

Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis

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Background: Several case reports show allergy and anaphylactic reactions to the fish parasite *Anisakis* in the domestic and occupational setting. Further research is needed on the prevalence and mechanisms of disease.

Objective: To determine the prevalence of *Anisakis* sensitization and related symptoms among workers in 2 fish-processing factories, and to use gene-deficient mice to determine the working mechanisms of *Anisakis* allergy.

Methods: A modified version of the European Community Respiratory Health Survey was used to interview 578 South African fish-processing workers. Sensitization to *Anisakis*, seafood, and common aeroallergens was determined by skin prick test. Lung function was measured by spirometry and methacholine challenge. Serum eicosapentaenoic acid levels were used as an index of seafood consumption. Sensitized wild-type, IL-4, or IL-4 receptor α -deficient mice were challenged orally with *Anisakis* extract. Allergic reactions, lung pathology, antibodies, cytokines, mast cell proteases, and histamine were evaluated.

Results: The prevalence of sensitization to *Anisakis* was higher than the prevalence of sensitization to fish (8% vs 6%).

Anisakis-specific IgE reactivity was associated with bronchial hyperreactivity and dermatitis, and significantly increased with fish consumption. In mice, *Anisakis* infective larvae (L₃) induced a striking T_H2/type 2 response. Food-allergic-type reactions induced by oral challenge with *Anisakis* extract were absent in IL-4 receptor α knockout mice.

Conclusion: *Anisakis* sensitization in fish-processing workers is associated with allergic symptoms and correlates with high levels of fish consumption. *Anisakis* proteins induce allergic reactions in sensitized mice by IL-4/IL-13-mediated mechanisms.

Clinical implications: *Anisakis* allergy should be considered in fish-processing workers with allergic symptoms. (J Allergy Clin Immunol 2006;117:1098-105.)

Key words: *Anisakis*, nematode, parasite, gene-deficient mice, food allergy, dermatitis, asthma, occupational allergy, IL-4, IL-13

Allergies, defined as harmful immunologic reactions to innocuous antigens (allergens), are a significant and widespread public health problem. In particular, food allergies are associated with considerable morbidity and mortality¹ and can affect as many as 4% of adults and 8% of children.² Fish and shellfish are among the 8 types of food that account for more than 90% of allergic reactions.² In the United States, the prevalence of self-reported seafood allergy has risen to 5.9%, almost double that of peanut allergy.³ However, the average consumer may have difficulty distinguishing between reactions to fish or seafood and reactions caused by contaminating substances such as histamine, toxins, and parasites. Larvae of the parasitic nematode *Anisakis* have been increasingly linked to adverse reactions on ingestion of contaminated fish.

Whereas adult worms of *Anisakis* sp parasitize sea mammals, larval stages must pass through several intermediate hosts. Infectious stage larvae (L₃) found within sea fish or cephalopods⁴ may be accidentally ingested by human beings. If ingested live, as a result of consumption of raw or undercooked fish, *Anisakis* L₃ larvae can cause the zoonotic disease known as anisakiasis. This is usually an acute and transient infection, with the worm dying within a few weeks. Within hours of being ingested, *Anisakis* L₃ penetrate the mucosal layers of the gastrointestinal tract, causing direct tissue damage that may lead to abdominal pain, nausea, and/or diarrhea.^{5,6} Furthermore, some patients develop an IgE-mediated "gastroallergic anisakiasis," which presents with clinical manifestations ranging from urticaria to life-threatening anaphylactic shock.⁷⁻¹⁰ This places gastroallergic anisakiasis on the borderline between parasitic infection and allergy.^{8,11} In the past, allergic reactions to *Anisakis* have been mistaken for other diseases such as acute urticaria or fish allergy.¹²

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Abbreviations used

IL-4R α : IL-4 receptor α
L₃: Third-stage larvae
MMCP-1: Mouse mast cell protease 1
OR: Odds ratio
SPT: Skin prick test

However, development of better diagnostic tools such as fiberoptic gastroscopy, skin prick testing, and *Anisakis*-specific IgE determination¹³ has resulted in an increase in frequency of reports in many parts of the world including the United States,¹⁴ Canada,¹⁵ Europe,⁸ and Japan.^{12,16} In a randomized study among almost 5 million Japanese,¹⁷ a higher prevalence of sensitization was found to *Anisakis* than to seafood. Moreover, a Spanish study found that *Anisakis* as a hidden food allergen was the leading cause of food allergy in Spain.¹⁸ However, it still remains highly controversial whether the development of *Anisakis*-induced allergic reactions requires previous infection with the living parasites or whether exposure to soluble larval antigens alone is capable of causing allergic reactions.^{4,19} Notably, recent studies showed that several *Anisakis* allergens are highly resistant to heat and pepsin treatment.²⁰ In addition, several case reports describe adverse reactions to *Anisakis* in people handling fish or fishmeal, with symptoms ranging from conjunctivitis to allergic asthma.^{21,22}

The Food and Agriculture Organization of the United Nations estimated that in 2002, fishery and aquaculture production activities provided direct employment and revenue for 38 million people worldwide (www.greenfacts.org/fisheries). The number of fishers and fish farmers has been growing at an average rate of 2.6% per year since 1990. Among these workers, approximately 52% work aboard fishing trawlers, 32% are involved in aquaculture production (marine and freshwater), and 16% work inland as capture fishers or in other land-based activities such as processing.²³

It has been estimated that 7% to 36% of fish-processing workers develop occupational asthma, whereas 3% to 11% have urticaria and atopic or protein contact dermatitis.²⁴ In fact, as many as 15% of adult asthma cases are related to occupational exposure.

In this study, we describe a cross-sectional epidemiologic analysis of 578 fish workers that revealed a high prevalence of sensitization to *Anisakis* associated with dermatitis and nonspecific bronchial hyperreactivity. Previous human studies have shown that *Anisakis* induces a T_H2/type 2 response, with upregulated IgE and IL-4.^{4,25}

To explore the underlying immunologic and allergic mechanisms of *Anisakis* sensitization, gene targeted mouse strains deficient in IL-4 or the IL-4 receptor α (IL-4R α) were used. The latter is the common receptor chain for IL-4 and IL-13 signaling.

METHODS

Human study

A cross-sectional study was conducted among 578 fish-processing workers. A modified version of the European Community Respiratory Health Survey questionnaire was used to interview workers. Lung function spirometry and methacholine challenge tests used American Thoracic Society guidelines. Skin prick tests (SPTs) were performed to determine the atopy status of the workers, using extracts of 8 common airborne allergens, 12 in-house fish products, and the fish parasite *Anisakis simplex*. The weight % $\mu\text{g/mL}$ (relative composition) of the major marine n3-polyunsaturated fatty acid, eicosapentaenoic acid (EPA; 20:5n-3), in workers' serum was used as an index of habitual seafood consumption. Data were analyzed by using Stata 8 statistical software (Stata Corporation, College Station, Tex). Logistic regression, adjusting for confounders, was used to determine the association between the predictors and the outcomes of interest. Ethical clearance of the protocol was obtained from the University of Michigan and the University of Cape Town's Research Ethics Committee.

Anisakis larvae and extract

Live *Anisakis pegreffii* larvae (L₃) were collected from parasitized fish (*Thyrsites atun*) shortly before infection and extensively washed in 4% acetic acid and PBS. For extract, L₃ were frozen and homogenized in PBS, then sonicated. The extract was centrifuged and filter-sterilized through a 0.20- μm filter (Sartorius, Goettingen, Germany). Protein concentration was measured by BCA Protein Assay Kit (Pierce, Rockford, Ill). Concentrated extracts were produced by centrifugation through 15-mL ultrafiltration units with a 10-kD cutoff (Amicon; Millipore, Cork, Ireland).

Infection and oral challenge

BALB/c mice (10–15 g, 8–10 weeks old), IL-4^{−/−},²⁶ and IL-4R α ^{−/−} BALB/c mice²⁷ 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. Mice were anaesthetized with ketamine (Anakot-V; Centaur Labs, Johannesburg, South Africa) and xylazine (Rompun; Bayer, Isando, South Africa), then injected intraperitoneally with 2 larvae at week 0, reinfected at week 8, and challenged orally with 5 mg *Anisakis* protein extract at week 11 using oral dosing cannulae (VetTech, Cheshire, United Kingdom). Allergic reactions were evaluated by using a modification of a previously described scoring system²⁸: 0, no symptoms; 1, scratching and rubbing around the nose and head, hypersensitivity to touch, irritability/aggression; 2, diarrhea, puffiness around the eyes and mouth, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, labored respiration, cyanosis around mouth and tail; 4, loss of consciousness; and 5, death.

Tissue analysis

Lungs were removed from killed mice and homogenized in TRIS buffer, then centrifuged for 20 minutes at 12,000 rpm. Supernatants were removed for mouse mast cell protease 1 (MMCP-1) ELISA. For histology, tissue samples were preserved in 4% phosphate-buffered formalin, cut in paraffin sections 5 to 7 μm , and stained with periodic acid-Schiff reagent for mucus.

In vitro cytokine production

Splenocytes were isolated from individual spleens of infected mice or sham-infected controls and cultured at 4×10^6 cells/mL in Iscove modified Dulbecco medium (Gibco, Paisley, Scotland)/10% FCS (Delta, Kempton Park, South Africa) in the presence or absence of anti-CD3 (clone 145-2C11; 10 $\mu\text{g/mL}$). Supernatants were

TABLE I. Prevalence of allergic sensitization, symptoms, and predictors of allergic health outcomes for fish and *Anisakis* among fish-processing workers (n = 578)

Allergens	Prevalence of sensitization (%)		Prevalence of allergic symptoms (%)				Association between symptoms, lung function, and sensitization (sensitization as predictor [OR, 95% CI]†)				Association between seafood intake‡ and sensitization (seafood intake as predictor [OR, CI]§)
	SPT	Skin	Ocular nasal	Chest	NSBH	Skin symptoms	Ocular-nasal symptoms	Chest symptoms	NSBH		High vs low
<i>Anisakis</i>	8%	3%	2%	1%	3%	1.90 (1.02-3.55)*	1.10 (0.54-2.24)	0.80 (0.34-1.92)	1.85 (0.91-3.77)		2.24 (1.01-4.97)*
Fish	6%	2%	2%	1%	2%	1.40 (0.68-2.91)	2.72 (1.30-5.70)**	0.79 (0.29-2.15)	0.94 (0.39-2.26)		1.22 (0.50-3.00)

NSBH, Nonspecific bronchial hyperresponsiveness = Positive methacholine challenge or postbronchodilator test.

* $P < .05$; ** $P < .01$.

†Adjusted for sex, factory, atopy, and smoking.

‡Seafood intake: eicosapentaenoic acid categories, low: ≤ 1.28 ; high: ≥ 2.23 weight % of 20:5n-3 omega fatty acid.

§Adjusted for factory and atopy.

collected after 48 hours, and cytokines were measured by using sandwich ELISA assays according to the manufacturer's protocol (BD Biosciences Pharmingen, San Jose, Calif).

Serum analysis

Blood samples were collected in separator tubes by tail vein bleeding and centrifuged for 15 minutes at 3000 rpm. *Anisakis*-specific antibodies were measured by indirect ELISA using *Anisakis* extract 5 $\mu\text{g/mL}$ (for IgG isotypes) or 1 mg/mL (for IgE) for coating and antimouse isotype specific antibodies (Southern Biotechnology, Birmingham, Ala) for detection. Absorbance was measured at 405 nm with 492 nm as a reference wavelength. Total IgE was measured by cytokine sandwich ELISA using clone 84.1C as a coat and antimouse IgE (Southern Biotechnology) for detection. MMCP-1 levels in serum were measured by ELISA according to the manufacturer's protocol (Moredun Scientific Ltd, Midlothian, United Kingdom). Histamine levels were quantified in serum taken 1 hour postchallenge using a histamine ELISA kit (IBL Immuno-Biological Laboratories, Hamburg, Germany).

Statistical analysis

Values are given as means $\pm$ SEMs, and significant differences were determined with the unpaired 2-tailed Student *t* test or ANOVA using computer software (GraphpadPrism 4; GraphPad Software Inc, San Diego, Calif). Values $P < .05$ were considered significant.

RESULTS

Increased risk to *Anisakis*-related adverse allergic reactions in fish-processing workers

A cross-sectional epidemiologic study of 578 workers employed in 2 large-scale (employing more than 200 workers) fish-processing workplaces in the Western Cape province of South Africa was undertaken to investigate work-related risk factors associated with bony fish-processing. As part of this investigation, associations of sensitization to bony fish and to *Anisakis* with allergic skin, ocular, nasal, and chest symptoms, as well as non-specific bronchial hyperreactivity, were investigated. *Anisakis* infests both pilchard and anchovy, which are processed on a large scale in these factories.

In this study, 30 workers (5%) reported allergic symptoms to seafood, with the majority reporting symptoms

after eating (87%), touching (40%), or smelling (17%) seafood. Although the overall prevalence of sensitization to fish (as determined by SPT; Table I) was 6%, a higher prevalence of sensitization (8%) was found to *Anisakis*. Detailed analysis of the nature of clinical outcomes indicated that the prevalence of skin symptoms and non-specific bronchial hyperresponsiveness associated with allergic sensitization to *Anisakis* was similar or slightly higher than that caused by fish (Table I). Analyses of fish consumption patterns among the entire workforce studied revealed a statistically significant association between high (versus low) consumption of fish (measured by serum levels of ω -3 fatty acid), and a 2-fold increased risk (odds ratio [OR], 2.24; 95% CI, 1.01-4.97) of sensitization to *Anisakis* but not to fish (OR, 1.22; 95% CI, 0.50-3.00; Table I). Furthermore, workers sensitized to *Anisakis* were almost twice as likely (OR, 1.9; CI, 1.0-3.6) to report skin symptoms on direct skin contact with *Anisakis*-infested fish (Table I). On the other hand, workers sensitized to fish were more likely (OR, 2.7; CI, 1.3-5.7) to report ocular-nasal allergic symptoms. Not all workers sensitized to *Anisakis* demonstrated allergic symptoms, but atopic workers (positive SPT to 1 or more common inhalant allergens) had a 3-fold or more increased risk of presenting with *Anisakis*-related allergic symptoms than nonatopic workers (data not shown), with the highest risk being for asthma-related allergic symptoms (OR, 10.7). In conclusion, this epidemiologic study demonstrated that workers involved in automated processing of fish are at increased risk of becoming sensitized to the fish parasite *Anisakis*, and that sensitization is an important risk factor for allergic disease. Moreover, the findings suggest that direct exposure to *Anisakis* in infested fish through skin contact or the oral or inhalational route are high-risk activities associated with adverse allergic health outcomes.

Anisakis infection induces a dominant T_H2 /type 2 immune response

An experimental murine model was established to explore possible mechanisms involved in sensitization to

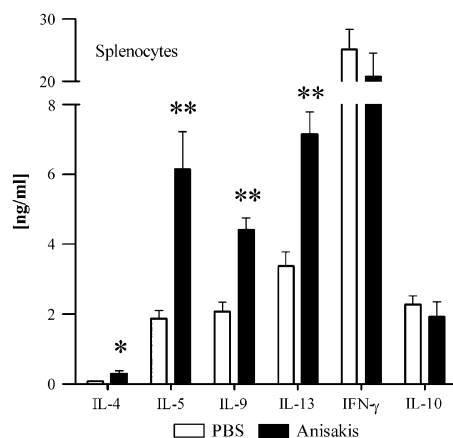


FIG 1. *Anisakis* induces T_H2 -dominant cytokine responses. Mice ($n = 5$) were injected intraperitoneally with 2 *Anisakis* larvae (black bar) or PBS (open bar) at week 0. Splenocytes were harvested at week 4 and restimulated with anti-CD3. Cytokines in supernatants were measured by ELISA ($n = 5$, individual spleens). Results are representative of 2 experiments. * $P < .05$; ** $P < .01$.

Anisakis and the apparent increased risk for allergic responses. Previous studies investigating the immune response of mice to live and dead *Anisakis* larvae have indicated considerable similarities to the human immune response at both the humoral²⁹ and cellular level.³⁰ Here BALB/c mice were infected intraperitoneally with 2 live *A. pegreffii* L₃ larvae, and their immunologic responses were analyzed over time. As in human beings,³¹ the infection was short-lived, with the worm dying within 3 weeks (data not shown). Restimulation of splenocytes harvested 4 weeks after infection showed a strong skewing toward a T_H2 /type 2 response (Fig 1) compared with sham-infected control mice, with significantly higher levels of IL-4, IL-5, IL-9, and IL-13 produced, but unaltered IFN- γ or IL-10. *Anisakis* infection furthermore resulted in raised levels of specific IgG₁ and IgE, which increased with time and after reinfection (Fig 2), whereas type 1 IgG_{2a} and IgG_{2b} antibodies remained low (data not shown).

***Anisakis* infection sensitizes mice for allergic symptoms on oral exposure to *Anisakis* proteins**

The outcome of the epidemiologic study suggested an increased risk of allergic sensitization caused by ingestion of *Anisakis*. It is controversial whether live *Anisakis* larvae are necessary to produce allergic reactions or whether exposure to proteins alone can cause reactions.⁴ Mice dosed orally with L₃ crude extract alone developed specific IgE and IgG₁, and restimulated splenocytes from these mice displayed a mildly increased IL-4/IFN- γ ratio; however, levels of T_H2 cytokines such as IL-5, IL-13, and IL-9 were not significantly raised compared with PBS-dosed controls, and the mice did not react to oral challenge (data not shown). To investigate further whether *Anisakis* proteins alone can cause allergic reactions, mice were infected with 2 *Anisakis* larvae, reinfected to boost

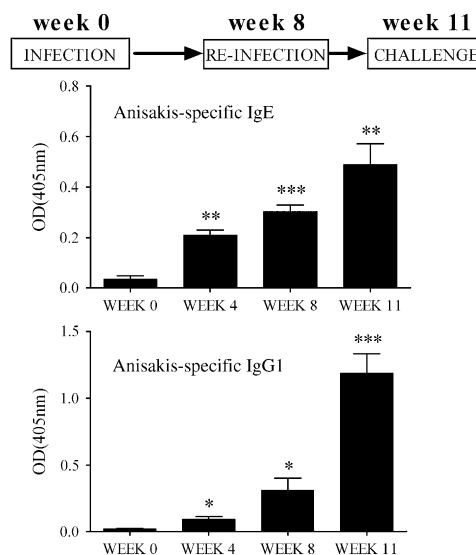


FIG 2. *Anisakis* induces specific IgE and specific IgG₁. Mice ($n = 4-5$) were infected with 2 live *Anisakis* L₃ larvae at week 0 and reinfected at week 8. Serum samples were taken by tail vein bleeding, and antibodies were measured by ELISA. Results are representative of 3 experiments. * $P < .05$; ** $P < .01$; *** $P < .001$.

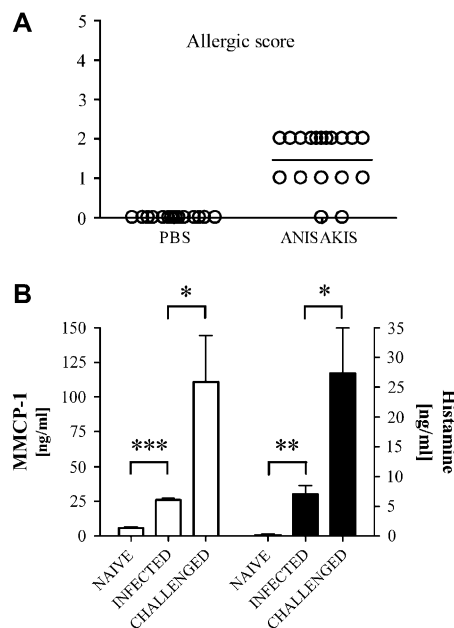


FIG 3. Allergic reactions in *Anisakis*-sensitized mice. Mice were infected with 2 *Anisakis* L₃ larvae (week 0), reinfected (week 8), and challenged orally with 5 mg *Anisakis* extract (week 11). **A**, Reactions were scored (Methods). Open circles represent individual mice (combined results of 3-4 experiments). **B**, MMCP-1 and histamine release in naive, infected, and challenged mice (representative of 2 experiments).

responses, and then orally challenged with 5 mg *Anisakis* protein extract in the absence of adjuvant (Fig 2).

Reinfection boosted the type 2 response observed in the primary infection, with increases in total IgE and *Anisakis* specific IgG₁ and IgE (Fig 2). Importantly, ingestion of

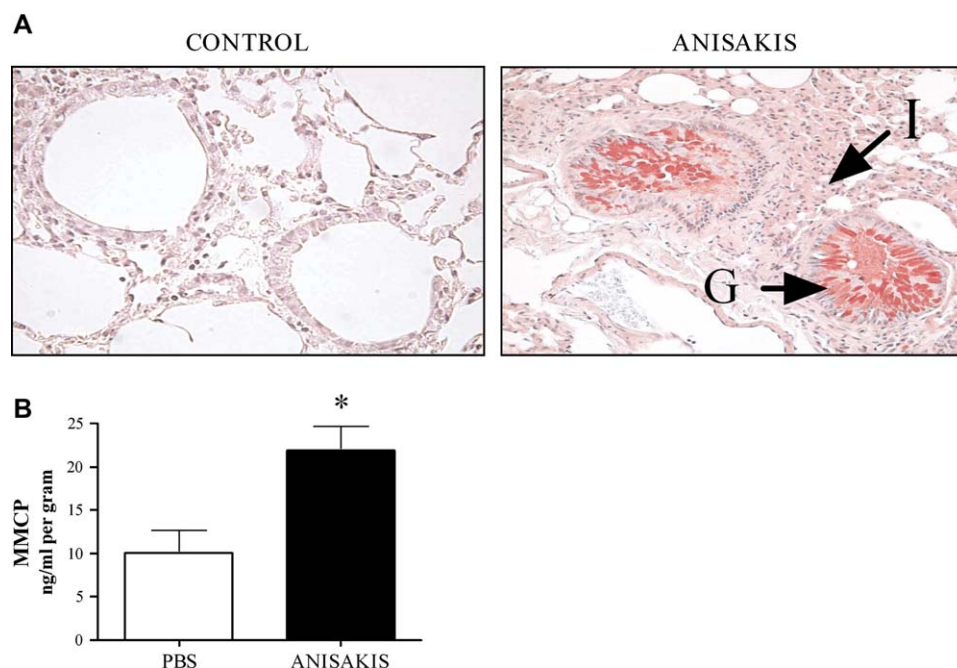


FIG 4. Lung histology and mast cell degranulation after *Anisakis* challenge. Lungs were taken from sham-sensitized (PBS) and *Anisakis*-sensitized mice after oral challenge with *Anisakis* extract. **A**, Lungs were stained with PAS for mucus production. **G**, Goblet cells; **I**, inflammatory infiltrate. **B**, MMCP-1 levels are increased in the lungs of challenged mice. * $P < .05$.

Anisakis proteins by previously infected mice induced signs of allergy within 1 hour, including scratching, irritability, reduced activity, diarrhea, and puffiness around eyes (Fig 3, A). In addition, markers for mast cell degranulation, such as plasma histamine levels and mouse mast cell protease 1 (MMCP-1), were increased after reinfection and further boosted during oral challenge (Fig 3, B). Lung histopathologic analysis showed that in sensitized mice, ingestion induced mucus hypersecretion by goblet cells and cellular infiltration (Fig 4, A), both common signs of airway hyperreactivity, as well as elevated levels of mast cell proteases in lung tissue (Fig 4, B). Together, these results demonstrate that infection with *Anisakis* predisposes to *Anisakis* allergy, and that proteins of the dead larvae alone are sufficient to cause allergic reactions in sensitized mice.

IL-4 and IL-13 are essential for *Anisakis*-induced hypersensitivity

Both human data²⁵ and our murine studies show up-regulated type 2/ T_H2 responses during anisakiasis. We used mice deficient in either IL-4 (IL-4^{-/-}) or responsiveness to IL-4 and IL-13 (IL-4R α ^{-/-}) to elucidate further the mechanisms underlying *Anisakis*-induced allergic reactions.

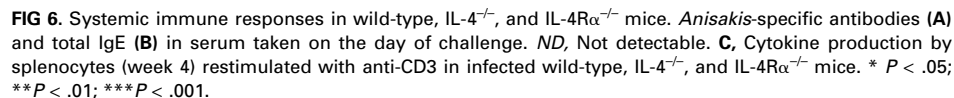
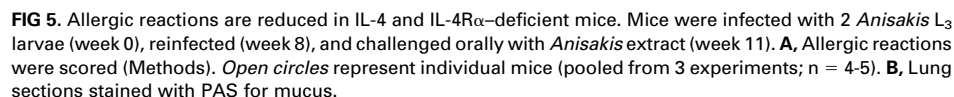
Infection with *Anisakis* followed by oral challenge with *Anisakis* extract resulted in noticeably reduced signs of allergy in IL-4^{-/-} mice compared with wild-type controls (Fig 5, A). Accordingly, lung histopathology with observed cellular infiltration and hyperplasia was present but less pronounced than in wild-type mice, and occurred

at a lower prevalence (Fig 5, B). In *Anisakis*-treated IL-4R α ^{-/-} mice, allergic reactions were abolished (Fig 5, A), and lung histology was identical to that of the sham control wildtype mice (Fig 5, B). Reduced and impaired allergic responses in IL-4^{-/-} and IL-4R α ^{-/-} mice, respectively, correlated with reduced and impaired antigen-specific IgG₁ responses but increased IgG_{2a} and IgG_{2b} responses (Fig 6, A). MMCP-1, histamine levels, and lung-specific IgE responses (data not shown) were strikingly impaired in both mutant mouse strains, with no total IgE detectable in the serum of either knockout strain (Fig 6, B). Furthermore, *Anisakis*-induced T_H2 cytokine responses were diminished in IL-4^{-/-} and IL-4R α ^{-/-} mice (Fig 6, C). These results demonstrate that IL-4 is necessary for *Anisakis*-induced T_H2 responses and suggest that both IL-4 and IL-13 play a crucial role in *Anisakis*-induced oral hypersensitivity.

DISCUSSION

This study presents the first large-scale epidemiologic analysis of occupational allergy to *Anisakis*, and uncovers mechanisms of *Anisakis*-induced allergy using relevant genetically engineered mouse models.

Zoonotic infections of human beings with larvae of the fish parasite *Anisakis simplex*³² have been described for more than 40 years,³³ occurring particularly in countries where seafood forms an important part of the diet, such as Japan and southern Europe.¹² More recently, *Anisakis* infection has also been associated with acute allergic



Furthermore, *Anisakis*-sensitized workers had a higher risk of allergic reactions, particularly skin symptoms and increased nonspecific bronchial hyperreactivity on methacholine challenge. These findings are consistent with case reports of *Anisakis*-related allergic symptoms when handling fish or fishmeal^{21,22} and strongly suggest that frequent occupational exposure to *Anisakis*-infested fish is a risk factor for developing sensitization to *Anisakis* and *Anisakis*-related allergic disease.

An experimental murine model was used to gain a better understanding of the underlying immunologic mechanisms controlling these allergic responses. The survival of L₃ was transient, similar to human *Anisakis* infection, with destruction of the worms occurring within 3 to 4 weeks accompanied by a peak in eosinophilia. Infection induced a marked IL-4-dependent T_H2/type 2 host immune response characteristic of anisakiasis patients.^{7,11,25} Subsequent oral challenge of sensitized mice with L₃ crude extract caused mast cell degranulation with histamine release, accompanied by food-allergic-type reactions. Histologic examination of lung tissue demonstrated that the airways of orally challenged mice were obstructed by inflammatory cells and displayed profound goblet cell hyperplasia and mucus. Respiratory symptoms are not uncommon in food allergy and are thought to be mediated by IgE.³⁴ Several of the T_H2 cytokines induced by *Anisakis* infection (IL-4, IL-5, IL-9, IL-13) have also been shown to play important roles in airway hyperactivity.³⁵ Unpublished data from our laboratory have shown that sensitization by oral administration of L₃ crude antigen extract alone does not lead to the striking T_H2-like response induced by live infection. This may indicate that live infection with *A. pegreffii* is able to bypass oral tolerance, inducing strong systemic type 2/T_H2-like responses that sensitize mice to develop allergic asthma on subsequent ingestion of *Anisakis* proteins. Taking into consideration that the sensitization was performed in the absence of an adjuvant (to reflect better a typical human situation), the observed allergic reactions in mice were relatively strong for a foodborne allergen. Most animal models for food allergies require adjuvants such as cholera toxin or aluminium hydroxide to trigger similar allergic reactions.^{28,36} However, *Anisakis* is unusual in that it is both an etiologic agent of allergies and a helminth. Although the immune response to parasites is generally mediated by the same T_H2 responses responsible for allergic disease, many studies have paradoxically indicated that parasite infection may protect against allergic responses.³⁷⁻³⁹ Mouse models indicate that this protection is mediated by IL-10 and/or regulatory T cells.^{40,41} However, in some studies, helminth infection shows no correlation with reduced atopy, and may even demonstrate a positive correlation.^{42,43} It has been suggested that a high intensity of infection eventually induces T-regulatory cells and IL-10, providing protection against allergies, whereas a low intensity of infection stimulates T_H2 responses, exacerbating allergy.⁴⁴ This hypothesis may explain why *Anisakis* causes allergies in human beings, because this parasite is not adapted to living in human beings, and infection is usually transient. Furthermore, the number of parasites ingested is usually low. In our animal model, the infection was similarly transient and consisted of only 2 larvae; this protocol resulted in potent T_H2 responses and type 2 antibody production.

The striking systemic antigen-specific IgE production may explain case reports of anaphylactic shock caused by *Anisakis* exposure. The majority of allergic reactions to food are IgE-dependent or IgE-associated,^{1,2,45} with

anti-IgE therapy raising tolerance thresholds in patients with severe peanut allergy.^{46,47} The prominence of the type 2/T_H2 response in our model of *Anisakis* allergy suggested an important role for T_H2 cytokines such as IL-4 and IL-13. Mice deficient in IL-4 or the IL-4R α , through which both IL-4 and IL-13 signal, were used to investigate the roles played by IL-4 and IL-13 in *Anisakis* allergy. Absence of endogenous IL-4 resulted in a reduction of allergic reactions after oral challenge of sensitized mice, accompanied by reduced local type 2 antibody responses. However, mucus hypersecretion was only partially depleted. In IL-4R α ^{-/-} mice, reactions were completely abrogated, suggesting an additional role for IL-13 in the allergic response. *Anisakis*-infected and orally challenged IL-13-deficient mice showed reduced T_H2/type 2 responses and did not develop respiratory pathology (data not shown). This supports recent findings that IL-13 plays a role in mucus hypersecretion and resulting airway hyperresponsiveness in mice.⁴⁸⁻⁵⁰ In human beings, the role of IL-13 may be even more significant because, in contrast with mouse B cells, human B cells possess a functional IL-13 receptor.⁵¹

Gene-deficient mice have not previously been used in models of food allergy. Our results indicate that the IL-4R α may play as crucial a role in food allergy as it does in allergic asthma, with important clinical implications.

Furthermore, the importance of the IL-4/IL-13-dependent T_H2/type 2 response in our model may explain why atopic workers presenting with *Anisakis* sensitization are at such a strongly increased risk of asthma-related allergic chest symptoms (data not shown). Polymorphisms in IL-13 receptor α , a subunit of both the human IL-4 receptor and the IL-13 receptor, have been associated with atopy.⁵²

It has long been debated whether infection with live *Anisakis* larvae is required for sensitization and allergic reactions.^{4,8,13} This study demonstrates that although infection certainly produces the potent T_H2/type 2 response likely to be responsible for sensitization to anaphylaxis in consumers, exposure to *Anisakis* proteins alone may be enough to elicit allergic reactions in sensitized individuals. Future studies are needed to identify key allergens in occupational *Anisakis*-related allergy.

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