ (46). Treatment of atopic dermatitis with rIFN- γ has improved rhinitis and conjunctivitis in patients with concurrent symptoms (47). Our study suggests that therapies designed to inhibit CD4⁺ T cell-specific IL-4/IL-13 responsiveness and/or increase numbers of IFN- γ -producing T cells may show promise in preventing severe anaphylactic reactions in allergic individuals.

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Disclosures

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