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A POSSIBLE ROLE OF CHITIN IN THE PATHOGENESIS OF ASTHMA AND ALLERGY

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Abstract: Chitin is the second most abundant polysaccharide in the world; it is found in insects, parasites and fungi. Chitinases break down chitin, and are a part of the defence mechanism against chitin-containing parasites in lower life forms. This review is based on the results of PubMed-searches using the search-terms: chitin, chitinase, allergy and asthma. Research in murine models has proved that chitin is a size-dependent microbial-associated molecular pattern, with the ability to induce an immunological response via pattern recognition receptors. Medium-sized chitin micro-particles (CMPs) have been shown to induce inflammation, while small-sized CMPs reduce inflammation. The amount of acidic mammalian chitinase correlates with asthma, and the enzyme has been shown to induce chemokine secretion in murine lungs. The high prevalence of asthma among people working with chitinous substances, such as crabs and fungi, supports the hypothesis that chitin might be an allergen playing a role of significance in the development of asthma. This new knowledge about chitin and chitinases, combined with the hygiene-hypothesis, may contribute to a model for the pathogenesis of allergic conditions where chitin and chitinases are potential therapeutic targets.

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CHITIN AND CHITINASES

Second only to cellulose, chitin is the most abundant polysaccharide world-wide. Chitin consists of N-acetylic glucosaminomolcules tied together via β-1,α-4 glycosidic bonds (Fig. 1). Chitin is a solid material comprising the exosceleton of insects, parasites and fungi.

Chitin is digested by chitinases. Organisms consisting of chitin use chitinases in the metabolic regulation of their chitinous structures. However, chitinases are also present in non-chitinous organisms where they represent a defence mechanism against chitinous parasites.

CHITIN, A PATTERN RECOGNITION RECEPTOR LIGAND

The immune system is divided into the innate and the adaptive parts. The innate immune system initiates a fast primary inflammatory response to infections and activates the adaptive immune system [22]. Innate immune cells use specific receptors to detect microbial patterns – pattern recognition receptors (PRRs). These microbial-associated molecular patterns (MAMPs) are essential structures in microorganisms, and are therefore maintained in a highly conserved condition during their evolution [19]. The PRR toll-like receptor-4 (TLR4) detects the MAMP lipopolysaccharid (LPS) from the Gram negative bacterial cell wall, when it is presented by MD2. The innate immune system is subsequently activated when LPS binds the TLR-4 MD2 complex [33, 34].

After the identification of TLRs, we attempted to identify more PRRs and their ligands, such as peptidoglycans, LPS and ß-glucans. The PRRs detect MAMPs and activate an adequate response to an infectious threat, but ignore the normal bacterial flora[27].

In 2008, chitin was identified as a MAMP, inducing IL-17-mediated inflammation in murine lungs. The inflammatory response was dependent on the size of the chitin

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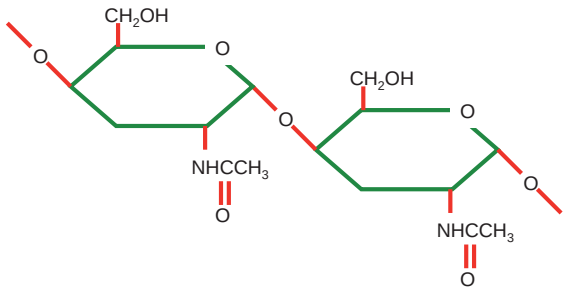

Figure 1. Chitin is a polysaccharide, consisting of N-acetylglucosamine molecules connected by β-1, α-4 glycosidic bonds. The figure illustrates two of the monomers from the polysaccharide.

micro-particles (CMPs) used: large particles (>70 µm) were ignored and induced no response; medium-sized CMPs (40–70 µm) induced an IL-17 and TNF-mediated inflammatory response. TLR2 knockout mice did not respond to CMP exposure, and it was concluded that chitin is a size-dependent MAMP that activates macrophages via TLR-2 [12]. A later study by the same group showed that small CMPs (<40 µm) induced the anti-inflammatory cytokine IL-10 (Fig. 2). The response to small CMPs was partly dependent on TLR-2, but other PRRs, e.g. dectin-1 and the mannose receptor had a more important role [11].

CHITIN AND ASTHMA

The experimental data on the association between chitins and asthma is divergent. In one study, CMPs were shown to induce airway inflammation mediated by TLR-2 and IL-17 in healthy mice [12]. Another study found that CMPs induced IL-4, eosinophilia and increased leukotrieneB4in macrophages[29]. Other studies have found that CMP treatment reduces the histopathology in the lungs of asthmatic mice [9, 26, 36]. CMP exposure has even been associated with a reduction of plasma IgE and reduced airway hyperresponsiveness in one of these murine models [36].

There could be many explanations for these conflicting results. An obvious factor of significance is the size of the CMPs used in the different studies. The study that showed that CMP exposure induced asthma [29] did not describe CMP size. If these were medium-sized CMPs, this would explain the observed inflammatory response [12]. The studies that reported reduced airway inflammation [9, 26, 36] used CMPs in the size range 1–20 µm. These small-sized CMPs have proved to be anti-inflammatory, inducing an IL-10 response [11]. The difference in the size of the CMPs used in these studies seems to be the most likely explanation for the divergent results. Another explanation could be that CMPs of 1–10 µm are phagocytised by macrophages, which subsequently release IL-12 [31, 32]. IL-12 induces INF-γ (Th1-cytokine) [14], resulting in a Th2- reducing Th1 response. This would explain why small CMPs reduce airway inflammation in asthmatic mice, since asthma is the result of a pathogenic Th2 inflammatory response (Fig. 3). Very small CMPs (<2 µm) have been shown to have no effect on the immune system [11, 29].

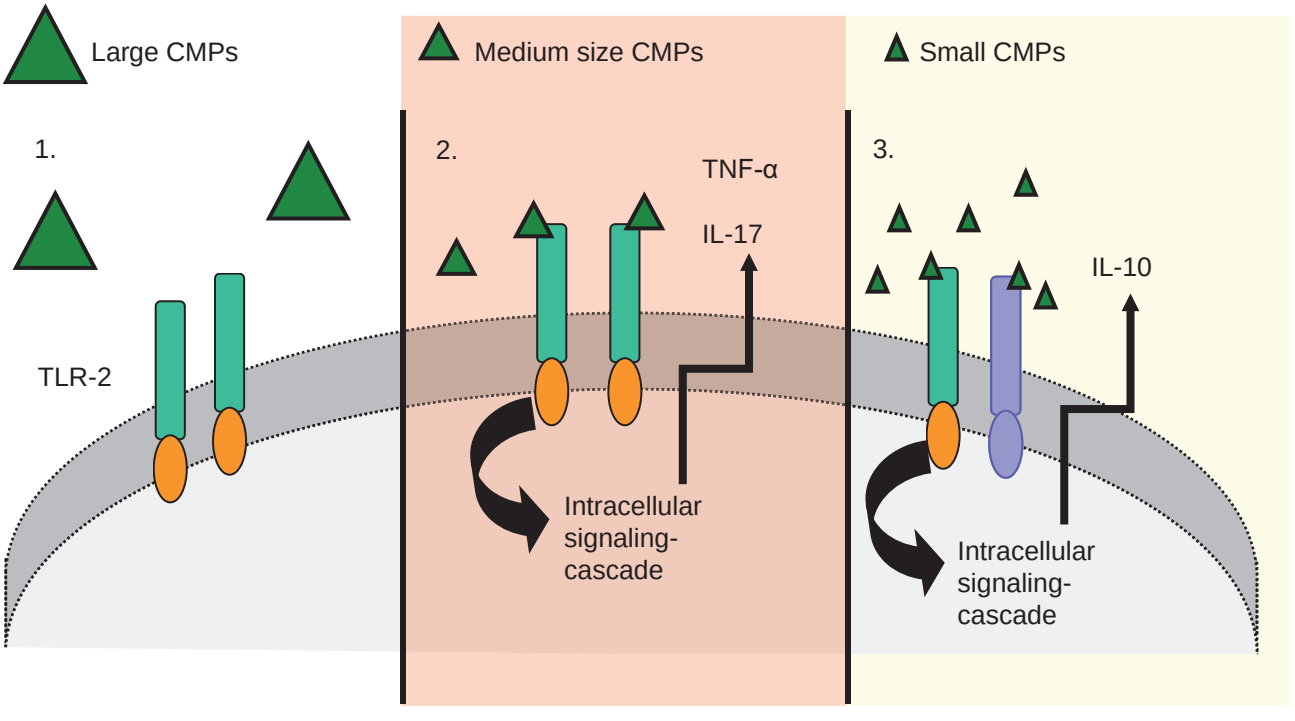

Figure 2. Chitin is a size-dependent microbial associated molecular pattern. The figure illustrates the interaction between chitin micro-particles (CMPs) and pattern recognition receptors (PRRs). 1. Large CMPs has no effect on alveolar macrophages in murine airways. 2. Medium size CMPs adhere to TLR-2 resulting in the excretion of pro-inflammatory cytokines, TNF-α and IL-17. 3. Small CMPs interact with several PRRs resulting in the release of the anti-inflammatory cytokine IL-10.

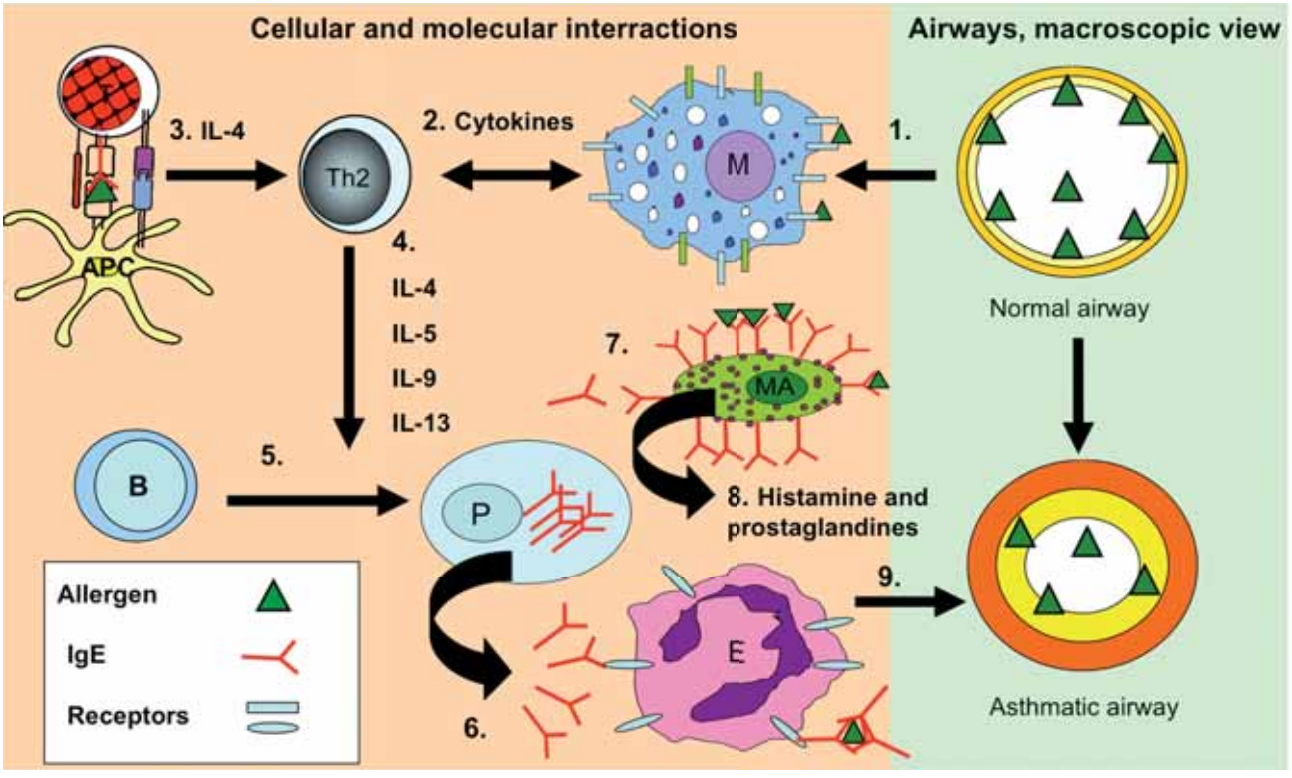

Figure 3. The pathogenesis of allergic asthma. 1. Airway allergens adhere to pattern recognition receptors on alveolar macrophages (M). 2. Macrophages secrete pro-inflammatory cytokines. 3. Naïve T-cells (T) differentiate to Th2 cells (Th2) after encountering antigen presenting cells (APC). 4. Th2-cells secrete interleukins. 5. These interleukins help convert B-cells (B) into plasma cells (P). 6. The plasma cells produce allergen specific IgE. 7. Allergen specific IgE molecules adhere to Fc-receptors on the mast cell (M). 8. The IgE primed mast cell degranulates on encounter with the allergen, releasing pro-inflammatory chemokines. 9. After the acute response of the mast cell, the eosinophil granulocytes (E) migrate to the airways, resulting in chronic inflammation and airway obstruction.

A possible source of error in these studies could be LPS contamination of the CMP material used. LPS exposure results in an INF-γ, mediated Th1 response [7], and would therefore lead to reduced Th2 response. Only one of the studies cited above deals with this problem and proves that the CMPmaterial used is free of LPS [29]. The presented study, reports that intranasal exposure to CMP results in airway eosinophilia even in TLR-4 knockout mice. The inflammatory response to LPS is TLR-4 dependent, proving that chitin induces eosinophilchemotaxia independent of LPS.

CHITINASES AND CHITINASE-LIKE PROTEINS

The existence of chitinases and chitinase-like proteins in insects and lower organisms has been known for decades. It has been generally accepted that they play a significant role in the immune defence against chitinous parasitic and fungi infections in these organisms [38].

In 2001 acidic mammalian chitinase (AMCase) was identified in mice. To-date, two chitinolyticchitinases have been identified in mammals, AMCase and chitotriosidase. Chitinases consist of a chitin-binding domain in the C-terminal, and an enzymatically-active domain in the N-terminal. AMCase was primarily found in the lungs and gastrointestinal tract. It has been assumed that the enzyme plays a role in digestion, and has a protective role in the lungs [5].

A correlation has been observed between AMCase and asthma in several studies on mice [39, 40, 41]. Human studies have found an association between polymorphisms in the AMCase gene with an attenuated chitinase and prevalence of asthma [4, 9]. AMCase initiates a Th2 response via IL-13 in mice, while the AMCase inhibitor allosamidine and AMCase specific antibodies reduce airway inflammation in asthmatic mice [41]. The level of upregulaion of AMCase in asthmatic mice was reduced in a dose-dependent manner when the mice were treated with glucocorticoids [39]. This indicates that one effect of glucocorticoid treatment of asthma may be a reduction in the level of AMCase. In 2008, AMCase was shown to be an auto- and paracrine agent that upregulates the chemokine secretion in epithelial cells (Fig. 4) [15]. This suggests that AMCase may have an effect also on leucocyticchemotaxia in the lungs of asthmatic mice. The importance of the chitinolytic effect of AMCase in the pathogenesis of asthma is still unclear. Mice exposed to CMPs that had been treated with AMCase experienced no inflammatory response [29]. AMCase breaks down chitin to very small CMPs (<2 μm), and these have been shown to have no effect on the immune

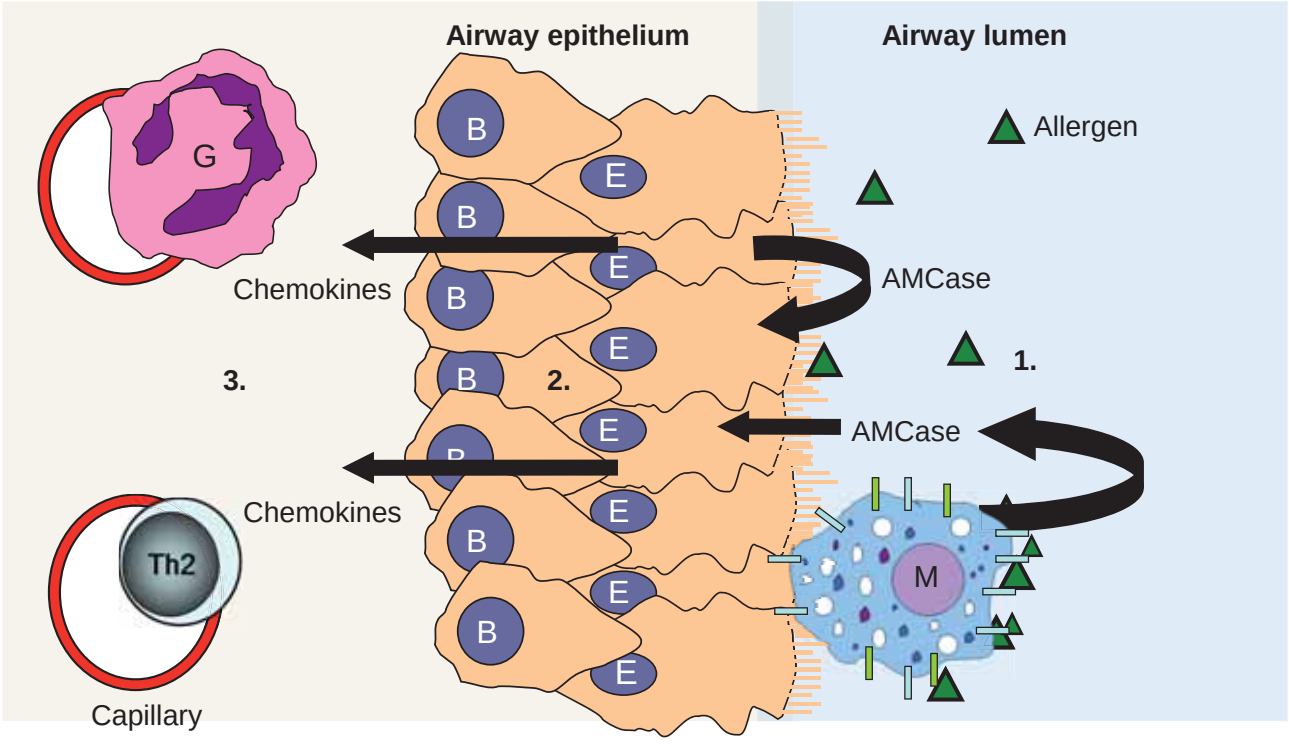

Figure 4. AMCase as a paracrine/autocrine mediator in asthma.1. AMCase is secreted by alveolar macrophages (M) and epithelial cells (E) in asthmatic airways. 2. In the extracellular matrix, AMCase is a paracrine/autocrinesignallingagent that enhances epithelial chemokine excretion. 3. The chemokines attract leukocytes from the circulation leading to chemotaxis.Basal cell (B), Granulocyte (G), Th2 Lymphocyte (L).

system [11]. Together, these results indicate that AMCase might be considered as a potential therapeutic target in the treatment of asthma.

A base duplication in the chitotriosidase gene with an allelic prevalence of 20% in Caucasians that results in a truncated enzyme has been found to be equally divided between asthmatics and controls. This makes a direct correlation between chitotriosidase and asthma unlikely [3]. Chitotriosidase breaks down the chitinous cell wall in fungi. Since exposure to some fungi can result in the development of asthma [13], chitotriosidase may be linked to asthma through its antifungal effect. Chitotriosidase may also play a role in the pathogenesis of other inflammatory conditions where the enzyme has been shown to be upregulated [2, 21, 38].

YKL-40, a chitinase like protein, is significantly upregulated in the plasma and lungs of patients with asthma [10]. A polymorphism in the promoter region og the YKL-40 gene has been associated with asthma [24]. These findings suggest that YKL-40 may also play a role in the pathogenesis of asthma.

CHITIN AND THE HYGIENE-HYPOTHESIS

The increase in asthma in the industrialised world is still insufficiently explained; and exposure to helminths, fungi and other infections may be important for the pathogenesis. The fact that many of these contain chitin suggests that chitin could be of significance in a multifactorial explanatory model. The high prevalence of asthma among people working with chitinous substances, such as crabs and fungi, is the strongest piece of evidence that chitin may be acting as an allergen [8, 18, 20, 25, 30, 37].

The hygiene-hypothesis, initially proclaimed by Strachan [35], explains the increase in allergic disease as a result of a lack of exposure to infections, symbiotic microorganisms and parasites in childhood, resulting in an "uneducated" immune system. This hypothesis is supported by many epidemiological studies that report a correlation between increased prevalence of allergy and asthma, and decreased interaction with microorganisms [1, 23]. The similarity between the pathological Th2 response in asthma and the Th2 response to parasitic infections also supports the hygiene-hypothesis [16, 17]. In industrialised countries, increased hygiene has resulted in a reduced rate of infections in general, and parasitic infections in particular. At the same time, a massive increase in allergy and autoimmune diseases has been observed. A lower prevalence of allergy has been found in the constantly waning population born and raised on farms in the industrialized areas of the world [28, 6]. In developing countries, with a high incidence of parasitic infections, a low prevalence of allergy, asthma and autoimmunity has been observed. A large proportion of parasites contain chitin, and it is possible that parasite-derived chitin modulates the immune system; the new findings that chitin is a MAMP supports this

hypothesis. Thus, a possible explanation for the observed difference in the prevalence of asthma between industrialised and development countries could be that infections with chitinous parasites results in hyposensitisation to chitin as an allergen.

CONCLUSION

Chitin as an environmental factor and chitinases as a part of the immune response may play an important role in the pathogenesis of inflammatory disease in general, and asthma in particular. Chitin can induce or reduce inflammation depending on CMP size. TLR-2 is pivotal to the pro-inflammatory response to medium-sized CMPs, while the anti-inflammatory response to small CMPs is dependent on other PRRs. AMCase upregulates chemokines in murine lungs; however, the specific role of chitinases and the significance of their chitinolytic effect for the pathogenesis of asthma needs to be further explored.

Most of the present results concerning chitin, chitinases and asthma are based on murine models. It is a matter of great importance to determine whether these results are also valid for humans, and studies with larger mammals could be a solution. The hygiene-hypothesis combined with new knowledge about chitin and chitinases should be considered in the context of a new multifactorial explanatory model for asthma. Chitin and chitinases are potential therapeutic targets in the future treatment of asthma.

REFERENCES

1. Araujo MI, Lopes AA, Medeiros M, Cruz AA, Sousa-Attaa L, Solé D, Carvalhoa EM: Inverse association between skin response to aeroallergen and Schistosoma mansoni infection. *Int Arch Allergy Immunol* 2000, **123**, 145–148.

2. Bargaglia E, Margolliccib M, Luddib A, Nikiforakisa N, Peraria MG, Grossob S, Perronea A, Rottoli P: Chitotriosidase activity in patients with interstitial lung diseases. *Respiration* 2007, **74**, 548–552.

3. Bierbaum S, Superti-Furga A, Heinzmann A: Genetic polymorphisms of chitotriosidase in Caucasian children with bronchial asthma. *Int J Immunogenet* 2006, **33**, 201–204.

4. Bierbaum S, Nickel R, Koch A, Lau S, Deichmann KA, Wahn U, Superti-Furga A, Heinzmann A: Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. *Am J Res Crit Care Med* 2005, **172**, 1505–1509.

5. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JM: Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J Biol Chem* 2001, **276**, 6770–6778.

6. Braun-Fahrländer C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, Vuille JC, Wutrich B: Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clin Exp All* 1999, **29**, 28–34.

7. Burton OT, Zaccone P: The potential role of chitin in allergic reactions. *Trends Immunol* 2007, **28**, 419–422.

8. Cartier A, Lehrer SB, Horth-Susin L, Swanson M, Neis B, Howse D, Jong M: Prevalence of crab asthma in crab plant workers in Newfoundland and Labrador. *Int J Circumpolar Health* 2004, **63**, 333–336.

9. Chatterjee R, Batra J, Das S, Sharma SK, Ghosh B: Genetic association of acidic mammalian chitinase with atopic asthma and serum total IgE levels. *J Allergy Clin Immunol* 2008, **122**, 202–208.

10. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA: A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Eng J Med* 2007, **357**, 2016-2027.

11. Da Silva CA, Chalouni C, Williams A, Hartl D, Lee CG, Elias JA: Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. *J Immunol* 2009, **182**, 3573–3582.

12. Da Silva CA, Hartl D, Liu W, Lee CG, Elias JA: TLR-2 and IL-17A in chitin-induced macrophage activation and acute inflammation. *J Immunol* 2008, **181**, 4279–4286.

13. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM: The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006, **27**, 615–626.

14. Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M: Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995, **182**, 1527–1536.

15. Hartl D, He CH, Koller B, Da Silva CA, Homer R, Lee CG, Elias JA: Acidic mammalian chitinase is secreted via an ADAM17/EGFR-dependent pathway and stimulates chemokine production by pulmonary epithelial cells. *J Biol Chem* 2008, **283**, 33472–334782.

16. Holt PG: Infections and the development of allergy. *Toxicol Lett* 1996, **86**, 205.

17. Holt PG: Parasites, atopy, and the hygiene hypothesis: resolution of a paradox? *Lancet* 2000, **356**, 1699.

18. Howsea D, Gautrinb D, Neisa B, Cartier B, Horth-Susin L, Jong M, Swanson MC: Gender and snow crab occupational asthma in Newfoundland and Labrador, Canada. *Environ Res* 2006, **101**, 163–174.

19. Janeway Jr CA: The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol Today* 1992, **13**, 11–16.

20. Jeebhay MF, Robins TG, Lehrer SB, Lopata AI: Occupational seafood allergy: a review. *Occup Environ Med* 2001, 58, 553–562.

21. Malaguarnera L, Rosa MD, Zambito AM, dell'Ombra N, Marco RD, Malaguarnera M: Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. *Am J Gastroenterol* 2006, **101**, 2060-2069.

22. Medzhitov R, Janeway Jr CA: Innate immunity. *N Eng J Med* 2000 **343**, 338–344.

23. Nyan OA, Walraven GE, Banya WA, Milligan P, Van Der Sande M, Ceesay SM, Del Prete G, McAdam KP: Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. *Clin Exp Allergy* 2001, **31**, 1672–1678.

24. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, Parry RR, Heinzmann A, Deichmann KA, Lester LA, Gern JE, Lemanske RF, Nicolae DL, Elias JA, Chupp GL: Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma and lung function. *N Eng J Med* 2008, 358, 1682–1691.

25. Ortega HG, Daroowalla F, Petsonk EL, Lewis D, Berardinelli S Jr, Jones W, Kreiss K, Weissman DN: Respiratory symptoms among crab processing workers in Alaska: epidemiological and environmental assessment. *Am J Ind Med* 2001, **39**, 598–607.

26. Ozdemir C, Yazi D, Aydogan M, Akkoc T, Bahceciler NN, Strong P, Barlan IB: Treatment with chitin microparticles is protective against lung histopathology in murine asthma model. *Clin Exp Allergy* 2006, **36**, 960–968.

27. Palm NW, Medzhitov R: Pattern recognition receptors and control of adaptive immunity. *Immunol Rev* 2009, **227**, 221–233.

28. Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G: Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy* 2002, **32**, 247–253.

29. Reese T, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM: Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* 2007, **447**, 92–96.

30. Senti G, Leser C, Lundberg M, Wüthrich B: Allergic asthma to shiitake and oyster mushroom. *Allergy* 2000, 55, 975-987.

31. Shibata Y, Foster LA, Bradfield JF, Myrvik QN: Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. *J Immunol* 2000, **164**, 1314–1324.

32. Shibata Y, Metzger WJ, Myrvik QN: Chitin particle-induced cell-mediated immunity is inhibited by soluble mannan: mannose receptor-mediated phagocytosis initiates IL-12 production. *J Immunol* 1997, **159**, 2462–2467.

33. Sigsgaard T, Bonefeld-Jørgensen EC, Hoffmann HJ, Bønløkke J, Krüger T: Microbial cell wall agents as an occupational hazard. *Toxicol Appl Pharmacol* 2005, **207(2 Suppl)**, 310–319.

34. Sigsgaard T, Hoffman HJ, Thorne PS: The role of innate immunity in occupational allergy: recent findings. *Curr Opin Allergy Clin Immunol* 2008, **8**, 120–125.

35. Strachan DP: Hay fever, hygiene, and household size. *BMJ* 1989, **299(6710)**, 1259–1260.

36. Strong P, Clark H, Reid K: Intranasal application of chitin microparticles down-regulates symptoms of allergic hypersensitivity to *Dermatophagoides pteronyssinus* and *Aspergillus fumigatus* in murine models of allergy. *Clin Exp Allergy* 2002, **32**, 1794–1800.

37. Tanaka H, Saikai T, Sugawara H, Takeya I, Tsunematsu K, Matsuura A, Abe S: Workplace-related chronic cough on a mushroom farm. *Chest* 2002, **122**, 1080-1085.

38. van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, Sugar A, Verhoeven AJ, Boot RG, Aerts JM: Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Int Immunol* 2005, **17**, 1505–1512.

39. Zhao J, Yeong LH, Wong WSF: Dexamethasone alters bronchoalveolar lavage fluid proteome in a mouse asthma model. *Int Arch Allergy Immunol* 2007, **142**, 219–229.

40. Zhao J, Zhu H, Wong CH, Leung KY, Wong WSF: Increased lungkine and chitinase levels in allergic airway inflammation. *Proteomics* 2005, **5**, 2799–2807.

41. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA: Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004, **304**, 1678–1682.