

Original article

Differential requirements for interleukin (IL)-4 and IL-13 in protein contact dermatitis induced by *Anisakis*

Background: Exposure to antigens of the fish parasite *Anisakis* is associated with the development of protein contact dermatitis in seafood-processing workers. Understanding the basic mechanisms controlling allergic sensitization through the skin is critical for designing therapies that will prevent the progression of allergic disease.

Objective: To investigate the roles of interleukin (IL)-4, IL-13 and the IL-4R α in both local skin pathology and systemic sensitization following epicutaneous exposure to *Anisakis* proteins.

Methods: BALB/c wild-type (WT) mice and mice deficient in IL-4, IL-13 or IL-4 and IL-13, as well as mice with cell-specific impairment of IL-4R α expression, were sensitized to *Anisakis* antigen by repeated epicutaneous application of *Anisakis* extract. Following this sensitization, skin pathology was recorded and systemic responses were investigated. Intravenous challenge with *Anisakis* extract was performed to test for the development of biologically relevant systemic sensitization.

Results: In WT mice, epicutaneous sensitization with *Anisakis* larval antigens induced localized inflammation, epidermal hyperplasia, production of T_H2 cytokines, antigen-specific IgE and IgG1. Intravenous challenge of sensitized mice resulted in anaphylactic shock. Interestingly, IL-13 deficient mice failed to develop epidermal hyperplasia and inflammation, whilst anaphylaxis was reduced only in strains deficient either in IL-4 only, or deficient in IL-4 and IL-13 concurrently, as well as in mice deficient in IL-4R α or with impaired IL-4R α expression on CD4⁺ T cells.

Conclusions: Interleukin-13 plays a central role in protein contact dermatitis associated with repeated epicutaneous exposure to *Anisakis* extract, whereas IL-4 drives systemic sensitization and resultant anaphylactic shock.

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Skin disorders are the most frequently reported type of occupational disease (1, 2). In our recent cross-sectional epidemiological study of 578 fish-processing workers in South Africa, individuals sensitized to the parasitic fish roundworm *Anisakis* were almost twice as likely to report skin symptoms upon direct contact with fish than nonsensitized workers [odds ratio (OR) 1.9; 95% CI 1.0–3.6] (3). *Anisakis* has been implicated in a range of allergic skin diseases including urticaria, contact dermatitis, protein contact dermatitis and atopic dermatitis (eczema) (3–8). The live larvae can also cause gastrointestinal disease in humans (9). Sensitization to *Anisakis*

may occur via ingestion of infected fish (4, 6), inhalation of airborne *Anisakis* allergens (10) or direct contact with *Anisakis* proteins in fish (7, 10). Whether direct epicutaneous exposure to *Anisakis* antigens can directly lead to systemic allergic sensitization is yet to be demonstrated.

Protein contact dermatitis is IgE-associated and appears likely to be a form of atopic dermatitis where sensitization occurs via the skin rather than by ingestion or inhalation of allergens (11), unlike allergic contact dermatitis, which is a CD8/Th1-mediated response to haptens or metal ions such as nickel, which are not allergenic in themselves but become allergenic after binding to proteins or peptides (12). Individuals with atopic dermatitis in early life are at an increased risk of developing work-related dermatitis (13). Atopic dermati-

Abbreviations: IL, interleukin; IL-4R α , IL-4 receptor alpha; MMCP-1, mouse mast cell protease 1; WT, wild type.

tis in humans can be triggered by ingestion, inhalation or contact with allergens (14), but the majority of mouse models for atopic dermatitis use application of protein extract to the skin. The clinical signs of atopic dermatitis often predate the development of other allergic diseases, leading to the hypothesis that cutaneous exposure may be an important route of sensitization to allergens (15–19). Indeed, defective skin barrier function is an important feature of atopic dermatitis, thought to allow allergen penetration into the dermis and subcutaneous tissue where immune cells reside (14). Skin inflammation most likely culminates from increased permeability to irritants, allergens and exposure to microbes such as *Staphylococcus aureus* and different yeasts (12). Epicutaneous exposure to allergenic proteins causes differentiation of T cells into CD4⁺ T_H2 cells secreting interleukin (IL)-4 and IL-13. These cells circulate through lungs and upper airways, which is thought to promote the development of allergic diseases such as asthma and rhinitis (16, 17).

Interleukin-4 and IL-13 share many biological functions, but also have distinct roles by virtue of receptor expression on different cell types (20, 21). The IL-4R α can pair with the common γ chain subunit (type 1 IL-4R), to mediate IL-4-signalling or with IL-13R α 1 (type 2 IL-4R), for IL-4- and IL-13-signalling (20). Studies on human patients have revealed specific polymorphisms in the genes for IL-4, IL-4R α and the IL-4R α -associated transcription factor STAT-6 that are significantly associated with atopic dermatitis (22, 23) and T_H2 cytokine production is significantly elevated in skin biopsies of patients with IgE-associated atopic dermatitis (14). In transgenic mice, over-expression of IL-4 in the basal epidermis caused increased IgE and chronic dermatitis (24). Together these data support the idea that IL-4 is central to the IgE-associated form of dermatitis, which often precedes atopic diseases such as allergic rhinitis, asthma and food allergies (25).

While increased expression of IL-13 mRNA is associated with atopic dermatitis and allergic contact dermatitis in humans (26, 27), the role of IL-13 in antigen-induced skin pathology has not been investigated. We studied *Anisakis* antigen-induced dermatitis using mouse strains deficient in either IL-4, or IL-13 or both of these concurrently. Results demonstrated that while IL-4 was more important for systemic sensitization, IL-13 was essential for pathological skin inflammation and affected both epidermal hyperplasia and cell infiltration into the dermis.

Methods

Mice

BALB/c mice (8- to 12-week-old) and IL-4^{-/-} (28), IL-13^{-/-} (29), IL-4/IL-13^{-/-} (21), IL-4R α ^{-/-} (30), CD4⁺ T-cell-specific IL-4R α ^{-/-} (Lck^{cre}IL-4R α ^{-/lox}) (31) and macrophage/neutrophil-specific IL-4R α ^{-/-} (LysM^{cre}IL-4R α ^{-/lox}) (32) mice, all on a BALB/C

background for at least nine generations, were housed under specific pathogen free barrier conditions using individually 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.

Anisakis extract

Anisakis pegreffii larvae (L3) were collected from the intestines of parasitized fish (*Thyrsites atun*) and extensively washed in 4% acetic acid and phosphate-buffered saline (PBS). Extract was prepared as previously described (3).

Sensitization schedule

Mice were sensitized by repeated epicutaneous application of 200 μ g *Anisakis* extract or chicken egg ovalbumin (OVA) (Sigma, Steinheim, Germany) according to previously described methods (33). Briefly, a 1 $\times$ 2-cm area of skin on the abdomen of each mouse was shaved and a gauze patch, containing PBS, *Anisakis* extract or OVA, attached to adhesive tape (Band Aid; Johnson and Johnson, Broadway, Australia) was affixed. Tape-stripping was not performed after shaving. Patches were left for 1 week and then removed for a 2-week rest period, following which the entire procedure was repeated twice more. Mice were killed 1 week after the final patch and local skin changes were assessed microscopically. Epidermal hyperplasia was quantified (27–45 measurements per mouse group) by a blinded observer using a SPT Diagnostics Imaging System and Simple PCI C-IMAGING Software (PCI C Imaging Systems, Cranberry Townships, PA, USA). To evaluate clinically relevant sensitization, mice were challenged intravenously with 750 μ g *Anisakis* extract or OVA following the final sensitization period in a shortened protocol using 5-day intervals between epicutaneous exposures. Control mice were sensitized with PBS and challenged with *Anisakis* extract.

Temperature measurements

Rectal temperature readings were taken every 10 min using a mouse rectal thermometer (VetTech, Cheshire, UK) lubricated with VaselineTM petroleum jelly (Unilever, La Lucia, South Africa).

Anaphylaxis scoring system

Mice were observed for 60–70 min post challenge, and signs of anaphylaxis were scored as previously described (34): 0, no symptoms; 1, irritability, scratching and/or hypersensitivity to touch; 2, reduced activity, pilar erecti, and/or diarrhoea; 3, lying flat but upright, weakness and/or cyanosis around the mouth and tail; 4, lying on side, no activity upon prodding, loss of grip, loss of consciousness with or without convulsions; 5, death.

Histology

Skin taken from the site of epicutaneous exposure was preserved in 4% phosphate-buffered formalin for at least 24 h and then embedded in wax. Paraffin sections of 5 μ m were cut and fixed to slides, and stained with haematoxylin and eosin or toluidine blue for mast cells. Immunohistochemistry with anti-CD3 (clone 145-2C11) was used to stain T cells. Cells per high power field were counted; 40–60 fields per strain at 400 $\times$ for T cells and leucocytes, 15 fields per strain at 100 $\times$ for mast cells.

Serum antibodies and mouse mast cell protease 1

Blood samples were collected into serum separator tubes (BD Microtainer™ SST; BD, Franklin Lakes, NJ, USA) and centrifuged for 15 min at 4500 *g* before being placed at -80°C for storage. Total IgE and *Anisakis*-specific antibodies were detected in the serum as previously described (3). Mouse mast cell protease 1 (MMCP-1) levels in serum were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Moredun Scientific Ltd, Midlothian, UK).

Cytokine production

Splenocytes were isolated from individual spleens after the final sensitization and cultured at 4×10^6 cells/ml in Iscove's Modified Dulbecco's medium (Gibco, Paisley, Scotland)/10% fetal calf serum

(Delta, Kempton Park, South Africa) containing 2 mM L-glutamine (Gibco), 100 $\mu\text{g}/\text{ml}$ penicillin (Gibco) and 100 $\mu\text{g}/\text{ml}$ streptomycin (Gibco), in the presence or absence of anti-CD3 (clone 145-2C11; 10 $\mu\text{g}/\text{ml}$) or *Anisakis* extract (100 $\mu\text{g}/\text{ml}$). Supernatants were collected after 48 h, and cytokines were measured using sandwich ELISA assays according to the manufacturer's protocol (BD Biosciences Pharmingen, San Diego, CA, USA).

Statistical analysis

Values are given as mean $\pm$ SEMs, and significant differences were determined by one-way ANOVA with Bonferroni's multiple comparison post-test using computer software (GRAPHPADPRISM 4.0; GraphPad Software Inc., San Diego, California, USA). The Kruskal–Wallis test with Dunn's post-test was used for allergic score graphs values $P < 0.05$ were considered significant.

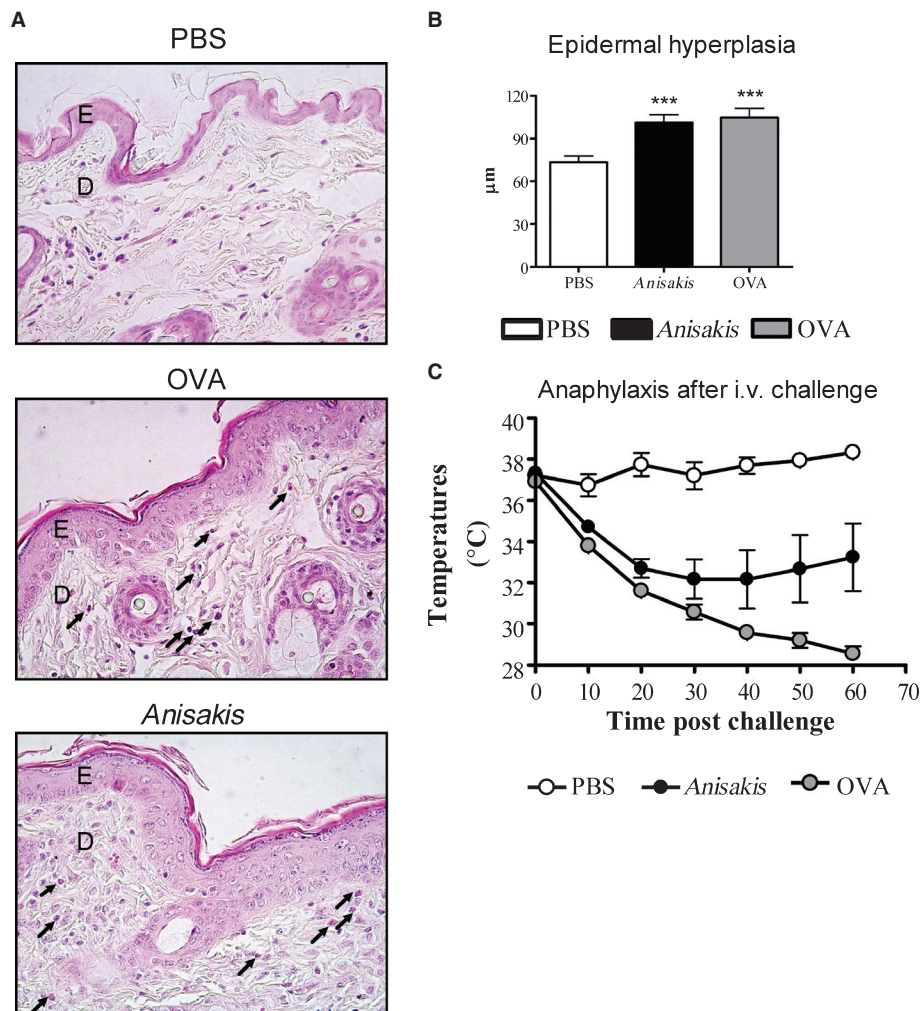


Figure 1. Epicutaneous exposure to *Anisakis* extract causes localized dermatitis. (A) After phosphate-buffered saline (PBS), *Anisakis* or OVA exposure, skin sections were stained with haematoxylin and eosin (400 $\times$ magnification). Black arrows point to eosinophils. E = epidermis; D = dermis. (B) Epidermal thickness was quantified using Simple PCI C-IMAGING Software. (C) After epicutaneous sensitization, mice were challenged intravenously with 750 μg of *Anisakis* extract or OVA, and rectal temperatures were measured. The PBS-sensitized mice were challenged with *Anisakis* extract as a control. *** $P < 0.001$ compared with the PBS control group. Data are representative of at least two experiments ($n = 4$ –5).

Results

Epicutaneous exposure to *Anisakis* extract induces localized dermatitis

Administration of *Anisakis* extract to wild-type (WT) mice via epicutaneous application resulted in a localized inflammatory response characterized by epidermal hyperplasia, dermal thickening and leukocyte infiltration (see Fig 1A). This phenotype resembled the allergic dermatitis elicited following treatment with chicken egg ovalbumin (OVA) in the control experiment and previous studies (19, 33, 35). Histological examination of the skin showed an inflammatory infiltrate consisting primarily of macrophages, neutrophils, lymphocytes and eosinophils. Epidermal hyperplasia was significantly increased in *Anisakis*- and OVA-treated mice as compared with PBS-treated mice (see Fig. 1B). Pathological changes in the skin after epicutaneous exposure to *Anisakis* extract were therefore highly similar to those induced by the model allergen OVA, which was confirmed by comparing OVA and *Anisakis*-induced dermatitis in WT, IL-4R α ^{-/-}, LysM^{cre}IL-4R α ^{-/-} and Lck^{cre}IL-4R α ^{-/-} mouse strains (data not shown).

To establish whether the systemic sensitization was biologically relevant, sensitized mice were given intravenous challenge with *Anisakis* antigen or OVA after the third epicutaneous exposure and monitored for signs of anaphylaxis, such as temperature loss and reduced activity (34) (see Fig. 1C). Three successive rounds of epicutaneous sensitization evoked anaphylactic responses in WT mice following both *Anisakis* and OVA challenge. Phosphate-buffered saline sensitized mice challenged intravenously with *Anisakis* extract did not react, demonstrating that reactions were antigen-specific.

Local pathology induced by epicutaneous *Anisakis* exposure is dependent on IL-13

To determine the roles for IL-4 and IL-13 in the observed skin pathology, IL-4^{-/-}, IL-13^{-/-} and IL-4/IL-13^{-/-} mice were exposed to PBS or *Anisakis* extract as described for WT mice, and skin biopsies were evaluated histologically (see Figs 2 and 3). We found that IL-4^{-/-} mice developed a similar degree of epidermal hyperplasia (Fig. 2A,B) and leucocyte infiltration (Fig. 3) to WT mice, although fewer infiltrating mast cells were present. In contrast, IL-13^{-/-} and IL-4/IL-13^{-/-} mice showed a marked decrease in recruitment of total leucocytes, T cells and mast cells (Fig. 3), combined with abrogated epidermal hyperplasia (Fig. 2B). This demonstrates that while both IL-4 and IL-13 cause inflammatory cell recruitment, it is the IL-13 which drives the pathological effects on the epithelium following epicutaneous application of *Anisakis* extract and specifically causes the recruitment of T cells.

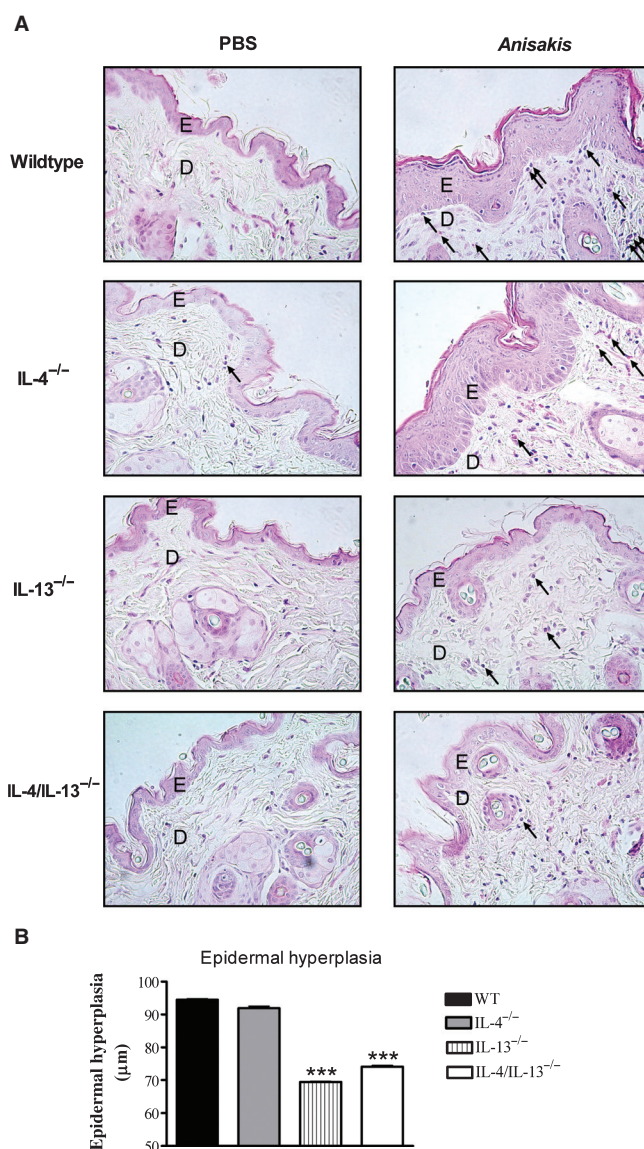


Figure 2. Histological features of wild-type (WT) and gene-deficient mouse skin epicutaneously exposed to PBS or *Anisakis* extract. (A) Sections were stained with H&E (400× magnification). Black arrows point to eosinophils. E = epidermis; D = dermis. (B) Epidermal hyperplasia after *Anisakis* extract exposure was quantified using Simple PCI C-IMAGING Software (see Methods). *** $P < 0.001$ compared with the WT group. Ns, not significant. Data are representative of two experiments ($n = 4-5$).

Systemic sensitization and Th2 skewing after epicutaneous antigen exposure is regulated by both IL-4 and IL-13

We sought to determine whether skin immunopathology was accompanied by IL-4- and/or IL-13-dependent systemic sensitization. Wild-type (WT), IL-4^{-/-}, IL-13^{-/-} and IL-4/IL-13^{-/-} mice were repeatedly epicutaneously exposed to *Anisakis* soluble antigen and serum levels of

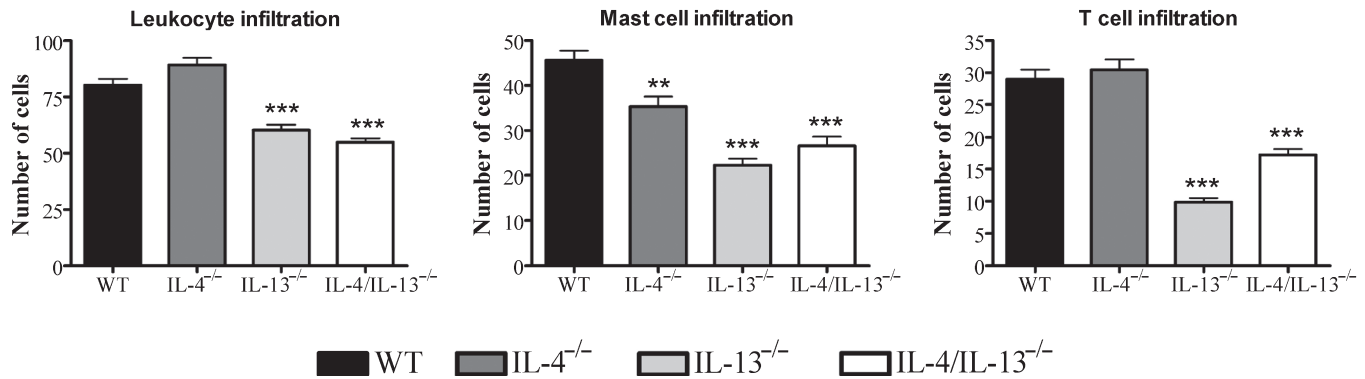


Figure 3. Cell infiltration into the skin. Skin sections were stained with haematoxylin and eosin (for total leucocytes), toluidine blue (for mast cells) or anti-CD3 (for T cells) and cells per high-power field were counted. ** $P < 0.01$; *** $P < 0.001$.

specific immunoglobulins were measured at the end of the sensitization period (week 7). The WT mice produced high titres of antigen-specific IgG1 and IgE, that were markedly reduced by the absence of IL-4 or IL-13, and virtually absent in IL-4/IL-13^{-/-} mice (Fig. 4A). Total IgE was raised in IL-13^{-/-} mice but not IL-4^{-/-} mice, although specific IgE was below the detection limit in both strains. Conversely, antigen-specific IgG2a was strongly elevated in the IL-4^{-/-} and IL-4/IL-13^{-/-} groups and lowest in the WT. The same patterns of antibody production were observed after a shortened sensitization protocol (see Methods), although antibody titres were lower in the latter case (data not shown). These data show that IL-4 rather than IL-13 is primarily responsible for allergic sensitization, i.e. IgE production, after epicutaneous allergen exposure in mice.

Splenocytes harvested at the end of the final sensitization period (week 7) were re-stimulated with anti-CD3 or *Anisakis* extract, and cytokine production was measured. This revealed a pattern consistent with the antibody results, with WT mice producing the highest amounts of IL-4, IL-5, and IL-13 compared with all other strains after both anti-CD3 and antigen-specific re-stimulation (see Fig. 4B,C). Conversely, IL-4^{-/-}, IL-13^{-/-} and IL-4/IL-13^{-/-} groups re-stimulated with anti-CD3 all produced significantly increased IFN- γ levels compared with WT mice, demonstrating a tendency to Th1 skewing in the absence of IL-4 and/or IL-13 signalling. Interleukin-10 levels were also decreased by gene-deficiency in IL-4 and/or IL-13. Together, the data indicate that both IL-4 and IL-13 contribute to systemic T_H2 sensitization after epicutaneous sensitization.

Systemic sensitization following epicutaneous exposure to *Anisakis* leads to IL-4/IL-13-dependent anaphylaxis

Figure 5 shows that IL-4/IL-13^{-/-} mice failed to develop hypothermia or any signs of anaphylactic shock after intravenous challenge with *Anisakis* extract, while IL-13^{-/-} mice experienced anaphylactic reactions similar

to those found in WT mice. However, IL-4^{-/-} mice were only partially protected. Neither IgE depletion nor Fc γ RII/III depletion abrogated anaphylaxis in WT mice, although both partially reduced it, suggesting that anaphylaxis could be mediated by both IgE and IgG antibodies in this model (data not shown).

Serum MMCP-1, a marker of mast cell degranulation, was dependent on IL-4 expression, as no differences were found between WT and IL-13^{-/-} mice whereas levels of MMCP-1 were significantly reduced in IL-4^{-/-} and IL-4/IL-13^{-/-} mice (see Fig. 5C). Therefore, raised MMCP-1 levels correlated with severe anaphylaxis (see Fig. 5A). Furthermore, partial depletion of mast cells by anti-ckit treatment reduced hypothermia in WT mice (data not shown). Taken together these data show that IL-4, as opposed to IL-13, drives anaphylactic pathways after epicutaneous sensitization.

Mice with impaired IL-4R α expression on CD4⁺ T cells or macrophages/neutrophils are not protected against dermatitis

Both IL-4 and IL-13 signal through the IL-4R α subunit. Similar to IL-4/IL-13^{-/-} mice, IL-4R α ^{-/-} mice showed reduced signs of dermatitis compared with WT mice after the application of epicutaneous antigen (see Fig. 6A). Interestingly, despite T cells and macrophages being the predominant cell types in dermatitis, cell-specific deletion of the IL-4R α on either CD4⁺ T cells or macrophages and neutrophils did not significantly reduce skin pathology. This raises the possibility that IL-13 may exert its effects via signalling on local skin cells such as keratinocytes. Re-stimulation of splenocytes from both cell-specific knockouts demonstrated that they are still capable of producing IL-13 (Fig. 6B), whereas IL-13 levels were significantly lowered in the global IL-4R α ^{-/-} mice. Both transgenic strains as well as the IL-4R α ^{-/-} mice showed significantly decreased production of IL-5 by re-stimulated splenocytes, whereas IL-4 production was reduced only in the CD4⁺ T cell-specific IL-4R α ^{-/-} mice (Lck^{cre}IL-4R α ^{-/lox}) and

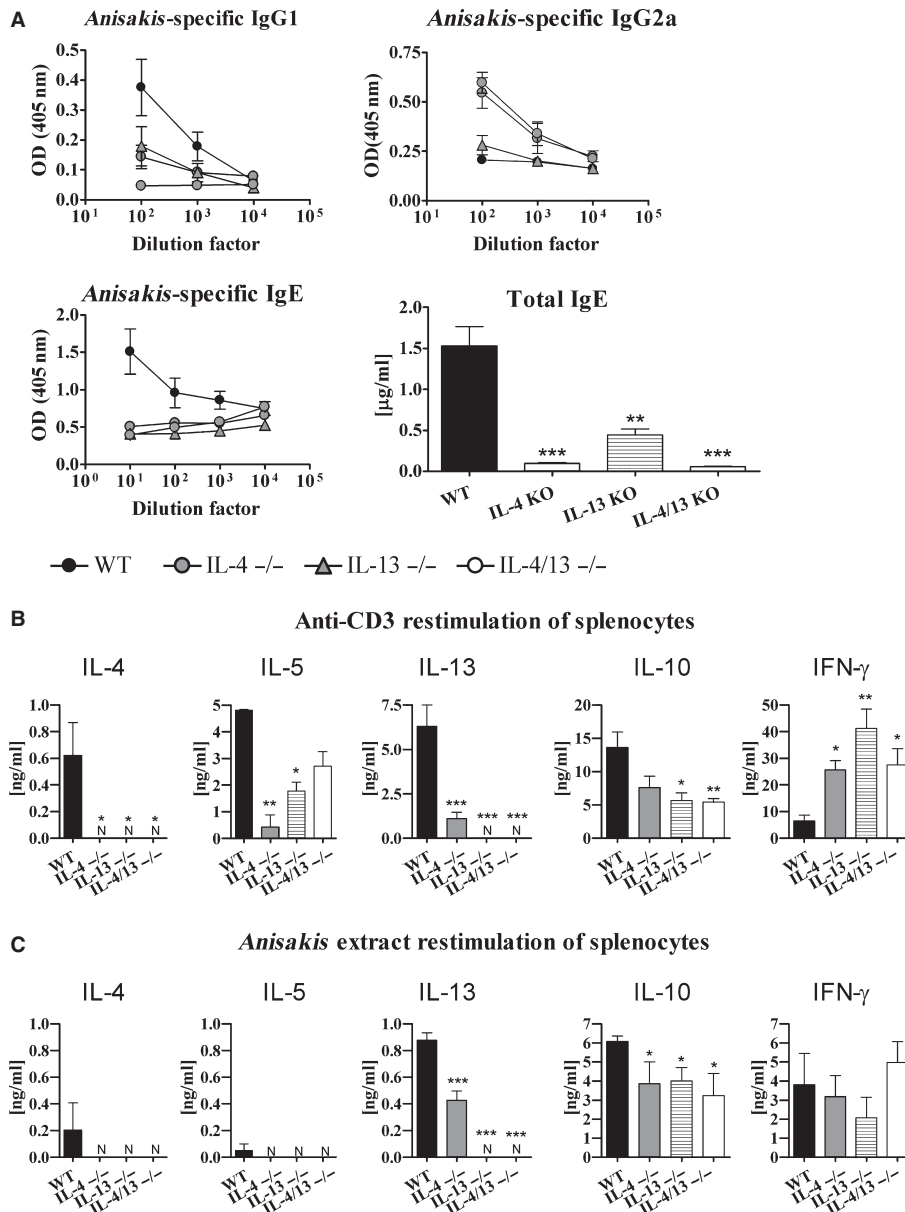


Figure 4. Systemic sensitization via epicutaneous exposure to *Anisakis* extract in WT and gene-deficient mice. (A) *Anisakis*-specific antibodies and total IgE were measured by ELISA ($n = 4$). ** $P < 0.01$; *** $P < 0.001$ compared with the wild-type. (B) Cells from individual spleens ($n = 4$) were re-stimulated with anti-CD3 or (C) *Anisakis* extract. Cytokines in supernatant were measured by enzyme-linked immunosorbent assay (ELISA). * $P < 0.05$; ** $P < 0.01$ compared with the wild-type. N, undetectable. Data representative of two experiments.

global knockouts. However, total IgE production was increased after sensitization in both cell-specific knockouts (Fig. 6C). After epicutaneous sensitization, intravenous antigen challenge was administered (see Fig. 6D). Similar to IL-4^{-/-} mice, CD4⁺ T cell-specific IL-4Rα^{-/-} mice showed a partial inhibition of anaphylaxis. This may have been caused by the decreased IL-4 production in this strain (Fig. 6B). Macrophage/neutrophil-specific IL-4Rα^{-/-} mice (LysM^{cre}IL-4Rα^{-/lox}) showed no inhibition of anaphylaxis.

Discussion

Understanding the immunological mechanisms surrounding skin-allergen interactions may help in designing strategies to treat allergic skin diseases and combat the progression of allergic disease. In this study, we have used mice deficient in IL-4, or in IL-13 or in both IL-4 and IL-13 to investigate mechanisms of allergic sensitization and dermatitis caused by epicutaneous exposure to clinically relevant antigens derived from the parasitic nematode

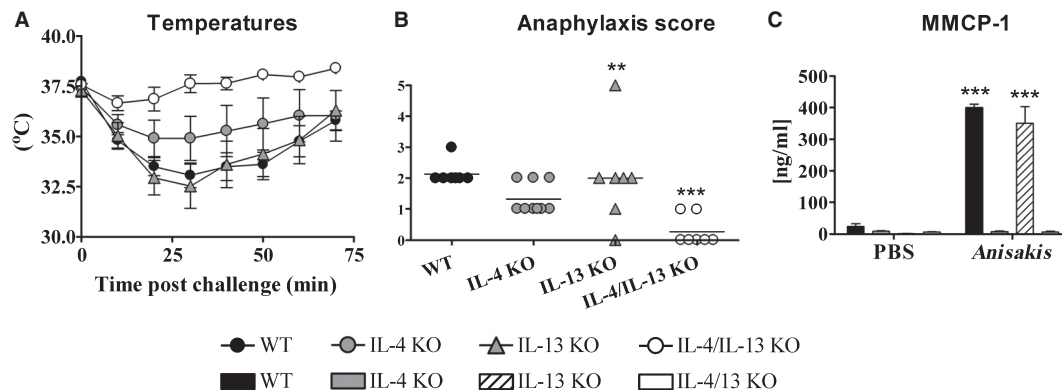


Figure 5. Anaphylactic responses after intravenous challenge of epicutaneously sensitized wild-type and gene-deficient mice. (A) Rectal temperatures were recorded following challenge. (B) Reactions were rated using a scoring system (see Methods). $**P < 0.01$; $***P < 0.001$ compared with the WT. (C) Mouse mast cell protease 1 (MMCP-1) levels were measured by ELISA in serum taken at day 0 and 80–90 min after challenge. $**P < 0.01$; $***P < 0.001$ compared with the PBS control groups. Data are representative of two experiments, $n = 4$ –5.

Anisakis. Our results reveal separate roles for these canonical T_H2 cytokines; IL-13 plays a central role in eczematous pathology, whilst IL-4 drives systemic anaphylaxis following skin sensitization.

Epicutaneous exposure to *Anisakis* extract caused dermatitis and systemic sensitization in WT BALB/c mice (see Fig. 1). The fact that *Anisakis* proteins appear capable of eliciting systemic sensitization via the skin highlights the importance of minimizing epicutaneous exposure to this allergen in fish-processing workers (2, 3). Indeed, *Anisakis*-exposed WT mice developed epidermal hyperplasia, dermal thickening and infiltration of immune cells into the dermis. Mice epicutaneously exposed to *Anisakis* extract developed *Anisakis*-specific IgG1 and IgE antibodies, providing clear evidence for systemic sensitization through the skin. Induction of systemic anaphylaxis following skin sensitization demonstrated the functional relevance of these antibodies.

This study demonstrates that IL-13 plays a central role in antigen-induced eczematous pathology in mice. In order to establish the specific roles of IL-4 and IL-13 in local skin inflammation, we utilized gene-deficient mice. Mice deficient in IL-4 possessed epidermal hyperplasia and leucocyte infiltration that was similar to that seen in WT mice, although less mast cells were observed; similar observations were made in a related study (35). In contrast, IL-13^{-/-} mice had a striking reduction in both epidermal hyperplasia and inflammatory cell recruitment following *Anisakis* antigen exposure. Likewise, IL-4/IL-13^{-/-} mice were protected against dermatitis, similar to IL-13^{-/-} mice.

Interleukin-4 was primarily important in mediating systemic reactions (anaphylaxis) following epicutaneous exposure to *Anisakis* extract (see Fig. 5). Herrick et al. investigated the role of IL-4 and IL-13 in airway hyper-reactivity following epicutaneous sensitization with OVA,

and concluded that IL-13 was the critical mediator in regulating T_H2 activation after epicutaneous sensitization (17). In this study, anaphylaxis after intravenous challenge was used to assess systemic sensitization to *Anisakis* extract. The results showed that epicutaneously sensitized IL-13^{-/-} mice did not have reduced anaphylactic responses after challenge. However, a possible role for IL-13 was indicated by the difference in temperature loss between mice that only lacked IL-4 vs mice that lacked both IL-4 and IL-13. Furthermore, both IL-4 and IL-13 were important in the induction of systemic Th2 responses (Fig. 4).

Interleukin-13^{-/-} mice produced both total IgE and *Anisakis*-specific IgG1, albeit at reduced levels, and showed serum MMCP-1 levels equivalent to those of WT mice, indicating that mast cell degranulation occurred after challenge (see Figs 4 and 5). In contrast, IL-4^{-/-} mice produced approximately the same levels of IgG1 as IL-13^{-/-} mice, but total IgE was strikingly decreased, as were the MMCP-1 levels in the serum. This suggests that IgE-mediated anaphylaxis was eliminated while IgG1-mediated anaphylaxis was retained. In contrast, IL-4/IL-13^{-/-} mice were completely protected against anaphylaxis. These mice had very low levels of *Anisakis*-specific IgG1, total IgE and MMCP-1 present in the serum, while IgG2a levels were raised. Antibody depletion experiments with anti-IgE, anti-FcγRII/III and anti-ckit suggested that anaphylaxis in this model can be mediated by either IgE or IgG antibody, and is at least partially dependent on mast cell degranulation (data not shown). As it has been demonstrated that IgG1 is the only anaphylactic IgG antibody in mice (36), it is possible that the IL-4/IL-13^{-/-} mice were protected because of the reduction in both IgE and IgG1 levels. Approximately half of all atopic dermatitis patients go on to develop asthma, while as many as two-thirds develop allergic rhinitis (16) and the severity of

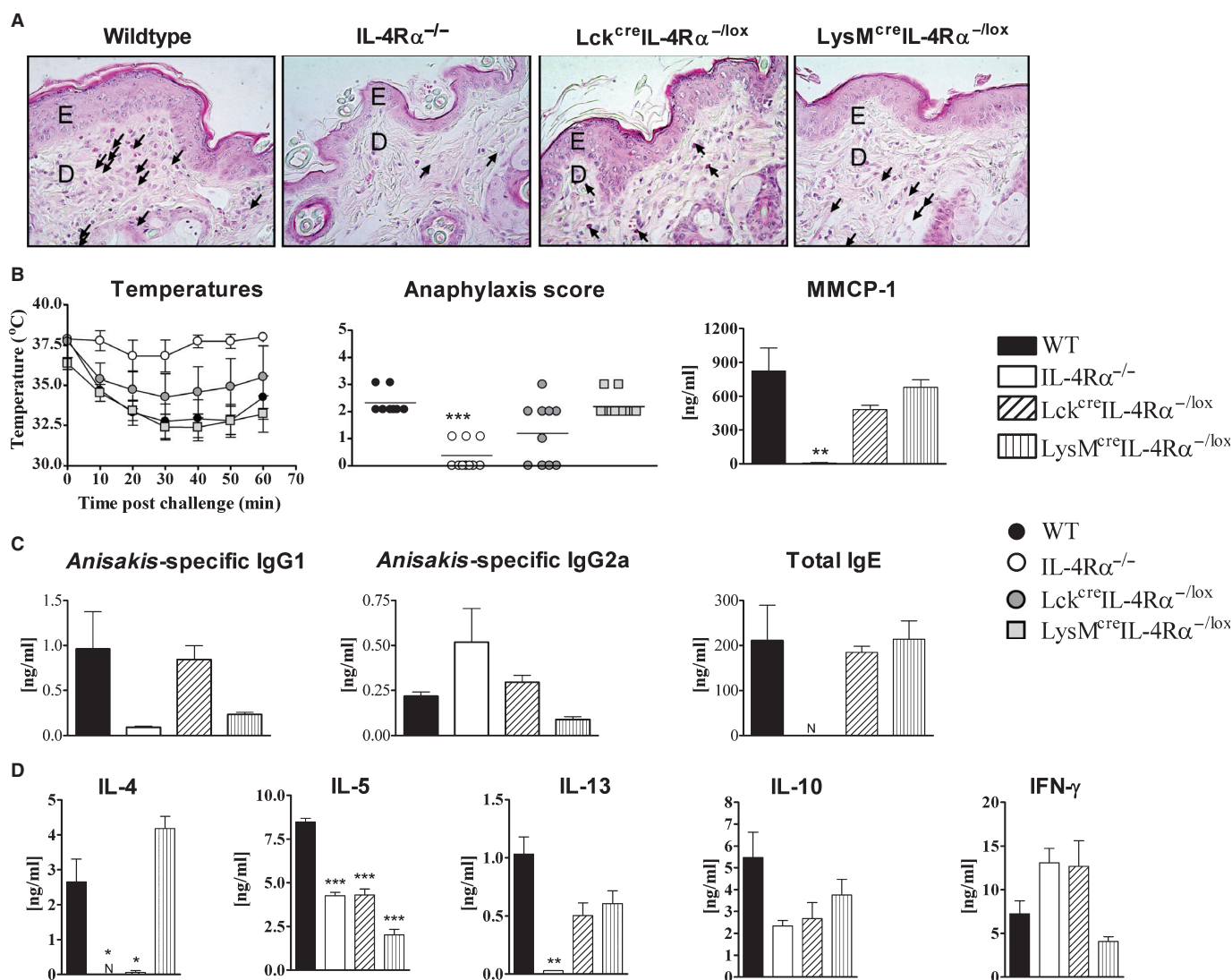


Figure 6. Dermatitis and epicutaneous sensitization in cell-specific IL-4Rα deficient mice. (A) Histological features of skin epicutaneously exposed to PBS or *Anisakis* extract. Sections were stained with H&E (400× magnification). Black arrows point to eosinophils. E = epidermis; D = dermis. (B) Anaphylactic responses after intravenous challenge: rectal temperatures were recorded following challenge, reactions were rated using a scoring system (see *Methods*) and serum MMCP-1 was measured 70 min post challenge. (C) *Anisakis* specific IgG and total IgE ($n = 4$). (D) Cytokine production from splenocytes re-stimulated with anti-CD3. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the WT. N, undetectable. Data are representative of two to four experiments, $n = 4-5$.

the dermatitis has been related to the likelihood of developing later respiratory allergy (37). Sensitization to food allergens may also occur through the skin (18). Thus, further investigation of mechanisms responsible for IL-4 mediated systemic sensitization following skin exposure to allergens may promote novel therapeutic strategies to prevent the atopic march.

The results of this study demonstrate clearly that IL-13 plays a central role in skin pathology caused by epicutaneous exposure to allergen. Increased expression of IL-13 mRNA occurs in atopic dermatitis and allergic contact dermatitis in humans (26, 27). Furthermore, a recent study demonstrates that transgenic expression of IL-13 in the skin induces a spontaneous dermatitis (38),

which strongly supports our findings. To further explore the mechanisms through which IL-13 signalling results in dermatitis, we examined mice with cell-specific impairment of IL-4Rα, through which IL-13 signals. While IL-4Rα^{-/-} mice showed protection against dermatitis, impairment of IL-4Rα expression on either CD4⁺ T cells or on macrophages and neutrophils did not abrogate dermatitis. Therefore, given that IL-13 signals primarily via the type 2 IL-4R (IL-4Rα/IL13Rα1), which facilitates marked effects upon non bone marrow-derived cells, it would be interesting to determine whether IL-13 acts directly upon keratinocytes and/or epithelial cells. Experimental models of asthma show that IL-13 has direct effects upon non immune cells such as epithelial cells,

endothelial cells, goblet cells and smooth muscle cells (39–41). Therefore, it is likely that IL-13 functions in a similar way to induce pathological changes in the skin. In previous studies, IL-13-stimulated human primary keratinocytes showed increased expression of MMP-9, an enzyme which can degrade the basement membrane of the skin, allowing migration of leucocytes into the epidermis (42). Interleukin-13 also up-regulated expression of CCL22, CCL2 and CCL5, chemokines, which attract leucocytes and contribute to inflammation (43). Furthermore, transgenic expression of IL-13 in mouse keratinocytes resulted in up-regulation of chemokine and cytokine genes and led to dermal infiltration by CD4⁺ T cells, mast cells, eosinophils, macrophages and Langerhans cells (38). Together, these findings strongly suggest that antagonists of IL-13 signalling may have therapeutic potential in protein contact dermatitis.

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