Seafood allergen-induced hypersensitivity at the microbiota-mucosal site: implications for prospective probiotic use in allergic response regulation

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Abstract

Food allergy is a serious disease worldwide; it can significantly lower the standard of living of affected individuals and may be life-threatening. In particular, hypersensitivity to seafood has been increasing in recent years owing to rising consumption. The mucosal immune system plays a critical role in the onset of seafood allergy and other allergic diseases. Recently, experimental and clinical evidence has shown that probiotics significantly modulate immune responses and

thus suppress allergic reactions. Therefore, in this review, we summarized the basic knowledge of seafood allergy, the mucosal immune system and probiotic activities. We also reviewed the critical immune factors involved in allergic reactions, as well as the potential mechanism and the potential use of probiotics to ameliorate seafood allergy. The elucidation of these topics may help us to develop preventive and therapeutic approaches for seafood allergy and other immune disorders in the future.

Keywords

Allergy, Seafood, Immunity, Mucosal, Probiotics

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Introduction

Food allergy is defined as an adverse health effect due to a specific immune response that occurs reproducibly on exposure to a given food(Panel, 2010); this condition endangers hundreds of millions of people worldwide and significantly diminishes their quality of life. According to the Food and Drug Administration (FDA), less than 2% of the adult population and 2 – 8% of children suffer from true food allergy, with children under 3 years old being the most common patients(Hajeb & Selamat, 2012; USFDA, 2010). The prevalence of fish and shellfish allergies, a major type of food allergy, is 0.2% and 0.6% worldwide, respectively(Sicherer, 2011). Moreover, since global seafood production and consumption have increased in the past few decades, the prevalence of seafood (especially fish and shellfish) allergy has also increased(Hajeb & Selamat, 2012; Panel, 2010; Sicherer & Sampson, 2014).

Through ingestion, skin contact and even inhalation, seafood allergens can induce hypersensitive reactions immediately within 2 hours(N. Y. Leung et al., 2014; Sicherer & Sampson, 2014). Symptoms after ingestion include coughing, itching, vomiting, abdominal pain, and swelling of the lips, mouth, and pharynx; skin contact may induce urticaria, angioedema, pruritus, and rash;

while long-term inhalation can cause respiratory illnesses(Boden & Wesley Burks, 2011; B. V. Gill et al., 2009; N. Y. Leung et al., 2014). Although most seafood allergies cause relatively mild and minor symptoms, some can induce anaphylaxis, a severe reaction that may be life-threatening(Hajeb & Selamat, 2012; Panel, 2010).

Despite the seriousness of seafood allergy and the major efforts that have been made to treat it, our knowledge of seafood allergy is still quite limited. Seafood allergens have not been fully elucidated, and the detailed mechanisms underlying seafood allergy are still unclear. Additionally, preventive and therapeutic methods for seafood allergy are not well established. The aim of this review is to summarize our current knowledge of seafood allergy, which may promote the development of seafood allergy studies. First, the major seafood allergens are presented, and then, the mucosal immune system involving intestinal microbiota is also introduced, which is the major site of seafood allergic responses. Finally, we discuss probiotics, a promising tool for modulating immune responses, including seafood allergy.

Seafood allergens

Species of seafood allergens

The major seafood allergens are parvalbumin in fish and tropomyosin in shellfish(N. Y. Leung et al., 2014; Lopata et al., 2010; Sharp & Lopata, 2014), which represent the largest two classes of animal-derived allergens according to the allergen database AllFam(Radauer et al., 2008). In addition to parvalbumin, other fish allergens, such as collagen and gelatin from fish skin or muscle(Hamada et al., 2001; Sakaguchi et al., 2000), the fish hormone vitellogenin(Perez-Gordo et al., 2008), and enzymes including aldolase and enolase(Kuehn et al., 2013), have also been identified. Interestingly, glyceraldehyde-3-phosphate dehydrogenase from cooked pilchard was presumed to be an allergen that could be inhaled through the respiratory tract(van der Ventel et al., 2011).

Multiple shellfish allergens other than tropomyosin have also been identified. Arginine kinase was reported in several types of crustaceans(N. Y. Leung et al., 2014), and myosin light chain(Ayuso et al., 2008), sarcoplasmic calcium-binding protein(Shiomi et al., 2008), troponin C and triosephosphate isomerase(Bauermeister et al., 2011) were also identified as shrimp allergens.

Novel mollusk allergens were also reported, but they have not been studied in detail, and their biochemical properties were not elucidated(N. Y. Leung et al., 2014). For example, Hal m 1 found in abalone(Lopata et al., 1997) and three other allergens found in common whelk(Lee & Park, 2004) were reported but not fully identified.

The major seafood allergens that have been reported are listed in **Table 1**.

Structure of seafood allergens: epitope and cross-reactivity

The first and best studied food allergen is Gad c 1, the major codfish allergen, which belongs to the parvalbumin family(Elsayed & Bennich, 1975; Jenkins et al., 2007; Lopata & Lehrer, 2009). Parvalbumins are calcium-transporting muscle proteins with molecular weights ranging from 10 to 13 kDa and can form oligomers up to 48 kDa(Das Dores et al., 2002; Rosmilah et al., 2005). Parvalbumins cross-react in 90% of patients with fish allergies(Swoboda et al., 2002). They belong to the largest group of animal-derived food allergens, the EF hand domain family, which contains more than 70 reported allergens(Radauer et al., 2008; Sharp & Lopata, 2014).

The parvalbumin family includes two different proteins, parvalbumin α and parvalbumin β. Although fish often express both parvalbumin proteins, parvalbumin β, but not α, is reported to

be the major allergen(Sharp & Lopata, 2014). Furthermore, parvalbumin β can divided into several isoforms termed parvalbumin β1, β2 and others, which further complicates fish allergen detection and diagnosis(Van Do et al., 2003).

Several studies have attempted to identified the IgE epitope on parvalbumin β, but each study obtained a different sequence, which did not share any identical residues with the others(Elsayed & Apold, 1983; Perez-Gordo et al., 2012; Untersmayr et al., 2006; Yoshida et al., 2008). These contradictory results may be due to the polyclonal nature of IgE from different patients and the varying techniques used by the investigators. Although the amino acid sequences of parvalbumin are not conserved among fish species, the higher structures are quite similar. Different fishes have a 50% probability of cross-reacting with another in allergic reactions(Sharp & Lopata, 2014); thus, there should be a conserved conformational epitope, but further efforts are needed to identify it. This is also the case for shellfish allergens, whose conserved allergic epitopes have not been identified, although epitopes for several species have been elucidated(Lopata et al., 2010).

Although no cross-reacting allergens between fish and shellfish have been reported(Lopata & Lehrer, 2009; Lopata et al., 2010), tropomyosin was identified as a major allergen among

different shellfish, including crustaceans and mollusks, and even other inhaled invertebrates, such as house dust mites and insects(Daul et al., 1994; Hoffman et al., 1981; P. S. Leung et al., 1994; Witteman et al., 1994). Tropomyosin is a component of the cytoskeleton that is present in both muscle and non-muscle cells and has a molecular weight of approximately 35 kDa. This protein can form homodimers, yielding a large protein complex. Tropomyosin was reported to be a panallergen among several types of arthropods, indicating that patients with shrimp allergies may also cross-react with other crustaceans, mollusks, insects (e.g., cockroaches) or house dust mites, and vice versa(Panel, 2010).

Similar to most food allergens, seafood allergens are generally heat stable. In fact, both parvalbumin and tropomyosin are characterized as heat-stable proteins(Hoffman et al., 1981; Kobayashi et al., 2006), and parvalbumin allergenicity may be increased after heating(Beale et al., 2009; van der Ventel et al., 2011). In addition to increased exposure of internal epitopes, heating with sugars may induce glycation of allergens, which enhances the uptake of allergens by antigen-presenting cells (APCs) through binding to scavenger receptors(Sharp & Lopata, 2014).

Mucosal immunity, the first step of seafood allergy

Composition and structure of the mucosal immune system

The mucosal surface is the largest area of the body and covers several hundred square meters in an adult, forming the first line of defense(Brandtzaeg, 2009). Most antigens that encounter the immune system enter the body through the mucosal surface, and this is the case for seafood allergens(Berin & Sampson, 2013; Montilla et al., 2004).

The mucosal immune system is the part of the immune system juxtaposed to mucosal surfaces. Compared to the peripheral immune system, the mucosal immune system produces more antibodies (more than 80% of all antibodies produced in the body) and has a more complex situation because the gastrointestinal tract must distinguish dangerous antigens (e.g., pathogens) and innocuous antigens (e.g., food and commensal microflora). The mucosal immune system has developed an immune tolerance mechanism to avoid responding to innocuous antigens, and any dysfunction in this mechanism may induce hypersensitivity, especially allergy.

The mucosal immune system is composed of mucosa-associated lymphoid tissue (MALT), which can be further separated into gut-associated lymphoid tissue (GALT), bronchus-associated

lymphoid tissue (BALT), nasopharynx-associated lymphoid tissue (NALT), the mammary and salivary glands, and the genitourinary organs(Cesta, 2006; Kuper, 2006; Montilla et al., 2004), among which GALT is the major tissue responsible for seafood allergy.

The GALT principally consists of Peyer's patches (PP), mesenteric lymph nodes (MLN), intraepithelial lymphocytes (IELs) and the lamina propria mononuclear cells (LPMC) of the mucosa (**Figure 1**). PP are macroscopic lymphoid aggregates found in the submucosa along the length of the small intestine(Mowat, 2003). These aggregates consist of B cell follicles and intervening T cell areas, which are covered by subepithelial dome (SED) and follicle-associated epithelium (FAE) and are thus separated from the intestinal lumen. FAE contains a type of specialized enterocyte termed M cells (microfold cells), which mediate antigen uptake(Neutra et al., 2001). This tissue also contains several types of APCs, such as dendritic cells (DCs) and macrophages. The mesenteric lymph node is the largest lymph node in the body and is suggested to be the crossroads between the peripheral and mucosal recirculation pathways(Mowat, 2003), whereas the lamina propria is the layer of connective tissue between the epithelium and the muscularis mucosa, which is infiltrated by lymphoid and myeloid cells (Montilla et al., 2004). B cells, especially IgA-secreting plasma cells, make up the major proportion of mononuclear cells

in human intestinal lamina propria.

The immune responses in GALT

When exogenous antigens pass through the FAE via M cells, the APCs in the SED uptake, process and present the antigen. Then, these cells move to the PP and prime naïve lymphocytes localized there, converting them into memory or effector cells. The primed T and B cells then migrate from GALT to MLN via the draining lymphatic vessels, where they undergo further differentiation and finally accumulate in the mucosa through the thoracic duct and bloodstream. Antigen or antigen-loaded APCs can migrate to MLN through draining lymphatic vessels and present antigens to lymphocytes(Huang et al., 2000; MacPherson & Liu, 1999).

When innocuous antigens are taken up by DCs, no inflammation occurs. These DCs are partially mature and further present antigens to naïve T cells and prime them into inhibitory regulatory T cells (Tregs), resulting in immune tolerance. By contrast, when pathogenic antigens are taken up by DCs, these DCs are complete mature and prime naïve T cells into pro-inflammatory T helper cells Th1 and Th2, inducing immune activation.

One of the most significant processes in the mucosal immune response is the secretion of

immunoglobulins. Primed B cells mature into plasma cells that produce immunoglobulin, and the predominant immunoglobulin is IgA in the intestinal lamina propria. In addition, IgM, IgG and IgE can also be produced, especially in some specific situations. IgA binds to antigens to neutralize them or eliminates pathogens by a process termed immune exclusion(Boullier et al., 2009), while IgM normally plays a compensatory role to IgA. IgE generally participates in allergic reactions, which will be discussed in the following sections.

Microbiota, the critical modulator of intestinal mucosal immunity

The gastrointestinal tract is the major site for microbial colonization, and the GALT is the first system to interact with microbiota. As described above, the mucosal immune system can distinguish between pathogens and commensal microflora; meanwhile, the microflora have also developed the ability to communicate with the host mucosal immune system. The interactions between the host and commensal microflora modulate the host immune system, reduce the risk of diseases, including seafood allergy, and may even enhance host longevity. In fact, both intestinal microbial colonization and the intestinal immune system are nonexistent at birth; later, they interact with each other, develop synchronously and mature around two years of

age(Delcenserie et al., 2008). The interaction between microbiota and the host predominantly affects the gut barrier function and the innate and adaptive immune systems. Notably, the function of the microbiota largely depends on the various strains, indicating that different microbial strains may have different or even opposite effects on the host immune system.

Probiotics are defined as live microorganisms that have a positive effect on the health of the host when administered in adequate amounts(O'Flaherty et al., 2010). Because they have the same biological characteristics as intestinal microbiota, probiotics can be easily introduced into natural host microbial communities. Owing to their biological characteristics and the convenient methods to produce and manipulate these organisms, probiotics are widely used in the studies on crosstalk and regulation of microbiota and the mucosal immune system (**Figure 3**).

Gut barrier function is vital for the maintenance of gut health, and barrier disruption may result in intestinal diseases, including Crohn's disease and irritable bowel syndrome (IBS) (O'Flaherty et al., 2010). A growing body of evidence has shown that microbiota are involved in gut barrier function maintenance. In murine models, application of probiotics prevents epithelial barrier cell apoptosis and maintains tight junction protein expression(Mennigen et al., 2009). Several studies have shown that only viable probiotics (*Lactobacillus acidophilus*) inhibit the decrease in

transepithelial resistance(Resta-Lenert & Barrett, 2003), but even the culture supernatant can help to maintain epithelial function in some situations. *Lactobacillus rhamnosus* GG, for example, secretes the proteins p40 and p75 into the medium, which inhibit epithelial cell apoptosis and intestinal barrier disruption(Ewaschuk et al., 2008). In addition, probiotics are able to induce the production of cytoprotective substances, such as heat shock proteins(Tao et al., 2006), defensin(Salzman et al., 2010) and mucin(Mack et al., 2003; Mack et al., 1999). Furthermore, probiotic-mediated modulation of intestinal permeability has also been observed in clinical trials(Zeng et al., 2008).

When crossing the intestinal barrier though M cells or epithelial cells, microbiota antigens are processed and presented and can then modulate immune activities. In the case of oral feeding of probiotics, the effect takes place in less than 10 minutes and lasts for several days(Galdeano & Perdigon, 2004).

Several types of cytokines are reported to be regulated by probiotics, with IL-8 being one of the best studied. In HT-29, a human colonic cell line, IL-8 production is induced by probiotic *E. coli* Nissle 1917 but not *Lactobacilli*(Lammers et al., 2002; Otte & Podolsky, 2004). However, when primed by TNF-α, HT-29 can respond to *L. plantarum* 299v and increase the production of IL-8

mRNA. This process requires adhesion between probiotics and host cells. IL-8 secretion is decreased after *L. plantarum* 299v treatment; thus, given the increase in the mRNA level, post-transcriptional regulation must be involved(McCracken et al., 2002). In addition to IL-8, other cytokines, such as IL-6, IL-1β, TGF-β and NGF, are also regulated by probiotics. Since most of these cytokines are produced by intestinal epithelial cells, it is likely that this process is an initiating event in probiotic immunomodulatory activities(Delcenserie et al., 2008).

In addition to cytokines, probiotics can also regulate the activity of innate immune cells, especially macrophages and natural killer (NK) cells. In 1988, *Lactobacillus* spp. were shown to stimulate the systemic immune response by inducing phagocytosis of macrophages(Perdigon et al., 1988). Further studies found that the probiotic *L. rhamnosus* GG upregulates neutrophil receptors, such as CR1, CR3, FcγRIII, FCαR and TLRs, which are important for phagocytosis(Pelto et al., 1998; Schwandner et al., 1999). Moreover, enhancement of phagocytic activity by probiotics was observed *ex vivo*(H. S. Gill et al., 2001; Sheih et al., 2001). Probiotics enhance NK cell activation, which is at least partially related to the induction of the cytokines IL-12 and IL-15(Ogawa et al., 2006; Takeda et al., 2006). Probiotic promotion of immunosurveillance of NK cells may endow probiotics with antiviral and antitumor

properties(Delcenserie et al., 2008; Olivares et al., 2007).

The adaptive immune system is composed of a variety types of immune cells, including DCs, T cells and B cells. These cells function cooperatively and finally result in adaptive immune responses, such as antigen-specific antibody production, especially IgA in the mucosal immune system. Many probiotic species have been reported to stimulate IgA production. *Lactobacillus* spp. increase serum IgA concentration as well as IgA-secreting cells in vaccinated individuals(Isolauri et al., 1995; Link-Amster et al., 1994). While some studies demonstrated the importance of vitality of probiotics, another study demonstrated that the metabolites of *L. helveticus* also showed the same effects(Leblanc et al., 2004). In addition to those in serum, IgA in GALT is also induced by probiotics (*B. bifidum*) and is physiologically significant(Park et al., 2002).

Microbiota regulate DC activation; in fact, owing to the importance of DCs in the immune system, it was hypothesized that microbiota modulate the immune response by influencing DC maturation(Delcenserie et al., 2008). Probiotics such as *L. rhamnosus* promote the maturation of DCs and alter the expression of cytokines and surface proteins of DCs, and in most cases, these DCs showed immunosuppressive activity and inhibited pro-inflammatory T cell

proliferation(Braat et al., 2004; Christensen et al., 2002; Hart et al., 2004). Different probiotic strains may have different effects on DCs. For example, *L. reuteri* and VSL#3 (a mixture of *Bifidobacteria*, *Lactobacilli*, and *Streptococcus salivarius*) reduced pro-inflammatory cytokine IL-12 expression and decreased inflammation, while *L. casei* subsp. Alactus and *B. longum* increased IL-12 expression and promoted Th1 differentiation(Christensen et al., 2002; Hart et al., 2004; Rigby et al., 2005). One of the consequences of DC modulation by probiotics is the development of Tregs. Moreover, microbiota can disrupt the Th1/Th2 equilibrium, which is involved in allergic responses. These processes will be described below.

Potential mechanism of seafood allergy

General allergic process

Allergic reactions are the results of a series of immune events in response to specific allergens. During this process, sundry immune cells, cytokines and other immune constituents (especially IgE) participate in and orchestrate the final anaphylactic symptoms (**Figure 2A**).

When individuals are exposed to a new allergen, the APCs, such as DCs and macrophages, take up and process the allergen and present it to naïve T cells by MHC class II. In the presence of

IL-4, naïve T cells are primed and differentiated into Th2 cells. Th2 cells produce cytokines, including IL-4, IL-5 and IL-13, which, together with a direct interaction between Th2 and B cells, drive the antigen-specific B cells to undergo class-switch recombination (CSR) and differentiate into IgE-producing plasma cells(Galli & Tsai, 2012; Gould & Sutton, 2008). The allergen-specific IgE produced by plasma cells then binds to its high affinity receptor FcεRI on mast cells or basophil cells, sensitizing them.

After exposure to the allergens again, multivalent allergens bind to IgE and induce the cross-linking of FcεRI, finally inducing mast cells and basophil cells to degranulate and release various mediators, such as histamine, heparin, tryptase, chymase, PGD2 and TNF-α. These processes lead to the “early phase” of the allergic reaction within minutes, including edema, pruritus, urticarial, and even vascular collapse. The cytokines and chemokines produced in the early phase further promote the recruitment of Th2 cells and other leukocytes, including neutrophils and eosinophils, leading to the “late phase” of allergic reaction in hours to days; the general symptoms include edema, pain and erythema(Boden & Wesley Burks, 2011; Gould & Sutton, 2008; Kumar et al., 2012).

Critical immune factors involved in allergic reactions

Although allergy is an adverse reaction, it is still tightly regulated. Sundry immune factors, especially immune cells, cytokines and receptors, control the initiation, development and termination of allergic reactions (**Figure 2B**).

Treg cells

At least 5 subsets of Tregs have been identified thus far, including nTregs, iTregs, Tr1 cells, $CD8^+$ Tregs and IL-17-producing Tregs. Most Tregs express CD4 and Foxp3 and secrete the inhibitory cytokines IL-10 and TGF-β(Zhang et al., 2014). A recent study identified a group of $CD4^+$ $CD25^+$ $CD127^-$ $Foxp3^+$ Tregs in tonsils with suppressive properties, which might serve as the first line of oral tolerance against allergens(Palomares et al., 2012).

Tregs are derived from naïve T cells under various conditions and play a crucial inhibitory role in controlling allergic diseases. Both generation and maintenance of allergen-specific Tregs are crucial for the induction of oral tolerance to allergens(Palomares, 2013).

To play an inhibitory role in seafood allergy, Tregs suppress other immune cells, such as effector T cells, B cells, eosinophils and mast cells. Tregs inhibit effector T cell differentiation and

function(Palomares et al., 2010) and block mast cell degranulation by OX40/OX40 ligand interactions but enhance IL-6 production via surface-bound TGF-β(Ganeshan & Bryce, 2012). Tregs can also inhibit the influx of eosinophils and effector T cells into inflamed tissues(Ring et al., 2006) and promote the generation of tolerogenic DCs(Wing et al., 2008). By directly regulating B cells, Tregs promote the production of allergen-specific IgG4 while inhibiting IgE production(Meiler et al., 2008).

Overall, by expression of soluble and membrane-bound suppressor factors, Tregs regulate almost all components in hypersensitivity reactions, indicating that they are a central controller in seafood allergy.

DCs

DCs are the most important APCs in the immune system. As described above, DCs play important roles in both oral tolerance and seafood allergy.

The main DCs that responsible for oral tolerance in intestinal mucosa express CD11c and CD103. These DCs express retinaldehyde dehydrogenase 2 that converts the vitamin A metabolite into retinoic acid (RA). RA, together with TGF-β that is also expressed by DCs, promote conversion

of naïve T cells into Treg cells(Coombes et al., 2007; Sun et al., 2007). RA also induces the expression of integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9 in naïve T cells, which promotes them homing in gut where $\alpha_4\beta_7$ ligand MadCAM-1 and CCR9 ligand CCL25 are expressed(Cassani et al., 2011; Hadis et al., 2011; Iwata et al., 2004). In addition, DCs produce IL-10 that induce the further production of IL-10 by Tregs, and help building a tolerogenic environment(Iwasaki & Kelsall, 2001; Manicassamy et al., 2010; Ruiter & Shreffler, 2012).

However, DCs are also required for seafood allergy(Hammad et al., 2010), and those DCs are either $CD11c^+$ $CD11b^+$ or $CD11c^+$ $CD103^+$(Smit et al., 2011). One of the most important function of DCs in seafood allergy is priming naïve T cells into Th2 cells(Ruiter & Shreffler, 2012). DCs uptake antigens that transported through M cells in PP or directly from gut lumen through the epithelial barrier(Rescigno, 2003; Rescigno et al., 2001). When stimulated by "weak signals" such as regular pro-allergic adjuvant cholera toxin (CT) or thymic stromal lymphopoietin (TSLP), DCs show a muted activation phenotype and present OX40L co-stimulation signaling to naïve T cells together with antigens presented by MHC class II, leading naïve T cells to differentiate into Th2 cells. In addition, these DCs also secrete IL-6 that further promotes Th2 activation while inhibits Tregs at the same time(Ahlers & Belyakov, 2010).

In addition to MHC class II and OX40L, T-cell immunoglobulin and mucin domain-containing proteins (TIMs) expressed on DCs, including TIM-3 and TIM-4, are also involved in Th2 cell activation. TIM-4 is a co-stimulation receptor, together with its ligand TIM-1 expressed on T cell, are required for Th2 cell differentiation and intestinal allergic diseases. Upregulation of TIM-4 by CT or other approaches promotes Th2 development and intestinal allergic responses, whereas blocking TIM-4 or TIM-1 does the opposite(Feng et al., 2008; Yang et al., 2007; Zhao et al., 2010). TIM-3 is upstream of TIM-4, stimulating TIM-3 with its ligand galectin-9 induces TIM-4 expression, promotes Th2 differentiation and suppress Th1 and Th17 differentiation, sustaining the allergic status in the intestine(Chen et al., 2011).

Cytokines

Immune cells communicate with each other and regulate immune responses principally through the secretion of cytokines. The major cytokines regulating allergic processes include IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ and TGF-β.

IL-4 produced by DCs assists in priming of T cells and promotes the differentiation of Th2 cells. Th2 cells can also produce IL-4, along with other cytokines, such as IL-5 and IL-13. IL-4 and

IL-13 further drive allergen-specific B cells to undergo CSR and clonal expansion, resulting in the production of IgE. IL-4, IL-5 and IL-13 contribute to the development of the allergy executors eosinophils and mast cells, leading to allergic reactions(Larche et al., 2006). By contrast, IL-12 produced by DCs skews towards Th1 differentiation and strongly inhibits oral sensitization(Zhang et al., 2014). IL-6 and TGF-β produced by APCs induce Th17 differentiation(Berin & Sampson, 2013; Coombes & Powrie, 2008), and Th17 cells further secrete cytokines, such as IL-17, which contribute to allergic disorders and autoimmune diseases(Oboki et al., 2008).

IL-10, IFN-γ and TGF-β are general inhibitory cytokines that are predominantly produced by Tregs. IL-10 impairs Th2 activation by inhibiting the maturation and expression of MHC class II and co-stimulatory molecules in DCs, promotes the production of allergen-specific IgG4 while inhibiting IgE production by B cells, and suppresses mast cell and eosinophil functions(Hawrylowicz & O'Garra, 2005). IFN-γ suppresses the development of Th2 cells, induces eosinophil apoptosis and blocks IgE isotype switch in B cells(Teixeira et al., 2005). TGF-β is a multifunctional cytokine in immune responses. It decreases inflammation by promoting Treg development and inhibiting Th1, Th2 and B cell development. However, TGF-β

leads to the rapid accumulation of macrophages and granulocytes and induces TH17 cell differentiation, which promote inflammation(Tirado-Rodriguez et al., 2014).

TLRs

Different pathogens may share some common components termed pathogen-associated molecular patterns (PAMPs), and different abnormal cells may also share some common components termed damage-associated molecular patterns (DAMPs). The innate immune system recognize PAMPs and DAMPs by a group of receptors known as pattern recognition receptors (PRRs) to initiate appropriate immune responses, such as Toll-like receptors (TLRs), a large family with a major role in immune responses, including seafood allergy.

The activity of TLRs may influence the polarization of Th1 or Th2 and finally alter allergic activity(Bauer et al., 2007). In general, TLR pathways induce the production of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, as well as regulatory cytokines, such as IL-12 and IL-18, which increase inflammation and promote Th1 differentiation(Wagner, 2001). As a result, the absence of MyD88, a critical adaptor in TLR pathways, leads to increased Th2 response and IgE production(Schnare et al., 2001). While strong TLR activity induces Th1

development, weak TLR activity, such as TLR4 activated by low concentration of LPS, may induce Th2 development(Eisenbarth et al., 2002; Kaisho et al., 2002). Epidemiological studies revealed that the upregulation of TLR2 and the activation of TLR4 decrease the risk of developing allergies, while TLR2 mutations are also correlated with the susceptibility and severity of allergic diseases(Ahmad-Nejad et al., 2004; Eder et al., 2004; Gereda et al., 2000; Lauener et al., 2002).

By contrast, TLRs expressed on Tregs may enhance allergic reactions. TLR2 and TLR8 activation was reported to reverse Treg functions(Peng et al., 2005; Sutmuller et al., 2006). Although these studies did not examine allergic diseases, they suggest that the suppression of Tregs by TLRs will increase the onset and severity of allergy.

Despite the effect of TLRs on Tregs, the overall role of TLRs appears to be allergy suppressors. Several TLR ligands, such as CpG DNA (ligand of TLR9) and R-848 (ligand of TLR7 and TLR8), have been studied as therapeutics for allergic diseases(Brugnolo et al., 2003; Horner et al., 2001; Quarcoo et al., 2004; Simons et al., 2004). Their underlying mechanism is the strong Th1 cytokine-inducing capacity and the inhibition of Th2 development.

Seafood-caused allergic response in murine models

In addition to the general mechanism of allergic reactions, the specific responses of seafood allergy were also investigated. Currently, four murine models have been established for seafood allergy studies(Liu et al., 2016). In the presence of antacids, administration of caviar proteins or recombinant parvalbumin sensitizes BALB/c mice, leading to increased specific IgE antibodies and T-cell reactivity, as well as a number of gastrointestinal eosinophils and mast cells. Using this model, Untersmayr et al. demonstrated that antacid medication inhibits the digestion of dietary proteins and thus enhances fish-allergic responses(Untersmayr et al., 2003). Swoboda et al. applied recombinant parvalbumin mutants to an *in vivo* skin prick testing and identified Mut-CD/EF as a hypoallergenic parvalbumin mutant, which showed reduced IgE reactivity but retained the ability to induce IgG. The induced IgG can further bind wild-type parvalbumin and thus may reduce fish allergic responses(Swoboda et al., 2007). By sensitizing mice with aerosolized fish proteins, van der Ventel et al. showed that glyceraldehyde-3-phosphate dehydrogenase was a new fish allergen. They also found different responses in Th2 cytokine production by natural or recombinant parvalbumin, suggesting the feasibility of testing recombinant allergens for immunotherapeutic potential(van der Ventel et al., 2011).

Another *in vivo* study introduced probiotics into the mouse model, demonstrating the benefit of probiotics in reducing allergic reactions(Capobianco et al., 2008; Schiavi et al., 2011). More details about probiotics and this study will be discussed in the following sections.

Role of gut microbiota in seafood allergy

As in the case of the intestinal immune system, oral tolerance is also established with the help of gut microbiota(Gourbeyre et al., 2011). Allergic patients and healthy individuals have diverse commensal microflora(He et al., 2001), and individuals with defective gut microbiota exhibited higher allergic tendencies or even failed to acquire oral tolerance(Bjorksten, 2008; Bjorksten et al., 1999; Sepp et al., 1997). As described above, Th2 is a major inducer of food allergy, which is consistent with our knowledge that gut microbiota inhibit seafood allergic reactions predominantly by reducing Th2 differentiation or activation.

A growing body of evidence has shown that probiotics inhibit Th2 function in a pro-inflammatory manner by skewing Th1 differentiation. Several species of *Lactobacilli* and *Bifidobacterium breve* M-16V were demonstrated to alter the Th1/Th2 ratio toward Th1 in an IL-12 dependent manner, resulting in the reduction of IL-4 and IL-5 and an increase in

IFN-γ(Inoue et al., 2009; Pochard et al., 2002; Shida et al., 2002). Although live *Lactobacilli* show stronger activity in modulating Th1 and Th2 function, heat-killed bacteria are also reported to inhibit Th2 development(Miettinen et al., 1996; Shida et al., 2002). Clinical studies showed that probiotics modulate the Th1/Th2 balance. In a double-blind clinical study of allergic children, *L. rhamnosus* GG reduced IL-4 secretion and increased IFN-γ production, indicating Th2 suppression(Pohjavuori et al., 2004). Although most studies investigated probiotic functions in peripheral blood mononuclear cells (PBMCs), local production of cytokines in GALT is also regulated, which confirmed its physiological significance(Maassen et al., 2000).

On the other hand, anti-inflammatory Treg cells play an important role in the immunomodulatory process by probiotics. *Lactobacillus rhamnosus* treatment significantly increased the $CD4^+CD25^+Foxp3^+$ Treg percentage in spleen and reduced Th1 and Th2 cytokines in the serum(Jang et al., 2012). *Bifidobacterium breve* M-16V(Inoue et al., 2009) and VSL#3(Schiavi et al., 2011) were also reported to induce Treg activation and promote IL-10 production, which subsequently led to immune tolerance, Th2 suppression and reduction of allergic reactions. This process is at least partially related to DCs because when human monocyte-derived DCs (MDCs) were incubated with *L. reuteri* and *L. casei*, but not *L. plantarum*, they were activated and

induced T cells to differentiate into Tregs. This resulted in the production of IL-10 and induction of immune tolerance(Smits et al., 2005). A mixture of probiotics (containing *L. acidophilus*, *L. casei*, *L. reuteri*, *B. bifidum*, and *S. thermophilus*) was also reported to upregulate $CD4^{+}Fox3^{+}$ Tregs through regulatory DCs (rDCs), resulting in both T-cell and B-cell hyporesponsiveness and down-regulation of Th1, Th2, and Th17 cytokines(Kwon et al., 2010).

In addition to disrupting the Th1/Th2 equilibrium or inducing anti-inflammatory Treg cells to suppress both Th1 and Th2 activation, some probiotics appear to stimulate both Th1 and Th2. For example, *L. rhamnosus* HNOOI, an inducer of IFN-γ, was shown to induce IL-4 and IL-5 during allergen sensitization; thus, this strain was considered to be an immunostimulant(Cross et al., 2002). Additionally, *L. acidophilus* enhanced the production of both IgG1 and IgG2a, the typical responses of Th1 and Th2, respectively(Perdigon et al., 2002). Interestingly, despite the phagocytosis inhibitory effects in allergic individuals, *L. rhamnosus* GG stimulated phagocytes in healthy individuals(Pelto et al., 1998). These results confirmed that the immunomodulatory activities of probiotics are strain- and context-dependent (**Figure 3**).

Prospective application of probiotics for seafood allergy

Prevention of allergic diseases by probiotics

Numerous studies have investigated the potential effects of different probiotic strains in preventing allergic diseases. Most of the studies were performed in perinatal conditions and focused on atopic eczema, the first manifestation of an atopic disease(Savilahti et al., 2008).

In some studies, the probiotics were administered only to the children. Lodinova-Zadnikova et al. demonstrated that a probiotic *Escherichia coli* strain significantly reduced the risk of allergy in infants(Lodinova-Zadnikova et al., 2010). In contrast, the probiotics *Lactobacillus* spp. and *Bifidobacterium* spp. were less efficient, although some studies detected anti-allergic activities, such as a reduction in IgE(Soh et al., 2009) or gastrointestinal infections(Prescott et al., 2008), but these results were not statistically significant. More frequently, no anti-allergic activities were detected(Scalabrin et al., 2009), and the opposite results were also observed(Taylor et al., 2007). These studies further confirmed that the immunomodulatory activities of probiotics are strain-dependent.

Other studies provided probiotics such as *Lactobacillus* spp. or *Bifidobacterium* spp. to both

mothers and children, and a protective effect on allergy was observed(Kalliomaki et al., 2001; Kim et al., 2010; Niers et al., 2009; Wickens et al., 2008). However, other studies that showed no significant effects have been reported(Kopp et al., 2008). Moreover, atopic eczema prevalence in children could be reduced to up to 7 years of age(Kalliomaki et al., 2001; Kalliomaki et al., 2003; Kalliomaki et al., 2007).

In addition to natural probiotics, recombinant lactic acid bacteria (LAB) have been suggested to be another effective strategy for prevention of allergic diseases. Recombinant LAB expressing specific allergens were shown to reduce hypersensitive reactions against that allergen(Adel-Patient et al., 2005; Charng et al., 2006; Daniel et al., 2006), and LAB producing inhibitory cytokines, such as IL-10(Frossard et al., 2007) and IL-12(Cortes-Perez et al., 2007; Wu et al., 2006), were also shown to induce oral tolerance. Unfortunately, all these studies were based on mouse models, and clinical evidence is lacking.

Consequently, these studies demonstrated that specific natural probiotic strains prevent infant allergic diseases, especially when provided to both mothers and children, and recombinant LAB is a powerful tool in allergic disease prevention, although more clinical studies are required.

Treatment of allergic diseases with probiotics

Mouse models have shown that probiotics exhibit therapeutic effects on allergies(Cortes-Perez et al., 2009; Hougee et al., 2010; Lim et al., 2009). In most human studies, probiotics also reduced allergic responses.

The first clinical report was on the management of food allergy and atopic dermatitis (AD) by *Lactobacillus* GG(Majamaa & Isolauri, 1997). Further studies reported that *Vitreoscilla filiformis* lysate significantly improved AD by both reducing *Staphylococcus aureus* colonization of the skin and directly modulating skin-associated immune responses(Gueniche et al., 2008). Furthermore, *Bifidobacterium lactis* Bb-12 or *Lactobacillus* strain GG also ameliorated atopic eczema(Isolauri et al., 2000), and *Lactobacillus fermentum* VRI-003 PCC improved the extent and severity of AD in young children(Weston et al., 2005). According to the Severity Scoring of Atopic Dermatitis (SCORAD) index, in addition to the beneficial effects described above, several other studies have also shown some moderate effects of probiotics(Gourbeyre et al., 2011; Rosenfeldt et al., 2003; Sistek et al., 2006; Viljanen et al., 2005). However, in another study, no clinical nor immunological effects of probiotics (*Lactobacillus rhamnosus*) in infants with AD were detected(Brouwer et al., 2006).

In conclusion, compared to prevention, therapeutic effects on allergic diseases are more significant and show less strain preference. Since different degrees of treatment effects were observed, there is an urgent need for further mechanistic studies to predict and improve the efficacy of probiotics.

Ameliorating seafood allergy by probiotics

As described above, Di Felice and colleagues developed a mouse model of seafood allergy by sensitizing C3H/HeJ mice via the oral route using purified shrimp tropomyosin and CT as an adjuvant(Capobianco et al., 2008). In this model, immune cell proliferation and cytokine production, as well as anaphylactic symptoms, are induced after sensitization. Using this model, they further investigated the role of probiotics in the treatment of seafood allergy. The probiotic preparation VSL#3 significantly reduced pro-inflammatory cytokine (IL-4, IL-5 and IL-13) production and increased anti-inflammatory protein (FOXP3, IL-27, IL-10, TGF-β and IFN-γ) expression. Furthermore, after probiotic treatment, the polarized Th2 response shifted to a Th1/Treg-type profile, and histamine release and anaphylactic symptoms were also relieved(Schiavi et al., 2011).

Recently, our studies showed that oral administration of five LAB strains (*B. coagulans* 09.712, *L. plantarum* 08.923, *B. infantis* 11.322, *L. rhamnosus* CGMCC 1.3724 and *S. thermophilus* CGMCC 1.2471) alleviated allergic symptoms caused by shrimp tropomyosin (ST) sensitization in mice (**Figure 4A**). The most effective anti-allergic strain, *B. coagulans* 09.712, strongly induced generation of $Foxp3^+$ Tregs in both the spleen and MLN (**Figure 4B**). The potential molecular mechanism of ST-induced allergy and its correlation with Treg induction by LAB strains are still unclear.

The above results shed light on the application of probiotics in seafood allergy, but many more studies, including clinical trials, are required for the practical application of this treatment.

Conclusions and future directions

Food allergy is a serious disease worldwide that significantly lowers the standard of living of affected individuals and can even be life-threatening. Seafood allergy is a major type of allergy and is increasing. As the first line of defense to interact with seafood, the mucosal immune system, especially the GALT, plays a critical role in the onset of allergic diseases. Following seafood allergen sensitization, specific immune cells are activated and cytokines are produced,

and these components function cooperatively to orchestrate the final anaphylactic symptoms. Probiotics can modulate mucosal immunity as well as the systemic immunity, indicating that they are promising candidates for preventing and treating seafood allergy. However, more research needs to be performed, as the practical use of probiotics has yet to be fully established.

For the development of probiotics to reduce allergic risk, it is important to further elucidate the underlying mechanisms and patterns of these organisms. Although many studies have been performed, the detailed mechanisms are still not fully understood. For example, most studies used whole bacteria instead of a characterized probiotic molecule; thus, the molecular mechanism of the interaction between probiotics and hosts cannot be determined, and it is not possible to develop a chemical medicine from probiotics. However, few studies have compared the immunomodulatory activity of different probiotic strains, and the strain specificity of probiotics is poorly clarified. Additionally, in the case of different allergens, it is impossible to identify the specific probiotic strain that is critical for an indicated allergen unless the allergic mechanism of that particular allergen is fully elucidated.

Additionally, compared to the commensal microflora in the intestinal tract, only a limited number of probiotic species (e.g. *Lactobacillus* GG and VSL#3) have been used and studied, and

most commensal bacteria, especially unculturable bacteria, were not assessed. More studies should investigate these uncharacterized species, as new mechanisms and effects may be discovered.

Furthermore, in clinical studies of the application of probiotics in allergic diseases, no significant effect was detected in a large proportion of the studies. Even using the same protocol, diverse results may be observed(Kalliomaki et al., 2001; Kopp et al., 2008). The factors that lead to the conflicting results require further investigation. Moreover, when the studies were extended, the preventive effect gradually decreased(Kalliomaki et al., 2007). Thus, extending the curative effect of probiotics should be a major aim of future studies.

Taken together, promising results from the use of probiotics in the treatment of seafood allergy were obtained in both experimental and pre-clinical studies, but more studies, particularly those investigating molecular mechanisms and probiotic strain specificity and clinical trials, are urgently needed.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China [grant number 31571770 and 31571913], the Zhejiang Provincial Natural Science Foundation of China [grant number LY14C200001 and LZ15C200001] and the Zhejiang Provincial Science and Technology Foundation of China [grant number 2016C32058].

References

Adel-Patient, K., Ah-Leung, S., Creminon, C., Nouaille, S., Chatel, J. M., Langella, P., & Wal, J. M. (2005). Oral administration of recombinant Lactococcus lactis expressing bovine beta-lactoglobulin partially prevents mice from sensitization. *Clin Exp Allergy,* **35**, 539-546.

Ahlers, J. D., & Belyakov, I. M. (2010). Molecular pathways regulating CD4(+) T cell differentiation, anergy and memory with implications for vaccines. *Trends Mol Med,* **16**, 478-491.

Ahmad-Nejad, P., Mrabet-Dahbi, S., Breuer, K., Klotz, M., Werfel, T., Herz, U., . . . Renz, H. (2004). The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol,* **113**, 565-567.

Ayuso, R., Grishina, G., Bardina, L., Carrillo, T., Blanco, C., Ibanez, M. D., . . . Beyer, K. (2008). Myosin light chain is a novel shrimp allergen, Lit v 3. *J Allergy Clin Immunol,* **122**, 795-802.

Bauer, S., Hangel, D., & Yu, P. (2007). Immunobiology of toll-like receptors in allergic disease. *Immunobiology,* **212**, 521-533.

Bauermeister, K., Wangorsch, A., Garoffo, L. P., Reuter, A., Conti, A., Taylor, S. L., . . . Reese, G. (2011). Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp Crangon crangon. *Mol Immunol,* **48**, 1983-1992.

Beale, J. E., Jeebhay, M. F., & Lopata, A. L. (2009). Characterisation of purified parvalbumin from five fish species and nucleotide sequencing of this major allergen from Pacific pilchard, Sardinops sagax. *Mol Immunol,* **46**, 2985-2993.

Berin, M. C., & Sampson, Hugh A. (2013). Mucosal Immunology of Food Allergy. *Current Biology,* **23**, R389-R400.

Bjorksten, B. (2008). Environmental influences on the development of the immune system: consequences for disease outcome. *Nestle Nutr Workshop Ser Pediatr Program,* **61**, 243-254.

Bjorksten, B., Naaber, P., Sepp, E., & Mikelsaar, M. (1999). The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy,* **29**, 342-346.

Boden, S. R., & Wesley Burks, A. (2011). Anaphylaxis: a history with emphasis on food allergy. *Immunol Rev,* **242**, 247-257.

Boullier, S., Tanguy, M., Kadaoui, K. A., Caubet, C., Sansonetti, P., Corthesy, B., & Phalipon, A. (2009). Secretory IgA-mediated neutralization of Shigella flexneri prevents intestinal tissue

destruction by down-regulating inflammatory circuits. *J Immunol,* **183**, 5879-5885.

Braat, H., van den Brande, J., van Tol, E., Hommes, D., Peppelenbosch, M., & van Deventer, S. (2004). Lactobacillus rhamnosus induces peripheral hyporesponsiveness in stimulated CD4+ T cells via modulation of dendritic cell function. *Am J Clin Nutr,* **80**, 1618-1625.

Brandtzaeg, P. (2009). Mucosal immunity: induction, dissemination, and effector functions. *Scand J Immunol,* **70**, 505-515.

Brouwer, M. L., Wolt-Plompen, S. A., Dubois, A. E., van der Heide, S., Jansen, D. F., Hoijer, M. A., . . . Duiverman, E. J. (2006). No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. *Clin Exp Allergy,* **36**, 899-906.

Brugnolo, F., Sampognaro, S., Liotta, F., Cosmi, L., Annunziato, F., Manuelli, C., . . . Parronchi, P. (2003). The novel synthetic immune response modifier R-848 (Resiquimod) shifts human allergen-specific CD4+ TH2 lymphocytes into IFN-gamma-producing cells. *J Allergy Clin Immunol,* **111**, 380-388.

Capobianco, F., Butteroni, C., Barletta, B., Corinti, S., Afferni, C., Tinghino, R., . . . Di Felice, G. (2008). Oral sensitization with shrimp tropomyosin induces in mice allergen-specific IgE, T cell response and systemic anaphylactic reactions. *Int Immunol,* **20**, 1077-1086.

Cassani, B., Villablanca, E. J., Quintana, F. J., Love, P. E., Lacy-Hulbert, A., Blaner, W. S., . . . Mora, J. R. (2011). Gut-tropic T cells that express integrin alpha4beta7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology,* **141**, 2109-2118.

Cesta, M. F. (2006). Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicologic Pathology,* **34**, 599-608.

Charng, Y. C., Lin, C. C., & Hsu, C. H. (2006). Inhibition of allergen-induced airway inflammation and hyperreactivity by recombinant lactic-acid bacteria. *Vaccine,* **24**, 5931-5936.

Chen, X., Song, C. H., Liu, Z. Q., Feng, B. S., Zheng, P. Y., Li, P., . . . Yang, P. C. (2011). Intestinal epithelial cells express galectin-9 in patients with food allergy that plays a critical role in sustaining allergic status in mouse intestine. *Allergy,* **66**, 1038-1046.

Christensen, H. R., Frokiaer, H., & Pestka, J. J. (2002). Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. *J Immunol,* **168**, 171-178.

Coombes, J. L., & Powrie, F. (2008). Dendritic cells in intestinal immune regulation. *Nature Reviews Immunology,* **8**, 435-446.

Coombes, J. L., Siddiqui, K. R., Arancibia-Carcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., &

Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med,* **204**, 1757-1764.

Cortes-Perez, N. G., Ah-Leung, S., Bermudez-Humaran, L. G., Corthier, G., Langella, P., Wal, J. M., & Adel-Patient, K. (2009). Allergy therapy by intranasal administration with recombinant Lactococcus lactis Producing bovine beta-lactoglobulin. *Int Arch Allergy Immunol,* **150**, 25-31.

Cortes-Perez, N. G., Ah-Leung, S., Bermudez-Humaran, L. G., Corthier, G., Wal, J. M., Langella, P., & Adel-Patient, K. (2007). Intranasal coadministration of live lactococci producing interleukin-12 and a major cow's milk allergen inhibits allergic reaction in mice. *Clin Vaccine Immunol,* **14**, 226-233.

Cross, M. L., Mortensen, R. R., Kudsk, J., & Gill, H. S. (2002). Dietary intake of Lactobacillus rhamnosus HNOO1 enhances production of both Th1 and Th2 cytokines in antigen-primed mice. *Med Microbiol Immunol,* **191**, 49-53.

Daniel, C., Repa, A., Wild, C., Pollak, A., Pot, B., Breiteneder, H., . . . Mercenier, A. (2006). Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy,* **61**, 812-819.

Das Dores, S., Chopin, C., Villaume, C., Fleurence, J., & Gueant, J. L. (2002). A new oligomeric parvalbumin allergen of Atlantic cod (Gad mI) encoded by a gene distinct from that of Gad cI. *Allergy,* **57 Suppl 72**, 79-83.

Daul, C. B., Slattery, M., Reese, G., & Lehrer, S. B. (1994). Identification of the major brown shrimp (Penaeus aztecus) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol,* **105**, 49-55.

Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y., & Roy, D. (2008). Immunomodulatory effects of probiotics in the intestinal tract. *Current Issues in Molecular Biology,* **10**, 37-53.

Eder, W., Klimecki, W., Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., . . . Martinez, F. D. (2004). Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol,* **113**, 482-488.

Eisenbarth, S. C., Piggott, D. A., Huleatt, J. W., Visintin, I., Herrick, C. A., & Bottomly, K. (2002). Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med,* **196**, 1645-1651.

Elsayed, S., & Apold, J. (1983). Immunochemical analysis of cod fish allergen M: locations of

the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy*, **38**, 449-459.

Elsayed, S., & Bennich, H. (1975). The primary structure of allergen M from cod. *Scand J Immunol*, **4**, 203-208.

Ewaschuk, J. B., Diaz, H., Meddings, L., Diederichs, B., Dmytrash, A., Backer, J., . . . Madsen, K. L. (2008). Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. *Am J Physiol Gastrointest Liver Physiol*, **295**, G1025-1034.

Feng, B. S., Chen, X., He, S. H., Zheng, P. Y., Foster, J., Xing, Z., . . . Yang, P. C. (2008). Disruption of T-cell immunoglobulin and mucin domain molecule (TIM)-1/TIM4 interaction as a therapeutic strategy in a dendritic cell-induced peanut allergy model. *J Allergy Clin Immunol*, **122**, 55-61, 61.e51-57.

Frossard, C. P., Steidler, L., & Eigenmann, P. A. (2007). Oral administration of an IL-10-secreting Lactococcus lactis strain prevents food-induced IgE sensitization. *J Allergy Clin Immunol*, **119**, 952-959.

Galdeano, C. M., & Perdigon, G. (2004). Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J Appl Microbiol*, **97**, 673-681.

Galli, S. J., & Tsai, M. (2012). IgE and mast cells in allergic disease. *Nat Med*, **18**, 693-704.

Ganeshan, K., & Bryce, P. J. (2012). Regulatory T cells enhance mast cell production of IL-6 via surface-bound TGF-beta. *J Immunol*, **188**, 594-603.

Garcia-Orozco, K. D., Aispuro-Hernandez, E., Yepiz-Plascencia, G., Calderon-de-la-Barca, A. M., & Sotelo-Mundo, R. R. (2007). Molecular characterization of arginine kinase, an allergen from the shrimp Litopenaeus vannamei. *Int Arch Allergy Immunol*, **144**, 23-28.

Gereda, J. E., Leung, D. Y., Thatayatikom, A., Streib, J. E., Price, M. R., Klinnert, M. D., & Liu, A. H. (2000). Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet*, **355**, 1680-1683.

Gill, B. V., Rice, T. R., Cartier, A., Gautrin, D., Neis, B., Horth-Susin, L., . . . Lehrer, S. B. (2009). Identification of crab proteins that elicit IgE reactivity in snow crab-processing workers. *J Allergy Clin Immunol*, **124**, 1055-1061.

Gill, H. S., Rutherfurd, K. J., Cross, M. L., & Gopal, P. K. (2001). Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. *Am J Clin Nutr*, **74**, 833-839.

Gould, H. J., & Sutton, B. J. (2008). IgE in allergy and asthma today. *Nat Rev Immunol*, **8**,

205-217.

Gourbeyre, P., Denery, S., & Bodinier, M. (2011). Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *Journal of Leukocyte Biology,* **89**, 685-695.

Gueniche, A., Knaudt, B., Schuck, E., Volz, T., Bastien, P., Martin, R., . . . Biedermann, T. (2008). Effects of nonpathogenic gram-negative bacterium Vitreoscilla filiformis lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. *Br J Dermatol,* **159**, 1357-1363.

Hadis, U., Wahl, B., Schulz, O., Hardtke-Wolenski, M., Schippers, A., Wagner, N., . . . Pabst, O. (2011). Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity,* **34**, 237-246.

Hajeb, P., & Selamat, J. (2012). A contemporary review of seafood allergy. *Clin Rev Allergy Immunol,* **42**, 365-385.

Hamada, Y., Nagashima, Y., & Shiomi, K. (2001). Identification of collagen as a new fish allergen. *Biosci Biotechnol Biochem,* **65**, 285-291.

Hammad, H., Plantinga, M., Deswarte, K., Pouliot, P., Willart, M. A., Kool, M., . . . Lambrecht, B. N. (2010). Inflammatory dendritic cells--not basophils--are necessary and sufficient for

induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med*, **207**, 2097-2111.

Hart, A. L., Lammers, K., Brigidi, P., Vitali, B., Rizzello, F., Gionchetti, P., . . . Stagg, A. J. (2004). Modulation of human dendritic cell phenotype and function by probiotic bacteria. *Gut*, **53**, 1602-1609.

Hawrylowicz, C. M., & O'Garra, A. (2005). Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol*, **5**, 271-283.

He, F., Ouwehand, A. C., Isolauri, E., Hashimoto, H., Benno, Y., & Salminen, S. (2001). Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. *FEMS Immunol Med Microbiol*, **30**, 43-47.

Hoffman, D. R., Day, E. D., Jr., & Miller, J. S. (1981). The major heat stable allergen of shrimp. *Ann Allergy*, **47**, 17-22.

Horner, A. A., Van Uden, J. H., Zubeldia, J. M., Broide, D., & Raz, E. (2001). DNA-based immunotherapeutics for the treatment of allergic disease. *Immunol Rev*, **179**, 102-118.

Hougee, S., Vriesema, A. J., Wijering, S. C., Knippels, L. M., Folkerts, G., Nijkamp, F. P., . . . Garssen, J. (2010). Oral treatment with probiotics reduces allergic symptoms in ovalbumin-sensitized mice: a bacterial strain comparative study. *Int Arch Allergy Immunol*, **151**,

107-117.

Huang, F. P., Platt, N., Wykes, M., Major, J. R., Powell, T. J., Jenkins, C. D., & MacPherson, G. G. (2000). A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med,* **191**, 435-444.

Inoue, Y., Iwabuchi, N., Xiao, J. Z., Yaeshima, T., & Iwatsuki, K. (2009). Suppressive effects of bifidobacterium breve strain M-16V on T-helper type 2 immune responses in a murine model. *Biol Pharm Bull,* **32**, 760-763.

Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., & Salminen, S. (2000). Probiotics in the management of atopic eczema. *Clin Exp Allergy,* **30**, 1604-1610.

Isolauri, E., Joensuu, J., Suomalainen, H., Luomala, M., & Vesikari, T. (1995). Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by Lactobacillus casei GG. *Vaccine,* **13**, 310-312.

Iwasaki, A., & Kelsall, B. L. (2001). Unique functions of CD11b+, CD8 alpha+, and double-negative Peyer's patch dendritic cells. *J Immunol,* **166**, 4884-4890.

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., & Song, S. Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. *Immunity,* **21**, 527-538.

Jang, S.-O., Kim, H.-J., Kim, Y.-J., Kang, M.-J., Kwon, J.-W., Seo, J.-H., . . . Hong, S.-J. (2012). Asthma Prevention by Lactobacillus Rhamnosus in a Mouse Model is Associated With CD4(+)CD25(+)Foxp3(+) T Cells. *Allergy, Asthma & Immunology Research*, **4**, 150-156.

Jenkins, J. A., Breiteneder, H., & Mills, E. N. C. (2007). Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *Journal of Allergy and Clinical Immunology*, **120**, 1399-1405.

Kaisho, T., Hoshino, K., Iwabe, T., Takeuchi, O., Yasui, T., & Akira, S. (2002). Endotoxin can induce MyD88-deficient dendritic cells to support T(h)2 cell differentiation. *Int Immunol*, **14**, 695-700.

Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., & Isolauri, E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet*, **357**, 1076-1079.

Kalliomaki, M., Salminen, S., Poussa, T., Arvilommi, H., & Isolauri, E. (2003). Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*, **361**, 1869-1871.

Kalliomaki, M., Salminen, S., Poussa, T., & Isolauri, E. (2007). Probiotics during the first 7 years

of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol,* **119**, 1019-1021.

Kim, J. Y., Kwon, J. H., Ahn, S. H., Lee, S. I., Han, Y. S., Choi, Y. O., . . . Ji, G. E. (2010). Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial. *Pediatr Allergy Immunol,* **21**, e386-393.

Kobayashi, A., Tanaka, H., Hamada, Y., Ishizaki, S., Nagashima, Y., & Shiomi, K. (2006). Comparison of allergenicity and allergens between fish white and dark muscles. *Allergy,* **61**, 357-363.

Kondo, Y., Komatsubara, R., Nakajima, Y., Yasuda, T., Kakami, M., Tsuge, I., & Urisu, A. (2006). Parvalbumin is not responsible for cross-reactivity between tuna and marlin: A case report. *J Allergy Clin Immunol,* **118**, 1382-1383.

Kopp, M. V., Hennemuth, I., Heinzmann, A., & Urbanek, R. (2008). Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics,* **121**, e850-856.

Kuehn, A., Hilger, C., Lehners-Weber, C., Codreanu-Morel, F., Morisset, M., Metz-Favre, C., . . .

Hentges, F. (2013). Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. *Clin Exp Allergy,* **43**, 811-822.

Kumar, S., Verma, A. K., Das, M., & Dwivedi, P. D. (2012). Molecular mechanisms of IgE mediated food allergy. *International Immunopharmacology,* **13**, 432-439.

Kuper, C. F. (2006). Histopathology of mucosa-associated lymphoid tissue. *Toxicologic Pathology,* **34**, 609-615.

Kwon, H. K., Lee, C. G., So, J. S., Chae, C. S., Hwang, J. S., Sahoo, A., . . . Im, S. H. (2010). Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. *Proc Natl Acad Sci U S A,* **107**, 2159-2164.

Lammers, K. M., Helwig, U., Swennen, E., Rizzello, F., Venturi, A., Caramelli, E., . . . Campieri, M. (2002). Effect of probiotic strains on interleukin 8 production by HT29/19A cells. *Am J Gastroenterol,* **97**, 1182-1186.

Larche, M., Akdis, C. A., & Valenta, R. (2006). Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol,* **6**, 761-771.

Lauener, R. P., Birchler, T., Adamski, J., Braun-Fahrlander, C., Bufe, A., Herz, U., . . .

Sennhauser, F. H. (2002). Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet*, **360**, 465-466.

Leblanc, J., Fliss, I., & Matar, C. (2004). Induction of a humoral immune response following an Escherichia coli O157:H7 infection with an immunomodulatory peptidic fraction derived from Lactobacillus helveticus-fermented milk. *Clin Diagn Lab Immunol*, **11**, 1171-1181.

Lee, B. J., & Park, H. S. (2004). Common whelk (Buccinum undatum) allergy: identification of IgE-binding components and effects of heating and digestive enzymes. *J Korean Med Sci*, **19**, 793-799.

Leung, N. Y., Wai, C. Y., Shu, S., Wang, J., Kenny, T. P., Chu, K. H., & Leung, P. S. (2014). Current immunological and molecular biological perspectives on seafood allergy: a comprehensive review. *Clin Rev Allergy Immunol*, **46**, 180-197.

Leung, P. S., Chu, K. H., Chow, W. K., Ansari, A., Bandea, C. I., Kwan, H. S., . . . Gershwin, M. E. (1994). Cloning, expression, and primary structure of Metapenaeus ensis tropomyosin, the major heat-stable shrimp allergen. *J Allergy Clin Immunol*, **94**, 882-890.

Lim, L. H., Li, H. Y., Huang, C. H., Lee, B. W., Lee, Y. K., & Chua, K. Y. (2009). The effects of heat-killed wild-type Lactobacillus casei Shirota on allergic immune responses in an allergy

mouse model. *Int Arch Allergy Immunol,* **148**, 297-304.

Link-Amster, H., Rochat, F., Saudan, K. Y., Mignot, O., & Aeschlimann, J. M. (1994). Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immunol Med Microbiol,* **10**, 55-63.

Liu, T., Navarro, S., & Lopata, A. L. (2016). Current advances of murine models for food allergy. *Mol Immunol,* **70**, 104-117.

Lodinova-Zadnikova, R., Prokesova, L., Kocourkova, I., Hrdy, J., & Zizka, J. (2010). Prevention of allergy in infants of allergic mothers by probiotic Escherichia coli. *Int Arch Allergy Immunol,* **153**, 201-206.

Lopata, A. L., & Lehrer, S. B. (2009). New insights into seafood allergy. *Curr Opin Allergy Clin Immunol*, **9**, 270-277.

Lopata, A. L., O'Hehir, R. E., & Lehrer, S. B. (2010). Shellfish allergy. *Clinical and Experimental Allergy,* **40**, 850-858.

Lopata, A. L., Zinn, C., & Potter, P. C. (1997). Characteristics of hypersensitivity reactions and identification of a unique 49 kd IgE-binding protein (Hal-m-1) in abalone (Haliotis midae). *J Allergy Clin Immunol*, **100**, 642-648.

Maassen, C. B., van Holten-Neelen, C., Balk, F., den Bak-Glashouwer, M. J., Leer, R. J., Laman, J. D., . . . Claassen, E. (2000). Strain-dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains. *Vaccine,* **18**, 2613-2623.

Mack, D. R., Ahrne, S., Hyde, L., Wei, S., & Hollingsworth, M. A. (2003). Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. *Gut,* **52**, 827-833.

Mack, D. R., Michail, S., Wei, S., McDougall, L., & Hollingsworth, M. A. (1999). Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. *Am J Physiol,* **276**, G941-950.

MacPherson, G. G., & Liu, L. M. (1999). Dendritic cells and Langerhans cells in the uptake of mucosal antigens. *Curr Top Microbiol Immunol,* **236**, 33-53.

Majamaa, H., & Isolauri, E. (1997). Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol,* **99**, 179-185.

Manicassamy, S., Reizis, B., Ravindran, R., Nakaya, H., Salazar-Gonzalez, R. M., Wang, Y. C., & Pulendran, B. (2010). Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. *Science,* **329**, 849-853.

McCracken, V. J., Chun, T., Baldeon, M. E., Ahrne, S., Molin, G., Mackie, R. I., & Gaskins, H. R. (2002). TNF-alpha sensitizes HT-29 colonic epithelial cells to intestinal lactobacilli. *Exp Biol Med (Maywood)*, **227**, 665-670.

Meiler, F., Klunker, S., Zimmermann, M., Akdis, C. A., & Akdis, M. (2008). Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy*, **63**, 1455-1463.

Mennigen, R., Nolte, K., Rijcken, E., Utech, M., Loeffler, B., Senninger, N., & Bruewer, M. (2009). Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol*, **296**, G1140-1149.

Miettinen, M., Vuopio-Varkila, J., & Varkila, K. (1996). Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. *Infect Immun*, **64**, 5403-5405.

Moneo, I., Caballero, M. L., Gómez, F., Ortega, E., & Alonso, M. J. (2000). Isolation and characterization of a major allergen from the fish parasite Anisakis simplex. *Journal of Allergy and Clinical Immunology*, **106**, 177-182.

Montilla, N. A., Blas, M. P., Santalla, M. L., & Villa, J. M. M. (2004). Mucosal immune system:

A brief review. *Inmunología,* **23**, 207-216.

Mowat, A. M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol,* **3**, 331-341.

Neutra, M. R., Mantis, N. J., & Kraehenbuhl, J. P. (2001). Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol,* **2**, 1004-1009.

Niers, L., Martin, R., Rijkers, G., Sengers, F., Timmerman, H., van Uden, N., . . . Hoekstra, M. (2009). The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy,* **64**, 1349-1358.

O'Flaherty, S., Saulnier, D. M., Pot, B., & Versalovic, J. (2010). How can probiotics and prebiotics impact mucosal immunity? *Gut Microbes,* **1**, 293-300.

Oboki, K., Ohno, T., Saito, H., & Nakae, S. (2008). Th17 and allergy. *Allergol Int,* **57**, 121-134.

Ogawa, T., Asai, Y., Tamai, R., Makimura, Y., Sakamoto, H., Hashikawa, S., & Yasuda, K. (2006). Natural killer cell activities of synbiotic Lactobacillus casei ssp. casei in conjunction with dextran. *Clin Exp Immunol,* **143**, 103-109.

Olivares, M., Diaz-Ropero, M. P., Sierra, S., Lara-Villoslada, F., Fonolla, J., Navas, M., . . . Xaus, J. (2007). Oral intake of Lactobacillus fermentum CECT5716 enhances the effects of influenza

vaccination. *Nutrition*, **23**, 254-260.

Otte, J. M., & Podolsky, D. K. (2004). Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol*, **286**, G613-626.

Palomares, O. (2013). The Role of Regulatory T Cells in IgE-Mediated Food Allergy. *Journal of Investigational Allergology and Clinical Immunology*, **23**, 371-382.

Palomares, O., Ruckert, B., Jartti, T., Kucuksezer, U. C., Puhakka, T., Gomez, E., . . . Akdis, C. A. (2012). Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol*, **129**, 510-520, 520.e511-519.

Palomares, O., Yaman, G., Azkur, A. K., Akkoc, T., Akdis, M., & Akdis, C. A. (2010). Role of Treg in immune regulation of allergic diseases. *Eur J Immunol*, **40**, 1232-1240.

Panel, N. I.-S. E. (2010). Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel. *Journal of Allergy and Clinical Immunology*, **126**, S1-S58.

Park, J. H., Um, J. I., Lee, B. J., Goh, J. S., Park, S. Y., Kim, W. S., & Kim, P. H. (2002). Encapsulated Bifidobacterium bifidum potentiates intestinal IgA production. *Cell Immunol*, **219**, 22-27.

Pelto, L., Isolauri, E., Lilius, E. M., Nuutila, J., & Salminen, S. (1998). Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. *Clin Exp Allergy,* **28**, 1474-1479.

Peng, G., Guo, Z., Kiniwa, Y., Voo, K. S., Peng, W., Fu, T., . . . Wang, R. F. (2005). Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. *Science,* **309**, 1380-1384.

Perdigon, G., de Macias, M. E., Alvarez, S., Oliver, G., & de Ruiz Holgado, A. P. (1988). Systemic augmentation of the immune response in mice by feeding fermented milks with Lactobacillus casei and Lactobacillus acidophilus. *Immunology,* **63**, 17-23.

Perdigon, G., Maldonado Galdeano, C., Valdez, J. C., & Medici, M. (2002). Interaction of lactic acid bacteria with the gut immune system. *Eur J Clin Nutr,* **56 Suppl 4**, S21-26.

Perez-Gordo, M., Lin, J., Bardina, L., Pastor-Vargas, C., Cases, B., Vivanco, F., . . . Sampson, H. A. (2012). Epitope mapping of Atlantic salmon major allergen by peptide microarray immunoassay. *Int Arch Allergy Immunol,* **157**, 31-40.

Perez-Gordo, M., Sanchez-Garcia, S., Cases, B., Pastor, C., Vivanco, F., & Cuesta-Herranz, J. (2008). Identification of vitellogenin as an allergen in Beluga caviar allergy. *Allergy,* **63**, 479-480.

Pochard, P., Gosset, P., Grangette, C., Andre, C., Tonnel, A. B., Pestel, J., & Mercenier, A. (2002). Lactic acid bacteria inhibit TH2 cytokine production by mononuclear cells from allergic patients. *J Allergy Clin Immunol,* **110**, 617-623.

Pohjavuori, E., Viljanen, M., Korpela, R., Kuitunen, M., Tiittanen, M., Vaarala, O., & Savilahti, E. (2004). Lactobacillus GG effect in increasing IFN-gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol,* **114**, 131-136.

Prescott, S. L., Wiltschut, J., Taylor, A., Westcott, L., Jung, W., Currie, H., & Dunstan, J. A. (2008). Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy,* **63**, 1481-1490.

Quarcoo, D., Weixler, S., Joachim, R. A., Stock, P., Kallinich, T., Ahrens, B., & Hamelmann, E. (2004). Resiquimod, a new immune response modifier from the family of imidazoquinolinamines, inhibits allergen-induced Th2 responses, airway inflammation and airway hyper-reactivity in mice. *Clin Exp Allergy,* **34**, 1314-1320.

Radauer, C., Bublin, M., Wagner, S., Mari, A., & Breiteneder, H. (2008). Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J Allergy Clin Immunol,* **121**, 847-852.e847.

Rescigno, M. (2003). Identification of a new mechanism for bacterial uptake at mucosal surfaces, which is mediated by dendritic cells. *Pathol Biol (Paris),* **51**, 69-70.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., . . . Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol,* **2**, 361-367.

Resta-Lenert, S., & Barrett, K. E. (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut,* **52**, 988-997.

Rigby, R. J., Knight, S. C., Kamm, M. A., & Stagg, A. J. (2005). Production of interleukin (IL)-10 and IL-12 by murine colonic dendritic cells in response to microbial stimuli. *Clin Exp Immunol,* **139**, 245-256.

Ring, S., Schafer, S. C., Mahnke, K., Lehr, H. A., & Enk, A. H. (2006). CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. *Eur J Immunol,* **36**, 2981-2992.

Rosenfeldt, V., Benfeldt, E., Nielsen, S. D., Michaelsen, K. F., Jeppesen, D. L., Valerius, N. H., & Paerregaard, A. (2003). Effect of probiotic Lactobacillus strains in children with atopic dermatitis. *J Allergy Clin Immunol,* **111**, 389-395.

Rosmilah, M., Shahnaz, M., Masita, A., Noormalin, A., & Jamaludin, M. (2005). Identification of major allergens of two species of local snappers: Lutjanus argentimaculatus (merah/ red snapper) and Lutjanus johnii (jenahak/ golden snapper). *Trop Biomed,* **22**, 171-177.

Ruiter, B., & Shreffler, W. G. (2012). The role of dendritic cells in food allergy. *J Allergy Clin Immunol*, **129**, 921-928.

Sakaguchi, M., Toda, M., Ebihara, T., Irie, S., Hori, H., Imai, A., . . . Inouye, S. (2000). IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *J Allergy Clin Immunol,* **106**, 579-584.

Salzman, N. H., Hung, K., Haribhai, D., Chu, H., Karlsson-Sjoberg, J., Amir, E., . . . Bos, N. A. (2010). Enteric defensins are essential regulators of intestinal microbial ecology. *Nat Immunol,* **11**, 76-83.

Savilahti, E., Kuitunen, M., & Vaarala, O. (2008). Pre and probiotics in the prevention and treatment of food allergy. *Curr Opin Allergy Clin Immunol,* **8**, 243-248.

Scalabrin, D. M., Johnston, W. H., Hoffman, D. R., P'Pool, V. L., Harris, C. L., & Mitmesser, S. H. (2009). Growth and tolerance of healthy term infants receiving hydrolyzed infant formulas supplemented with Lactobacillus rhamnosus GG: randomized, double-blind, controlled trial. *Clin*

Pediatr (Phila), **48**, 734-744.

Schiavi, E., Barletta, B., Butteroni, C., Corinti, S., Boirivant, M., & Di Felice, G. (2011). Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. *Allergy,* **66**, 499-508.

Schnare, M., Barton, G. M., Holt, A. C., Takeda, K., Akira, S., & Medzhitov, R. (2001). Toll-like receptors control activation of adaptive immune responses. *Nat Immunol,* **2**, 947-950.

Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., & Kirschning, C. J. (1999). Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem,* **274**, 17406-17409.

Sepp, E., Julge, K., Vasar, M., Naaber, P., Bjorksten, B., & Mikelsaar, M. (1997). Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr,* **86**, 956-961.

Sharp, M. F., & Lopata, A. L. (2014). Fish Allergy: In Review. *Clin Rev Allergy Immunol,* **46**, 258-271.

Sheih, Y. H., Chiang, B. L., Wang, L. H., Liao, C. K., & Gill, H. S. (2001). Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium Lactobacillus rhamnosus HN001. *J Am Coll Nutr,* **20**, 149-156.

Shida, K., Takahashi, R., Iwadate, E., Takamizawa, K., Yasui, H., Sato, T., . . . Kaminogawa, S. (2002). Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin Exp Allergy,* **32**, 563-570.

Shiomi, K., Sato, Y., Hamamoto, S., Mita, H., & Shimakura, K. (2008). Sarcoplasmic calcium-binding protein: identification as a new allergen of the black tiger shrimp Penaeus monodon. *Int Arch Allergy Immunol,* **146**, 91-98.

Sicherer, S. H. (2011). Epidemiology of food allergy. *J Allergy Clin Immunol,* **127**, 594-602.

Sicherer, S. H., & Sampson, H. A. (2014). Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *Journal of Allergy and Clinical Immunology,* **133**, 291-+.

Simons, F. E., Shikishima, Y., Van Nest, G., Eiden, J. J., & HayGlass, K. T. (2004). Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J Allergy Clin Immunol,* **113**, 1144-1151.

Sistek, D., Kelly, R., Wickens, K., Stanley, T., Fitzharris, P., & Crane, J. (2006). Is the effect of probiotics on atopic dermatitis confined to food sensitized children? *Clin Exp Allergy,* **36**, 629-633.

Smit, J. J., Bol-Schoenmakers, M., Hassing, I., Fiechter, D., Boon, L., Bleumink, R., & Pieters, R. H. (2011). The role of intestinal dendritic cells subsets in the establishment of food allergy. *Clin Exp Allergy,* **41**, 890-898.

Smits, H. H., Engering, A., van der Kleij, D., de Jong, E. C., Schipper, K., van Capel, T. M., . . . Kapsenberg, M. L. (2005). Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol,* **115**, 1260-1267.

Soh, S. E., Aw, M., Gerez, I., Chong, Y. S., Rauff, M., Ng, Y. P., . . . Shek, L. P. (2009). Probiotic supplementation in the first 6 months of life in at risk Asian infants--effects on eczema and atopic sensitization at the age of 1 year. *Clin Exp Allergy,* **39**, 571-578.

Sun, C. M., Hall, J. A., Blank, R. B., Bouladoux, N., Oukka, M., Mora, J. R., & Belkaid, Y. (2007). Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med,* **204**, 1775-1785.

Sutmuller, R. P., den Brok, M. H., Kramer, M., Bennink, E. J., Toonen, L. W., Kullberg, B. J., . . . Adema, G. J. (2006). Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Invest,* **116**, 485-494.

Swoboda, I., Bugajska-Schretter, A., Linhart, B., Verdino, P., Keller, W., Schulmeister, U., . . . Spitzauer, S. (2007). A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. *J Immunol*, **178**, 6290-6296.

Swoboda, I., Bugajska-Schretter, A., Verdino, P., Keller, W., Sperr, W. R., Valent, P., . . . Spitzauer, S. (2002). Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. *J Immunol*, **168**, 4576-4584.

Takeda, K., Suzuki, T., Shimada, S. I., Shida, K., Nanno, M., & Okumura, K. (2006). Interleukin-12 is involved in the enhancement of human natural killer cell activity by Lactobacillus casei Shirota. *Clin Exp Immunol*, **146**, 109-115.

Tao, Y., Drabik, K. A., Waypa, T. S., Musch, M. W., Alverdy, J. C., Schneewind, O., . . . Petrof, E. O. (2006). Soluble factors from Lactobacillus GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. *Am J Physiol Cell Physiol*, **290**, C1018-1030.

Taylor, A. L., Dunstan, J. A., & Prescott, S. L. (2007). Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol*, **119**, 184-191.

Teixeira, L. K., Fonseca, B. P., Barboza, B. A., & Viola, J. P. (2005). The role of interferon-gamma on immune and allergic responses. *Mem Inst Oswaldo Cruz,* **100 Suppl 1**, 137-144.

Tirado-Rodriguez, B., Ortega, E., Segura-Medina, P., & Huerta-Yepez, S. (2014). TGF- beta: an important mediator of allergic disease and a molecule with dual activity in cancer development. *J Immunol Res*, **2014**, 318481.

Untersmayr, E., Scholl, I., Swoboda, I., Beil, W. J., Forster-Waldl, E., Walter, F., . . . Jensen-Jarolim, E. (2003). Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol,* **112**, 616-623.

Untersmayr, E., Szalai, K., Riemer, A. B., Hemmer, W., Swoboda, I., Hantusch, B., . . . Jensen-Jarolim, E. (2006). Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Mol Immunol,* **43**, 1454-1461.

USFDA. (2010). Food allergies rare but risky.

van der Ventel, M. L., Nieuwenhuizen, N. E., Kirstein, F., Hikuam, C., Jeebhay, M. F., Swoboda, I., . . . Lopata, A. L. (2011). Differential responses to natural and recombinant allergens in a murine model of fish allergy. *Mol Immunol,* **48**, 637-646.

Van Do, T., Hordvik, I., Endresen, C., & Elsayed, S. (2003). The major allergen (parvalbumin) of codfish is encoded by at least two isotypic genes: cDNA cloning, expression and antibody binding of the recombinant allergens. *Mol Immunol*, **39**, 595-602.

Viljanen, M., Savilahti, E., Haahtela, T., Juntunen-Backman, K., Korpela, R., Poussa, T., . . . Kuitunen, M. (2005). Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy*, **60**, 494-500.

Wagner, H. (2001). Toll meets bacterial CpG-DNA. *Immunity*, **14**, 499-502.

Weston, S., Halbert, A., Richmond, P., & Prescott, S. L. (2005). Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child*, **90**, 892-897.

Wickens, K., Black, P. N., Stanley, T. V., Mitchell, E., Fitzharris, P., Tannock, G. W., . . . Crane, J. (2008). A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol*, **122**, 788-794.

Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., . . . Sakaguchi, S. (2008). CTLA-4 control over Foxp3+ regulatory T cell function. *Science*, **322**, 271-275.

Witteman, A. M., Akkerdaas, J. H., van Leeuwen, J., van der Zee, J. S., & Aalberse, R. C. (1994). Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects.

Int Arch Allergy Immunol, **105**, 56-61.

Wu, C., Yang, G., Bermudez-Humaran, L. G., Pang, Q., Zeng, Y., Wang, J., & Gao, X. (2006). Immunomodulatory effects of IL-12 secreted by Lactococcus lactis on Th1/Th2 balance in ovalbumin (OVA)-induced asthma model mice. *Int Immunopharmacol,* **6**, 610-615.

Yang, P. C., Xing, Z., Berin, C. M., Soderholm, J. D., Feng, B. S., Wu, L., & Yeh, C. (2007). TIM-4 expressed by mucosal dendritic cells plays a critical role in food antigen-specific Th2 differentiation and intestinal allergy. *Gastroenterology,* **133**, 1522-1533.

Yoshida, S., Ichimura, A., & Shiomi, K. (2008). Elucidation of a major IgE epitope of Pacific mackerel parvalbumin. *Food Chemistry,* **111**, 857-861.

Zeng, J., Li, Y. Q., Zuo, X. L., Zhen, Y. B., Yang, J., & Liu, C. H. (2008). Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther,* **28**, 994-1002.

Zhang, H. Y., Kong, H., Zeng, X. N., Guo, L. Y., Sun, X. Y., & He, S. H. (2014). Subsets of regulatory T cells and their roles in allergy. *Journal of Translational Medicine,* **12**, 11.

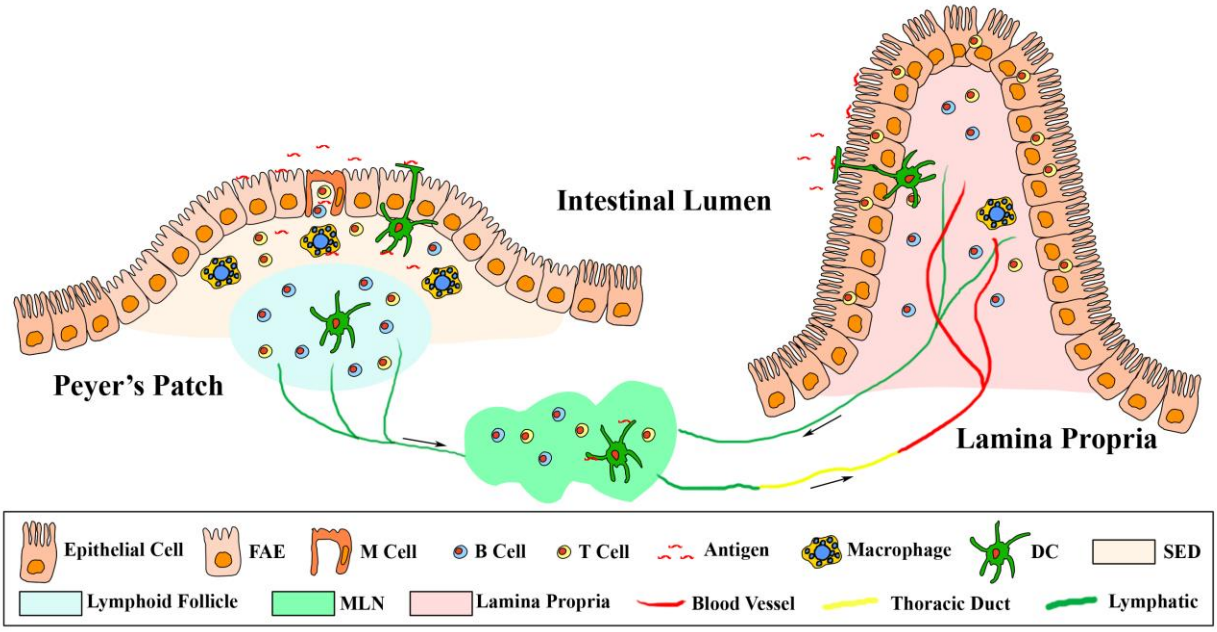
Zhao, C. Q., Li, T. L., He, S. H., Chen, X., An, Y. F., Wu, W. K., . . . Yang, P. C. (2010). Specific immunotherapy suppresses Th2 responses via modulating TIM1/TIM4 interaction on dendritic

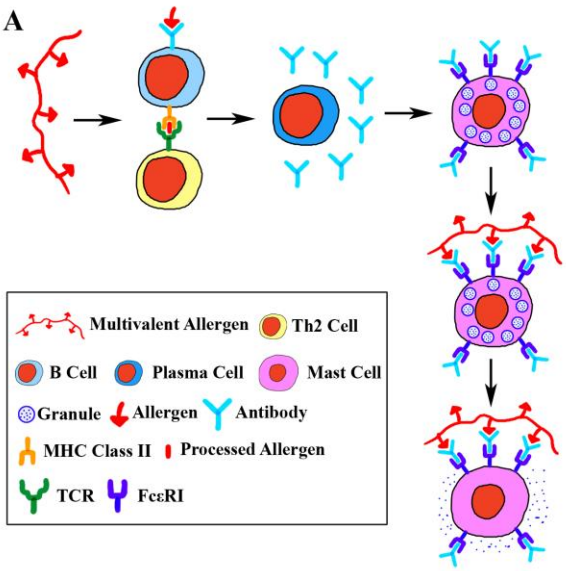
cells. *Allergy,* **65**, 986-995.

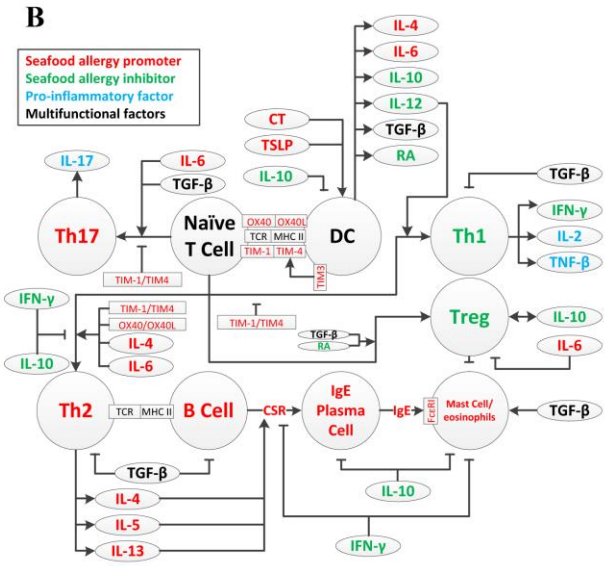
Table 1 Major seafood allergens.

Allergen identified isoform	Rough MW (kDa)	Species implicated	Reference
Parvalbumin β	12	Fishes	(Elsayed & Bennich, 1975)
Collagen	120	Bigeye tuna	(Hamada et al., 2001)
Vitellogenin	118	Beluga (caviar)	(Perez-Gordo et al., 2008)
Transferrin	94	Tuna and marlin	(Kondo et al., 2006)
Enolase β	50	Cod, salmon and tuna	(Kuehn et al., 2013)
Aldolase A	40	Cod, salmon and tuna	(Kuehn et al., 2013)
glyceraldehyde-3-phosphate dehydrogenase	36	Pilchard (cooking vapors)	(van der Ventel et al., 2011)
Kunitz-type trypsin inhibitor	24	*Anisakis simplex* (fish parasite)	(Moneo et al., 2000)
Tropomyosin	35	Shellfishes	(Hoffman et al., 1981)
Arginine kinase	40	Whiteleg shrimp	(Garcia-Orozco et al.,

			2007)
Myosin light chain	20	White pacific shrimp	(Ayuso et al., 2008)
Sarcoplasmic calcium-binding protein (Hal-m-1)	20 49	Black tiger shrimp Abalone	(Shiomi et al., 2008) (Lopata et al., 1997)
Troponin C	21	North Sea shrimp	(Bauermeister et al., 2011)
Triosephosphate isomerase	28	North Sea shrimp	(Bauermeister et al., 2011)







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