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Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea

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Abstract

Rationale—Intestinal anaphylaxis (manifested by acute diarrhea) is dependent on IgE and mast cells.

Objective—We aimed to define the respective roles of IL-4 and IL-13 and their receptors in disease pathogenesis.

Methods—Wild-type mice and mice deficient in IL-4, IL-13, and IL-13Rα1 (part of the type 2 IL-4R) were sensitized with ovalbumin (OVA)/alum and subsequently given repeated intragastric OVA exposures. IL-4Rα chain was targeted with anti-IL-4Rα mAb prior to or after intragastric OVA exposures.

Results—IL-4$^{-/-}$ (and IL-4/13$^{-/-}$) mice produced almost no IgE and were highly resistant to OVA-induced diarrhea, whereas allergic diarrhea was only partially impaired in IL-13$^{-/-}$ and IL-13Rα1$^{-/-}$ mice. IL-13Rα1-deficient mice developed decreased IgE despite having normal baseline IL-4 levels. Intestinal mast cell accumulation and activation also depended mainly on IL-4 and to a lesser extent on IL-13. Prophylactic anti-IL-4Rα mAb treatment, which blocks all IL-4 and IL-13 signaling, suppressed development of allergic diarrhea. However, treatment with anti-IL-4Rα mAb for 7 days only partially suppressed IgE and did not prevent intestinal diarrhea.

Conclusion—Endogenously-produced IL-13 supplements the ability of IL-4 to induce allergic diarrhea by promoting oral allergen sensitization rather than the effector phase of intestinal anaphylaxis.

Keywords

allergy; anaphylaxis; IL-4; IL-13; IL-13Ralpha1; intestine; mast cell

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INTRODUCTION

Currently 2–6% of the population in the U.S. suffers from food allergy;[1] a disease characterized by elevated total and Ag-specific IgE eosinophilia, mastocytosis and gastrointestinal dysfunction (e.g. vomiting, diarrhea and failure-to-thrive). The development of experimental models of gastrointestinal hypersensitivity has provided important insight into the immunological mechanisms responsible for this disease.[1, 2]

Allergen-induced acute diarrhea, which develops in mice sensitized intraperitoneally (i.p.) with OVA/alum followed by repeated intragastric (i.g.) OVA administration, is dependent on IgE, mast cells and mast cell-generated vasoactive mediators.[3] The mild systemic features observed in this model led us to use the term "intestinal anaphylaxis" to describe the IgE-mediated mast cell degranulation that occurs in the small intestine and leads to increased intestinal permeability and acute diarrhea without shock.[3, 4]

Although increased quantities of both IL-4 and IL-13 are produced in the small and large intestine in this model, the roles of these cytokines and their receptors in the pathogenesis of intestinal anaphylaxis have not been explored.[3, 5, 6] IL-4 and IL-13 both signal through receptors that contain IL-4Rα chain and activate STAT6, but only IL-4 signals through the type 1 receptor, whereas both cytokines signal through the type 2 receptor (composed of the IL-4Rα and IL-13Rα1 polypeptides). The relative roles of these two receptors can be distinguished by genetic deletion of the IL-13Rα1 chain, since such genetically engineered mice have an intact type 1 IL-4R, but lack the type 2 IL-4R.[7, 8] T cell responses should not be directly affected by IL-13Rα1 deletion, because T cells lack the type 2 receptor.[9] Most murine B cells also express little or no type 2 IL-4R;[9] however, IL-4 and IL-13 signaling through this receptor might potentially influence the sensitization phase of allergic diarrhea by affecting the function of macrophages and dendritic cells.[4, 10] Based on their role in expulsion of nematode parasites,[4] IL-4 and IL-13 might also be involved in the effector phase of allergic diarrhea. Indeed, IL-4Rα positive non-bone marrow-derived cells have been implicated in parasite expulsion.[11] Subsequent work by Shea-Donohue and colleagues has demonstrated parasite-induced STAT6 dependent alterations in both intestinal epithelial cell function and smooth muscle contractility.[12, 13] Collectively, these studies suggest a role for IL4 and IL13 in the effector phase of the disease by increasing the sensitivity of intestinal tissues smooth muscle, epithelium, and vasculature to mediators released by mast cells.[12–14]

Defining the specific involvement of IL-4 and IL-13 is particularly important since therapeutic agents that block these cytokines or their common receptor (IL-4Rα) are being actively developed.[15, 16] These approaches are particularly timely since safety concerns have been raised by an anti-IgE clinical trial for peanut allergy. [17]

Using mice genetically deficient in IL-4, IL-13 or their receptors, we now demonstrate a central role for IL-4 in antigen-triggered intestinal mastocytosis and allergic diarrhea. Importantly, IL-13 and IL-13Rα1 are also shown to have a significant role.

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MATERIALS AND METHODS

Animals

IL-4-deficient mice (BALB/c background) were obtained from Jackson Laboratory (Bar Harbor, ME). IL-13-deficient and IL-4/IL-13 double-deficient BALB/c background mice were originally obtained from Andrew McKenzie (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK).[18] IL-13Rα1-deficient mice were generated at Regeneron by Velocogene Technology as recently reported,[7] and backcrossed into the BALB/c background for at least 6 generations. Animals involved in these studies were housed under specific pathogen-free conditions and treated in a humane manner according to institutional guidelines.

Intestinal Anaphylaxis Model

Mice were primed i.p. with OVA/alum and challenged repeatedly with OVA by oral gavage as previously described.[3]

In vivo cytokine capture assay

The in vivo cytokine capture assay (IVCCA) was used to monitor in vivo production of IL-4 as previously described.[19]

Intestinal mast cell quantification

Jejunum sections were stained for mast cells with chloroacetate esterase and numbers of mast cells/mm^3 of jejunum were determined as previously described.[3]

ELISA

Mouse mast cell protease 1 (MMCP-1) and total IgE plasma levels were measured according to manufacturers' instructions (respectively, Moredun Scientific, Midlothian, United Kingdom and BD Biosciences-Pharmingen, San Diego, California, USA).

Antibody treatment

IL-4Rα was blocked with 2 mg of an antibody (4-3, anti-IL-4 receptor α chain hybrid IgG1 mAb) given either i.p. or i.v. respectively 24 h or 3 h prior to OVA exposure.

Statistical analysis

Data are expressed as mean ± SD. Statistical significance comparing different sets of mice was determined by Student's unpaired t-test or the non-parametric Mann Whitney U-test.

RESULTS

Allergen-induced diarrhea is mediated by IL-4 and potentially IL-13

In order to determine the respective importance of IL-4 and IL-13 in intestinal anaphylaxis, we sensitized mice deficient in IL-4, IL-13 or both cytokines i.p. with OVA/alum and challenged them repeatedly i.g. with OVA. IL-4 deficiency almost completely protected against allergic diarrhea; two IL-4/IL-13-double-deficient mice developed diarrhea on the

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10th OVA exposure (Figure 1A). Induction of allergic diarrhea required a significantly greater number of allergen challenges in IL-13-deficient mice than in wild type mice (Figure 1A).

IgE and mast cell responses depend mainly upon IL-4

Because IgE and mast cells are essential for the development of allergic diarrhea,[3] we compared serum IgE levels, jejunal mast cell numbers, and serum levels of MMCP1 (an enzyme released by degranulating mast cells) in wild-type, IL-4-, IL-13- and IL-4/IL-13-deficient mice that had been primed and challenged with OVA. Serum IgE levels were ~2 logs lower in IL-4- and IL-4/IL-13-deficient than in wild-type mice and ~1 log lower in IL-13-deficient than in wild-type mice (Figure 1B). Jejunal mast cell numbers following OVA immunization were decreased 2-3-fold in IL-4-, and IL-4/IL-13-deficient mice (Figure 1C). Most importantly, MMCP1 responses in OVA-immunized IL-4- and IL-4/IL-13-deficient mice were 2-3-logs lower than MMCP1 responses in similarly treated wild-type mice (Figure 1D), although OVA immunization stimulated an ~10-fold increase in MMCP1 levels even in the absence of IL-4. Despite non-significantly altered intestinal mast cell levels, MMCP1 levels were significantly lower in IL-13-deficient than in wild-type mice (Figure 1D).

IL-13Rα1$^{-/-}$ mice demonstrate a role for IL-13 in IgE and mast cell mediated allergic diarrhea

Because IL-13-deficient mice produce subnormal amounts of IL-4 (a consequence of deletion of an IL-4 promoter sequence in the IL-13 gene),[20] the delayed development of intestinal anaphylaxis in IL-13$^{-/-}$ mice might result from diminished IL-4 production rather than the absence of IL-13. Indeed, we found that IL-4 production was reduced in naïve IL-13$^{-/-}$ mice compared to wild type mice as determined by in vivo cytokine capture assay (IVCCA) (66.1±23.1 vs. 115.1±39.3 pg/ml; $p<0.01$). This difference was not a direct effect of the absence of IL-13 signaling, because mice with defective IL-13 signaling (IL-13Rα1$^{-/-}$) had comparable levels of IL-4 (187.9±31.4 vs. 170.7±22.0 pg/ml for –/– and +/+, respectively). Consequently, we evaluated the ability of IL-13Rα1-deficient mice to develop allergic diarrhea and found that it was also significantly impaired (Figure 2A).

The decrease in serum IgE level in IL-13Rα1-deficient mice was ~2-fold and did not quite reach statistical significance (Figure 2B). These data suggest that IL-13 has a modest stimulatory effect on IgE production in OVA-immunized mice, resulting in impaired mast cell stimulation in the absence of IL-13 signaling, as shown by MMCP1 levels that were ~1 log lower in IL-13Rα1-deficient mice than in wild-type mice.

Prophylactic targeting of IL-4rα alleviates allergic diarrhea

In order to inhibit the effects of IL-4 and IL-13 in experimental intestinal anaphylaxis, 4-3, a mAb to IL-4Rα that blocks both IL-4 and IL-13 effects in vitro and in an in vivo mouse model of allergic airway disease (unpublished data) was used. Initial experiments tested whether a single dose of 4-3, injected 1 day prior to the initiation of i.g. OVA administration, could inhibit the development of allergic diarrhea. This single dose strongly inhibited the development of allergic diarrhea after 4–6 i.g. doses of OVA (Figure 3A). This

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delay in the development of allergic diarrhea was associated with impaired intestinal mast cell accumulation (Figure 3B–C), decreased MMCP-1 plasma levels (Figure 3D), and lower plasma IgE (Figure 3E).

IL-4 and IL-13 are not required for the effector phase of allergic diarrhea

The ability of IL-4 and IL-13 to enhance smooth muscle contractility and epithelial permeability and secretion and the ability of these cytokines to increase sensitivity to mediators released by activated mast cells suggested that IL-4 and IL-13 might be contributing to the effector as well as the sensitization phase of intestinal anaphylaxis.[12–14] However, administration of up to 3 doses of anti-IL-4Rα mAb over a 7 day period, starting after OVA-immunized mice had already developed allergic diarrhea, failed to decrease the incidence of diarrhea following high dose OVA challenge (Figure 4A), although it decreased total IgE levels (Figure 4B), intestinal mast cell numbers (Figure 4C) and MMCP1 blood levels (Figure 4D).

DISCUSSION

Taken together, our observations demonstrate that not only IL-4 but also IL-13 has a significant role in intestinal anaphylaxis. Our finding that IL-4-deficient mice are protected from OVA-induced diarrhea is supported by an earlier study using a different model, which shows that mice pre-treated with an anti-IL-4 antibody prior to sensitization failed to develop diarrhea.[6] Confirmation of the delayed development of allergic diarrhea observed in IL-13-deficient mice with studies in IL-13Rα1-deficient mice was important because it could result from their decreased production of IL-4,[20] which would be expected to decrease IgE and mast cell responses.[21] In contrast, IL-4 production appears to be normal in IL-13Rα1-deficient mice. Although it could be argued that the delayed development of diarrhea in these mice might reflect the lack of IL-4, rather than IL-13, signaling through the type 2 IL-4 receptor, this seems unlikely, given the greater production of IL-13 than IL-4[5] and the more potent signaling of IL-13 than IL-4 through the type 2 receptor.[22] In addition, this explanation cannot account for the accelerated development of allergic diarrhea in IL-13Rα2-deficient mice (data not shown).

Surprisingly, in contrast to observations made in allergic airway disease models,[23] IL-13 is important in the induction, rather than the effector phase of intestinal anaphylaxis. This point is of importance, since the only available data so far in the gastrointestinal tract suggested that IL-13 and IL-4 had a significant impact on effector functions (e.g.; parasite expulsion).[4] Blocking both IL-4 and IL-13 signaling with a high dose of a potent anti-IL-4Rα mAb for as long as 7 days had little effect on allergic diarrhea induced by a high dose of allergen. It is unlikely that this negative result reflected inadequate IL-4Rα blockade, because even a single dose of the 4-3 mAb suppressed allergic diarrhea for > 2 weeks in the prophylactic model.

These observations raise the question of how IL-13 contributes to the induction phase of intestinal anaphylaxis, inasmuch as T cells lack the type 2 IL-4R and IL-4 is much more potent than IL-13 at activating mouse mast cells and inducing isotype switching by mouse B cells.[10, 24] One possibility is that IL-13 directly stimulates isotype switching by a small, but

important B cell subset.[25] This is consistent with the recent observation that baseline IgE levels are significantly lower in IL-13Rα1-deficient mice than in wild-type mice[7] and earlier observations of increased IgE levels in IL-13Rα2-deficient mice and in IL-4-deficient mice that overproduce IL-13.[26, 27] Alternatively, IL-13 effects on antigen presenting cells may indirectly promote IgE production and mastocytosis by contributing to Th2 cytokine production. In this regard, it is noteworthy that while both IL-4 and IL-13 can activate dendritic cells, only IL-4 stimulates their production of IL-12, which can inhibit Th2 cytokine production and B cell isotype switching to IgE by stimulating IFN-γ production.[28]

The observation that 10% of IL-4/IL-13-double-deficient mice developed diarrhea by the 10th allergen exposure indicates that the IL-4 requirement for induction of allergic diarrhea is not absolute. Detectable IgE serum levels have been observed in naïve IL-4Rα and IL-4/IL-13 deficient mice.[21] Furthermore, OVA-specific IgE levels were observed in IL-4 and IL-4Rα deficient mice following OVA sensitization either through the i.p. route or through repeated intranasal instillations.[29] This would support our findings that IgE is present in IL-4 and IL-4/IL-13-deficient mice following sensitization, albeit at barely detectable levels. Although we did not measure a significant induction of total plasma IgE levels following repeated intestinal allergen exposures, mast cell-bound OVA-specific IgE may have increased without a detectable increase in circulating levels of IgE in multiply-immunized IL-4- and IL-4/IL-13-deficient mice. We observed a 2-fold increase in mast cell accumulation associated with a log increase in MMCP-1 plasma levels in these mice, indicating that repeated immunization could induce mast cell activation through an IL-4/IL-13-independent pathway. Similar results were observed with STAT6-deficient mice (data not shown). Interestingly, intestinal mastocytosis is actually increased more in STAT6-deficient than in wild-type mice following infection with some nematodes.[30]

Finally, one would expect that blocking IL-4Rα should 1) normalize smooth muscle contractions and epithelial cell functions 2) reduce Th2-related antibody responses (IgE, IgG1), 3) decrease intestinal inflammation; and 4) impair mast cell degranulation with release of MMCP1, serotonin and PAF in allergen-immunized mice. Indeed, our findings demonstrate that a prophylactic approach blocks diarrhea development. Although treatment with anti-IL-4Rα for 7 days failed to block the diarrheal response to allergen challenge, it significantly decreased IgE, mast cell and MMCP1 responses despite continuing allergen administration. This suggests that a longer period of treatment with this mAb might suppress allergic diarrhea. The ability of this mAb to decrease IgE production may make it particularly effective as a treatment for food allergy when paired with a second biological agent, such as a non-activating anti-IgE mAb.

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Abbreviations used

Alum	aluminum potassium sulfate
i.p.	intraperitoneal
i.g.	intragastric
IVCCA	in vivo cytokine capture assay
mAb	monoclonal antibody
MMCP1	mouse mast cell protease 1
OVA	ovalbumin
PAF	platelet activating factor

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Key Messages

Using mice genetically deficient in IL-4 and IL-13 signaling, we demonstrate a central role for IL-4 in antigen-triggered intestinal mastocytosis and allergic diarrhea; however, IL-4 and IL-13 are not absolutely required for the development of intestinal anaphylaxis. IL-13 contributes to allergic diarrhea and does so by promoting oral allergen sensitization rather than the effector phase of intestinal anaphylaxis.

Short-term treatment of established allergic diarrhea with an inhibitor of both IL-4 and IL-13 is not effective.

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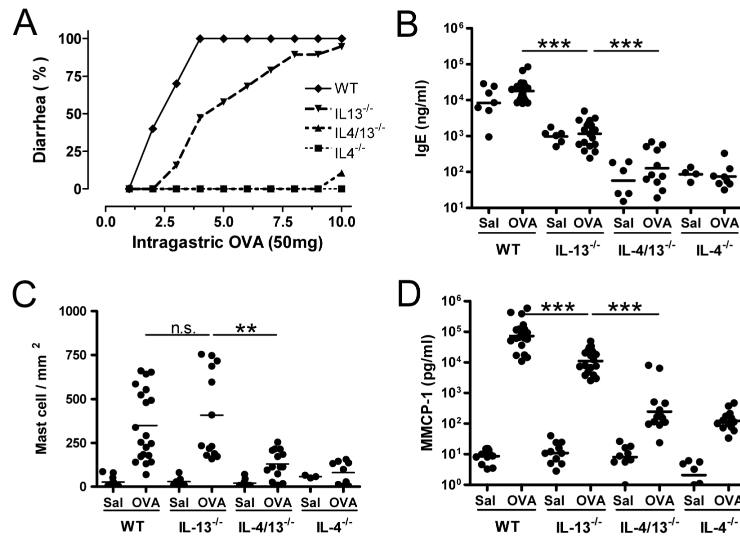

Figure 1. Ablated intestinal anaphylaxis in the absence of IL-4 and IL-13

(**A**) Occurrence of diarrhea was assessed in OVA/alum-primed wild type, IL-13$^{-/-}$, IL-4/13$^{-/-}$ and IL-4$^{-/-}$ mice (n=9–14 mice/group) up to 60 minutes after 1–10 i.g. inoculations with 50 mg of OVA and (**B**) Jejunum mast cells numbers were assessed by morphometric analysis of chloroacetate esterase-stained cells. (**C**) IgE and (**D**) MMCP1 levels were measured by ELISA in blood drawn 60–90 minutes after the last saline or OVA inoculation. Serum IgE and MMCP1 levels were 490±204ng/ml and 9.7±7.8pg/ml respectively in naïve wild type mice, which had 35±25 mast cells/mm^3 of jejunum. (** $p < 0.01$; *** $p<0.001$).

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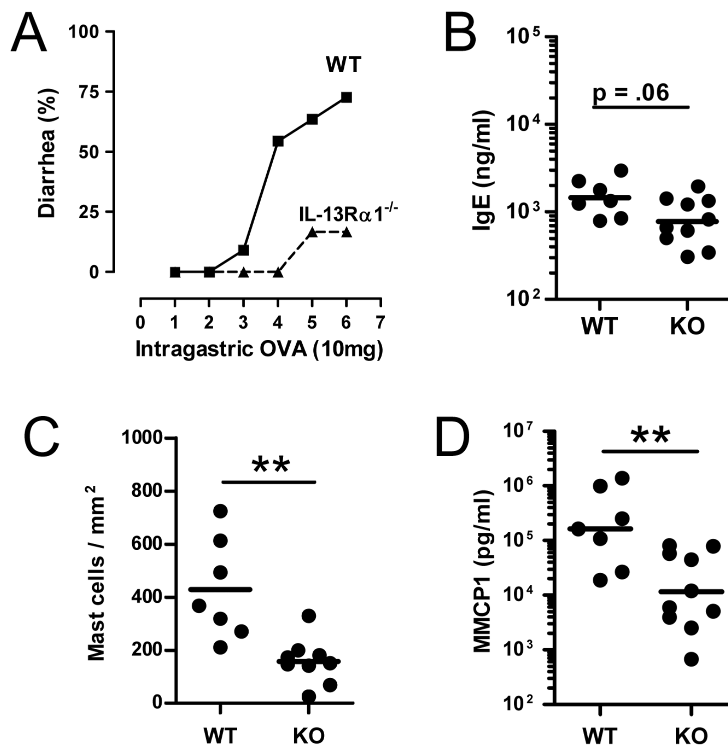

Figure 2. IL-13 contributes to IgE and mast cell allergic responses

(**A**) Diarrhea occurrences in OVA-alum primed wild-type and IL-13Rα1$^{-/-}$ mice after 1–6 i.g. inoculations with 10 mg of OVA. (**B**) Serum IgE levels, (**C**) jejunum mast cell numbers, and (**D**) serum MMCP1 levels were compared in wild-type and IL-13Rα1-deficient mice (7–18/group; ** $p < 0.01$; *** $p<0.001$).

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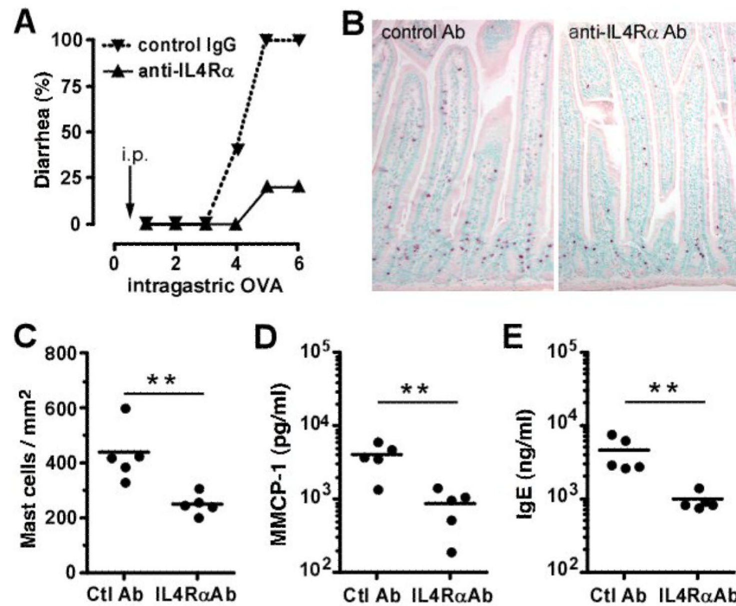

Figure 3. Prophylactic effects of anti-IL-4Rα mAb

OVA/alum-primed BALB/c mice (5/group) were injected i.p. with either 2 mg of 4–3 anti-IL-4Rα mAb or a control IgG1 mAb 1 day before the first i.g. inoculation with OVA. **(A)** Development of diarrhea was assessed during the 60 minutes after 1–6 i.g. OVA inoculations. **(B)** Representative jejunum section of antibody treated mice stained with chloroacetate esterase. (**C**) Intestinal mast cell numbers were assessed by morphometric analysis. (**D**) Blood MMCP-1 and (**E**) total IgE levels were assessed 1-2 hours following the last allergen exposure (* $p<0.05$; ** $p < 0.01$).

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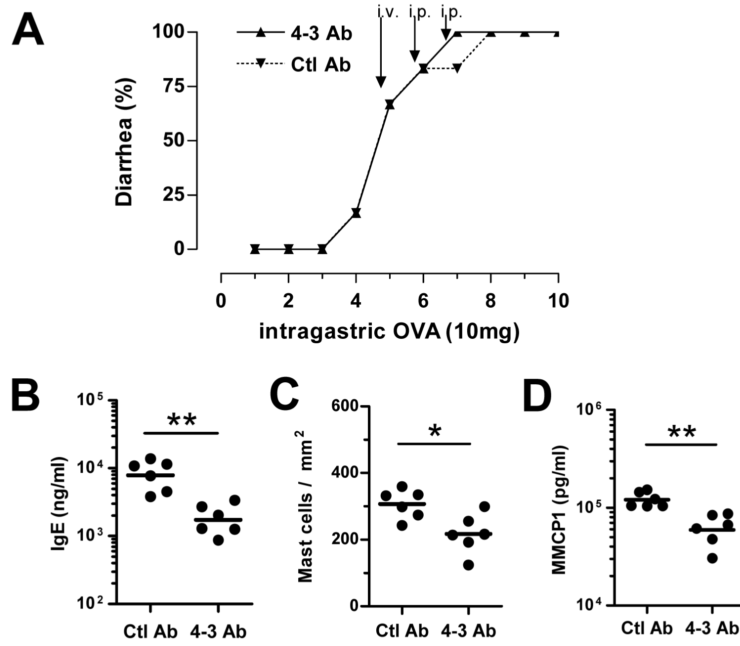

Figure 4. IL-4 and IL-13 are not required during the effector phase of allergic diarrhea

(**A**) Once diarrhea had developed (after 4 i.g. challenges with OVA), BALB/c mice (6/group) were injected with 2 mg of 4–3 or control IgG1 mAb before the 5th, 6th and 7th OVA inoculations (n=6 mice/group). (**B**) One week and three OVA inoculations later, mice were sacrificed and plasma IgE levels were determined. (**C**) Jejunum mast cell numbers and (**D**) MMCP-1 blood levels were assessed 1–2 hours after the last OVA inoculation (* $p<0.05$; ** $p < 0.01$).