The role of chitin, chitinases, and chitinase-like proteins in pediatric lung diseases

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Abstract

Chitin, after cellulose, the second most abundant biopolymer on earth, is a key component of insects, fungi, and house-dust mites. Lower life forms are endowed with chitinases to defend themselves against chitin-bearing pathogens. Unexpectedly, humans were also found to express chitinases as well as chitinase-like proteins that modulate immune responses. Particularly, increased levels of the chitinase-like protein YKL-40 have been associated with severe asthma, cystic fibrosis, and other inflammatory disease conditions. Here, we summarize and discuss the potential role of chitin, chitinases, and chitinase-like proteins in pediatric lung diseases.

Keywords: Inflammation; Lung diseases; Asthma; Cystic fibrosis; Chitin; Chitinases; Chitinase-like proteins; Chitotriosidase; AMCase; CHIT1; YKL-40; BRP-39; Fungi; M2 macrophages

Introduction

The role of chitin and chitinases has been firmly established in the field of plant and microbial immunity by demonstrating that host-derived chitinases cleave chitin to protect against invading chitin-bearing pathogens, such as fungi. Although mammals lack endogenous chitin or chitin synthases, chitinases and chitinase-like proteins are endogenously expressed in their lung and other organs. Particularly, chitinase-like proteins have been described as dysregulated in a variety of diseases characterized by chronic inflammation and tissue remodeling, yet their potential role for humans has just recently begun to evolve [1,2]. Chitin is a major component of a variety of allergy-triggering environmental components, including house-dust mites or fungal spores, and fungal asthma is increasingly appreciated as an under-diagnosed disease entity [3]. Thus, an understanding of the complex immunological and pathophysiological implications of chitin-chitinase interactions in the human body is of high relevance for identifying new biomarkers and therapeutic targets for fungal diseases and other conditions, where chitin-coated microbial derivatives play a critical role. Here, we provide an overview of an emerging, yet complex field of research. Of particular interest are interspecies differences with resulting specific nomenclatures. Subsequent to an overall introduction of chitin, the role of chitinases and chitinase-like proteins in pediatric lung diseases are reviewed, leading up to a summary of ideas how these mechanisms could be exploited to improve diagnostics and therapeutics in lung diseases in childhood and beyond (Figure 1).

Review

Chitin

Chitin, a polymer of *N*-acetylglucosamine and the second most abundant polysaccharide in nature following cellulose, is an essential component of fungi, house-dust mites, exoskeletons of crabs, shrimp and insects, parasitic nematodes, and digestive tracts of many insects [1]. Chitin protects these microbes from their environment, and its turnover is regulated by biosynthesis and degradation through endogenous chitinases.

The first immune stimulatory activity of chitin and chitin derivatives in mammals was discovered and extensively explored in the middle to late 1980s, as reviewed recently [2,1]. These early studies clearly indicated that chitin has important immunologic effects *in vitro* and *in vivo*, initially highlighted by Shibata et al. who

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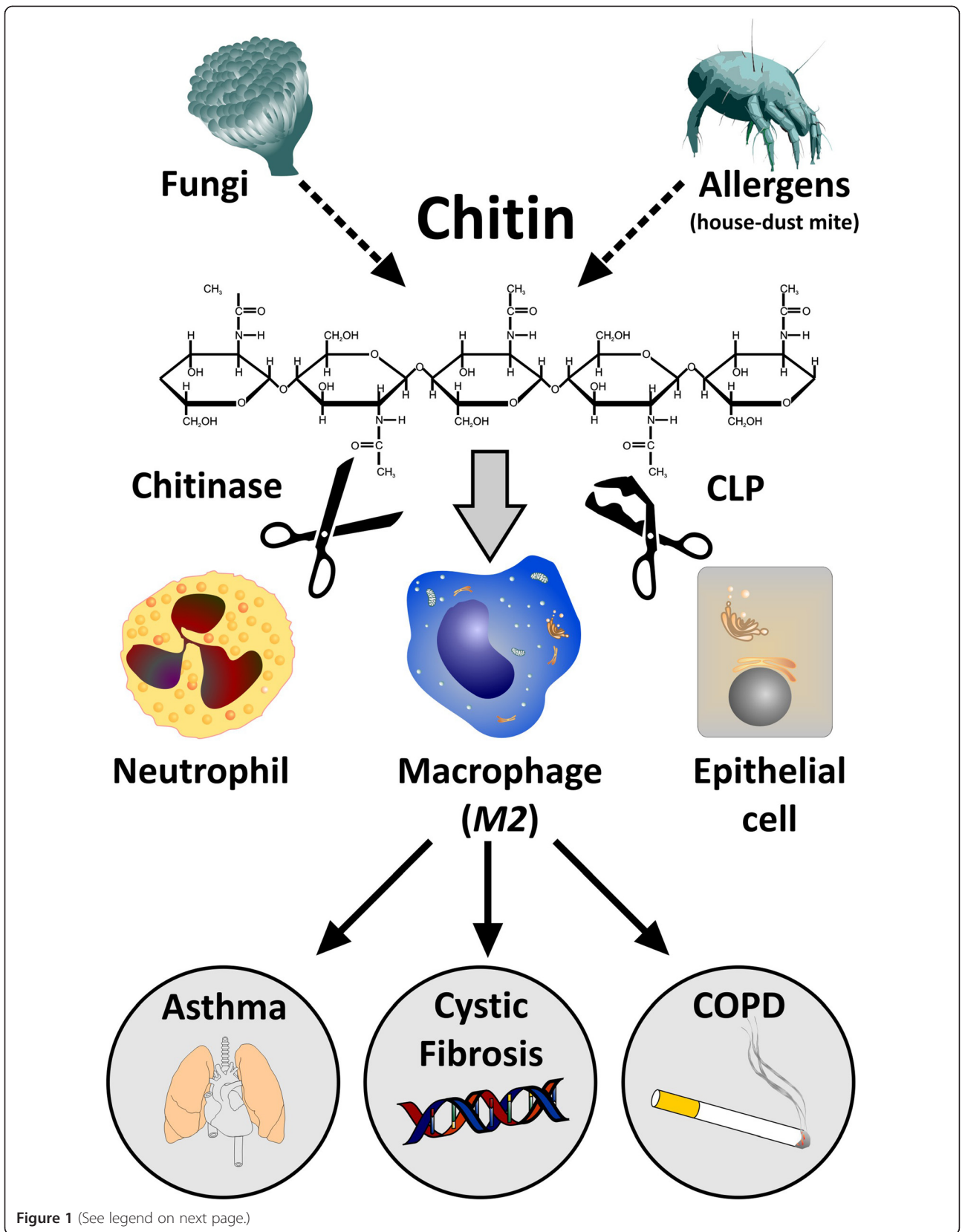Full list of author information is available at the end of the article

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Figure 1 (See legend on next page.)

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Figure 1 The proposed role of chitin, chitinases and chitinase-like proteins (CLPs) in lung diseases. Chitin is a common component of allergy-triggering environmental components, including fungal spores and house-dust mites, which trigger an innate immune response, including chitinases (cleaving chitin; scissors) and chitinase-like proteins (binding, but not cleaving chitin; damaged scissors). Chitinases and chitinase-like proteins are mainly secreted by neutrophils, alternatively activated macrophages (M2 macrophages) and epithelial cells. The interplay of M2 macrophages, neutrophils, and epithelial cells drives inflammation and remodeling in chronic lung diseases, particularly asthma, cystic fibrosis, and COPD.

demonstrated that chitin activates peritoneal/alveolar macrophages and natural killer (NK) cells to express a number of pro-inflammatory cytokines such as interleukin-1β (IL-1β), colony-stimulating factor (CSF), and gamma interferon (IFN-γ) [4]. More recent studies by Reese et al. addressed the *in vivo* immune effects of chitin in mice [5]. They noted that after several hours of chitin exposure, innate immune cells were recruited to the lung and/or the peritoneum. These studies also demonstrated that chitin induced alternative macrophage activation and that macrophage depletion with clodronate liposome treatment prevented the recruitment of eosinophils. Van Dyken et al. further demonstrated that fungal chitin from asthma-associated home environments induced allergic eosinophilic lung inflammation [6]. Collectively, these studies strongly suggest that chitin can contribute to the development of allergic type 2 (Th2) inflammation by activating innate immune cells. Beyond these findings, chitin has also been proposed to serve as an immunoadjuvant. In murine asthma models, Shibata et al. demonstrated that orally administered chitin suppressed the production of Th2 cytokines and IgE [7] and, when used as an adjuvant, chitin caused similar effects as a Th1-promoting adjuvant [8,9]. Accordingly, one can speculate that the immune system senses chitin and that chitin can skew the Th1/Th2 immune response in a bidirectional way.

The studies noted above suggest that the size of the chitin fragment is a crucial determinant of the effector responses that it elicits. This can be seen in comparisons of large chitin polymers that are biologically inert and intermediately sized fragments, which trigger IL-17, IL-23, and TNF-α production [1], while even smaller fragments enhance the production of the anti-inflammatory cytokine IL-10. Therefore, chitin has size-dependent effects on murine immune cell function and may bind to different receptors in a size-dependent manner, similar to findings in plants [10,11]. The observation that large chitin polymers are inert, smaller fragments are pro-inflammatory, and even smaller fragments exert an anti-inflammatory effect allows for an interesting hypothesis regarding the importance of the size-dependent effects of chitin in this response. However, in all of these studies, it is difficult to rule out that chitin preparations contained mixtures of differently long chitin polymers or that 'large' chitin preparations were containing contaminating 'smaller' chitin fragments. Clearly, a more thorough study using well-defined chitin fragments is warranted. Collectively, it is tempting to

speculate that chitin recognition by pattern recognition receptors triggers the induction of chitinases, leading to the generation of small-sized chitin particles that are taken up by the host cell. Recently, fungal chitin was found to dampen inflammation through IL-10 induction mediated by activation of the intracellular receptors NOD2 and TLR9 [12]. Despite these intriguing insights, the precise molecular recognition principles of chitin perception remain incompletely understood. Interestingly, this hypothesis is very similar to the established biology of another polysaccharide, hyaluronin that also serves as an alarm signal after degradation. Thus, the ability of appropriately sized polysaccharides to induce inflammation may be a more general principle of glycobiology [2,1]. When viewed in combination, chitin is a central component of potential pro-allergic microbes (e.g., *Aspergillus fumigatus* and house-dust mite) and has been shown to drive Th2-associated immune responses. Mechanisms that interfere with chitin metabolism are therefore of high relevance for allergic diseases and infections with chitin-bearing pathogens such as fungi.

Chitinases and chitinase-like proteins
Chitin-degrading enzymes, known as chitinases, are produced by humans and other mammals and are part of the 18-glycosyl-hydrolase family that encompasses both enzymatically active chitinases and chitinase-like proteins, the latter also termed chi-lectins, which lack enzymatic activity. In humans, acidic mammalian chitinase (AMCase), chitotriosidase, oviductin, and human cartilage glycoprotein (HcGP)-39/YKL-40 and YKL-39 have been described, while YM-1, YM-2, AMCase, oviductin, and breast regression protein (BRP-39) have been identified in mice [13,1,14-17]. Humans express two functional chitinases, chitotriosidase (*CHIT1*) and AMCase (*CHIA*) with an acidic pH optimum, both able to degrade chitin polymers. In mammals, chitinases [18,19] and chitinase-like proteins [20] are mainly expressed and secreted by phagocytes (mainly neutrophils and macrophages) and are induced at sites of inflammation, infection, and tissue remodeling, suggesting that these proteins play active roles in anti-infective defense and repair responses. Specifically, both chitinases and chitinase-like proteins have been linked to an alternative activation (M2) phenotype of macrophages [21,1,22], which is found in asthma and other chronic diseases, such as cystic fibrosis (CF), providing a rationale for chitinases and chitinase-like proteins to play a role in these disease conditions. While

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chitotriosidase is used as disease biomarker for Gaucher disease, a disease characterized by the accumulation of lipid-laden macrophages, acidic mammalian chitinase has been linked to allergic asthma and hypersensitivities [23]. However, the major evidence for an involvement in lung diseases exists for chitinase-like proteins. Therefore, we will focus on their role in pediatric lung diseases in the chapters below.

The chitinase-like protein YKL-40 and its involvement in lung diseases

YKL-40, or also called HcGP-39 or BRP-39 in mice, lacks measurable enzymatic chitinase activity, due to mutations in its highly conserved putative active sites [17]. In mice, BRP-39 has been associated with cell growth, breast cancer, and tissue remodeling. Recent evidence from BRP-39 knock-out mice indicates that BRP-39 plays a role in T-cell, macrophage and dendritic cell responses as well as cell apoptosis and tissue repair [21,1]. In human cells, HcGP-39/YKL-40 has been shown to regulate apoptosis/proliferation/cell survival, MAPK, and cytokine pathways [24,2].

The first indication that YKL-40 could be linked to human lung diseases came from a multi-center study quantifying YKL-40 serum levels in 253 adult patients with asthma [25]. This study showed that YKL-40 serum levels were mainly increased in adult patients with severe asthma and correlated with disease severity and airway remodeling. These studies in adults were, on the one hand, confirmed for children with severe, therapy-resistant asthma [26], but, on the other hand, challenged by another study [27]. Besides increased levels of YKL-40, elevated *bona fide* chitinase activities were also found in bronchoalveolar-lavage (BAL) fluids from children with asthma [28]. YKL-40 was also found to be increased in BAL fluid after segmental allergen challenge, indicating local production of this chitinase-like protein in response to allergens [29]. Gavala et al. confirmed this finding and further demonstrated that segmental allergen challenge also increased chitinase activities [30]. Clinically, YKL-40 serum levels remained increased in patients in spite of long-term inhaled corticosteroids, which could imply that YKL-40 production is resistant to current asthma treatments and might represent an alternative therapeutic target for severe asthma. YKL-40 is mainly released by activated neutrophils [20], and neutrophilic asthma is well known to be corticosteroid-resistant [25]. Thus, increased YKL-40 levels may be a hallmark of neutrophilic asthma. A follow-up study analyzing single nucleotide polymorphisms (SNPs) in the *YKL-40/CHI3L1* gene showed a genetic association with increased susceptibility to asthma, increased bronchial hyperresponsiveness, and reduced lung function [31]. The role of *YKL-40/CHI3L1* SNPs for asthma was further confirmed in a Taiwanese population

[32]. Another study found a different *YKL-40/CHI3L1* variant associated with asthma [33]. Given the enigmatic role of YKL-40, the functional importance of these variants awaits further investigation. Moreover, a Chinese study found that YKL-40 levels were increased in asthmatic patients and correlated with exacerbation, eosinophils, and immunoglobulin E [34], while a study from Poland found increased YKL-40 levels in asthma, but no correlation with disease severity or total IgE levels [35]. Besides a positive correlation between YKL-40 levels and age in subjects with asthma across all age groups, Santos et al. found no difference in circulating YKL-40 levels among asthma severities in children nor a correlation with IgE levels [27]. When viewed in combination, some but not all studies support the notion that YKL-40 is increased in severe and/or neutrophilic asthma, yet the clear relationship with age/disease progression (pediatric vs adult) and atopy remains to be defined in future studies.

Beyond asthma, other pulmonary or lung-associated disease conditions where YKL-40 levels were found to be increased were chronic obstructive pulmonary disease (COPD) [36,37], idiopathic pulmonary fibrosis [38], tuberculous pleural effusions and pneumonia [39], small-cell lung cancer [40], non-small-cell lung cancer [41], bronchiolitis obliterans syndrome [42], hyperoxic acute lung injury [43], sarcoidosis [44], allergic rhinitis [45], and CF lung disease [46]. Despite the heterogeneity of these disease conditions, alternative (M2) macrophage activation is a common feature of the majority of them, suggesting that increased YKL-40 levels reflect M2 macrophage polarization and disease conditions featuring M2 activation [24,22,2,47].

In CF, a chronic neutrophilic inflammatory disease [48,49], YKL-40 BALF levels were found to reflect airway inflammation and infection in early CF lung disease [50] and correlated inversely with lung function in adult CF patients, where YKL-40 levels were also found to be increased systemically [46]. The potential role of YKL-40 for the pathogenesis of CF lung disease is further supported by findings in *Scnn1b*-transgenic mice, a murine model of CF-like lung disease [51,52]. In this model, airway-specific overexpression of the β-subunit of the epithelial Na^+ channel ENaC mimics airway surface dehydration characteristic for CF airways and produces a CF-like lung disease with early onset of mucus obstruction, chronic airway inflammation, slowed bacterial clearance, and progressive structural lung damage [53]. Similar to patients with CF, levels of the murine homologue of YKL-40, BRP-39, were significantly increased in BALF and showed an inverse correlation with pulmonary function in *Scnn1b*-transgenic mice [46]. Collectively, these studies suggest that YKL-40/BRP-39 may be implicated in the pathogenesis of chronic airway inflammation and airflow obstruction and thus

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serve as a potential biomarker of disease severity in patients with CF.

Recent studies in this mouse model of CF-like lung disease also provided first mechanistic insights on how chitinases/chitinase-like proteins (CLPs), including BRP-39, may be upregulated in CF lung disease. These studies demonstrated that CF-like airway surface dehydration causing mucociliary dysfunction and mucus obstruction [54,55] provides a robust stimulus for macrophage activation, even when *Scnn1b*-transgenic mice are kept in a germ-free environment [54, 56]. More recently, a series of gene expression studies identified signatures of alternatively activated macrophages (M2), including *Ym1, Ym2,* and *BRP-39,* in whole lungs and isolated macrophages from *Scnn1b*-transgenic mice [57, 58]. These results suggest alternative macrophage activation in mucostatic airways as a mechanism underlying elevated expression of a range of chitinases and CLPs, even in the absence of chitin-containing parasites or allergens, in the airways of patients with CF and potentially other muco-obstructive lung diseases. While the pathogenic role of chitinases/CLPs in (dys)regulation of inflammation in CF airways remains poorly understood, observations in lungs from *Scnn1b*-transgenic mice demonstrate that some proteins of the chitinase/CLP family, such as *Ym1* and *Ym2,* are expressed at such high levels that they precipitate and form sharp crystals often greater than 100 μm in size [54]. These may cause chronic mechanical irritation and injury of airway epithelial cells and phagocytes thus contributing to chronic airway inflammation. Additionally, it is intriguing to speculate that such crystals may activate the NLRP3 inflammasome, a pattern recognition receptor able to trigger inflammation in response to other crystalline or aggregated endogenous substances such as cholesterol or uric acid crystals [59,60,61].

Beyond asthma and CF, circulating YKL-40 has been further associated with decline of lung function in the general population and has been proposed as a biomarker of susceptibility to the long-term effects of cigarette smoking [62]. Studying chitinases in New York City firefighters after World Trade Center exposure revealed that increased serum chitotriosidase reduced the odds of developing pulmonary obstruction after World Trade Center-particulate matter exposure and was associated with recovery of lung function. The underlying mechanisms remain unclear [63]. As chitin is a structural component of fungi, which are well known for their role in environmental asthma, a study investigated whether the exposure to environmental fungi modulates the effect of chitinases in individuals with asthma. The study demonstrated that environmental exposure to fungi modified the effect of *CHIT1* SNPs on severe asthma exacerbations [64].

In order to dissect the cellular sources of YKL-40 in human airways and the mechanisms regulating YKL-40 expression, Park and coworkers identified human airway epithelial cells as a source of YKL-40 and demonstrated that mechanical stress potently induces CHI3L1 expression leading to increased secretion of YKL-40 protein in an EGFR and MEK1/2-dependent pathway, suggesting that mechanical stress contributes to enhanced YKL-40 levels in asthmatic lungs [65]. A further mechanistic study found that YKL-40 increased the proliferation and migration of bronchial smooth muscle (BSM) cells through PAR-2-, AKT-, ERK-, and p38-dependent mechanisms and demonstrated that YKL-40 epithelial expression was positively correlated with BSM mass in asthma [66]. Other studies showed that YKL-40 induced IL-8/CXCL8 expression from bronchial epithelium via MAPK (JNK and ERK) and NF-κB pathways [67]. Taken together, these results suggest that YKL-40-mediated IL-8 production could be related to BSM remodeling [68]. Further studies showed that the allergen ovalbumin increased YKL-40 expression in tracheal epithelial cells [69] and demonstrated that YKL-40 increased mucin5AC production in human bronchial epithelial cells [70]. Collectively, these studies extend the view that YKL-40 is mainly a marker of neutrophilic inflammation by demonstrating modulatory effects of this chitinase-like protein on airway epithelial cells. Despite these intriguing insights into the biological effects of chitinase-like proteins, their precise functional role in biological processes and disease conditions in humans still remains largely unclear.

Conclusions

Chitin, chitinases, and chitinase-like proteins remain enigmatic terms for human diseases. However, after the second look into the pathophysiology of allergic and chronic lung diseases, these ancient, insect glycoprotein-associated pathways attract high relevance as potential biomarkers and therapeutic targets. Particularly, fungal chitin-linked asthma is increasing, but treatment options and successful clinical trials are scarce, necessitating further therapeutic developments. Before those approaches can be applied clinically, several key questions remain to be answered:

- How is chitin recognized by the human immune system? Is it a novel pattern recognition receptor ligand? And if so, can this interaction be targeted and exploited therapeutically?
- What is the primary role of enzymatically active chitinase found in the human body? To defend against chitin-bearing pathogens or to skew the immune system?
- Is a dysregulation of chitin sensing or YKL-40 induction pathways associated with altered

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susceptibility for diseases like fungal asthma or house-dust mite allergy?
- Is YKL-40 a potential biomarker of severe asthma, a marker of neutrophilic innate immune activation, or a marker reflecting tissue remodeling (or all of these)?
- Does YKL-40 play a causative role in these disorders? Can YKL-40 be neutralized pharmacologically? And if so, which diseases would benefit?
- Considering YKL-40 as a therapeutic target in human diseases, what is the physiologic function of this protein?

These issues remain to be solved and pave the way for an exciting novel field in pediatric immunology, bridging a gap between insects, fungi, immune cells, and the lung.

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References
1. 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
2. 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
3. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, Delhaes L, Sergejeva S (2014) Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy 4:14, doi:10.1186/2045-7022-4-14
4. 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
5. 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
6. Van Dyken SJ, Garcia D, Porter P, Huang X, Quinlan PJ, Blanc PD, Corry DB, Locksley RM (2011) Fungal chitin from asthma-associated home environments induces eosinophilic lung infiltration. J Immunol 187(5):2261–2267, doi:10.4049/jimmunol.1100972
7. 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
8. Strong P, Clark H, Reid K (2002) 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 32(12):1794–1800
9. Ozdemir C, Yazi D, Aydogan M, Akkoc T, Bahceciler NN, Strong P, Barlan IB (2006) Treatment with chitin microparticles is protective against lung histopathology in a murine asthma model. Clin Exp Allergy 36(7):960–968, doi:10.1111/j.1365-2222.2006.02515.x
10. Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, Kim SY, Stacey MG, Stacey G (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell 20(2):471–481, doi:10.1105/tpc.107.056754
11. Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc Natl Acad Sci U S A 104(49):19613–19618, doi:10.1073/pnas.0705147104
12. 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
13. Zheng T, Rabach M, Chen NY, Rabach L, Hu X, Elias JA, Zhu Z (2005) Molecular cloning and functional characterization of mouse chitotriosidase. Gene 357(1):37–46
14. Herrera-Estrella A, Chet I (1999) Chitinases in biological control. EXS 87:171–184
15. Boot RG, Renkema GH, Strijland A, van Zonneveld AJ, Aerts JM (1995) Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem 270(44):26252–26256
16. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JM (2001) Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem 276(9):6770–6778
17. Bleau G, Massicotte F, Merlen Y, Boisvert C (1999) Mammalian chitinase-like proteins. EXS 87:211–221
18. van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, Sugar A, Verhoeven AJ, Boot RG, Aerts JM (2005) Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol 17(11):1505–1512, doi:10.1093/intimm/dxh328
19. van Eijk M, Scheij SS, van Roomen CP, Speijer D, Boot RG, Aerts JM (2007) TLR- and NOD2-dependent regulation of human phagocyte-specific chitotriosidase. Febs Lett 581(28):5389–5395, doi:10.1016/j.febslet.2007.10.039
20. Volck B, Price PA, Johansen JS, Sorensen O, Benfield TL, Nielsen HJ, Calafat J, Borregaard N (1998) YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Physicians 110(4):351–360
21. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, Humbles A, Kearley J, Coyle A, Chupp G, Reed J, Flavell RA, Elias JA (2009) Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. J Exp Med 206(5):1149–1166, doi:jem.20081271
22. Hartl D, Lee CG, Da Silva CA, Chupp GL, Elias JA (2009) Novel biomarkers in asthma: chemokines and chitinase-like proteins. Curr Opin Allergy Clin Immunol 9(1):60–66, doi:10.1097/ACI.0b013e32831f8ee0
23. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA (2004) Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 304(5677):1678–1682
24. 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
25. 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 (2007) A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med 357(20):2016–2027
26. Konradsen JR, James A, Nordlund B, Reinius LE, Soderhall C, Melen E, Wheelock AM, Lodrup Carlsen KC, Lidegran M, Verhoek M, Boot RG, Dahlen B, Dahlen SE, Hedlin G (2013) The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. J Allergy Clin Immunol 132(2):328–335, e325. doi:10.1016/j.jaci.2013.03.003
27. Santos CB, Davidson J, Covar RA, Spahn JD (2014) The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children. Ann Allergy Asthma Immunol 113(3):263–266, doi:10.1016/j.anai.2014.05.024

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28. Goldman DL, Li X, Tsirilakis K, Andrade C, Casadevall A, Vicencio AG (2012) Increased chitinase expression and fungal-specific antibodies in the bronchoalveolar lavage fluid of asthmatic children. Clin Exp Allergy 42(4):523–530, doi:10.1111/j.1365-2222.2011.03886.x

29. Kuepper M, Bratke K, Virchow JC (2008) Chitinase-like protein and asthma. N Engl J Med 358(10):1073–1075, author reply 1075. doi:10.1056/NEJMc073406

30. Gavala ML, Kelly EA, Esnault S, Kukreja S, Evans MD, Bertics PJ, Chupp GL, Jarjour NN (2013) Segmental allergen challenge enhances chitinase activity and levels of CCL18 in mild atopic asthma. Clin Exp Allergy 43(2):187–197, doi:10.1111/cea.12032

31. 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 Jr, Nicolae DL, Elias JA, Chupp GL (2008) Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 358(16):1682–1691

32. Tsai Y, Ko Y, Huang M, Lin M, Wu C, Wang C, Chen Y, Li J, Tseng Y, Wang T (2014) CHI3L1 polymorphisms associate with asthma in a Taiwanese population. BMC Med Genet 15:86, doi:10.1186/1471-2350-15-86

33. Rathcke CN, Holmkvist J, Husmoen LL, Hansen T, Pedersen O, Vestergaard H, Linneberg A (2009) Association of polymorphisms of the CHI3L1 gene with asthma and atopy: a populations-based study of 6514 Danish adults. Plos One 4(7):e6106, doi:10.1371/journal.pone.0006106

34. Tang H, Fang Z, Sun Y, Li B, Shi Z, Chen J, Zhang T, Xiu Q (2010) YKL-40 in asthmatic patients, and its correlations with exacerbation, eosinophils and immunoglobulin E. Eur Respir J 35(4):757–760, doi:10.1183/09031936.00034409

35. Specjalski K, Jassem E (2011) YKL-40 protein is a marker of asthma. J Asthma 48(8):767–772, doi:10.3109/02770903.2011.611955

36. Matsuura H, Hartl D, Kang MJ, Dela Cruz CS, Koller B, Chupp GL, Homer RJ, Zhou Y, Cho WK, Elias JA, Lee CG (2011) Role of breast regression protein-39 in the pathogenesis of cigarette smoke-induced inflammation and emphysema. Am J Resp Cell Mol 44(6):777–786, doi:10.1165/rcmb.2010-0081OC

37. Letuve S, Kozhich A, Arouche N, Grandsaigne M, Reed J, Dombret MC, Kiener PA, Aubier M, Coyle AJ, Pretolani M (2008) YKL-40 is elevated in patients with chronic obstructive pulmonary disease and activates alveolar macrophages. J Immunol 181(7):5167–5173, doi:181/7/5167

38. Furuhashi K, Suda T, Nakamura Y, Inui N, Hashimoto D, Miwa S, Hayakawa H, Kusagaya H, Nakano Y, Nakamura H, Chida K (2010) Increased expression of YKL-40, a chitinase-like protein, in serum and lung of patients with idiopathic pulmonary fibrosis. Respir Med 104(8):1204–1210, doi:10.1016/j.rmed.2010.02.026

39. Kim HR, Jun CD, Lee KS, Cho JH, Jeong ET, Yang SH, Lee YJ, Park DS (2012) Levels of YKL-40 in pleural effusions and blood from patients with pulmonary or pleural disease. Cytokine 58(3):336–343, doi:10.1016/j.cyto.2012.03.001

40. Xu CH, Yu LK, Hao KK (2014) Serum YKL-40 level is associated with the chemotherapy response and prognosis of patients with small cell lung cancer. Plos One 9(5):e96384, doi:10.1371/journal.pone.0096384

41. Thom I, Andritzky B, Schuch G, Burkholder I, Edler L, Johansen JS, Bokemeyer C, Schumacher U, Laack E (2010) Elevated pretreatment serum concentration of YKL-40-An independent prognostic biomarker for poor survival in patients with metastatic nonsmall cell lung cancer. Cancer 116(17):4114–4121, doi:10.1002/cncr.25196

42. Jaksch P, Taghavi S, Klepetko W, Salama M (2014) Pretransplant serum human chitinase-like glycoprotein YKL-40 concentrations independently predict bronchiolitis obliterans development in lung transplant recipients.
J Thorac Cardiovasc Surg 148(1):273–281, doi:10.1016/j.jtcvs.2014.02.059

43. Sohn MH, Kang MJ, Matsuura H, Bhandari V, Chen NY, Lee CG, Elias JA (2010) The chitinase-like proteins breast regression protein-39 and YKL-40 regulate hyperoxia-induced acute lung injury. Am J Respir Crit Care Med 182(7):918–928, doi:10.1164/rccm.200912-1793OC

44. Kruit A, Grutters JC, Ruven HJ, van Moorsel CC, van den Bosch JM (2007) A CHI3L1 gene polymorphism is associated with serum levels of YKL-40, a novel sarcoidosis marker. Respir Med 101(7):1563–1571, doi:10.1016/j.rmed.2006.12.006

45. Park SJ, Jun YJ, Kim TH, Jung JY, Hwang GH, Jung KJ, Lee SH, Lee HM, Lee SH (2013) Increased expression of YKL-40 in mild and moderate/severe persistent allergic rhinitis and its possible contribution to remodeling of nasal mucosa. Am J Rhinol Allergy 27(5):372–380, doi:10.2500/ajra.2013.27.3941

46. Hector A, Kormann MSD, Mack I, Latzin P, Casaulta C, Kieninger E, Zhou Z, Yildirim AO, Bohla A, Rieber N, Kappler M, Koller B, Eber E, Eickmeier O, Zielen S, Eickelberg O, Griese M, Mall MA, Hartl D (2011) The Chitinase-Like Protein YKL-40 Modulates Cystic Fibrosis Lung Disease. Plos One 6 (9). doi:ARTN e24399 DOI 10.1371/journal.pone.0024399

47. Elias JA, Homer RJ, Hamid Q, Lee CG (2005) Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. J Allergy Clin Immunol 116(3):497–500, doi:10.1016/j.jaci.2005.06.028

48. Mall MA, Hartl D (2014) CFTR: cystic fibrosis and beyond. Eur Respir J 44(4):1042–1054, doi:10.1183/09031936.00228013

49. Hartl D, Gaggar A, Bruscia E, Hector A, Marcos V, Jung A, Greene C, McElvaney G, Mall M, Doring G (2012) Innate immunity in cystic fibrosis lung disease. J Cyst Fibros 11(5):363–382, doi:10.1016/j.jcf.2012.07.003

50. Fantino E, Gangell CL, Hartl D, Sly PD, Arest CF (2014) Airway, but not serum or urinary, levels of YKL-40 reflect inflammation in early cystic fibrosis lung disease. BMC Pulm Med 14:28, doi:10.1186/1471-2466-14-28

51. Zhou Z, Duerr J, Johannesson B, Schubert SC, Treis D, Harm M, Graeber SY, Dalpke A, Schultz C, Mall MA (2011) The ENaC-overexpressing mouse as a model of cystic fibrosis lung disease. J Cyst Fibros 10(Suppl 2):S172–S182, doi:10.1016/S1569-1993(11)60021-0

52. Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC (2004) Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med 10(5):487–493, doi:10.1038/nm1028nm1028

53. Mall MA, Graeber SY, Stahl M, Zhou-Suckow Z (2014) Early cystic fibrosis lung disease: role of airway surface dehydration and lessons from preventive rehydration therapies in mice. Int J Biochem Cell Biol 52:174–179, doi:10.1016/j.biocel.2014.02.006

54. Mall MA, Harkema JR, Trojanek JB, Treis D, Livraghi A, Schubert S, Zhou Z, Kreda SM, Tilley SL, Hudson EJ, O'Neal WK, Boucher RC (2008) Development of chronic bronchitis and emphysema in beta-epithelial Na+ channel-overexpressing mice. Am J Respir Crit Care Med 177(7):730–742, doi:200708-1233OC

55. Mall MA (2008) Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J Aerosol Med Pulm D 21(1):13–24, doi:10.1089/jamp.2007.0659

56. Livraghi-Butrico A, Kelly EJ, Klem ER, Dang H, Wolfgang MC, Boucher RC, Randell SH, O'Neal WK (2012) Mucus clearance, MyD88-dependent and MyD88-independent immunity modulate lung susceptibility to spontaneous bacterial infection and inflammation. Mucosal Immunol 5(4):397–408, doi:10.1038/mi.2012.17

57. Trojanek JB, Cobos-Correa A, Diemer S, Kormann M, Schubert SC, Zhou-Suckow Z, Agrawal R, Duerr J, Wagner CJ, Schatterny J, Hirtz S, Sommerburg O, Hartl D, Schultz C, Mall MA (2014) Airway mucus obstruction triggers macrophage activation and MMP12-dependent emphysema. Am J Respir Cell Mol Biol. doi:10.1165/rcmb.2013-0407OC

58. Saini Y, Dang H, Livraghi-Butrico A, Kelly EJ, Jones LC WKON, Boucher RC (2014) Gene expression in whole lung and pulmonary macrophages reflects the dynamic pathology associated with airway surface dehydration. BMC Genomics 15(1):726, doi:10.1186/1471-2164-15-726

59. Samstad EO, Niyonzima N, Nymo S, Aune MH, Ryan L, Bakke SS, Lappegard KT, Brekke OL, Lambris JD, Damas JK, Latz E, Mollnes TE, Espevik T (2014) Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. J Immunol 192(6):2837–2845, doi:10.4049/jimmunol.1302484

60. De Nardo D, De Nardo CM, Latz E (2014) New insights into mechanisms controlling the NLRP3 inflammasome and its role in lung disease. Am J Pathol 184(1):42–54, doi:10.1016/j.ajpath.2013.09.007

61. Gross O, Thomas CJ, Guarda G, Tschopp J (2011) The inflammasome: an integrated view. Immunol Rev 243(1):136–151, doi:10.1111/j.1600-065X.2011.01046.x

62. Guerra S, Halonen M, Sherrill DL, Venker C, Spangenberg A, Carsin AE, Tares L, Lavi I, Barreiro E, Martinez-Moratalla J, Urrutia I, Sunyer J, Anto JM, Martinez FD (2013) The relation of circulating YKL-40 to levels and decline of lung function in adult life. Respir Med 107(12):1923–1930, doi:10.1016/j.rmed.2013.07.013

63. Cho SJ, Nolan A, Echevarria GC, Kwon S, Naveed B, Schenck E, Tsukiji J, Prezant DJ, Rom WN, Weiden MD (2013) Chitotriosidase is a biomarker for the resistance to World Trade Center lung injury in New York City firefighters. J Clin Immunol 33(6):1134–1142, doi:10.1007/s10875-013-9913-2

64. Wu AC, Lasky-Su J, Rogers CA, Klanderman BJ, Litonjua AA (2010) Fungal exposure modulates the effect of polymorphisms of chitinases on emergency department visits and hospitalizations. Am J Respir Crit Care Med 182(7):884–889, doi:10.1164/rccm.201003-0322OC

65. Park JA, Drazen JM, Tschumperlin DJ (2010) The chitinase-like protein YKL-40 is secreted by airway epithelial cells at base line and in response to compressive mechanical stress. J Biol Chem 285(39):29817–29825, doi:10.1074/jbc.M110.103416

66. Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, Thumerel M, Ousova O, Kolbeck R, Coyle AJ, Woods J, Tunon de Lara JM, Marthan R,

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Berger P (2012) Role of YKL-40 in bronchial smooth muscle remodeling in asthma. Am J Respir Crit Care Med 185(7):715–722, doi:10.1164/rccm.201105-0915OC

67. Tang H, Sun Y, Shi Z, Huang H, Fang Z, Chen J, Xiu Q, Li B (2013) YKL-40 induces IL-8 expression from bronchial epithelium via MAPK (JNK and ERK) and NF-kappaB pathways, causing bronchial smooth muscle proliferation and migration. J Immunol 190(1):438–446, doi:10.4049/jimmunol.1201827

68. Tang H, Shi Z, Xiu Q, Li B, Sun Y (2012) YKL-40-mediated interleukin 8 production may be closely associated with remodeling of bronchial smooth muscle cells. Am J Respir Crit Care Med 186(4):386, author reply 386–387. doi:10.1164/ajrccm.186.4.386a

69. Ben SQ, Qiu YL, Zhou J, Zhou XY, Zhang S, Wu Y, Ju SQ, Ni SS (2014) Ovalbumin enhances YKL-40, IL-5, GM-CSF, and eotaxin expression simultaneously in primarily cultured mouse tracheal epithelial cells. In Vitro Cell Dev Biol Anim 50(3):243–250, doi:10.1007/s11626-013-9698-x

70. Liu C, Li Q, Zhou X, Kolosov VP, Perelman JM (2013) The chitinase-like protein YKL-40 increases mucin5AC production in human bronchial epithelial cells. Exp Cell Res 319(18):2866–2873, doi:10.1016/j.yexcr.2013.08.009