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Diving deep into fish allergen immunotherapy: Current knowledge and future directions

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Abstract

Fish allergy is one of the "big nine" categories of food allergens worldwide, and its prevalence is increasing with the higher demand for this nutritious food source. Fish allergies are a significant health concern as it is a leading cause of food anaphylaxis, accounting for 9% of all deaths from anaphylaxis. The gaps in treating fish allergies at present are the incomplete identification of fish allergens, lack of component-resolved diagnosis of fish allergens in the clinical setting, and the variability in sensitization profiles based on different fish consumption practices. Allergen immunotherapy (AIT) improves tolerance towards accidental consumption of fish and is longer lasting than pharmacotherapy. Current practice or research of fish AIT ranges from the use of whole fish via oral desensitization, to the use of purified recombinant parvalbumin and its hypoallergenic variant, passive IgG immunization, and modifying the allergenicity of parvalbumin by changing the diet of farmed fish. However, the focus of fish allergen-based studies in the context of AIT has been restricted to parvalbumins. More research is required to understand the involvement of other fish allergens, and several other strategies of AIT including peptide vaccines, DNA vaccines, hybrid allergens, and the use of nanobodies that have the capacity to treat multiple allergens have been proposed. For AIT, other important aspects to consider are the route of desensitization, and the biomarkers to assess the success of immunotherapy. Finally, we also address several clinical considerations for fish AIT.

Key words: Fish allergy, Immunotherapy, Anaphylaxis, Allergen, Vaccine, Component resolved diagnostics

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Introduction

Fish allergy is one of the "big nine" categories of food allergens worldwide. The increase in global consumption of fish has caused a steady increase in fish allergies reported. Alarmingly, fish allergies are the leading cause of food anaphylaxis, accounting for 9% of anaphylaxis-related deaths.[1] Fish allergies usually persist from childhood to adulthood.[2] Coastal Nordic countries such as Finland and Norway have reported a higher prevalence of fish allergy, at 7% and 3% respectively, compared to land-locked European countries (0.2–1.3%).[3] Similarly in Asia, Kolkata in India and Vietnam reported the highest levels of prevalence at 4.6% and 3.7% respectively, while the prevalence in the other Asian countries ranged between 0–1.6%.[4]

The median age at first reaction was reported between 12–16 months of age.[5,6] Natural tolerance to fish allergy has been shown to develop with age, with up to 45% of the children tested developing tolerance at their adolescence.[6] Most children with fish allergies were able to tolerate at least one fish species, which provides alternative dietary options for them.[5,6] Fish allergy prevalence is often based on patient-reported questionnaires, as this method is quick and able to gather a large number of responses.[7]

However, these studies may risk overestimating true allergic cases due to the overlapping symptoms of fish allergies to non-allergic conditions such as scombroid poisoning, reactions to fish parasites, or others.[8] On the other hand, diagnostic methods such as skin prick tests could under-report the actual level of fish allergies in these countries if the incorrect extract is used. For example, the utilization of cod fish extract among Asian patients, where cod is not commonly eaten, has been shown to underestimate the prevalence of fish allergies within this population.[4]

Traditionally, treatment of IgE-mediated food allergy (including fish) is the complete avoidance of all seafood. Given it is always not possible to eliminate seafood allergens from patients' surroundings, it became important to search for other treatment modalities. Allergen immunotherapy (AIT) has been studied to this end in recent years in various forms. The more commonly researched four major routes of AIT are oral immunotherapy (OIT), sublingual immunotherapy (SLIT), subcutaneous immunotherapy (SCIT), and epicutaneous immunotherapy (EPIT). Currently, the only FDA-approved food allergen AIT is for peanuts, an OIT. Despite many advancements, there are still considerable knowledge gaps about how best to perform AIT and more well-designed AIT trials are required.

Gaps in Knowledge

Incomplete fish allergen spectrum

In allergy research, component-resolved diagnosis (CRD) is the latest approach in identifying the complete allergen reactivity profile.[9-11] Currently, twelve fish allergen groups have been identified (**Table 1**). In addition to allergens listed in **Table 1**, which was retrieved from the WHO/IUIS Allergen Nomenclature Database (www.allergen.org), other allergens such as alpha-parvalbumin have been identified in cartilaginous fish such as rays and sharks but have not been deposited in the database.[12,13] Alpha parvalbumins have also been identified in crocodiles and frogs, which may be a putative cross-reactive food source.

The clinical diagnosis of fish allergies is mainly performed using whole extracts. The use of CRD in the diagnosis of fish allergies has been mainly done in research-based laboratories, its application for clinical use is limited.[14] Group 1 allergen of fish, parvalbumin was discovered in 1969 in cod.[15] Since then, many other fish parvalbumin allergens have been identified (**Table 1**), with the prevalence of IgE reactivity ranging from 70–100% among fish-allergic individuals.[16,17] Specific IgE binding to β-enolase and aldolase A was 63% and 50% respectively among fish-allergic individuals, with limited cross-reactivity among homologous allergens from different fish species.[18]

Table 1. List of fish allergens identified to date.

Allergen group	Biochemical function	Molecular weight (kDa)	Frequency of IgE reactivity (%)	Number of registered allergens	Potentially cross-reactive allergens		
					Food allergens	Airborne allergens	Other allergens
1	β-parvalbumin	11–14	70–100	17	Frog, crocodile	-	-
2	β-enolase	47–50	63	5	Chicken	Cockroach, ragweed pollen, fungus, yeast, wormwood, mold, grass pollen, tree pollen, yeast	Latex
3	Aldolase A	40	50	4	Chicken	-	-
4	Tropomyosin alpha	33–37	6–32	3	Crustacean, mollusk, anisakis, worm	Dust mite, cockroach, silverfish, termite, moth	Mosquito, midge
5	Vitellogenin	18	100[#]	1	-	-	-
6	Collagen alpha	130–140	21–50	2	-	-	-
7	Creatine kinase	43	9–14	2	-	-	-
8	Triosephosphate isomerase	25	21–34	2	Crustacean, wheat	Mold, dust mite, tree pollen	Mosquito
9	Pyruvate kinase PKM-like	65	8	1	-	-	-
10	L-lactate dehydrogenase	34	15	1	-	-	-
11	Glucose 6-phosphate isomerase	60	6	1	-	-	-
13	Glyceraldehyde-3-phosphate dehydrogenase	36	8	1	-	-	-

All allergens listed were compiled from the www.allergen.org database (accessed on 26 August 2023).
[#]Data was obtained from patients who were allergic to chum salmon roe.

Fish tropomyosin allergen was first identified among patients with allergic sensitization to tilapia,[19] and more recent studies among pediatric fish-allergic patients demonstrate that 6–32% had IgE binding to tropomyosin allergens depending on the raw or heated forms tested.[20] IgE reactivity to collagen was reported in 21–50% of the fish-allergic patients.[21,22]

We identified several pitfalls in fish allergy research. First, it is not clear if the complete CRD panel for fish allergens have been identified. Additionally, the extent of cross-reactivity with homologous allergens from other sources has not been determined. Second, the majority of fish allergen research has been concentrated on parvalbumins (PV), while most other fish allergens remain poorly characterized. Third, the total number of fish species that have been investigated for food allergies remains a small subset of the actual diversity consumed around the world.[3] Hence, more research is required to gain a better understanding of a more complete CRD profile of the commonly consumed fish species around the world.

Diagnosis of fish allergies

The gold standard of diagnosis for food allergies (including fish) is the double-blind placebo-controlled food challenge (oral food challenge; OFC), which must be administered by trained personnel as there is a risk of an anaphylaxis reaction. Although effective, OFCs are time and resource intensive, and can only be performed for a limited number of suspected fish allergens. In addition, OFCs are a stressful intervention for the patient, especially if they already experienced severe allergic reactions to fish previously. An alternative diagnostic test for fish allergies is the use of skin prick tests (SPT). This method still carries the risk of an anaphylactic reaction and therefore needs to be performed with caution.[23] Given that the number of commercially available SPT fish extracts is limited, prick-to-prick tests with the fresh fish species could be a solution for improved diagnosis.[3]

Different fish consumption patterns

There are a variety of ways to consume fish; raw, or cooked in several ways – steamed, fried, or grilled. The differences in the cooking processes can affect the protein structure, and therefore the allergenicity. Raw fish contains both heat-stable and heat-labile proteins, while cooked fish would only retain the heat-stable proteins as potential allergen sensitizers. Raw fish consumption has been linked to collagen sensitization. Almost half of the subjects who consumed raw fish were sensitized to collagen,[24] compared to those who ate cooked fish.[21] Another allergen, vitellogenin which is found primarily in fish roe was allergenic among half of the subjects who consumed it.[25]

As fish is very susceptible to spoilage due to decomposition, microbial growth, and rancidity, it is commonly preserved to increase its shelf life. The effects of different preservation methods (e.g., salting, drying, smoking, pickling, and fermentation) on its allergenicity have been recently reviewed.[26] Other methods of prolonging the shelf life of fish involve thermal processes such as canning, cooking/boiling, and hot smoking. Processes involving heating or chemical hydrolysis reduce fish allergenicity.[26] To date, the extent of fish preservation techniques on the allergenicity of the diverse fish species is not well studied. Such data provide additional options for low-allergenic fish that may be suitable for certain atopic individuals.

Fish can be divided into bony fish (Osteichthyes) or cartilaginous fish (Chondrichthyes). Based on available research, cartilaginous fish are less allergenic compared to bony fish.[12] It could be possible to recommend a diet comprising cartilaginous fish in those tolerant to it but are allergic to bony fish to maintain a nutritious protein source in their diets.

Current Practices in Fish Immunotherapy

Fish Immunotherapy using whole fish or fish extracts

A case study on a 20-year-old Japanese female presenting with urticaria from handling fish and gut and throat discomfort from eating fish was successfully treated with a 2-year oral immunotherapy protocol using cooked mackerel fillet, whereby she could tolerate larger amounts of mackerel compared to before treatment.[27] In another case, a 13-month infant with an anaphylactic reaction to fish ingestion was found to be allergic to rosefish and jack mackerel, but was tolerant to tuna, salmon, cod, sardine, Japanese jack mackerel and chub mackerel. For the next 16 months, he was given a diet of the tolerant fish species. Following this, the repeated challenge with the allergenic fish species was negative.[28] Hence, this can be considered as another method to promote fish tolerance. Canned fish generally demonstrate lower allergenic capacity compared to fresh fish, owing to denaturation of IgE epitopes upon heating,[29] and this has been confirmed for PV allergen.[30] The effects of heating on other fish allergens are presently unknown. In addition, while industrially canned fish sounds like an attractive starting point to build up fish tolerance, more studies are required to ensure the safety of this approach.

The first evidence of a life-threatening fish allergy treated with immunotherapy was reported on a 39-month girl, who was treated with commercial cod extracts subcutaneously.[31] Attempts of oral desensitization using extracts of boiled cod fish were only partially effective and resulted in adverse effects.[32,33] A combination of dehydrated and boiled cod fish

was successful in the treatment of a 11-year-old boy, as confirmed by a negative oral food challenge.[34] Oral desensitization has also been successful in a pediatric patient with multiple fish sensitization. Treatment of this patient with hake extract (primary sensitizer) provided tolerance to other fish species, likely due to the similarities of its IgE epitopes.[35] Based on the EAACI guidelines, OIT for fish allergies is not yet recommended, due to the limited studies, and the presence of adverse effects.[36]

Clinical trials of fish immunotherapy

To date, several clinical trials have been conducted for fish immunotherapy, with 3 completed, while the remaining two are in the enrolment phase or still in progress as listed on https://clinicaltrials.gov/ (**Table 2**). Of the five studies, two are on the efficacies of cod parvalbumin (Cyp c 1), two on ADP101 (a multi-allergen oral immunotherapy candidate containing a combination of 5 allergic foods including fin fish), and one on codfish oral immunotherapy. None of the studies have published the findings of the trials so far.

Table 2. Study design and outcome measures of clinical trials on fish immunotherapy.

Clinical trial registration number	Phase	Outcome Measures	Study design and sample size	Treatment	Status
NCT02017626	1 and 2	Primary outcome: Safety in the form of number and severity of adverse events Secondary outcomes: 1. Specific IgE 2. IgG4 3. Skin prick test	**Primary Purpose:** Treatment **Allocation:** Randomized **Interventional Model:** Parallel Assignment **Masking:** Double (Participant, Investigator) Sample size: 15	Biological: mCyp c 1	Completed
NCT02382718	2	Primary outcome: Efficacy of subcutaneous immunotherapy with mCyp c 1 for the treatment of fish allergy (change from baseline in the threshold of fish protein that induces an allergic reaction) Secondary outcomes: 1. Safety (recording of adverse events) - Number of participants with adverse events and recording of the nature of adverse events 2. Severity of reaction in food challenge 3. Skin prick test (SPT) reactivity 4. Serum specific IgE, IgG, IgG4 and IgA antibodies 5. Biological activity of IgE	**Primary Purpose:** Treatment **Allocation:** Randomized **Interventional Model:** Parallel Assignment **Masking:** Double (Participant, Investigator) Sample size: 45	Biological: mCyp c 1	Completed
NCT04856865	1 and 2	Primary outcome: Food Allergy Desensitization Secondary outcome: Incidence of adverse events, including serious adverse events during the study period (Safety and Tolerability)	**Primary Purpose:** Treatment **Allocation:** Randomized **Interventional Model:** Parallel Assignment **Masking:** Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Sample size: 73	Biological: ADP101 vs Placebo Dose Regimen A Biological: ADP101 vs Placebo Dose Regimen B ADP101 is given in high or low doses.	Completed
NCT05243719	1 and 2	Primary outcome: Long-term safety and tolerability of ADP101 Secondary outcome: Food Allergy Desensitization	**Primary Purpose:** Treatment **Allocation:** N/A **Interventional Model:** Single Group Assignment **Masking:** None (Open Label) Sample size: 45	Biological: ADP101	In progress

Table 2. (Continued)

Clinical trial registration number	Phase	Outcome Measures	Study design and sample size	Treatment	Status
NCT05590299	N/A	Primary outcome: Proportion of participants who achieve full desensitisation (passed T1 challenge) in OIT vs placebo. [Time Frame: T1 - One Day after final day of maintenance treatment] Secondary outcomes: 1. Proportion of participants with 8-week sustained unresponsiveness (passed T1 and T2 challenges) in OIT vs placebo 2. The cumulative dose tolerated during the T1 challenge in OIT vs placebo. 3. Skin prick test wheal size and fish-specific IgE to fish in OIT vs placebo. 4. Exposure-adjusted incidence rate and severity of treatment emergent adverse events (TEAEs) in OIT vs placebo.	**Primary Purpose:** Treatment **Allocation:** Randomized **Interventional Model:** Parallel Assignment **Masking:** Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Sample size: 70	Other: Fish oral immunotherapy (codfish) Other: Placebo oral immunotherapy	Enrolment phase

Recombinant allergens and hypoallergens

Recombinant allergens with altered sequences to reduce their allergenic activity are referred to as hypoallergens.[37] Hypoallergens have been generated for different food allergens[38-40] and are a good treatment option for food allergies, as they reduce the risk of unwanted adverse reactions following treatment. Among fish allergens, hypoallergen research has been focused on parvalbumin (PV), the major fish allergen.[41] Several studies have focused on the cod PV hypoallergen characterization, mutations to both of its calcium-binding domains demonstrated reduced IgE reactivity, and the specific IgG generated post-immunization could block IgE binding to the wild-type allergen (**Table 3**). Given its structural stability and low allergenicity, it may be possible to administer hypoallergenic PV of cod at sufficiently higher doses to achieve a therapeutic maintenance dose at a faster pace compared to the natural allergen, with lesser side effects.[42] Although both wild-type and hypoallergen cod PV share the same major T cell epitope and secondary structural

Table 3. Animal-based studies using recombinant or glycated fish allergens.

Allergen (Route)	Dosage	Duration	Main Results	Ref.
Recombinant Hypoallergenic Molecules				
Allergen: mCyp c 1 Route: SC	10 µg mCyp c 1 (Mut-CD/EF) with alum	16 weeks	Immunization with mCyp c 1 (Mut-CD/EF) raised IgG1 antibodies in mice while reduced IgE binding and histamine levels.	(44)
Allergens: Raw / cooked pilchard extract, purified pilchard parvalbumin, or rCyp c1.01 Routes: IP, IN	Sensitization: 50 µg of pilchard extract (raw/cooked) in 200 µl of PBS, Route: intraperitoneal Immunization: 50 µl of 100 µg rCyp c 1.01	24 days	1. Cooked pilchard extract mainly sensitized mice to parvalbumin and induced specific IgG1 and IgE antibodies against both pilchard parvalbumin and rCyp c1.01. 2. Mice sensitized with raw extract recognized an additional 36 kDa allergen, glyceraldehyde-3-phosphate dehydrogenase. 3. Mice challenged with cooked extract and purified pilchard parvalbumin showed increased Th2 cytokine production, but mice challenged with rCyp c1.01 did not. Instead, mice challenged with rCyp c 1.01 showed an increase in IFN-γ levels.	(45)
Allergens: natural (n) Cyp c 1, mutant (m) Cyp c 1. Route: SC, IG	Mice were sensitized followed by immunization with 20 µg nCyp c 1. Allergen challenge was performed with 10 mg nCyp c 1 or mCyp c 1.	204 days	1. mCyp c 1 was more sensitive to enzymatic digestion in contrast to natural Cyp c 1. 2. A single high-dose of oral administration of nCyp c 1 induced long-term tolerance without allergic symptoms. These outcomes were not observed in mice administered with mCyp c 1.	(43)
Allergens: rCyp c 1, mCyp c 1 Routes: SC, IN	C3H/HeJ mice were sensitized with recombinant wildtype Cyp c 1 or carp extract by intragastric gavage.	50 days	Antisera developed against hypoallergenic Cyp c 1 (mCyp c 1) was able to protect mice when challenged with wild-type Cyp c 1 (rCyp c 1), by inhibiting IgE binding, basophil degranulation and resulting allergic symptoms.	(46)

Table 3. (Continued)

Allergen (Route)	Dosage	Duration	Main Results	Ref.
Glycated Hypoallergen				
Allergens: Parvalbumin treated with Maillard reaction and pressure. Route: IP	150 µL of purified parvalbumin followed by 2 mg of Maillard reaction combined with pressure treated parvalbumin (MPT-PV).	29 days	1. MRPT-PV treated mice showed lower levels of specific IgE, IgG1, IgG2a and histamine. 2. Maillard reaction - pressure treatment may be used to reduce food allergenicity.	(47)

SC, subcutaneous; IP, intraperitoneal; IN, intranasal; IG, intragrastric;

properties, only the hypoallergen is sensitive to gastric enzyme digestion.[43] The digestion-resistant of wild-type PV favors its use in oral AIT routes, but its usage must be carefully assessed against potential adverse effects.[43]

Allergen Glycation

Glycation of allergen involves the addition of sugar molecules that may cause structural changes. The glycation of parvalbumin reduces its allergenicity, as seen by the decrease of IgE and IgG reactivity, as well as cellular molecules such as IL-4 and TNF-α (**Table 3**). Studies by several researchers indicate that several conditions can influence the glycation process that would increase or decrease parvalbumin allergenicity, including the type of sugar used, and the duration and temperature of the process.[48,49] Apart from parvalbumin, glycation has been shown to reduce the allergenicity of vitellogenin.[50] At present, the use of glycation as an industrial process to treat fish is limited as it is deemed a sensitive process.[48]

Passive Immunization with IgG Antibodies

The passive transfer of anti-sera from rabbits immunized with mCyp c 1 protected against allergic reactions induced by oral allergen challenge in mice models of fish allergy.[51] Passive transfers of allergen-specific antibodies were successful in Phase II/III clinical trials for cat and birch pollen allergens.[52] These antibodies had IgE-blocking activities, which prevented acute allergic symptoms upon challenge, and even longer-term improved lung function and higher tolerance to respiratory allergens.[52] The blocking antibody action prevented both direct and facilitated IgE-allergen interaction.[52] Administration of allergen-specific IgG as a mode of passive immunotherapy has the advantage of providing high titers of specific IgE-blocking antibodies, without the risk of adverse effects.

Changing diets of farmed fish

The main fish allergen, parvalbumin, is a calcium-binding protein. The IgE epitopes of parvalbumin are dependent on its three-dimensional conformation, wherein the modification of its calcium-binding regions disrupts its IgE epitopes, and results in negligible IgE reactivity.[53] Using this principle an innovative approach to manage food allergies has been developed, fish feed is supplemented with a calcium chelator, which reduces the IgE-binding capacity of parvalbumin.[54] This area of fish feed modulation needs to be explored further, and even if it is successful, we must remain cognisant that there are patients who react to other allergens besides parvalbumins, for whom this fish may remain equally allergenic as the "wild type."

Biomarkers – Measuring the success of AIT

Biomarkers are important in measuring clinical safety, tracking clinical improvement, and assessing the efficacity of AIT (**Table 4**). Key biomarkers that are used in clinical practice are the measurement of the levels of IgE using blood tests or the presence of IgE cross-linking in SPT or food provocation tests. Other classes of immunoglobulins (IgG2, IgG4 and IgA), cytokines and chemokines remain to be explored to be used more routinely in the clinics. Cellular-based tests (enumeration of lymphocyte and DC subpopulations) are still restricted to research usage, while the exploration of microbiome signatures as a marker of AIT success is still in the early stages of research (**Table 4**).

Looking ahead, future research should focus on investigating a multiplex or combinatorial approach to biomarkers that can assess several key aspects, including: i) distinguishing between responders and non-responders to AIT, ii) determining the appropriateness of AIT dosing or the need for dose escalation, iii) identifying the optimal timing to initiate the maintenance phase of AIT, and iv) determining the appropriate time to discontinue AIT. It is essential to implement continuous patient monitoring, to assess if a booster AIT is necessary following the development of tolerance towards the treated allergens.

Table 4. Clinical significance of soluble, cellular, and microbial-based biomarkers for AIT.

Type	Source	Biomarker	Clinical significance
Soluble	Serum	IgE	A transient increase of specific IgE (usually between 0-3 months), followed by a decline towards pre-AIT levels.[55] Facilitated allergen binding CD23 is an alternative mode of simulating the allergenic pathway.[56] The production of blocking IgG4 antibodies also inhibits the IgE-FAB pathway, resulting in diminished clonal T-cell proliferation,[57] suggesting that IgE-FAB pathway inhibition is a promising biomarker of successful AIT.[58]
		IgG2	IgG2 is a newly identified biomarker of AIT. Patients treated for grass pollen and dust mite allergies have demonstrated an increase in IgG2 titres following,[59,60] which significantly correlated to high-responder individuals.[60]
		IgG4	Production of IgG4 is favoured in subcutaneous immunotherapy (SCIT).[61] Specific IgG4 generated post-AIT has the capacity to block IgE binding to the specific allergen.[62]
		IgA	Sublingual immunotherapy (SLIT) elicits both IgG and IgA antibodies.[61] Specific IgA generated post-AIT has the capacity to block IgE binding to the specific allergen.[62]
		Cytokines	Following immunotherapy, the T-cell milieu is modulated towards Th1 and Treg cells. Consequently, there is a shift from Th2 cytokines (IL-4, IL-13, IL-9) to Th1 (IFN-gamma) and T-reg (IL-10).[59]
			Innate lymphoid cells 2 (ILC2) produce IL-5, IL-13, and IL-9 in response to high levels of IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) following AIT in an allergen-independent manner.[58]
		Chemokines	Reduced levels of eotaxin, which plays a role in eosinophil recruitment following AIT.[63]
Cellular	Peripheral blood	Regulatory T, B and DCs	Increase frequencies of $CD4^+$ Th1, Treg, Breg, and DC reg subsets post-AIT compared to pre-AIT.[64]
		Basophils	Basophil activation test (BAT) is based on the measurement of basophil degranulation upon *in vitro* stimulation by a specific allergen. It has been described as a low-risk test for food allergens, as the gold-standard OFC may induce anaphylaxis or adverse effects.[65] Diagnostically, it is able to differentiate between a clinically reactive versus a tolerant individual.[66]
Microbiome	Faecal samples	Gut microbiome	The changes in gut microbiota between allergic and non-allergic individuals could also be used as a 'biomarker' for AIT. For instance, Lachnospiraceae and Ruminococcaceae are found in high abundance in milk-allergic children compared to controls[67] while Streptococcaceae was more abundant in the guts of children with egg allergy.[68] Children who develop tolerance to their respective food allergies have unique microbiome signatures,[69,70] which may be harnessed to understand the resolution of food allergies following AIT.

Clinical perspectives

Sustained unresponsiveness: is it the practical endpoint?

The optimal goal of AIT would be tolerance which entails the permanent resolution of the allergy. However, this outcome cannot be established within the context of a clinical trial. Thus, researchers have rationalised towards achieving sustained unresponsiveness (SU) or state of remission, (the long-lasting ability to tolerate a standard amount of food (fish) even after a period of treatment withdrawal) as a practical endpoint of treatment.

Currently, OIT for cow's milk, egg white and peanut may be useful[71] and appears more effective than SLIT. The original intent of OIT was to prevent anaphylaxis on accidental exposure to traces of the causative allergen. The need to assess the usefulness of OIT to fish becomes important as fish allergy is a major cause of anaphylaxis, on the background that fish allergy is life-long or rarely resolved. However, there is a scarcity of published data on fish OIT warranting further studies to establish the efficacy of this route of AIT.

In a study using hypoallergenic decomposed fish meat, five patients were enrolled in a pilot to undergo OIT.[72] After 5-11 months, 4 patients could eat 20 g salmon meat compared to less than 2 g pre-OIT. Three patients could also eat horse mackerel and not just salmon. One patient was able to eat 10 times salmon meat after 2 months. There were no adverse reactions throughout that study. This study may suggest that OIT using hypoallergenic decomposed fish meat was effective and safe. However, further studies would be required to ascertain whether long-term tolerance could be achieved or if this is just another case of SU. Any form of fish allergy treatment would ideally entail the clinically non-reactive immunological state to persist, and provide the patient with an improved quality of life in the long run.[73]

An EU-funded FAST (food allergy-specific immunotherapy) project in 2008 investigated one part of FAST which aimed at the development of a SCIT for fish allergy based on an alum-adsorbed hypoallergenic mutant of the parvalbumin fish allergen.[53] Mutant (m) Carp parvalbumin (mCyp c 1) was tested in a first-in-man Phase I/IIa randomized double-blinded placebo-controlled clinical trial involving 16 fish-allergic subjects. A low level of side effects and positive immunological response were sufficiently present to warrant further study on the efficiency of the vaccine.

Hence, the FAST project proceeded with a Phase IIb trial[74] where the primary endpoint was the change in threshold for fish protein in a double-blind placebo-controlled food challenge from baseline to post-treatment. After an up-dosing phase of 6 weeks, the patients received 5 maintenance doses (one every 4 weeks) of alum-absorbed mCyp c 1. The conclusion of this 4-month subcutaneous immunotherapy with rCyp c 1 mutant was it was safe and well-tolerated.[75] The vaccine was able to reduce SPT wheal size, while robustly increasing protective serum IgG, in particular serum IgG4, to fish allergen. There were no significant changes in clinical reactivity, however, in the post-hoc analysis, results were skewed significantly by a response to placebo of patients with subjective symptoms only. Overall, even though the primary outcome was negative, the researchers were confident that there was enough ground suggesting that the molecule was a promising treatment of fish allergy.

Allergen sensitization profiles

The outcome of AIT may be influenced by various factors, including the allergen and/or epitope profiles, the types and/or patterns of sensitization, and the preparation of the fish. Parvalbumin, being one of the major proteins in fish, varies among different species, sharing structural homologies ranging from 60% to 80% across different species of fish.[76] Despite the high structural resemblance of parvalbumin, only around 60% of people with allergies to parvalbumin have a response to multiple fish species. This implied that 40% of patients tolerate one or more fish species.[77] Whilst parvalbumin has been considered a panallergen, however, parvalbumin species-specific epitopes have now been identified.[78]

Other fish allergens including enolase and aldolase have been identified as major heat-labile fish allergens. In these circumstances, there is a clinically relevant sensitization for enolase or aldolase but in the absence of parvalbumin-specific IgE, and this appears to be associated with a species-specific fish allergy.[101] Hence, when evaluating fish allergy, it is helpful to categorize patients with fish allergy into three clusters: (A) polysensitized patients who respond to all types of fish on the basis of cross-reactions of β-parvalbumin, and often enolase and aldolase, (B) mono-sensitized patients with a selective allergic reaction for one individual fish species based on a specific epitope of β-parvalbumin, and (C) oligo-sensitized patients who respond to several specific fish based on enolase and aldolase, without IgE for β-parvalbumin.[78-80] This cluster categorization of sensitization based on allergens and/or allergen epitopes may influence the clinical outcome of AIT leading to SU or tolerance.

Patient education and adherence

It is most apparent the key to success in the management of food allergy – food avoidance, treatment of anaphylaxis, allergen immunotherapy – the importance of patient education, and adherence to these treatment modalities needs constant emphasis.

The availability of approved adrenaline autoinjectors (AAI) since the mid-eighties has made these devices crucial in the management of autoinjectors. However, the frequency of patients carrying AAI is as low as 57% and errors in their usage are around 40%.[81] These numbers are worrying, and a study was initiated to improve the carrying frequency of AAI, as well as the knowledge of indications and accurate usage.[81] The initiative consisted of a closed-loop education, redesigned workflow, electronic medical record (EMR) reminder-based interventions, and educational materials. Despite the limitations of the study, the percentage of patients who carried AAI at all times increased from 55% to 93% in 6 months. The knowledge of AAI indications also improved from 22% to 91% and technique demonstration scores of AAI increased from 21% to 91%. Thus, overall, the quality improvement interventions demonstrated a significant improvement of more than 80% in AAI carriage frequency, knowledge of indications, and proper device technique.

On that note, we would be more reassured in initiating AIT, lest there be an anaphylaxis to the treatment. Similarly, ensuring good patient education for those undergoing AIT is imperative as adherence can influence its outcome.[36] Patients' adherence to treatment is critical for AIT success, and there is little data on real-life experience confirming the results of published study protocols. Most AIT clinical trials assessing effectiveness may not consider adherence problems in the first instance, although such issues remain critical in real-life settings. Indeed, AIT (in any form) is a very demanding therapeutic option, and its efficacy depends on patients' adherence to treatment. Thus, appropriate patient selection represents a cornerstone to increase the treatment's probability of success and adherence. The latter as alluded appears difficult, and it involves medical (e.g., sensitization, history of reactions, adherence to control asthma) but also human factors, e.g., motivation of the patients and family. In fact, poor adherence is an absolute contraindication to AIT.

Future Perspectives on Fish Allergen Immunotherapy

Precision diagnostics

Precision treatment strategies using recombinant allergens as immunotherapy molecules, require the understanding of the offending allergen(s) using precision diagnostics, using purified fish allergens instead of extracts to measure the levels of specific IgE. As previously demonstrated for other food allergens, the level of specific IgE reactivity to recombinant allergens is correlated with its clinical reactivity, hence the use of component-resolved diagnostics may also reduce the need for avoidance of oral food challenges that carry a risk of anaphylaxis.[82] Currently available 'allergen chips' have very limited fish allergens or extracts; Allergy Explorer, ALEX2 test contains several fish extracts, while the MeDALL chip and ImmunoCAP ISAC only has one recombinant fish allergen, the codfish parvalbumin (Gad c 1).[83,84]

Precision therapeutics and improving immunotherapy delivery

Improvement of AIT molecules focuses on the immunogenic properties that induce the tolerogenic response, while avoiding any adverse effect requires the modification of the natural wild-type allergen. These modifications can be done in multiple ways (**Figure 1**, **Table 5**). Besides modifying the allergen immunotherapy molecules, the use of other agents with adjuvant properties can further boost the desired immune response (**Table 5**). Certain adjuvants such as liposomes and virus-like particles can also act in improving and targeting the delivery of the AIT molecule to certain cell types, which further improves tolerance development (**Table 5**). Other innovative strategies in AIT such as dendritic cell engineering,[85,86] microbiome modulation by the administration of prebiotics,[87-89] probiotic administration[90-92] or short-chain fatty acids (SFCA)[93,94] have shown promising results in pre-clinical studies, but yet to be tested for fish allergies.

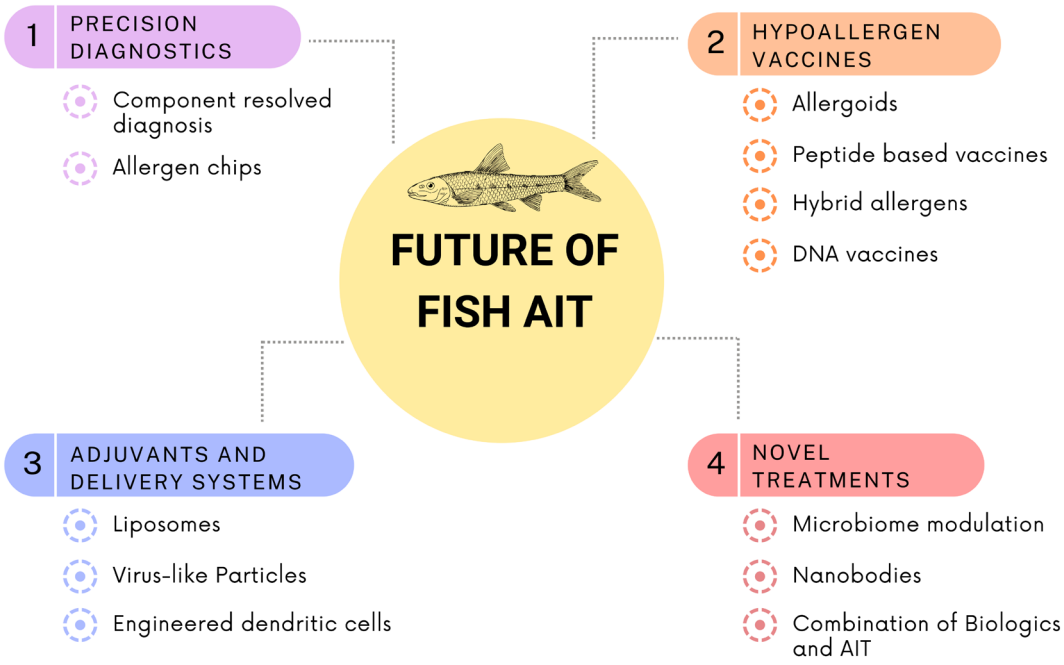

Figure 1. Future perspectives of fish allergen immunotherapy.

Table 5. Strategies in precision therapeutics, and innovative strategies in allergen immunotherapy.

Therapy	Description and brief mechanism	Allergen	Significant clinical findings
Hypoallergens			
Allergoids	Chemically altered allergens that result in the disruption of conformational IgE epitopes while leaving the linear T-cell epitopes intact	Ovalbumin	Ovalbumin allergoid AIT showed reduced IgE binding and IgE cross-linking on mast cells, and induced the production of blocking IgG antibodies, increased IFN-gamma levels, and reduced allergic symptoms post-immunization.[95,96]
		Peanut	Peanut allergoid AIT demonstrated reduced allergenicity, and a shift into the Th1-profile.[97,98]
Peptide-based vaccines	Peptide immunotherapy uses allergen peptides consisting of either T-cell or B-cell epitopes to induce allergen-specific tolerance	Cat	Phase III clinical trial on cat peptide immunotherapy failed to meet the primary endpoint and was subsequently discontinued.[99]
		Peanut	The peanut peptide immunotherapy candidate, PVX108, has completed phase 2 clinical trials with a good safety profile, and the ability to provide desensitization for more than 17 weeks post discontinuation in most of the individuals tested.[100]
		Cow's milk	Peptides of aS1-casein (Bos d 9) demonstrated reduced allergenicity, but retained immunogenicity in pre-clinical testing, suggesting a safer putative immunotherapy molecule.[101]
		Grass pollen	A B-cell epitope-based peptide vaccine for grass pollen allergens, BM32, only required half the number of injections to induce blocking IgG antibodies at comparable levels to the whole allergen extract, and was shown to induce the tolerance mechanism.[102]
		Fish	Five parvalbumin mimotopes (structural epitope mimics) were identified,[103] but their potential as an AIT vaccine has not been tested.

Table 5. (Continued)

Therapy	Description and brief mechanism	Allergen	Significant clinical findings
Hybrid allergens	Hybrid allergens, consisting of two or more proteins, possibly with modifications to its IgE epitope can lead to a standardized molecule that provides reliable therapeutic outcomes, while being hypoallergenic.	Dust mite (*Blomia tropicalis*)	Hypoallergenic hybrid constructs of two major *Blomia tropicalis* allergens Blo t 5/21 (called BTH2) stimulated the generation of blocking IgG antibodies, increased levels of IL-10 and IFN-gamma compared the Th2 cytokines, and reduced eosinophil infiltration compared to the wild type allergens when immunized in mice, hence suggesting promising therapeutic outcomes.[104,105]
		Dust mite (*Dermatophagoides pteronyssinus*)	Mice immunized with a hybrid protein (DPx4) composed of four *D. pteronyssinus* allergens produced blocking IgG antibodies that could inhibit patient IgE binding *D. pteronyssinus* protein extracts.[106] Co-culture of DPx4 with peripheral blood mononuclear cells from sensitized patients resulted in elevated IL-10 levels when compared to co-culture with mite extract, suggesting potential utility as an AIT molecule.[106]
		Bet v 1 and its food allergen homologues	A hybrid protein consisting of allergens from birch, apple and hazelnut, MBC4, had negligible IgE binding to sera of allergic individuals, stimulated T cell proliferation, and could induce blocking IgG antibodies, suggesting its potential as a simultaneous treatment of Bet v 1 and its food allergen homologues.[107]
DNA Vaccines	DNA-based vaccines are based on the introduction of the DNA of an allergen in a plasmid vector to the allergic individual. Thereafter, the host cells translate the DNA vaccine into allergenic protein *in vivo*.	Peanut	A peanut DNA vaccine based on Ara h 2 demonstrated the potential as both a therapeutic and prophylactic vaccine.[108]
		Shrimp	A multivalent shrimp DNA vaccine encoding for three shrimp allergens produced specific IgG2a antibodies against all three allergens, a Th1 cytokine profile, reduced mast cell activation, and significant suppression of anaphylactic reaction in a mouse shrimp allergy model.[109]
Adjuvants and Delivery Systems			
Liposomes	Synthetic self-assembling vesicles made of lipids. Protects allergen from degradation.[110]	Dust mite (Der p 1)	A clinical trial in dust mite-sensitized asthmatic patients with liposome-encapsulated Der p 1 demonstrated improved clinical and medication scores, as well as the increased threshold to methacholine challenge compared to the control group.[111]
		Dust mite (extract)	Fifty-five dust mite allergic individuals treated with AIT using modified D. pteronyssinus extracts encapsulated in liposomes reported improved FEV1 compared to the placebo-treated group following 1 year of treatment.[112]
		Egg (ovalbumin)	Liposomes co-formulated with neoglycolipids, and ovalbumin were administered intranasally for AIT in a mouse model of food allergy. Treated mice had reduced allergic symptoms, that was accompanied by elevated IL-10 cytokine levels, increased OVA-specific IgG1, IgG2a and IGA levels, suppressed OVA-specific IgE levels and an increase in CD8+ and Foxp3+ T cells in the mesenteric lymph nodes.[113]
			OVA-liposome preparation administered sublingually improved the tolerance induction in mice compared to OVA alone.[110]
Virus-like Particles	Consist of viral structural proteins but are devoid of infectious genetic material. VLPs provide the adjuvant effect and are highly immunogenic and may be used in an allergen-independent or allergen-specific manner.	Peanut	Peanut-sensitized mice immunized with a chemically coupled major peanut allergens and the modified cucumber mosaic virus VLP. This construct was able to stimulate the production of specific blocking IgG, reduced eosinophil and mast cell infiltration in the gastrointestinal tissue, and protection against anaphylactic shock.[114]

Combination of Biologics and AIT

Biologicals targeting the Th2 pathway such as anti-IgE, anti-IL5/IL5-R and anti-IL13/IL4 and antibodies against epithelial alarmins such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) were developed to downregulate the allergy-related Th2 immune mechanisms. In food allergies, the use of AIT may result in adverse effects, including life-threatening anaphylaxis. In these situations, a combination of biologics with AIT could improve its safety profile by reducing background Th2 cytokines, and/or levels of IgE, which either avoids or delays any potential adverse reactions from the AIT administration.[115] So far, the most studied biologics to be used in combination with AIT is omalizumab, with studies in peanut, cow's milk and egg allergies showing promising results.[116] However, more studies are needed to understand the criteria of patient selection, optimal dosage and duration of biological adjuvant therapy, and the economics of the treatment. Besides this, a further understanding of the use of other biologicals apart from anti-IgE antibodies could also provide alternatives for patients who need the combination of both therapies.

Conflict of interests

All authors declare no conflicts of interests.

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