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Differential responses to natural and recombinant allergens in a murine model of fish allergy

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ABSTRACT

Aerosolized fish proteins are an important cause of allergic airway reactions in both the domestic and the occupational environment. The aim of this study was to investigate inhalant fish-induced allergy in a mouse model and compare immune responses generated by raw and heat-treated fish extracts as well as natural and recombinant forms of the major fish allergen parvalbumin. Mice were sensitized with raw or cooked pilchard extract and challenged intranasally with cooked pilchard extract, purified natural pilchard parvalbumin or recombinant carp parvalbumin (rCyp c1.01). Cooked pilchard extract predominantly sensitized mice to parvalbumin and induced specific IgG1 and IgE antibodies against both pilchard parvalbumin and rCyp c1.01, whereas additional allergens were recognized by mice sensitized with raw extract, including a 36 kDa allergen that was also recognized by fish processing workers and was identified as glyceraldehyde-3-phosphate dehydrogenase. Mice challenged with cooked extract and purified pilchard parvalbumin had increased Th2 cytokine production in mediastinal lymph node cells and splenocytes, whereas mice challenged with rCyp c1.01 did not. This study identifies a new IgE-binding protein that may be important in occupational allergy to fish and demonstrates the feasibility of testing recombinant allergens for immunotherapeutic potential in vivo.

1. Introduction

Fish is one of eight foods responsible for the majority of food allergic reactions (Sicherer and Sampson, 2006). Various studies have estimated the prevalence of fish allergy to be between 0.2% and 0.4% of the general population (Ostblom et al., 2008; Osterballe et al., 2005; Rona et al., 2007; Sampson, 2004; Sicherer et al., 2004), although the incidence can be higher in children and fish processing workers or in populations with a high level of fish consumption (Aas, 1987; Douglas et al., 1995; Jeebhay et al., 2001; Nieuwenhuizen et al., 2006; Priftis et al., 2008). Allergic symptoms occurring after ingestion of fish can also include the respiratory tract, with the most commonly reported ingestion-related symptoms being dyspnea, wheeze, tightness of the throat, urticaria, oedema, oral pruritis and light-headedness (Sicherer et al., 2004). Other manifestations include vomiting, diarrhea and coughing, while the most extreme form of reaction is life-threatening anaphylactic shock. Patients with food allergy to fish can also react to aerosolized proteins produced by cooking or processing fish at home or in restaurants (Anibarro et al., 2007; Crespo et al., 1995; Eigenmann and Zamora, 2002; James and Crespo, 2007; Jeebhay et al., 2008; Roberts et al., 2002; Sicherer et al., 2004). Asthma appears to be a risk factor for fatal anaphylaxis to food (Bock et al., 2001), and conversely, food allergy is a risk factor for life-threatening asthma (Priftis et al., 2008; Roberts et al., 2003).

The Food and Agriculture Organization, reports that over 38 million people are directly involved in fishery and aquaculture production worldwide (www.greenfacts.org/fisheries), making work-related reactions to fish an important consideration. Fish has been identified as an important cause of occupational asthma in this working population. Recently, we showed that up to 7% of

Abbreviations: coPE, cooked pilchard extract; IVC, individually ventilated cages; IMDM, Iscove's Modified Dulbecco's medium; Pparv, pilchard parvalbumin; rawPE, raw pilchard extract; rCyp c1.01, recombinant carp parvalbumin.

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pilchard and anchovy processing workers in two South African fish processing factories were sensitized to fish and demonstrated a 3-fold increased risk of having ocular-nasal reactions, with 2% having occupational asthma due to fish (Jeebhay et al., 2001, 2008; Nieuwenhuizen et al., 2006). Other studies conducted among processors of fish and fishmeal have reported the prevalence of occupational asthma at <7% and 8% respectively (Bang et al., 2005; Douglas et al., 1995).

Management of food allergy is generally directed at the avoidance of the offending foods and prompt recognition and treatment of acute allergic reactions. However, reactions to hidden allergens in foods or through inhalation of the food of close contacts can also pose problems (Anibarro et al., 2007; James and Crespo, 2007). In a recent study, 22.7% of 530 food-related reactions were due to hidden allergens, with 35% of fish allergic patients having reacted to fish proteins hidden in other foods or to fish vapours (Anibarro et al., 2007). Desensitization to fish through immunotherapy has been reported (Casimir et al., 1997), but immunotherapy for food allergies is not usually recommended due to the high risk of adverse reactions (Nelson et al., 1997). Therefore, current approaches include the development of hypoallergenic recombinant derivatives of allergens so that immunotherapy may be carried out without side effects (Swoboda et al., 2007; Valenta and Niederberger, 2007). One target for immunotherapy to fish is the muscle protein parvalbumin, which is a major allergen recognized by over 90% of patients with food allergies to fish (Bugajska-Schretter et al., 1998; Lopata and Lehrer, 2009; O'Neil et al., 1993; Swoboda et al., 2007). Patients who mount IgE antibodies to one parvalbumin often react to parvalbumins of other fish species as well, demonstrating the importance of parvalbumins as cross-reactive fish allergens (Bugajska-Schretter et al., 1998). Approximately one third of children and two thirds of adults react to multiple types of fish (Bernhisel-Broadbent et al., 1992; Helbling et al., 1999; Lim et al., 2008; Sicherer et al., 2004).

Recently we were able to characterize and sequence the major allergen parvalbumin from pilchard (Beale et al., 2009), which was shown to cause seafood allergy in seafood consumers. However, other as yet unidentified allergenic proteins seem to be present in raw and/or cooked fish that are responsible for causing occupational allergy and asthma following inhalation of fish aerosols.

We have developed the first mouse model of inhalant fish allergy and used it to characterize detailed immune responses to raw and cooked fish in comparison to natural parvalbumin and recombinant parvalbumin (rCyp c1.01). Heat treatment of proteins causes denaturation and precipitation of many proteins, which may consequently be recognized by people exposed to raw fish proteins only. Mice sensitized with raw fish recognized different allergens to those sensitized with cooked fish, including a 36 kDa protein identified as glyceraldehyde-3-phosphate dehydrogenase, which was also recognized by sera from fish processing workers. Natural pilchard parvalbumin and recombinant Cyp c1.01 both caused airway reactions after intranasal challenge, but specific immune responses to the two parvalbumins were different. We suggest that this model of fish allergy could be used to test recombinant allergens intended for specific immunotherapy against work-related fish allergy as well as accidental or unavoidable exposure to hidden fish allergens or fish aerosols during fish preparation in domestic environments.

2. Materials and methods

2.1. Sera from fish processing workers

Sera was chosen from nine fish processing workers who had had tested positive for specific IgE against pilchard (*Sardina pilchardus*) (f308) using the UNICAP system (ImmunoCAP, Phadia, Sweden) during an epidemiological study of fish processing factories in South Africa (Jeebhay et al., 2008).

2.2. Preparation of pilchard extracts

Frozen pilchard (*Sardinops sagax*) was filleted and homogenized in phosphate-buffered saline (PBS). The extract was centrifuged and the supernatant was filtered through 8.0 μm and 0.45 μm filter paper (Whatman, International Ltd., Maidstone, England). For cooked extract, raw pilchard extract was heated for 20 min at 95 °C. Precipitated proteins were removed by centrifugation and the supernatant was recovered and concentrated by centrifugation through 15 ml ultrafiltration units with a 5 kDa cut-off (Amicon; Millipore, Cork, Ireland). Protein concentrations were quantified using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and aliquots were stored at −20 °C before use.

2.3. Purification of parvalbumins

Parvalbumin was purified from pilchard extract as previously described (Bugajska-Schretter et al., 2000). Briefly, cooked pilchard extract was subjected to ammonium sulphate precipitation at 70% saturation and the supernatant was collected and dialysed (Pierce, USA) against 10 mM Tris, pH 7.5 at 4 °C then concentrated using an Amicon centrifugal filter device with a molecular weight cut off of 5 kDa (Millipore, Cork, Ireland). Recombinant carp parvalbumin (rCyp c1.01) was generated as previously described (Swoboda et al., 2002b). The endotoxin content in the purified recombinant and natural protein preparations as well as in the protein extract was determined with the Limulus-Amebocyte-Lysate Assay (BioWhittaker, Walkersville, MD).

2.4. Mice

BALB/c mice (5–6-week-old females) were housed under specific pathogen free barrier conditions using individual ventilated cages (IVC) and maintained on a fish-free diet. All experiments were approved by the University of Cape Town's Animal Ethics Committee.

2.5. Sensitization and challenge protocol

A modified version of a previously published model was used (Kuperman et al., 2005). Mice were sensitized intraperitoneally with 50 μg of raw or cooked pilchard extract in 200 μl PBS-1.5% alum (Sigma, Germany) at days 0, 7 and 14. Control mice were injected with PBS-1.5% alum. On days 21, 22 and 23, mice were anaesthetized with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa) and xylazine (Rompun; Bayer, Isando, South Africa) and challenged intranasally with 50 μl of 100 μg raw pilchard extract, cooked pilchard extract, purified pilchard parvalbumin, rCyp c1.01 or PBS only for controls. Mice were killed by CO_2 administration on day 24.

2.6. Serum analysis

Blood samples were collected in serum separator tubes 60–80 min after the final challenge. Antigen-specific antibodies were measured by ELISA using pilchard extracts and parvalbumins (100 μg/ml) for coating and anti-mouse isotype specific antibodies (Southern Biotechnology, Brimingham Ala) for detection. Plates were developed with p-nitro-phenyl phosphate (PNP) substrate and absorbance was measured at 405 nm with 492 nm as a reference wavelength.