

Chitin and Its Effects on Inflammatory and Immune Responses

Daniel Elich Ali Komi^{1,2} · Lokesh Sharma³ · Charles S. Dela Cruz^{3,4}

Published online: 1 March 2017
© Springer Science+Business Media New York 2017

Abstract Chitin, a potential allergy-promoting pathogen-associated molecular pattern (PAMP), is a linear polymer composed of *N*-acetylglucosamine residues which are linked by β -(1,4)-glycosidic bonds. Mammals are potential hosts for chitin-containing protozoa, fungi, arthropods, and nematodes; however, mammals themselves do not synthesize chitin and thus it is considered as a potential target for recognition by mammalian immune system. Chitin is sensed primarily in the lungs or gut where it activates a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes). Chitin induces cytokine production, leukocyte recruitment, and alternative macrophage activation. Intranasal or intraperitoneal administration of chitin (varying in size, degree of acetylation and purity) to mice has been applied as a routine approach to investigate chitin's priming effects on innate and adaptive immunity. Structural chitin present in microorganisms is actively degraded by host true chitinases, including acidic mammalian chitinases and chitotriosidase into smaller fragments that can be sensed by mammalian receptors such as FIBCD1, NKR-P1, and RegIIIc. Immune recognition of chitin also involves pattern recognition receptors, mainly via TLR-2 and Dectin-1,

to activate immune cells to induce cytokine production and creation of an immune network that results in inflammatory and allergic responses. In this review, we will focus on various immunological aspects of the interaction between chitin and host immune system such as sensing, interactions with immune cells, chitinases as chitin degrading enzymes, and immunologic applications of chitin.

Keywords Chitin · Chitinase · Immune system · Innate immunity · Adaptive immunity

Abbreviations

PAMP	Pathogen-associated molecular pattern
PBMCs	Peripheral blood mononuclear cells
AMCase	Acidic mammalian chitinase
Chit1	Chitotriosidase
CLPs	Chitinase like proteins

Introduction

Chitin is a poly(β -(1-4)-*N*-acetyl-D-glucosamine) that was first identified in 1884 and is a natural polysaccharide of major biological importance [1]. Chitin is a nontoxic, biodegradable, and biocompatible polymer [2, 3] with poor solubility at neutral pH [4]. Owing to its chemical properties, chitin has found many applications in biomedicine and biotechnology such as enzyme immobilizer in the food industry [5], immobilization of antibody in the presence of alginate [6], wound dressing and healing [7], anticancer agent [8], tissue engineering, and cancer diagnosis [9]. Chitin and its deacetylated derivative chitosan have been shown to possess immunostimulating properties in mammals and plants [2]. Chitin is a structural component in cell walls of bacteria and fungi, the exoskeleton

✉ Charles S. Dela Cruz
Charles.delacruz@yale.edu

¹ Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, East Azerbaijan, Iran

² Department of Immunology, Tabriz University of Medical Sciences, Tabriz, East Azerbaijan, Iran

³ Section of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA

⁴ Department of Microbial Pathogenesis, Yale School of Medicine, Cedar Street, New Haven, CT TACS441D, USA

of crustaceans [10], insects [11], and eggshells of nematodes [12]. Indeed, such organisms benefit from possessing chitin for the purpose of protection from harsh environmental conditions or from the host anti-parasite/pathogen immune responses [11]. Chitin can be sensed by innate immune system and have immunomodulatory effects on the adaptive immunity which will be discussed in this review.

Structure and General Properties

Chitin polymers are synthesized intracellularly by chitin synthase, translocated across the plasma membrane and coalesce to form rigid crystallites which finally shape the chitinous parts of microorganisms [13]. Chitin has three different crystalline allomorphs namely the α -chitin (the most prevalent form), β -chitin, and γ -chitin (found in mushrooms) which all differ in the orientation of their microfibrils [14]. Chitinase and β -*N*-acetylglucosaminidase mediate the complete hydrolysis of chitin to GlcNAc units [15]. Fungi benefit from glucan cross-linked chitin fibers to reinforce their cell wall. The alkali insoluble core of the *Aspergillus fumigatus* cell wall contains chitin-associated to β -1,3 glucans [16, 17], whereas insects use protein-chitin cross-linked fibers in their exoskeleton. Bacteria and fungi secrete chitinases and chitosanases to recycle large quantities of chitin/chitosan to GlcNAc/GlcN [18]. House dust mites not only benefit from chitin as a component of their cell wall but also release chitin in the environment since their fecal pellets are covered with chitinous materials [19]. Avoiding environmental chitin exposure is impossible due to the large quantities of chitin produced by microorganisms such as house dust mite that induces T helper type 2 (Th2) airway inflammatory diseases such as asthma in sensitized subjects [20].

Chitin Sensing by Immune System

Chitin/chitosans are potential targets for recognition by mammalian immune system since mammals lack such biopolymers naturally. Mammalian chitinases and chitinase-like proteins (C/CLPs) upon exposure to chitin-bearing microbes bind to chitin, while true chitinases such as acidic mammalian chitinases and chitotriosidase can also actively degrade chitin [21, 22]. FIBCD1, NKR-P1, and RegIIIc have been identified as mammalian chitin-binding receptors [18]. Furthermore, Toll-like receptor (TLR) 2, dectin-1 (a β -glucan receptor which mediates T helper type 17 (Th17) development and consequently recruitment of neutrophils), and mannose receptor also participate in mediating immune responses to chitin [18, 23, 24]. Mannose receptor (CD206) is known as an endocytic receptor present in the recycling endocytic compartment. CD206-recognition is pH-dependent in which once acidification takes place in the endosomal compartment, mannose receptors dissociate from their ligands and the empty

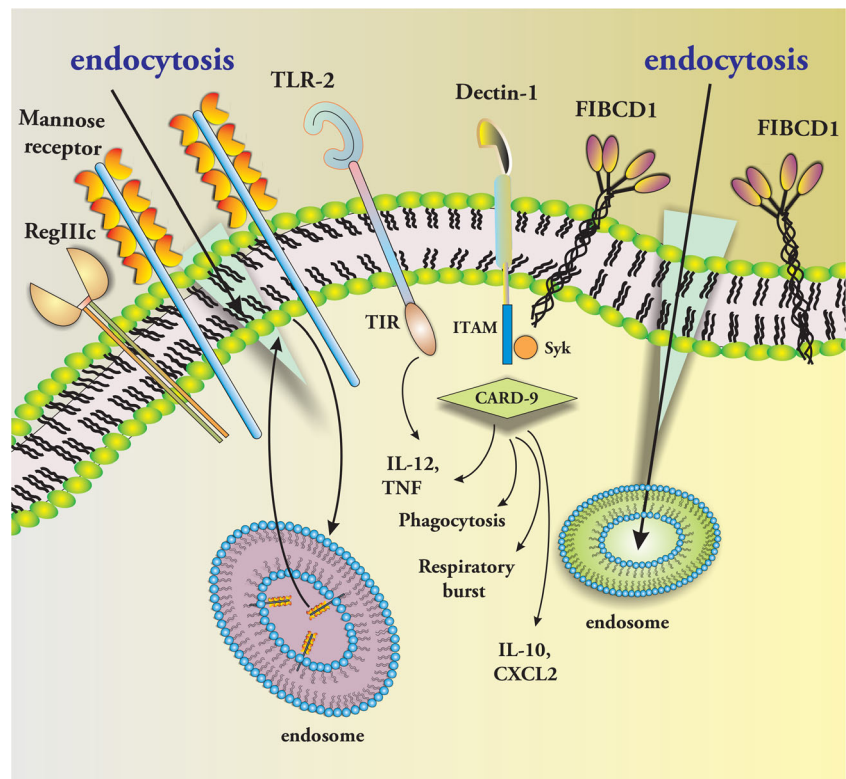
receptors recycle back to the plasma membrane [25] (Fig. 1). Semenuk and colleagues reported NKR-P1, a surface receptor from animal C-lectins superfamily, present on natural killer (NK) cells that acts as chito-oligomer receptor [26].

Chitin-binding activity of RegIII γ , a secreted C-type lectin, and its human counterpart HIP/PAP are crucial for their function as pattern-recognition proteins [27]. Schlosser et al. reported a chitin receptor, FIBCD1, to be structurally as a homotetrameric 55-kDa type II transmembrane protein. FIBCD1 is highly expressed in the gastrointestinal tract [28]. Immunohistochemistry findings revealed FIBCD1 to have highly polarized localization to the apical surface corresponding to the brush border of small and large intestine epithelial cells. This group also reported FIBCD1 as a calcium-dependent acetyl group-binding molecule capable of binding to chitin, but not to other PAMPs [29]. In plants, CERK1 [30] and CEBiP (a chitin elicitor binding protein) contribute to chitin signaling [31]. In addition, some insects such as mosquito *Anopheles gambiae* have been reported to possess Sp22D, a modular chitin-binding protease associated with hemocytes and hemolymph [32].

Several physical variables of chitin including size, shape, source, and purification method affect the immune recognition, cytokine profiles, and inflammatory cell recruitment [33, 34]. Pretreatment of mice with purified chitin particles from *C. albicans* was reported to enhance their survival after experimental infection by *C. albicans*. In this experiment, the candidacidal activity of peritoneal macrophages was enhanced by the increased generation of reactive oxygen species [35]. However, other studies reported that ultrapure chitin from *C. albicans* failed to induce significant immune response when incubated with human peripheral blood mononuclear cells [33]. Large size chitin polymers (such as those of chitin containing pathogens) are biologically inert, while the small fragments of chitin have been shown to effectively induce immune responses. The anti-parasitic responses during exposure result in chitin fragmentation and the initiation of immune responses [11].

Ultrapurified fungal chitin does not directly stimulate production of cytokines from PBMCs but chitin fragments of 40- to 70- μ m size were able to stimulate TNF- α production through a phagocytosis-independent mechanism [36]. The intermediate fragments produced by the catalytic activity of chitinases interact with TLR-2, dectin-1, and mannose receptors on the surface of macrophages to produce pro-inflammatory cytokines and mediators that include IL-17, IL-18, IL-23, TNF- α , and LTB4, which in turn stimulate the production of the C/CLPs. Further, the catalytic activity of AMCase results in the production of smaller fragments which are sensed by Dectin-1 and induce IL-10 and YKL-40 (known also as CHI311 and BRP-39) [11] (Fig. 2). The difference in inflammatory response elicited by different sized fragments might be due to utilization of different combinations of TLR2, dectin-1,

Fig. 1 Chitin-binding receptors from various superfamilies sense chitin fragments through different pathways and activate signaling. FIBCD1 binds chitin and directs acetylated components for degradation in the cytoplasmic endosomes through endocytosis. Dectin-1 through its signaling induces the respiratory burst and phagocytosis. TLR-2 signaling mediated by TIR domain results in inducing IL-12 and TNF production similar to Dectin-1. Mannose receptors participate in endocytosis of chitinous materials along with formation of endosomes. Mannose receptors in a pH-dependent manner dissociate from ligands and recycle back to the plasma membrane



and to a lesser extent, mannose receptor, and phagocytosis to activate distinct signaling pathways that differentially regulate the production of TNF- α and IL-10 [37]. Da Silva et al. studied the stimulating effects of chitin exposure on macrophage IL-17 production via TLR pathway [38]. Through this study on chitin-stimulated macrophages from wild-type mice and mice with null mutations of MyD88, TLR-2, or TLR-4, it was revealed that MyD88 and TLR-2, but not TLR-4, have a key role in the ability of chitin to stimulate IL-17AR [38].

Chitin and Innate Immunity

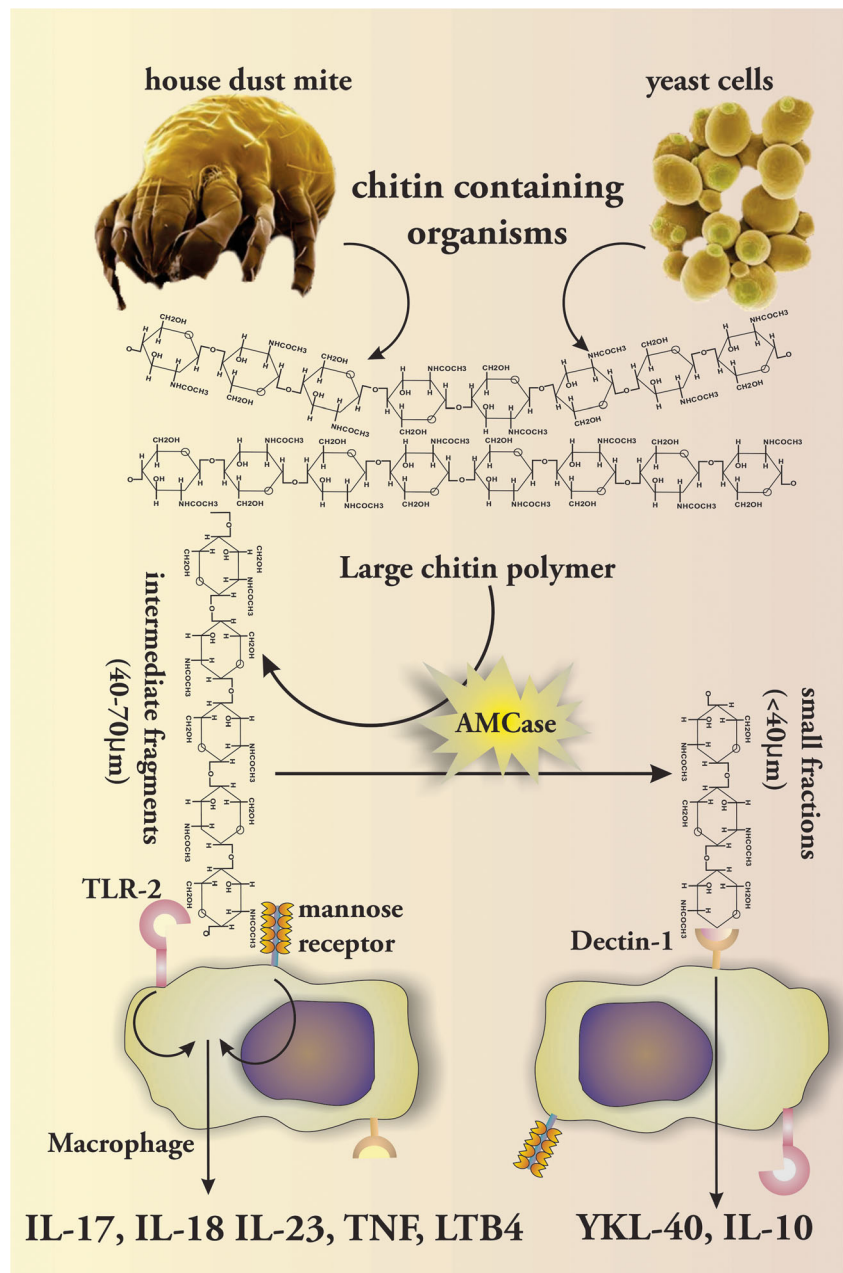
Chitin is sensed by the immune system as a PAMP through specific membrane-bound receptors and plays a key role in defense against pathogens [39]. Expressing chitin-binding receptors on the surface of innate immune cells such as macrophages makes them master cells in chitin-immune system interactions. After innate immune cells, sense chitin as a PAMP, various molecular signaling cascades are triggered to alter the cytokine profiles and cellular phenotype. The *A. fumigatus* cell wall consists of covalently bound β -glucan (recognized by the C-type lectin receptor dectin-1), chitin, galactomannan, and α -glucan that are absent in mammals and present prime targets for recognition as PAMPs by PRRs on host cells. Dectin-1 activation promotes phagocytosis, ROS production, and inflammatory cytokine production. Chitin exposure increases the expression of CCL2, IL-25, IL-33, and TLSP by lung epithelial cells to induce type 2 innate lymphoid cells

(ILC2) to secrete IL-5 and IL-13 cytokines that are essential for accumulation of eosinophils and alternatively activated or M2 macrophages [40].

It has been reported that chitin-induced secretion of IL-17A and TNF- α from macrophages are dependent on the TLR-2/MyD88 and dectin-1/TLR2 pathway, respectively [34]. Intranasal administration of chitin particles into the lung activated alveolar macrophages to induce the expression of cytokines including IL-12, tumor necrosis factor TNF- α , and IL-18, resulting in IFN- γ production mainly by NK cells [11]. Moreover, this chitin treatment significantly increases oxidative burst of macrophages when later stimulated in vitro with phorbol myristate acetate (PMA) [41]. Reese et al. showed the ability of chitin to induce the accumulation of IL-4-expressing innate immune cells in tissues, including eosinophils and basophils, when given to mice. In these experiments, chitin was reported to mediate alternative macrophage activation suggesting that these cells might represent sensors for chitin in tissues. Chitin can also enhance T cell functions, NK cell activity, and interferon (IFN- γ) production by NK cells [2].

Arginase I (argI) is a signature gene induced in alternatively activated macrophages. Reese and colleagues generated mice containing an IRES-driven enhanced yellow fluorescent protein (eYFP) reporter to assess the ability of chitin to induce alternative macrophage activation in vivo. There were no eYFP-positive macrophages identified in the lungs or peritoneum of ArgI reporter mice (YARG mice) under resting conditions, whereas on the 9th day after *N. brasiliensis* infection,

Fig. 2 Large chitin polymers from chitin containing organisms are inert but under chitinolytic activity of AMCase produce intermediate fragments successfully sensed by chitin-binding receptors on the surface of macrophages. Each receptor depending on activation pathway induces specific cytokines



a large number of eYFP positive CD11b⁺, CD11c⁻, and Gr1⁻ macrophages were present in both tissues. Thus, chitin exposure during parasitic infection elicits the accumulation of arginase-I-positive macrophages [42]. Upon chitin exposure, macrophages often express alternatively activated phenotype or M2 markers, characterized by Arg1, Ym1 (also known as chitinase-like 3 or Chil3 or homologous to the eosinophil chemotactic factor), Fizz1, mannose receptor, production of IL-10, and chemokines such as CCL17 and CCL24 [43] and leukotriene B4 which acts as eosinophil chemoattractant [12]. Although chitin exposure induces M2 polarization in vivo, macrophages fail to acquire an M2 phenotype in vitro when exposed to chitin and instead secrete TNF- α

suggesting that macrophages require a factor for in vivo polarization. Roy et al. performed in vivo and in vitro experiments using AMJ2-C11 and LA-4 murine macrophage-derived cell lines as well as CCR2 and CCL2 knockout (KO) mice. They reported that CD326⁺ airway epithelial cells secrete CCL2 in response to chitin, which is a key factor in alternative activation of macrophages and allergic inflammation in vivo [44].

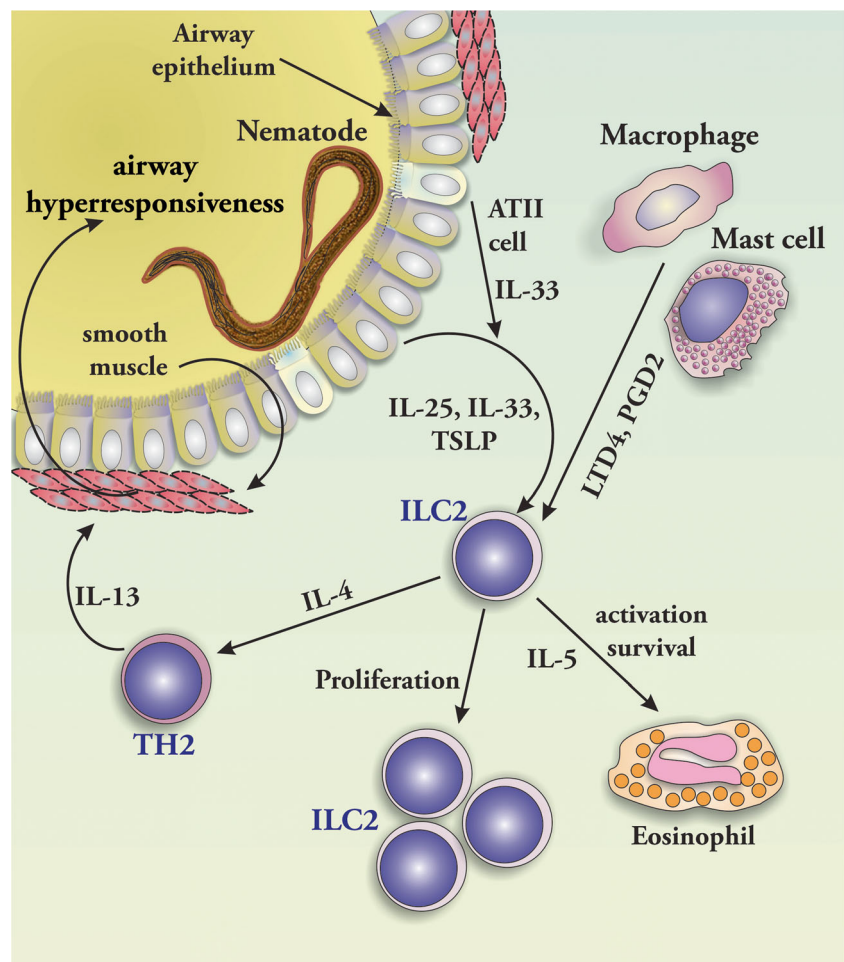
Type II alveolar epithelial (ATII) cells, which are small, cuboidal shaped cells, are crucial for the repair of the injured alveolus by differentiating into alveolar epithelial type I cells [45]. These ATII cells express IL-33 during infection with chitin-bearing nematode parasites such as *Strongyloides*

venezuelensis. ATII cells-derived IL-33 induces ILC2 proliferation and activates them to produce IL-5 and IL-13, which in combination induce eosinophilic inflammation in the lung [46]. The epithelial cytokines IL-25, IL-33, and TSLP activate ILC2 cells to produce high levels of Th2 cytokines IL-5 and IL-13. Furthermore, mast cells and macrophages release PGD2 and leukotriene D4 (LTD4) and stimulate additional ILC2 cells [45]. ILC2-derived IL-5 stimulates eosinophil activation and survival, whereas IL-13 induces airway hyperresponsiveness, and in concert with IL-9, promotes mucus production [47] (Fig. 3). Yasuda et al. demonstrated the role of IL-33 in the induction of eosinophilia upon administration of chitin in mice. They demonstrated that the number of IL-33-expressing ATII cells and IL-33 protein levels increased in the bronchoalveolar lavage fluid of wild-type mice that were expected to develop pulmonary eosinophilia after chitin treatment, while no eosinophilia was observed in IL33-deficient mice. Researchers in this study also investigated whether *S. venezuelensis* infection induced pulmonary eosinophilia without the help from Th2 cells using infected WT and Rag2^{-/-} mice (to exclude the contribution of T cells). Both types of mice equally exhibited increased numbers of IL-33-

expressing ATII cells and developed lung eosinophilia; thus, the adaptive immune cells are not essential for IL-33-induced eosinophil accumulation in the lungs [46].

Since chitin is expressed by microorganisms that are involved in many skin allergies, the keratinocyte-chitin interactions maybe important in the regulation of epidermal immunity. Koller and colleagues investigated the bioactivity and the innate immune modulation responses of chitin on keratinocytes upon exposure to chitin fragments using both immortalized keratinocyte cell line HaCaT (which express TLR1–5 and TLR10) and primary human keratinocytes HEK cells (which express TLR2, TLR3, TLR5, and TLR9). Their results revealed that chitin induces the secretion of CXCL8, IL-6, and TSLP in a dose-dependent fashion in both keratinocyte cell lines. Blocking TLR2 prior to chitin treatment abrogated the chitin-induced inflammatory cytokine production. Assessment of TLR or NOD gene expression levels showed that chitin is a potent modulator of the pattern recognition receptors, especially TLR4 [48] (Fig. 4). The macrophages were shown to initiate the production of IL-12, IL-18, and TNF- α , all of which are extracellular signaling cytokines for IFN- γ production upon phagocytosing bacteria such as heat-killed (HK) *Mycobacterium bovis* BCG or

Fig. 3 Infection by chitin-containing nematode results in the release of IL-33, IL-25, and TSLP from airway epithelium which activate ILC-2 cells. The later cells produce Th2 cytokines IL-4, IL-5, and IL-13 by which Th2 and eosinophil activation occur. Macrophage and mast cell contribute to further ILC2 activation by producing PGD2 and LTD4



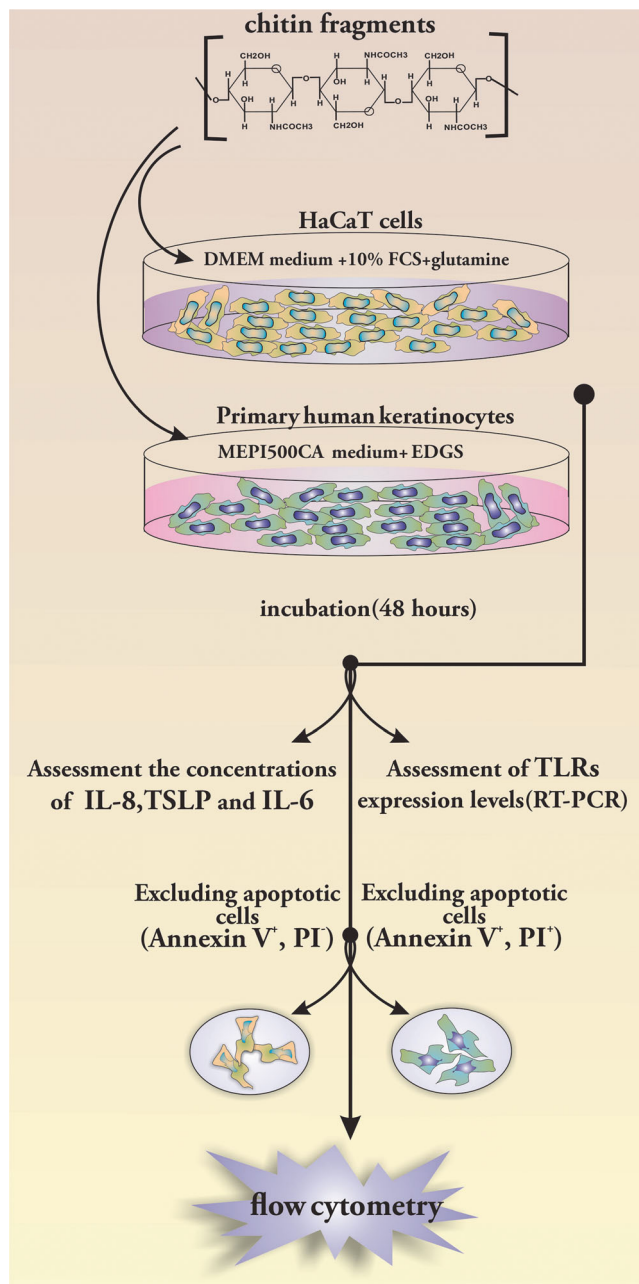


Fig. 4 TLR expression and cytokine production patterns after chitin exposure to evaluate chitin bioactivity

Propionibacterium parvum or their products through mannose receptors. Yoshimi et al. used 1–10- μ m chitin fragments to mimic its microbiologic property to downregulate allergic inflammation in vivo, which similarly could be recognized and ingested by macrophages through the mannose receptors. In their mouse models, both BALB/c and C57BL/6 mice were injected with endotoxin-free ragweed allergen, and allergic responses were assessed by the measurements of IgE levels and ex vivo splenocyte-stimulation assays. Chitin was shown to stimulate spleen cells from ragweed-sensitized mice to produce IFN- γ and IL-10, but not IL-4 or IL-5 resulting in a shift of T helper

cell responses in favor of Th1. Chitin-treated mice were also shown to have significantly reduced IgE levels. Interestingly, ragweed-specific IgE levels were also reduced significantly after chitin treatment, whereas ragweed-specific IgG2a levels were enhanced. Challenge of ragweed allergen in ragweed-immunized mice was accompanied by migration of lymphocytes, eosinophils, and neutrophils in BAL fluid, while chitin treatment abrogated these inflammatory effects. Moreover, compared with non-chitin-treated control group, the peribronchial, the perivascular, and total lung inflammation were inhibited in the chitin-treated group [49]. Wagener et al. similarly analyzed the immunological properties of purified chitin derived from *Candida albicans* and determined that mannose receptor, NOD2 and TLR9 to recognize chitin and participate in mediating an anti-inflammatory response through the secretion of the cytokine IL-10 [50].

Chitosan, the most studied chitin derivative, is capable of also activating the NLRP3 inflammasome in primed bone marrow-derived macrophages resulting in the induction of a robust IL-1 β response. Such immune activating property has implications for chitosan in translational applications; for instance, its employment as a drug or vaccine delivery system could result in a potent, targeted inflammatory response [51]. Chitin not only activates innate immune cells but also activates the complement system. To delineate the mechanism of chitin-induced complement activation, Roy et al. added chitin to the serum in the presence of neutralizing antibody against C1q for the purpose of blocking the classical activation pathway or against factor B to block the alternative pathway. Interestingly, neutralizing antibodies to factor B, but not to C1q, blocked the activation of complement by chitin particles in serum, suggesting that alternative pathway is the major mechanism in complement activation in the presence of chitin [52].

Immunomodulatory effects of dietary chitin on gilthead seabream (*Sparus aurata* L.) innate immunity revealed that chitin is capable of increasing the respiratory burst activity, natural hemolytic complement activity and cytotoxic activity while it failed to increase the phagocytic and serum lysozyme activity [53]. House dust mites (HDMs) are considered as the main source of inhaled allergens known to induce Th2-type airway inflammatory diseases, including asthma in sensitized individuals. It is reported that airway sensitization with HDM-derived chitin plus OVA-enhanced OVA-induced airway inflammation was associated with the increased expression of Th1, Th2, and Th17 cytokines when compared with OVA alone. Interestingly, OVA-specific Th2 cell response, enhanced by chitin, was abolished by the treatment of chitinase. It is also concluded that HDM-derived chitin enhances airway hypersensitivity to inhaled allergens, via the TLR2-dependent pathway. Chitin-induced TNF- α is also reported as a key mediator in the development of Th2 cell response to inhaled allergens [20].

Chitin and Adaptive Immunity

During fungal infections, type 1 T helper (Th1) responses are generally protective, while Th2 responses lead to poor disease outcomes. O’Dea et al. compared the T helper responses in experimental aspergillosis using two fungal isolates with different chitin contents, namely Af293 and Af5517 (with higher chitin content). They found that CD4⁺ T cells in the bronchoalveolar lavage fluid of Af5517-aspirated mice displayed decreased IFN- γ secretion but increased interleukin-4. Moreover, recruitment of eosinophils was correlated with the level of chitin exposure during fungal germination and was decreased by constitutive lung chitinase expression [54]. Most recently, Wiesner et al. studied Th2 responses to cryptococcal infection and provided new evidence that chitin is involved in specific pulmonary fungal immunity. They reported that chitin recognition via chitotriosidase leads to the initiation of harmful Th2 cell differentiation by contribution of CD11b⁺ lung-resident dendritic cells in response to pulmonary fungal infection. During their cryptococcal experimental infection in mice, chitinase activity in lung homogenates was significantly elevated compared with controls.

To test the hypothesis that Th2 cell-associated disease depends on chitinases, these researchers infected wild-type, Chit1KO, and AMCCaseKO mice with *C. neoformans* and quantified the Th2 cell response. They found that infected Chit1KO mice comparing with WT controls had tenfold less Th2 cells in their lungs, while AMCCase deficiency did not impact the number of Th2 cell [55]. Chitin micro particles (CMPs) also have been reported by Nagatani et al. to have positive effects in the control of intestinal inflammation. They administered CMPs (1.5 mg/day/orally) or PBS to acute and chronic colitis models every 3 days to C57Bl/6 WT and C57Bl/6 TCR α KO mice at weaning age. This group induced colitis using dextran sulfate sodium (DSS) in C57Bl/6 IL-10 reporter mice and later sacrificed mice at day 12 after DSS administration for histological assessment of the intestinal tissues. Splenic, mediastinal lymph node (MLN), and colonic cells were analyzed by flow cytometry for CD4 and IFN- γ production. Mouse bone marrow-derived dendritic cells (JAWSII) were also incubated with 100 μ g CMPs and used as antigen presenting cells for the in vitro CD4⁺ stimulation assay. They reported that mediastinal lymph node (MLN)-derived CD4⁺ T cells from CMP-treated TCR α KO mice produced sevenfold higher amount of IFN- γ in the presence of mouse dendritic cells (DCs) and CMPs, in comparison to PBS-treated control mice. Upregulating IFN- γ by oral administration of CMPs ameliorated DSS-induced acute colitis. Moreover, there was IL-10 production in the inflamed colon [56].

Bae et al. investigated the role of chitin and chitosan in inhibition of food allergic responses to peanuts. They treated C3H/HeJ mice with α -chitin, β -chitin, and β -chitosan for 6 weeks starting 1 week before peanut sensitization. They evaluated the allergic symptoms 30–40 min after the oral

ground whole peanut (GWP) challenge and reported the capability of chitin and chitosan to suppress the anaphylaxis symptoms from peanut-induced hypersensitivities. Moreover, peanut-specific IgE levels were reduced in mice treated with α -chitin and β -chitosan. The cytokine profile of cultured splenocytes obtained from mice showed that chitin and chitosan group produced much lower levels of IL-5 and IL-10, the Th2 cytokines critical in the infiltration and activation of eosinophils [57].

Chitin and Chitinases

Both chitin and chitinases have been reported to be involved in the host immune responses against microbe in a wide range of organisms including plants, insects, and mammals [58]. Mammals including humans, in spite of lacking chitin biosynthesis, express chitinases of which Chit1 and AMCCase possess chitinolytic activity [59]. Enzymatically active mammalian chitinases (possess endo/exochitinase activity to hydrolyze the β -1,4-linkages in chitin) [60, 61] and CLPs belong to the 18 glycosyl hydrolase family which contain a chitin binding domain [62]. CLPs unlike true chitinases do not possess the typical chitin-binding domain that contains six cysteine residues, but they still can bind to chitin with high affinity [63]. Chitinases also are important in modulating the immune response. Increased secretion of chitinases is closely associated with pathophysiological conditions dominated by T-helper type 2 cells (Th2) including infection, fibrosis, allergy, and asthma [64, 65]. Chitin exposure directly stimulates expression of the pro-Th2 mediators AMCCase and eotaxin-3 in cultures of sinonasal epithelial cells [66]. Owing to their enzymatic function, different sized chitin fragments are produced that trigger innate immunity pattern recognition receptors (PRRs) to induce tumor necrosis factor TNF- α , IL-17, and/or IL-10 [21]. However, some fungal parasites such as *Magnaporthe oryzae* evade the plant immune system through masking chitin with β -1,3-glucan which interferes with the enzymatic digestion of chitin [67]. Various fungal pathogens such as *Cladosporium fulvum* secrete LysM (proteins with lysine motifs) effectors to scavenge chitin fragments and thus avoid recognition by host immune receptors [68].

Immunologic Application

Immunological activity of chitin derivatives and their effect as adjuvant have been assessed nearly three decades ago [69, 70]. Subsequently, the Th1 adjuvant effect of chitin microparticles in inducing viral specific immunity was reported by Hamajima et al. [64]. Da Silva et al. studied the adjuvant properties of chitin to induct adaptive Th2, Th1, and Th17 immune responses using in vivo and in vitro models. They used five groups of mice including TLR2-, TLR4-, MyD88-, IL17A-deficient mice and C57BL/6 wild-type mice as control group.

The mice received two intraperitoneal injections of chitin plus ovalbumin (OVA) or alum plus OVA to compare the adjuvant activity of chitin. Alum and chitin were both similarly found to be adjuvants for OVA-induced BAL and tissue inflammation, eosinophilic infiltration, and OVA-specific IgE induction. MyD88 and IL-17 played critical roles in the chitin-mediated responses, whereas TLR2 had a more limited role with only modest decrease in alum-related responses when TLR2 was not present. However, the adjuvant effects of chitin in T cell proliferation were mediated by TLR2. The induced IL-4, IL-5, and IL-13 production was observed both in OVA/chitin-primed and OVA/alum-primed T cells. Interestingly, the adjuvant capacity of chitin was completely abrogated in IL-17A null mice. Further investigations also revealed that TLR2-dependent pathway involving IL-17A mediates the adjuvant effects of chitin [71] (Fig. 5). Chitosan also has been widely studied as an adjuvant in DNA and protein-based vaccines [72]. In this

regard, chitosan benefits from multiple mechanisms that include antigen protection, depot formation, enhanced antigen uptake and presentation, and direct modulation of immune responses for its adjuvant capacity when used in injectable and mucosal vaccines [73].

Clinical Implications of Chitin

A wide variety of medical implications for chitin and its deacetylated derivative chitosan have been reported over the last few decades [74]. Chitin made sutures have been shown to resist attack in bile, urine, and pancreatic juice. Moreover, several patented wound dressing including Beschitin W, composed of chitin nonwoven fabric were reported to be beneficial in clinical practice [75]. Tissue engineering benefits from chitin in variety of forms including hydrogels, fibrous scaffolds, or porous sponges, within which the appropriate cell types

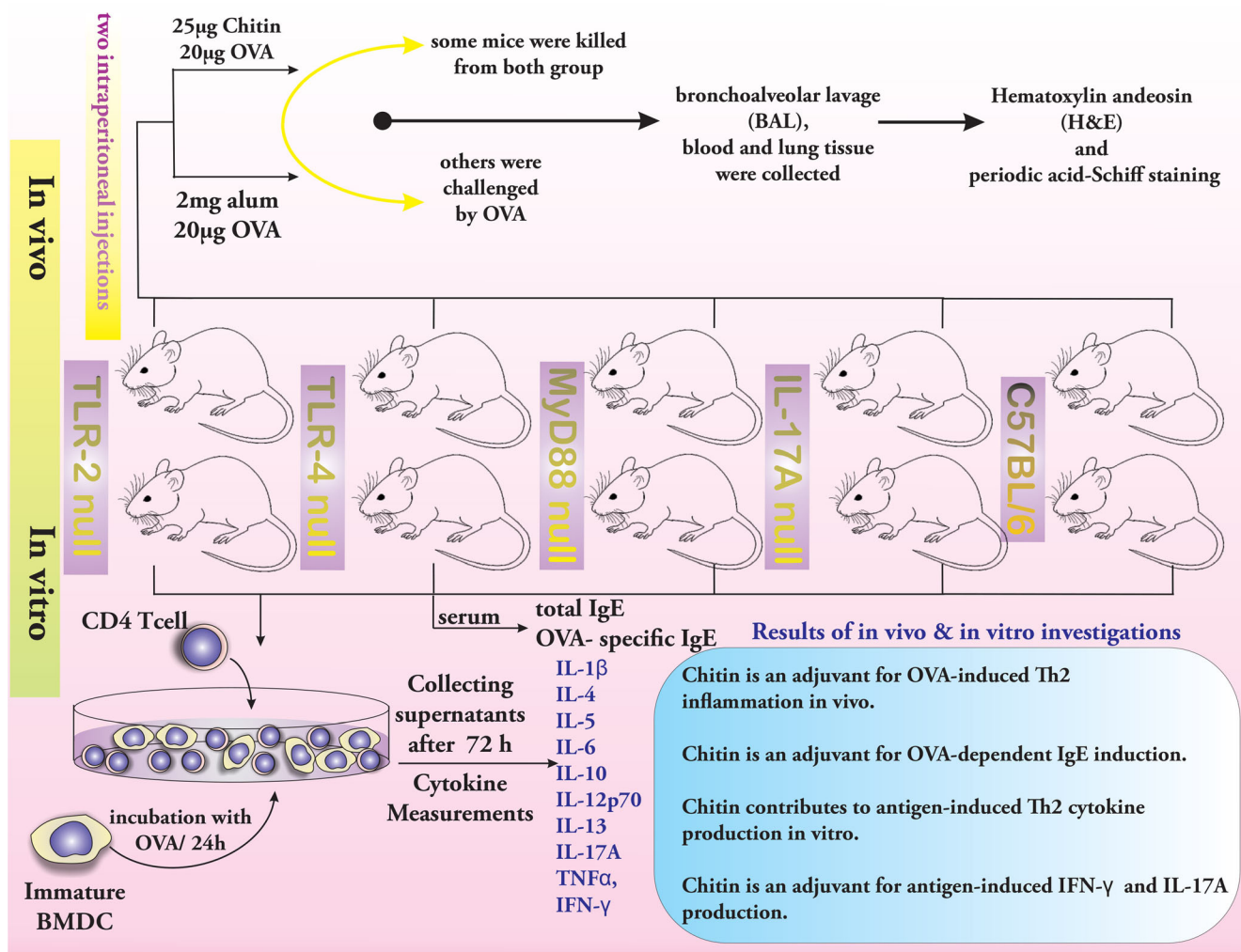


Fig. 5 Adjuvant property of chitin assessed using TLR2, TLR4, MyD88, IL-17A null mice, and control C57BL/6. In vivo and in vitro investigations were carried on each group after being exposed with alum or chitin fragments. MyD88 and IL-17 were found to have a key

role in chitin responses. Further cytokine assays revealed that chitin possess a high capacity to act as adjuvant to induce adaptive Th2, Th1, and Th17 immune responses

could be cultured for the purpose of repairing injured body parts [76]. The antimicrobial activity of chitin and its derivatives against bacteria, yeast, and fungi has received considerable attention in recent years. The proposed mechanisms of antimicrobial activity include cell lysis, breakdown of the cytoplasmic membrane barrier, and the chelation of trace metal cations by the chitosan [77]. Additionally, some chitin derivatives have been investigated for their capacity in drug delivery. Carboxymethyl chitin (CMC) nanoparticles for example were successfully applied in sustained and controlled release of 5-fluorouracil at pH 6.8 [78]. Moreover, unique biologic characterization of chitin and its derivatives resulted in finding new medical applications. For instance, chitin and especially chitosan act as the inhibitors of angiotensin-converting enzyme, the enzyme associated with hypertension [79]. Interestingly, chitosan owing to its fat binding property actively interferes with the absorption of dietary lipids from the gastrointestinal tract. Additionally, it has been reported to increase serum leptin concentrations while decreasing C-reactive protein (CRP), sensitive marker of inflammation [80].

Conclusion

The significance of chitin and its derivatives on immune responses has not been fully appreciated. Such responses are reflection of not only chitin but also chitinases and chitinase-like proteins during natural or experimental exposure, each with their own mechanisms to induce and regulate immune responses. There are many aspects of chitin-immune system interactions which are not thoroughly understood yet. Chitin receptors on myeloid cells that induce a phagocytic response have yet to be definitively identified. Moreover, full-length, insoluble chitin may not be recognized by receptors but soluble by-products of chitin digestion can be recognized. The differential effects of chitin and chitin receptor interaction on immune modulation require more exploration. Commercial shellfish chitin has been used in most chitin immunology studies, and our knowledge remains incomplete regarding other sources of chitin such as fungal chitin in similar studies. The results obtained from each chitin source may differ from others due to their structural differences as a consequence of variable attachment of chitin to other immunologically active materials. Fungal chitin structurally is linked to glucans and glycosylated proteins that potently elicit and modify specific innate responses. Chitin in microorganisms naturally is linked with other cell wall components, and their elimination involves a challenging process. Lacking novel methods for chitin purification may explain the conflicting data in the literature of immune responses to chitin. Wide distribution of chitin makes its exposure inevitable; however, the avoidance of chitin exposure needs to be investigated.

Compliance with Ethical Standards I hereby state that none of the coauthors and the corresponding author of this paper have a conflict of interest and it has been prepared for publication without using any fund. Moreover, the paper does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest Daniel Elieh Ali Komi, Lokesh Sharma, and Charles S. Dela Cruz declare that they have no conflict of interest.

References

1. Younes I, Rinaudo M (2015) Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine drugs* 13(3):1133–1174. doi:10.3390/md13031133
2. Cuesta A, Esteban MA, Meseguer J (2003) In vitro effect of chitin particles on the innate cellular immune system of gilthead seabream (*Sparus aurata* L.) *Fish & shellfish immunology* 15(1):1–11
3. Esteban MA, Mulero V, Cuesta A, Ortuno J, Meseguer J (2000) Effects of injecting chitin particles on the innate immune response of gilthead seabream (*Sparus aurata* L.) *Fish & shellfish immunology* 10(6):543–554. doi:10.1006/fsim.2000.0271
4. Jung WJ, Park RD (2014) Bioproduction of chitooligosaccharides: present and perspectives. *Marine drugs* 12(11):5328–5356. doi:10.3390/md12115328
5. Zdarta J, Klapiszewski L, Wysokowski M, Norman M, Kolodziejczak-Radzimska A, Moszynski D, Ehrlich H, Maciejewski H, Stelling AL, Jesionowski T (2015) Chitin-lignin material as a novel matrix for enzyme immobilization. *Marine drugs* 13(4):2424–2446. doi:10.3390/md13042424
6. Badwan AA, Rashid I, Omari MM, Darras FH (2015) Chitin and chitosan as direct compression excipients in pharmaceutical applications. *Marine drugs* 13(3):1519–1547. doi:10.3390/md13031519
7. Azuma K, Izumi R, Osaki T, Ifuku S, Morimoto M, Saimoto H, Minami S, Okamoto Y (2015) Chitin, chitosan, and its derivatives for wound healing: old and new materials. *Journal of functional biomaterials* 6(1):104–142. doi:10.3390/jfb6010104
8. Azuma K, Osaki T, Minami S, Okamoto Y (2015) Anticancer and anti-inflammatory properties of chitin and chitosan oligosaccharides. *Journal of functional biomaterials* 6(1):33–49. doi:10.3390/jfb6010033
9. Jayakumar R, Deepthy M, Manzoor K, Nair SV, Tamura H (2010) Biomedical applications of chitin and chitosan based nanomaterials-A short review. *Carbohydrate Polymers*
10. Ayyaru G, Venkatesan A (2006) Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Elsevier
11. Lee CG, Da Silva CA, Lee JY, Hartl D, Elias JA (2008) Chitin regulation of immune responses: an old molecule with new roles. *Curr Opin Immunol* 20(6):684–689. doi:10.1016/j.coi.2008.10.002
12. Brodaczewska K, Donskow-Lysoniewska K, Doligalska M (2015) Chitin, a key factor in immune regulation: lesson from infection with fungi and chitin bearing parasites. *Acta parasitologica / Witold Stefanski Institute of Parasitology, Warszawa, Poland* 60(2):337–344. doi:10.1515/ap-2015-0047
13. Cohen E (2001) Chitin synthesis and inhibition: a revisit. *Pest Manag Sci* 57(10):946–950. doi:10.1002/ps.363
14. Elieh-Ali-Komi D, Hamblin MR (2016) Chitin and chitosan: production and application of versatile biomedical nanomaterials. *International journal of advanced research*
15. Purushotham P, Arun PV, Prakash JS, Podile AR (2012) Chitin binding proteins act synergistically with chitinases in *Serratia*

- proteamaculans 568. PLoS One 7(5):e36714. doi:10.1371/journal.pone.0036714
16. Barreto-Bergter E, Figueiredo RT (2014) Fungal glycans and the innate immune recognition. Front Cell Infect Microbiol 4:145. doi:10.3389/fcimb.2014.00145
 17. Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latge JP (2000) Molecular organization of the alkali-insoluble fraction of aspergillus fumigatus cell wall. J Biol Chem 275(52):41528
 18. Buetter CL, Specht CA, Levitz SM (2013) Innate sensing of chitin and chitosan. PLoS Pathog 9(1):e1003080. doi:10.1371/journal.ppat.1003080
 19. Gregory LG, Lloyd CM (2011) Orchestrating house dust mite-associated allergy in the lung. Trends Immunol 32(9):402–411. doi:10.1016/j.it.2011.06.006
 20. Choi JP, Lee SM, Choi HI, Kim MH, Jeon SG, Jang MH, Jee YK, Yang S, Cho YJ, Kim YK (2016) House dust mite-derived chitin enhances Th2 cell response to inhaled allergens, mainly via a TNF- α -dependent pathway. Allergy, Asthma Immunol Res 8(4):362–374. doi:10.4168/aa.2016.8.4.362
 21. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA (2011) Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol 73:479–501. doi:10.1146/annurev-physiol-012110-142250
 22. Dela Cruz CS, Liu W, He CH, Jacoby A, Gornitzky A, Ma B, Flavell R, Lee CG, Elias JA (2012) Chitinase 3-like-1 promotes Streptococcus Pneumoniae killing and augments host tolerance to lung antibacterial responses. Cell Host Microbe 12(1):34–46. doi:10.1016/j.chom.2012.05.017
 23. Dong B, Li D, Li R, Chen SC, Liu W, Liu W, Chen L, Chen Y, Zhang X, Tong Z, Xia Y, Xia P, Wang Y, Duan Y (2014) A chitin-like component on sclerotic cells of Fonsecaea pedrosoi inhibits dectin-1-mediated murine Th17 development by masking beta-glucans. PLoS One 9(12):e114113. doi:10.1371/journal.pone.0114113
 24. Dostert C, Tschopp J (2007) DETECTING fungal pathogens. Nat Immunol 8(1):17–18. doi:10.1038/ni0107-17
 25. Taylor PR, Gordon S, Martinez-Pomares L (2005) The mannose receptor: linking homeostasis and immunity through sugar recognition. Trends Immunol 26(2):104–110. doi:10.1016/j.it.2004.12.001
 26. Semenuk T, Krist P, Pavlicek J, Bezouska K, Kuzma M, Novak P, Kren V (2001) Synthesis of chito-oligomer-based glycoconjugates and their binding to the rat natural killer cell activation receptor NKR-P1. Glycoconj J 18(10):817–826
 27. Cash HL, Whitham CV, Behrendt CL, Hooper LV (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science (New York, NY) 313(5790):1126–1130. doi:10.1126/science.1127119
 28. Thomsen T, Schlosser A, Holmskov U, Sorensen GL (2011) Ficolins and FIBCD1: soluble and membrane bound pattern recognition molecules with acetyl group selectivity. Mol Immunol 48(4):369–381. doi:10.1016/j.molimm.2010.09.019
 29. Schlosser A, Thomsen T, Moeller JB, Nielsen O, Tornoe I, Mollenhauer J, Moestrup SK, Holmskov U (2009) Characterization of FIBCD1 as an acetyl group-binding receptor that binds chitin. Journal of immunology (Baltimore, Md : 1950) 183(6):3800–3809. doi:10.4049/jimmunol.0901526
 30. Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K, Molinaro A, Kaku H, Shibuya N (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. Proc Natl Acad Sci U S A 111(3):E404–E413. doi:10.1073/pnas.1312099111
 31. Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiya C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci U S A 103(29):11086–11091. doi:10.1073/pnas.0508882103
 32. Danielli A, Loukeris TG, Lagueux M, Muller HM, Richman A, Kafatos FC (2000) A modular chitin-binding protease associated with hemocytes and hemolymph in the mosquito *Anopheles gambiae*. Proc Natl Acad Sci U S A 97(13):7136–7141
 33. Alvarez FJ (2014) The effect of chitin size, shape, source and purification method on immune recognition. Molecules 19(4):4433–4451. doi:10.3390/molecules19044433
 34. Amarsaikhan N, Templeton SP (2015) Co-recognition of beta-glucan and chitin and programming of adaptive immunity to aspergillus fumigatus. Front Microbiol 6:344. doi:10.3389/fmicb.2015.00344
 35. Suzuki K, Okawa Y, Hashimoto K, Suzuki S, Suzuki M (1984) Protecting effect of chitin and chitosan on experimentally induced murine candidiasis. Microbiol Immunol 28(8):903–912
 36. Mora-Montes HM, Netea MG, Ferwerda G, Lenardon MD, Brown GD, Mistry AR, Kullberg BJ, O'Callaghan CA, Sheth CC, Odds FC, Brown AJ, Munro CA, Gow NA (2011) Recognition and blocking of innate immunity cells by *Candida albicans* chitin. Infect Immun 79(5):1961–1970. doi:10.1128/iai.01282-10
 37. Da Silva CA, Chalouni C, Williams A, Hartl D, Lee CG, Elias JA (2009) Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. Journal of immunology (Baltimore, Md : 1950) 182(6):3573–3582. doi:10.4049/jimmunol.0802113
 38. Da Silva CA, Hartl D, Liu W, Lee CG, Elias JA (2008) TLR-2 and IL-17A in chitin-induced macrophage activation and acute inflammation. Journal of immunology (Baltimore, Md : 1950) 181(6):4279–4286
 39. Klausner D, Flury P, Boller T, Bartels S (2013) Several MAMPs, including chitin fragments, enhance AtPep-triggered oxidative burst independently of wounding. Plant Signal Behav 8(9). doi:10.4161/psb.25346
 40. Van Dyken SJ, Mohapatra A, Nussbaum JC, Molofsky AB, Thornton EE, Ziegler SF, McKenzie AN, Krummel MF, Liang HE, Locksley RM (2014) Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and $\gamma\delta$ T cells. Immunity 40(3):414–424. doi:10.1016/j.immuni.2014.02.003
 41. Shibata Y, Foster LA, Metzger WJ, Myrvik QN (1997) Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. Infect Immun 65(5):1734–1741
 42. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM (2007) Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature 447(7140):92–96. doi:10.1038/nature05746
 43. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, Akira S (2010) The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nat Immunol 11(10):936–944. doi:10.1038/ni.1920
 44. Roy RM, Wuthrich M, Klein BS (2012) Chitin elicits CCL2 from airway epithelial cells and induces CCR2-dependent innate allergic inflammation in the lung. Journal of immunology (Baltimore, Md : 1950) 189(5):2545–2552. doi:10.4049/jimmunol.1200689
 45. Wang D, Haviland DL, Burns AR, Zsigmond E, Wetsel RA (2007) A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 104(11):4449–4454. doi:10.1073/pnas.0700052104
 46. Yasuda K, Matsumoto M, Nakanishi K (2014) Importance of both innate immunity and acquired immunity for rapid expulsion of *S. venezuelensis*. Front Immunol 5:118. doi:10.3389/fimmu.2014.00118

47. Lund S, Walford HH, Doherty TA (2013) Type 2 innate lymphoid cells in allergic disease. *Curr Immunol Rev* 9(4):214–221. doi:10.2174/1573395510666140304235916
48. Koller B, Muller-Wiefel AS, Rupec R, Korting HC, Ruzicka T (2011) Chitin modulates innate immune responses of keratinocytes. *PLoS One* 6(2):e16594. doi:10.1371/journal.pone.0016594
49. Shibata Y, Foster LA, Bradfield JF, Myrvik QN (2000) Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. *J Immunol* 164(3):1314–1321
50. Wagener J, Malireddi RK, Lenardon MD, Koberle M, Vautier S, MacCallum DM, Biedermann T, Schaller M, Netea MG, Kanneganti TD, Brown GD, Brown AJ, Gow NA (2014) Fungal chitin dampens inflammation through IL-10 induction mediated by NOD2 and TLR9 activation. *PLoS Pathog* 10(4):e1004050. doi:10.1371/journal.ppat.1004050
51. Bueter CL, Lee CK, Wang JP, Ostroff GR, Specht CA, Levitz SM (2014) Spectrum and mechanisms of inflammasome activation by chitosan. *Journal of immunology* (Baltimore, Md : 1950) 192(12):5943–5951. doi:10.4049/jimmunol.1301695
52. Roy RM, Paes HC, Nanjappa SG, Sorkness R, Gasper D, Sterkel A, Wuthrich M, Klein BS (2013) Complement component 3C3 and C3a receptor are required in chitin-dependent allergic sensitization to *aspergillus fumigatus* but dispensable in chitin-induced innate allergic inflammation. *MBio* 4(2). doi:10.1128/mBio.00162-13
53. Esteban MA, Cuesta A, Ortuno J, Meseguer J (2001) Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish & shellfish immunology* 11(4):303–315. doi:10.1006/fsim.2000.0315
54. O'Dea EM, Amarsaikhan N, Li H, Downey J, Steele E, Van Dyken SJ, Locksley RM, Templeton SP (2014) Eosinophils are recruited in response to chitin exposure and enhance Th2-mediated immune pathology in *aspergillus fumigatus* infection. *Infect Immun* 82(8):3199–3205. doi:10.1128/iai.01990-14
55. Wiesner DL, Specht CA, Lee CK, Smith KD, Mukaremera L, Lee ST, Lee CG, Elias JA, Nielsen JN, Boulware DR, Bohjanen PR, Jenkins MK, Levitz SM, Nielsen K (2015) Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal infection. *PLoS Pathog* 11(3):e1004701. doi:10.1371/journal.ppat.1004701
56. Nagatani K, Wang S, Llado V, Lau CW, Li Z, Mizoguchi A, Nagler CR, Shibata Y, Reinecker HC, Mora JR, Mizoguchi E (2012) Chitin microparticles for the control of intestinal inflammation. *Inflamm Bowel Dis* 18(9):1698–1710. doi:10.1002/ibd.22874
57. Bae MJ, Shin HS, Kim EK, Kim J, Shon DH (2013) Oral administration of chitin and chitosan prevents peanut-induced anaphylaxis in a murine food allergy model. *Int J Biol Macromol* 61:164–168. doi:10.1016/j.ijbiomac.2013.06.017
58. Beckerman AP, de Roij J, Dennis SR, Little TJ (2013) A shared mechanism of defense against predators and parasites: chitin regulation and its implications for life-history theory. *Ecology and evolution* 3(15):5119–5126. doi:10.1002/ece3.766
59. Vega K, Kalkum M (2012) Chitin, chitinase responses, and invasive fungal infections. *International journal of microbiology* 2012:920459. doi:10.1155/2012/920459
60. Funkhouser JD, Aronson NN Jr (2007) Chitinase family GH18: evolutionary insights from the genomic history of a diverse protein family. *BMC Evol Biol* 7:96. doi:10.1186/1471-2148-7-96
61. Kanneganti M, Kamba A, Mizoguchi E (2012) Role of chitotriosidase (chitinase 1) under normal and disease conditions. *Journal of epithelial biology & pharmacology* 5:1–9
62. Lee CG (2009) Chitin, chitinases and chitinase-like proteins in allergic inflammation and tissue remodeling. *Yonsei Med J* 50(1):22–30. doi:10.3349/ymj.2009.50.1.22
63. Kawada M, Hachiya Y, Arihiro A, Mizoguchi E (2007) Role of mammalian chitinases in inflammatory conditions. *The Keio journal of medicine* 56(1):21–27
64. Muzzarelli RA (2010) Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Marine drugs* 8(2):292–312. doi:10.3390/md8020292
65. Komi DE, Kazemi T, Bussink AP (2016) New insights into the relationship between chitinase-3-like-1 and asthma. *Current allergy and asthma reports* 16(8):57. doi:10.1007/s11882-016-0637-2
66. Lalaker A, Nkrumah L, Lee WK, Ramanathan M, Lane AP (2009) Chitin stimulates expression of acidic mammalian chitinase and eotaxin-3 by human sinonasal epithelial cells in vitro. *American journal of rhinology & allergy* 23(1):8–14. doi:10.2500/ajra.2009.23.3256
67. Fujikawa T, Sakaguchi A, Nishizawa Y, Kouzai Y, Minami E, Yano S, Koga H, Meshi T, Nishimura M (2012) Surface alpha-1,3-glucan facilitates fungal stealth infection by interfering with innate immunity in plants. *PLoS Pathog* 8(8):e1002882. doi:10.1371/journal.ppat.1002882
68. Sanchez-Vallet A, Saleem-Batcha R, Kombrink A, Hansen G, Valkenburg DJ, Thomma BP, Mesters JR (2013) Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. *elife* 2:e00790. doi:10.7554/eLife.00790
69. Li X, Min M, Du N, Gu Y, Hode T, Naylor M, Chen D, Nordquist RE, Chen WR (2013) Chitin, chitosan, and glycosylated chitosan regulate immune responses: the novel adjuvants for cancer vaccine. *Clinical & developmental immunology* 2013:387023. doi:10.1155/2013/387023
70. Nishimura K, Nishimura S, Nishi N, Saiki I, Tokura S, Azuma I (1984) Immunological activity of chitin and its derivatives. *Vaccine* 2(1):93–99
71. Da Silva CA, Pochard P, Lee CG, Elias JA (2010) Chitin particles are multifaceted immune adjuvants. *Am J Respir Crit Care Med* 182(12):1482–1491. doi:10.1164/rccm.200912-1877OC
72. Levitz SM, Huang H, Ostroff GR, Specht CA (2015) Exploiting fungal cell wall components in vaccines. *Semin Immunopathol* 37(2):199–207. doi:10.1007/s00281-014-0460-6
73. Carroll EC, Jin L, Mori A, Munoz-Wolf N, Oleszycka E, Moran HB, Mansouri S, McEntee CP, Lambe E, Agger EM, Andersen P, Cunningham C, Hertzog P, Fitzgerald KA, Bowie AG, Lavelle EC (2016) The vaccine adjuvant chitosan promotes cellular immunity via DNA sensor cGAS-STING-dependent induction of type I interferons. *Immunity* 44(3):597–608. doi:10.1016/j.immuni.2016.02.004
74. Dutta PK, Dutta J, Tripathy VS (2004) Chitin and chitosan: chemistry, properties and applications. *Journal of Scientific & Industrial Research*
75. Kumar MNVR (2000) A review of chitin and chitosan applications. Elsevier Ltd
76. Venkatesan J, Vinodhini PA, Sudha PN, Kim SK (2014) Chitin and chitosan composites for bone tissue regeneration. *Adv Food Nutr Res* 73:59–81. doi:10.1016/b978-0-12-800268-1.00005-6
77. Park BK, Kim MM (2010) Applications of chitin and its derivatives in biological medicine. *Int J Mol Sci* 11(12):5152–5164. doi:10.3390/ijms11125152
78. Ashish Deva JCM, Ashish Deva JCM, Sreeja V, Tamurab H, Patzkec GR, Hussainc F, Weyenethd S, Naira SV, Jayakumar R (2010) Novel carboxymethyl chitin nanoparticles for cancer drug delivery applications. Elsevier Ltd
79. Senevirathne M, Kim SK (2012) Utilization of seafood processing by-products: medicinal applications. *Adv Food Nutr Res* 65:495–512. doi:10.1016/b978-0-12-416003-3.00032-9
80. Walsh AM, Sweeney T, Bahar B, O'Doherty JV (2013) Multifunctional roles of chitosan as a potential protective agent against obesity. *PLoS One* 8(1):e53828. doi:10.1371/journal.pone.0053828