Chitinases and Chitinase-like proteins in asthma

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ABSTRACT

Despite the lack of endogenous chitin synthesis, mammalian genomes encode two enzymatically active true chitinases (chitotriosidase and acidic mammalian chitinase) and a variable number of chitinase-like proteins (CLPs) that have no enzyme activity but bind chitin. Chitinases and CLPs are prominent components of type-2 immune response-mediated respiratory diseases. However, despite extensive research into their role in allergic airway disease, there is still no agreement on whether they are mere biomarkers of disease or actual disease drivers. Functions ascribed to chitinases and CLPs include, but are not limited to host defense against chitin-containing pathogens, directly promoting inflammation, and modulating tissue remodeling and fibrosis. Here, we discuss in detail the chitin-dependent and -independent roles of chitinases and CLPs in the context of allergic airway disease, and recent advances and emerging concepts in the field that might identify opportunities for new therapies.

Abbreviations:

AM	alveolar macrophage
AAM	alternatively activated macrophage
AMCase	acidic mammalian chitinase
BAL	bronchoalveolar lavage
CHIT1	chitotriosidase
CLP	chitinase-like protein
COPD	Chronic obstructive pulmonary disease
DAMP	damage-associated molecular pattern
DC	dendritic cell
GH18	family 18 of glycosyl hydrolases
GlNAc	N-acetyl-glucosamine
HA	hyaluronan
HDM	house dust mite
IgE	immunoglobulin E
OVA	ovalbumin
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
WT	wild-type

Introduction

Despite the lack of endogenous chitin synthesis, mammalian genomes encode two enzymatically active true chitinases, chitotriosidase (Chit1; encoded by *CHIT1* in humans/*Chit1* in mice) and acidic mammalian chitinase (AMCase; encoded by *CHIA i*n humans/*Chia1* in mice) that bind and degrade chitin, in addition to a variable number of chitinase-like proteins (CLPs) that have no enzyme activity but bind chitin (Table 1) (1). Chitinases belong to the evolutionary conserved family 18 of glycosyl hydrolases (GH18), a gene family found in all six kingdoms, that catalyse the hydrolysis of chitin to simple sugars (2). They are produced for different purposes ranging from morphogenesis and nutrition to immune defence against chitin-containing pathogens (2-4).

Besides their potential host defense function in mammals (5), chitinases and CLPs have not only been associated with type-2 immune response-mediated respiratory diseases, but proposed as potential biomarkers and drivers of allergic asthma (4, 6). Allergic asthma is a Th2-mediated, chronic inflammatory disease of the lung that is triggered by the exposure to allergens and associated with aberrant expansion of Th2 lymphocytes, airway eosinophilia, elevated serum immunoglobulin E (IgE), and excessive airway mucus production. Non-allergic asthma also exists, some forms also associated with eosinophilia driven by innate type-2 lymphocytes, and others with more neutrophilic inflammation or little immune cell influx (7). While asthma prevalence has increased to epidemic proportions over the last few decades, the development of targeted therapies to treat this debilitating disease has not kept pace, and the economic burden of disease morbidity and control continues to escalate. Recent years have seen renewed interest in chitinases and CLPs as critical regulators of type-2 immunity not only in response to chitin-containing allergenic sources (i.e., house dust mites (HDM)) but also in chitin-independent processes driving type-2 immunity (3, 8). This is supported by the discovery of polymorphisms and major variations in the expression of the chitinase enzymes and CLPs in the human population which have been correlated with enhanced/decreased activity, reduced lung function and increased susceptibility to bronchial asthma (4, 9).

Here, we discuss the chitin-dependent and -independent roles of chitinases and CLPs in the context of allergic airway disease and recent advances and emerging concepts in the field that might identify opportunities for new therapies.

Chitin

Chitin is an insoluble, linear polymer of N-acetyl-glucosamine units, which are linked by ß(1,4)-bonds. It is structurally similar to cellulose, except that one hydroxyl group of each subunit is replaced by an N-acetyl group. The resulting increased hydrogen bonding between separate polymers makes it one of the strongest and most resilient natural materials on earth. In contrast to α-chain carbohydrates such as the readily digestible glycogen and starch, these ß-linked polymers are highly resistant to degradation. Chitin is the second most abundant polysaccharide on earth after cellulose, and is produced by fungi, nematodes, and arthropods (such as crustaceans, insects and house dust mites). Due to its physical properties, it provides excellent structural rigidity to these invertebrates, just like cellulose provides structural rigidity to plants.

Presumably, chitin is the evolutionary precursor of hyaluronan (HA), the major component of the extracellular matrix of many vertebrates. HA is composed of repeating disaccharides containing N-acetyl-glucosamine and glucuronic acid, which are connected exclusively by ß-bonds, like chitin and cellulose. Despite having similar structures, chitin and HA considerably differ in physical properties. While chitin excludes water, HA attracts large volumes of water, allowing cells to travel through the extracellular matrix. In humans, chitin synthase genes have been lost, leading to the assumption that mammals do not produce chitin. However, this view has recently been challenged by the observation that short chitin oligomers, remnants of chitin synthesis, are produced during the initiation of HA synthesis in vertebrates (10). In addition, chitin-like polysaccharides in Alzheimer's disease brains have now been documented (11). *In vitro*, HA synthases make small chitin oligomers that potentially serve as endogenous primers for the initiation of HA synthesis (10). These short chitin oligomers might act as a hydrophobic needle that penetrates the cell plasma membrane, dragging the hydrated HA chain into the extracellular space to allow its unrestrained growth.

Chitin is considered non-self and as described above, it has a structure analogous to HA, which can be recognized as a damage-associated molecular pattern (DAMP) by TLR4 (12), suggesting that chitin might also be recognized by the innate immune system as a pathogen-associated molecular pattern (PAMP). Indeed, chitin has been shown to

activate the mammalian immune system. However, as there is no consensus yet on which pattern recognition receptors (PRR) sense chitin, chitin is considered an “orphan” PAMP. Several PRRs have been implicated in chitin signalling in immune cells (Figure 1), such as TLR2 (13-15), TLR9 and NOD2 (16), mannose receptor (14, 17), and dectin-1 (14, 18). However, direct receptor-chitin interactions have only been firmly demonstrated for TLR2 (15). That study also showed that six subunit long chitin chains were identified as the smallest, immunologically active motif. Chitin oligomers of 5 or less subunits are immunologically inactive, and even inhibit the binding of larger chitin particles to TLR2 in a dose dependent manner; potentially this short conformation fails to induce cross-linking of receptors.

The fact that small chitin oligomers are immunologically silent could be evolutionary beneficial, allowing the distinction between self and non-self, as endogenous chitin oligomers are produced during HA synthesis. In addition to TLR2, other direct chitin-receptor interactions have also been described. The transmembrane receptor FIBCD1 binds chitin (19), but is mainly expressed in the intestinal epithelium, limiting its relevance for chitin sensing in the lungs. In contrast, LYSMD3 is expressed on airway epithelial cells and also directly binds chitin (20). Chitin binding to LYSMD3 induced receptor signalling with consequent cytokine secretion, which could be reversed by knockdown or knockout of LYSMD3. However, further studies are required to determine its role *in vivo*. Finally, chitin has also been shown to directly activate the alternative complement pathway (21).

The immune responses induced by chitin are rather complex and depend on the chitin particle size (Figure 1). Initial studies focused on chitin sensing and uptake by macrophages (22). For macrophages to engulf chitin, chitin polymers need to have the appropriate size to be phagocytosed and to result in a proper cytokine response (14, 23). Phagocytosis-dependent immune responses only occur with small chitin, and have been suggested to be dependent on the mannose receptor (14, 16). In the absence of the mannose receptor, chitin can still be phagocytosed, however this phagocytosis does not induce a cytokine response. There is some controversy about the phagocytosis-induced cytokine response. While Da Silva et al. report that phagocytosis induces TNFα secretion (14), Wagener et al. observed inhibition of TNFα secretion and induction of IL-10 secretion

(16). Next to phagocytosis, chitin recognition by PRR might activate macrophages to secrete type-1 cytokines with consequent IFN-γ production by NK cells (13, 14, 17, 22). TLR2 and consequent NF-kB pathway activation, and dectin-1 and Syk activation have been implicated in chitin sensing by macrophages (13, 14).

These early reports mostly analyzed type-1-biased immune responses initiated by macrophages. Importantly, the majority of these studies were done *in vitro*, and might not reflect the *in vivo* situation. Indeed, chitin elicits much more complex immune responses *in vivo*. In 2007, Reese et al. reported for the first time that chitin administered to mouse lungs induced the accumulation of IL-4-expressing innate immune cells in tissues, including basophils and eosinophils (24). They found that chitin induced alternatively activated macrophages (AAMs) that produce leukotriene B4, leading to eosinophil recruitment. Macrophages fail to acquire an AAM phenotype upon *in vitro* stimulation with chitin and instead secrete TNFα (25), suggesting an additional factor is required for *in vivo* AAM polarization. Roy et al. found airway epithelium-derived CCL2 to be needed for driving AAM activation (25). Indeed, more recent studies have shifted focus from macrophages as chitin sensors to the airway epithelium. These studies show that inhalation of chitin induces different patterns of epithelial cytokine secretion leading to a mixed eosinophilic-neutrophilic infiltrate in the airways (26, 27). Van Dyken et al. demonstrated that chitin triggers IL-33, IL-25, and TSLP secretion by the epithelium, which non-redundantly activate IL-5/IL-13-secreting ILC2 and eosinophil recruitment (26). At the same time, chitin also induced the expression of IL-1β, TNFα, and IL-23, which lead to IL-17A production by γδ T cells, and consequent neutrophilia. Of note, chitin induced epithelial responses might be influenced by macrophages. Kim et al. demonstrated that chitin phagocytosis by macrophages resulted in the activation of caspase-7, which inactivates epithelial-derived IL-33 thereby resolving type-2 immune responses (23).

It is striking that most chitin-containing organisms are known to trigger eosinophil-rich type-2 immune responses in mammalian hosts, such as the anti-parasite response against gastro-intestinal nematodes and the allergic response against allergenic sources, such as HDM, cockroaches, and fungi. Moreover, asthma is a common occupational hazard among people working with chitinous substances, such as shellfish processors

(28, 29). These observations have led to the hypothesis that chitin might be implicated in the pathogenesis of allergic diseases. The induction of chitin-binding antibodies has been described in mice (30). Immunization of mice with certain bacteria, such as *Streptococcus pyogenes*, induced anti-N-acetyl-glucosamine (GlcNAc) antibodies which were shown to bind chitin. However, these antibodies were rather protective, instead of contributing to allergic airway disease, as chitin-induced airway inflammation was greatly reduced by administration of anti-GlcNAc antibodies, suggesting that chitin stimulates airway inflammation. The current view is that the development of asthma and allergic inflammation involves innate immune responses that promote allergic sensitization, and chitin might act as an adjuvant in these responses. In animal models of airway sensitization, chitin was proven to act as an adjuvant for adaptive allergic responses elicited by ovalbumin (OVA) and by *Aspergillus* antigens (21, 31, 32). Chitin induced allergen-specific Th2 cells and allergen-specific antibodies. Complement activation and IL-33-induced DC activation have been implicated in the adjuvant effect of chitin (21, 31). Yet, the exact molecular pathways remain to be elucidated. As B and T lymphocytes might express TLR2 and other PRRs, it is plausible that they might be directly activated by chitin. Except for one report showing chitin to be a polyclonal B cell activator (33), the fine molecular details remain to be elucidated.

In contrast to the studies described above, chitin has also been reported to downregulate allergic features in mouse models. Intranasal application of chitin before and during allergen challenges reduced inflammation in the lungs and systemic IgE levels in some models of intraperitoneal sensitization (34, 35). Two other studies administered chitin orally and found that this reduced allergic features in a model of peanut-induced anaphylaxis and in a model of intraperitoneal sensitization to ragweed allergen followed by intratracheal challenges (36, 37). These findings are seemingly at odds with studies demonstrating the adjuvant effect of chitin.

In conclusion, chitin can elicit a broad range of innate immune responses, leading to the initiation of/or influencing adaptive immune responses. Yet the underlying molecular pathways are not fully understood. Conflicting observations may result from different

routes and timing of sensitization and chitin administration, different chitin preparations (degree of acetylation, size and endotoxin content), and experimental design.

Chitinases

Structurally, members of the chitinase family consist of a catalytic domain, a hinge region and a chitin-binding cleft (38). These enzymes catalyse the cleavage of chitin ß-bonds, consequently resulting in the depolymerization and degradation of chitin (38). Chitin bearing organisms express various chitinases for the remodelling of their complex chitin structures (38). While chitin synthase genes have been lost during evolution, chitinase genes were highly conserved across species with multiple duplication events (39). In mammals, two active endochitinases are expressed: Chit1 and AMCase. These proteins resulted from an ancient gene duplication event (39). While AMCase is very acid-stable and has a pH optimum of 2, Chit1 is most active at a more neutral pH (40).

Considerable differences exist between mice and men in the expression of active chitinases across tissues. It is well established that AMCase mediates chitinase activity in the mouse lung and that its expression is increased by an IL-13/STAT-6-dependent pathway, resulting in increased chitinolytic activity during allergic inflammation (41, 42). Chit1 levels are very low to absent in the mouse lung. In contrast, in humans, chitinolytic activity in the airways is exclusively attributed to CHIT1 (43). Although AMCase is expressed in the human lung, it is an inactive splice variant which has no chitinolytic activity (43). Active AMCase is expressed in the stomach of humans (43). Because of expression in the barrier tissues of the airways and gastro-intestinal tract of mammals, it is plausible that chitinases confer protection against chitin-bearing organisms. However, chitinases seem to have more complex functions, and might either contribute to or inhibit inflammation depending on the context. Extensive research has been conducted on the potential value of chitinases as biomarkers and therapeutic targets of airway inflammation and remodelling, as discussed below.

Chitotriosidase

CHIT1 was the first active chitinase to be described in humans (44). It is produced, stored, and secreted mainly by phagocytes, such as macrophages and neutrophils (5). Two CHIT1 isoforms exist: a secreted 50 kDa protein and a 39 kDa protein which is found in the lysosomes (5). In the healthy human lung, chitotriosidase is expressed by alveolar macrophages (45), while Chit1 mRNA is often undetectable in the mouse lung at steady state (45-48).

Originally, Chit1 was believed to confer protection from chitin-bearing pathogens, such as fungi. *In vitro* exposure of chitin-containing fungi to recombinant Chit1 results in growth inhibition (5). The *in vitro* activity of recombinant Chit1 was also observed in *in vivo* models of systemic Candidiasis and systemic Aspergillosis (5). In contrast to these observations, *Chit1* knockout mice had prolonged survival due to decreased Th2 induction (49, 50). A common polymorphism in *CHIT1* exists in which a 24-bp duplication results in aberrant splicing, leading to an in-frame deletion making the expressed CHIT1 inactive (51). Homozygous individuals have no CHIT1 chitinolytic activity, while heterozygous individuals have reduced activity. This defect is frequently encountered in different ethnic groups, suggesting that CHIT1 chitinolytic activity is not (any longer) indispensable for the defence against chitin-containing pathogens. The divergent frequencies of the 24-bp duplication allele in different populations led to speculations that active CHIT1 might still have benefits in host defence (52). However, no strong or consistent support for this hypothesis exists. While some studies associated this 24-bp duplication polymorphism with increased susceptibility to *Plasmodium falciparum* and filariasis (53, 54), others could not confirm this (55). It might be hypothesized that active CHIT1 is no longer required in evolution because of the duplication event that resulted in a second active chitinase, *AMCase*. However, their different expression profiles (45) and the inactive splice variant of AMCase expressed in the human airway (43) do not support this hypothesis.

To date, CHIT1 is thought to have more functions than only host defence. Increased levels of CHIT1 are observed in a variety of granulomatous and fibrotic lung diseases associated with tissue remodelling, such as tuberculosis, sarcoidosis, idiopathic pulmonary fibrosis, scleroderma-associated interstitial lung diseases, and chronic

obstructive pulmonary disease (COPD) (56). *CHIT1* is expressed by a specific subset of recruited macrophages that also express other profibrotic genes, and co-express *CHIL3L1* (YKL-40) (57). This was also observed in yet unpublished single cell RNA-sequencing data from COVID-19 patients included in the SARPAC trial (58). The elevation of CHIT1 in these patients reflects chronically activated tissue macrophages and has been suggested to also contribute to tissue inflammation and remodelling (2). One study using mice genetically deficient in *Chit1* reported that Chit1 was instrumental in bleomycin- and IL-13-induced pulmonary fibrosis by augmenting TGF-ß and MAPK signalling in mice (59). However, the role of Chit1 in inflammatory and remodelling responses remains to be elucidated, as only few functional studies are available.

To date, no strong evidence supports CHIT1 being a major player in asthma pathogenesis. Two independent studies showed that CHIT1 concentrations in the bronchoalveolar lavage (BAL) fluid of asthmatics did not differ from those observed in healthy controls (43, 60). Rather, a smoking habit and COPD were observed to result in increased chitotriosidase activity in the BAL fluid. Similarly, another study found serum CHIT1 levels were only very modestly increased in asthmatics compared to healthy controls, while much more pronounced increases were observed in COPD patients (61). Conversely, CHIT1 levels were observed to be locally increased in the lungs after segmental allergen challenge during bronchoscopy in patients with atopic asthma (62). However, this study did not include healthy controls, meaning the transient increase in CHIT1 expression might have been due to phagocyte activation, possibly also observed in healthy controls. Most genetic association studies failed to find an association between *CHIT1* polymorphisms and asthma/atopy (52, 63-65). Only one study reported that a single polymorphism in *CHIT1* was associated with atopy in Korean children (66). While not all reports found *Chit1* expression in the lungs of mice, those that did detect *Chit1* expression found it was not altered upon induction of allergic airway inflammation (42, 46), making Chit1 unlikely to play an exacerbating role in allergy and asthma in mice. In contrast, Hong and colleagues reported CHIT1 concentrations were higher in the sputum of asthmatic children compared to age-matched controls, and that the Chit1 concentration was increased in the lungs from OVA sensitized and challenged mice (67). Using *Chit1* knockout mice, they claimed that Chit1 inhibits allergic airway disease through induction

of Foxp3^{+} regulatory T cells via regulation of TGF-ß signalling (ref.). These findings need confirmation, as no other reports on the functional role of chitotriosidase in asthma are available to date.

AMCase

AMCase is expressed in the stomach and to a lesser extent in the lungs (45). While being inactive in the human lung, AMCase is the major chitinase contributing to airway chitinolytic activity in mice (67). Airway epithelial cells are the main source of AMCase in mouse airways, while AMCase was found to be mainly produced by macrophages in human lung (43). Also in mice, AMCase was reported to be expressed by alveolar macrophages (23, 42), but this appears to be under the control of specific stimuli as not all studies could confirm this (48). Kim et al. found both epithelial cells and macrophages to contribute to biologically significant amounts of AMCase (23), while Van Dyken et al. found the epithelium to be the main producer of AMCase (48).

Its high expression in the gastro-intestinal tract, together with its acid stability and low pH optimum, suggest that AMCase might be implicated in the processing of chitin in the gut. However, it seems that humans and rodents do not use chitin as a dietary source. Instead, chitinase expression in the gut is likely to confer protection against chitin-bearing parasites. Vannella et al. showed that mice genetically lacking AMCase had decreased clearance of gastro-intestinal parasites due to profound defects in type-2 immunity (46). The authors hypothesized that the chitinolytic activity of AMCase in the stomach aids in disrupting, releasing, and processing parasite-associated chitinous antigens that are critical for the initiation of protective immune responses in the gut.

In analogy with its function in the gastro-intestinal tract, it is plausible that AMCase also protects the airways from chitin-containing organisms and chitin-induced inflammation. Yet, its function in the airways seems to be much more complex than in the gut, possibly depending on both its chitinolytic activity and chitinolytic-independent effects. As many sources of aeroallergens, such as fungi, HDM and cockroaches, contain chitin, AMCase might also influence the development of atopic asthma. Zhu et al. were the first

to report AMCase is increased in the lungs of asthmatics and claimed a crucial role for this chitinase in asthma based on their mouse studies (42). Since then, extensive research on AMCase and allergy pathogenesis was conducted.

In accord with increased AMCase levels in the lungs of asthmatics (42), AMCase expression is also increased during other manifestations of allergic inflammation, such as allergic rhinitis, allergic rhinoconjunctivitis, and nasal polyposis (68-70), as well as in the lungs of allergen-challenged mice (23, 42, 47). Increased airway chitinolytic activity upon allergen challenges in mice is dependent on IL-13 and STAT6 signalling (41, 42). Some polymorphisms in the *CHIA* gene are associated with pediatric asthma (71), while polymorphisms associated with increased chitinase activity *in vitro* were protective against asthma (72). However, other genetic association studies failed to find associations between *CHIA* polymorphisms and asthma (65).

Several mouse models have been used to investigate the functional roles of AMCase in allergies, yielding highly conflicting results. Zhu et al. observed that the inhibition of AMCase ameliorated pulmonary inflammation and airway hyperreactivity in a chitin-independent model of OVA-induced allergic airway disease and IL-13 overexpressing mice (42). While not affecting IL-13 levels, AMCase inhibition diminished the expression of chemokines normally induced by IL-13, suggesting that AMCase contributes to the downstream chemokine responses to IL-13. Later studies using different chitinase inhibitors and AMCase-silencing RNA also observed that blocking AMCase ameliorates features of allergic airway disease in the OVA-induced asthma model (73-75). In addition, in a model of HDM-driven allergic asthma, targeting AMCase with a specific inhibitor also reduced lung inflammation and serum IgE concentrations (76). One could question whether the systemic administration of chitinase inhibitors is ideal to study airway chitinase biology, as this strategy might have broader systemic effects. Moreover, chitinase inhibitors have targets other than AMCase. They might block the other active chitinase Chit1, and have the potential to bind a range of CLPs, possibly interfering with their function as well. As the above-described studies were mainly performed with non-chitinous allergens and adjuvants, AMCase is suggested to exert its effects through a mechanism that is independent of its chitinolytic activity, including the protection of airway

epithelial cells from apoptosis (77). Yet, it should be noted that two separately generated AMCase transgenic mice exhibit normal lung function and do not develop lung disease in the steady state, meaning that high concentrations of AMCase in the airways are not sufficient to trigger or potentiate allergic inflammation. However, this does not rule out the possibility that AMCase contributes to inflammation once the appropriate stimuli are present.

In contrast, in the presence of chitin, AMCase does have a protective role in airway inflammation through its capability to degrade chitin (23, 24, 48, 78, 79). This was first proposed by Reese et al., who observed that chitin-induced airway eosinophilia was ameliorated in mice overexpressing AMCase (24). Conversely, AMCase-deficient mice exhibit premature morbidity and mortality, concomitant with accumulation of environmentally derived chitin polymers in the airways and increased pulmonary inflammation (48). The inflammatory infiltrate observed in these aged AMCase-deficient mice resembled the profile of lung cellular infiltrates induced after acute inhalation of purified chitin in wild-type (WT) mice (26). The inability to degrade chitin resulted in the development of pulmonary fibrosis, which could be reversed by restoring chitinase activity in the airways. AMCase was also protective against allergic airway inflammation driven by chitinous aeroallergens. Constitutive overexpression of AMCase protected mice from airway eosinophilia in response to fungal challenges (78, 79). Conversely, treatment with the chitinase inhibitor allosamidin prolonged the duration of tissue eosinophilia (79). In addition, mice expressing enzymatically inactive AMCase showed enhanced type-2 immune responses upon intratracheal sensitisation and challenges with HDM extract (23). The authors of this study showed that the inability to cleave chitin prevented macrophages from phagocytosing chitin; uncleaved chitin remains outside the cells, continuing to cause tissue damage and IL-33 release. These inactive AMCase expressing mice showed no difference from WT mice in airway inflammation in the chitin-independent OVA-alum model, confirming that the observed effects of AMCase are mediated through its chitinolytic activity, and suggesting that AMCase does not influence allergic airway disease via a chitinolytic-independent mechanism, in contrast to the earlier reports. In contrast with the above-described studies, mice congenitally lacking AMCase demonstrated little to no role for the enzyme in establishing type-2 immunity in models of

HDM- or OVA-induced allergy in the lung, despite using several asthma models, with both chitinous and non-chitinous allergens (46, 47). This might possibly be due to chitin contents being too low in the HDM extracts used.

The seemingly contrasting observations in mouse models on the role of AMCase might have several reasons. First, AMCase might have distinct functions depending on the context. It clearly is protective against chitin-induced airway inflammation, while in the absence of chitin, AMCase only exerts functions independent of its chitinolytic activity. However, it remains to be elucidated if these functions exist and what they are. Of note, it might be questioned how relevant models using purified chitin or filtered, crushed allergen extracts, are for studying the function of AMCase in airway inflammation. Usually, chitin is not inhaled as a pure polymer, but rather complexed with other sugars and protein antigens. When intact particulate antigen is inhaled, chitinases might act to create additional free chitin ends in particles, promoting chitin recognition by TLR2 (15). However, additional chitinase activity might generate small chitin fragments, which were shown to inhibit TLR2 signalling (15). Moreover, it can be hypothesized that chitinases might promote the release of allergens associated with chitin, promoting the induction of more profound adaptive type-2 immune response.

A second reason for the controversy on AMCase is the use of different strategies to block its function. Thirdly, the hypothesis has been raised that when AMCase is absent, Chit1, the other active chitinase, might compensate for its loss and maintain airway chitinolytic activity. This hypothesis was introduced with the observation that AMCase-deficient mice still had low levels of chitinolytic activity in the serum, which might be attributed to Chit1 (47). This idea has been challenged by some studies that could not find Chit1 expression in the lungs of either WT or AMCase-deficient mice (47, 48). However, Vannella et al., who did not observe different asthmatic features in WT and AMCase deficient mice, did observe expression of Chit1 in the lungs (46). The controversial observation of Chit1 expression in mouse lungs might be due to different activation status of lung macrophages, possibly influenced by housing conditions and microbiome.

The potential significance of targeting AMCase in human asthma remains uncertain, since AMCase was shown to minimally contribute to chitinolytic activity in the

418 human lung (43). Inactive AMCase retains its ability to bind chitin, and as such, might still
419 influence inflammation and tissue remodeling in analogy with CLPs, as described below.
420 Hence, future research should try to unravel the possible functions of AMCase that are
421 independent of its chitinolytic activity.

422

423 **Chitinase-like proteins**

424 Mammalian CLPs have a chitin binding domain, yet lack enzymatic activity: YKL-
425 39 (*CHI3L2*) and YKL-40 (C*HI3L1*) in humans and Ym1 (*Chil3*), Ym2 (*Chil4*) and BRP-39
426 (*Chil1*/*Chi3l1*) in the mouse (Table 1). In CLPs, the substitution of an essential glutamic
427 acid to leucine, isoleucine or glutamine within the highly conserved enzyme site accounts
428 for the lack of chitinolytic activity. In both species, CLPs have been implicated in an
429 enormous variety of pathologies, suggesting broad generalized functions (8). Among
430 human and murine CLPs, YKL-40 retained the property of binding chitin with high affinity
431 (6), while Ym1 does not bind chitin but chitin-like saccharides such as glucosamine
432 oligosaccharides, heparin and heparan sulfate (80). The fact that some CLPs retain chitin-
433 binding ability suggests they contribute to the recognition and immune signaling of chitin-
434 associated PAMPs, or might control the substrate specificity of real chitinolytic enzymes
435 by competitive or synergistic binding. However, CLPs have been evolving at a remarkably
436 rapid rate, potentially associated with the acquisition of novel, chitin-independent
437 regulatory functions. It is therefore no surprise that the role of CLPs in host immunity,
438 especially their contribution to type-2 immunity, has received great attention in recent
439 years. CLPs are expressed in immune and structural cells in steady state, while type 2-
440 polarized immune responses associated with repair and regeneration boost their
441 expression. Dysregulated CLP expression is often associated with inflammatory
442 conditions including allergic asthma and related obstructive lung diseases, as will be
443 discussed in detail below. In addition, at least in mice, decreased lung CLP transcripts are
444 a genetic determinant of lung function associated with lower basal pulmonary capacity
445 (81).

446

447 **YKL-40/BRP-39**

In humans, several CLPs have been identified. Among these, YKL-40 is the most prominently expressed during lung inflammation and tissue remodelling (11), with increased expression associated with many pathologies including allergic asthma (8). In fact, YKL-40 is increased in the serum and lungs of patients with asthma, correlated with disease severity and asthma exacerbation rate and inversely correlated with lung function (82-84). In these settings, YKL-40 can be produced by a multitude of cells of both hematopoietic and non-hematopoietic origin as reviewed in (85). YKL-40 was shown to contribute to the differentiation of monocytes to activated macrophages in inflamed tissues (86), facilitating the late stages of human macrophage maturation (87). Enhanced expression of YKL-40 protein is also found in bronchiolar epithelial cells and alveolar macrophages adjacent to fibrotic lesions (88) and YKL-40 levels in bronchial smooth muscle cells show a clear correlation with the thickness of bronchial epithelial basement membrane in asthmatic patients (89). Experimental studies then showed enhanced proliferation and migration of bronchial smooth muscle cells in response to YKL-40 (90). This might be due to the binding of YKL-40 to type I collagen and subsequent formation of collagen fibrils and stimulation of pro-fibrotic and inflammatory mediators (91, 92).

Thus, YKL-40 seems to be closely related to the pathogenesis of asthma, especially airway remodeling. It is hence not surprising that variation in *CHI3L1,* the gene that encodes the YKL-40 protein, is closely linked to asthma susceptibility and reduced lung function, and that YKL-40 is a significant biomarker for asthma severity (4, 93). A correlation between *CHI3L1* SNPs and asthma is evident as *CHI3L1* polymorphisms have been associated with increased serum YKL-40 levels, reduced pulmonary function, bronchial hyperresponsiveness and airway remodeling (93-97). One of the most recent meta-analyses investigating a total of 13 articles showed that the serum concentration of YKL-40 in asthmatic patients was significantly higher than in healthy subjects and YKL-40 tracked with asthma severity (98). However, the correlation between YKL-40 and Th2-related inflammation in asthma is still controversial (98) as asthma is a highly heterogeneous disease presenting with different endotypes and inflammatory signatures that may be broadly viewed as type-2-high or type-2-low (7, 99). Initial studies suggested that YKL-40 may enhance allergen sensitization and IL-4/IL-13 Th2 cytokine responses (100). More recent work rather points to a connection of elevated YKL-40 levels in non-

eosinophilic and paucigranulocytic asthma as compared to type-2 high, eosinophilic asthma. This is likely through elevated YKL-40 expression by various cell types including neutrophils and macrophages (101). These findings are in line with other reports of significant positive relationships between YKL-40 levels, blood neutrophil numbers, asthma severity and reduced lung function (102-104), strengthening the association of serum YKL-40 with non-eosinophilic, type-2-low asthma.

However, while this seems to be true for adult-onset asthma, data on elevated YKL-40 levels and asthma severity in childhood asthma are conflicting. While Konradsen et al. found that compared with healthy controls, serum YKL-40 levels were higher in children with severe, therapy-resistant asthma and were associated with the *CHI3L1* promoter single nucleotide polymorphism (105), another study found no association of elevated YKL-40 levels with asthma severity, lung function or type-2 inflammation in asthmatic children (106).

In mice, BRP-39 is often referred to as the 'prototypical' CLP, because it is the genetic orthologue of YKL-40 in humans (8). Elias et al. generated and characterized BRP-39-deficient mice (*Chil3l1*$^{-/-}$), YKL-40 transgenic mice, and *Chil3l1*$^{-/-}$ mice that simultaneously produced transgenic human YKL-40 only in their respiratory epithelium to overcome any contributing effects of the mouse ortholog BRP-39 (100). BRP-39 was significantly increased after OVA-induced allergic airway inflammation, predominantly in type 2 alveolar cells and alveolar macrophages. Others found BRP-39 levels not significantly altered following allergic inflammation, claiming that Ym1/2 proteins mimic the biological effects of YKL-40 more closely (8, 107, 108).

Loss of BRP-39 resulted in substantial reductions in all the hallmarks of allergic asthma, along with increased apoptosis of CD4 T cells and eosinophils. In addition, BRP-39 potently stimulated AAM polarization and the production of the Th2 chemokines CCL17 and CCL22 from alveolar macrophages. Overexpression of YKL-40 in BRP-39-deficient mice rescued type-2 responses to levels comparable to those seen in WT animals (100, 109). Mechanistically, BRP-39 was driven by IL-13 and as such seems to be a critical downstream target of IL-13 effector responses (109). Following up on this, the authors could demonstrate that both YKL-40 and BRP-39 bind to the interleukin 13 receptor alpha

2 (IL-13Rα2), in concert with IL-13, although this finding still needs validation by other groups (109). Since then, numerous YKL-40/BRP-39 binding partners have been identified including Prostaglandin D2 receptor, receptor for advanced glycation end product and type I collagen (8).

Similarly diminished type-2 inflammation in *Chil3l1*$^{-/-}$ mice could be observed after HDM exposure (110) and in a model of fungal-associated allergic airway inflammation (111). However, despite significantly lower type-2 responses to fungal exposure, mice showed significantly increased airway hyperresponsiveness, indicating that BRP-39 protects against airway hyperresponsiveness during fungal asthma despite contributing to type-2 inflammation (111). The authors also found higher serum YKL-40 concentration in fungal-sensitized asthmatics (111), which is, as already discussed, often associated with more severe disease, reduced lung function and features of type-2 low asthma. Along these lines, BRP-39 has been shown to support IL-17A production by γδ T cells and both total numbers of γδ T cells and IL-17A-producing γδ T cells were significantly lower in *Chil3l1*$^{-/-}$ mice than in wild-type mice (108). However, levels of IFN-γ and IL-17A were unaffected by the absence of BRP-39 during fungal asthma as were the levels of neutrophils in the lung (111).

In summary, the studies demonstrate that correlation of asthma endotypes and *CHI3L1* SNPs is warranted when looking at the contribution of YKL-40 to allergic asthma (98). Whether YKL-40 participates in the pathogenesis of asthma and/or is a biomarker of asthma severity remains an open question, given the divergent evidence at present (83).

Ym1/Ym2

Ym1 and Ym2 proteins, encoded by the genes *Chil3* and *Chil4*, respectively, on mouse chromosome 3, are rodent-specific CLPs with no true human orthologs (4). They are highly homologous, sharing up to 91% amino acid sequence identity, indicating a relatively recent gene duplication event (8). Nevertheless, Ym1 and Ym2 show obvious tissue expression specificity (112, 113) and have fairly distinct cellular sources (107).

Under steady state conditions, Ym1 is the most prominently expressed CLP in the mouse lung, while Ym2 is barely detectable. Allergens (107, REF, 108, 114), particulate matter (115), helminthic parasites (108), and cancer (116) significantly increase expression of Ym2, which then becomes the predominant lung CLP. While Ym2 is largely expressed by airway epithelial cells (107) under these conditions, Ym1 is mainly restricted to the myeloid compartment (117-120). This is a very recent finding because due to the lack of specific tools, Ym1 and Ym2 could not be clearly distinguished and the literature is confounded by observations that can be attributed to both Ym1 and Ym2.

A distinct feature of Ym proteins is that they are commonly found to form crystals during 'type-2-high' responses including allergic airway inflammation (114, 121). Recent findings suggest that a hyperactivated IL-33/ILC2 axis might drive Ym1/Ym2 crystallopathies in mice (122), like Charcot-Leyden crystallopathy in humans during hypereosinophilic inflammation (123). In this context, Ym proteins are best known as IL-4/IL-13-inducible (114, 117, 124) hallmark genes of AAMs, which is mediated by STAT6 (118) and further facilitated by PPARγ signaling (125). The dependence on IL-4/IL-13 might also partly explain the occurrence of Ym crystals in viable motheaten mice that harbor a mutant Src homology protein tyrosine phosphatase that, amongst others, negatively regulates IL-4Rα signaling, leading to uncontrolled Ym protein expression in these mice (121). However, Ym1/ Ym2 protein expression is far more than just a mere marker of type-2-high responses although their detailed functions during such so far remain poorly understood.

While Ym1 was initially described as a chemotactic factor for eosinophils (known as eosinophil chemotactic factor or ECF-L) (126), this chemotactic activity could later not be confirmed in *in vitro* and *in vivo* settings (80, 114). There is, however, consensus that Ym protein expression is highly increased in the mouse lung and BAL fluid during experimental models of allergic asthma (112, 114, 124). In response to IL-13, dendritic cells (DC) upregulate Ym1/2 expression to enable Th2 cytokine production by $CD4^+$ T cells; Ym1/2 supplementation of IL-13-deficient DCs restored the ability to stimulate IL-5/IL-13 secretion in DC/CD4 T cell cocultures by inhibiting the production of 12-hydroxyeicosatetraenoic acid by 12/15(S)-lipoxygenase (124). In turn, blocking Ym1/2 signaling during the induction of allergic airways disease with an anti-Ym1/2 antibody or

targeted delivery of siRNA attenuated IL-5 and IL-13 production in the mediastinal lymph nodes (124) and protected from allergen-induced allergic airway inflammation (127, 128), underscoring the relevance of Ym1/Ym2 in the enhancement of type 2 responses. However, there was no experimental distinction between Ym1 and Ym2 proteins and it was not clear whether both or one of the two was triggering type-2 cytokines.

In addition, a growing body of literature has accumulated around the role of Ym1 in IL-17-mediated neutrophil recruitment in allergic lung inflammation (108, 129). Plasmid-directed overexpression of Ym1 protein increased neutrophilia in the lungs, while eosinophilia was significantly decreased, dependent on IL-17 production from γδ T cells. By blocking Ym proteins, the number and proportion of neutrophils in the lung were decreased, along with reduced expression of IL-17A and IL-17A target genes in OVA-challenged mice (108). In mice infected with *N. brasiliensis*, blocking Ym1 in the early innate immune stage could reduce the amount of Th2 cytokines in mice; while during the adaptive type-2 response, blocking Ym1 significantly enhanced type-2 cytokine production from both ILCs and $CD4^+$ T; indicating that Ym1 is important for limiting the magnitude of type-2 responses. More importantly, Ym1 could directly contribute to lung repair via enhanced RELMα production (129).

Recent publications underscore the role of Ym1 in tissue repair while also reinforcing its function in type-2 immune responses and AAM polarization. Mice harboring a natural polymorphism in the *Chil3* promoter, which results in partial Ym1-deficiency, show reduced OVA-induced allergic inflammation, and demonstrate an enhanced AAM phenotype, indicating that Ym1 may control or limit the alternative activation of macrophages (112). Inhibition of $Ym1^+$ AAMs by cynaropicrin, a galectin-3 pathway inhibitor, dampened eosinophilic lung inflammation in a model of HDM-induced allergic airway inflammation, while simultaneously inducing neutrophil influx. This resulted in worsened airway hyperresponsiveness to methacholine but significantly lower collagen deposition (130). In addition, tissue injury leads to the recruitment of $Ym1^+Ly6C^{hi}$ monocytes from the bone marrow, which are associated with the resolution of inflammation and tissue healing, and exhibit an immunoregulatory phenotype (120). One of the postulated functions of Ym1 is binding to components of the extracellular matrix, facilitating matrix deposition and tissue repair (80, 131). Differences in deposition of

specific collagen subtypes have been noticed between mouse strains (107), and Ym1 expression differs considerably between distinct inbred and wild-derived mouse strains due to natural polymorphism in their respective promotor regions (112). Hence, Ym1 polymorphisms might be correlated with differential susceptibility to type-2 inflammation-related tissue remodeling (112).

In summary, Ym proteins are generally recognized as fundamental players in allergic airway disease in mice, contributing to type-2 responses, AAM polarization and tissue repair. The effects ascribed to Ym proteins are, however, multifaceted, and even contradictory. One of many reasons is the lack of specific tools to clearly distinguish Ym1 and Ym2 proteins in the lung; as such, the literature is confounded by observations that could be attributed to either Ym1 or Ym2. However, they clearly differ in their spatial (myeloid vs. epithelial cells) and temporal (constitutive vs induced) expression patterns, and hence can be assumed to perform distinct roles in allergic airway inflammation. In addition, bi-phasic upregulation of Ym1 has been observed, which might play diametrically opposite roles in the early vs. late phase of inflammation and explain type-2 polarization vs. Th17 responses but also the different cellular sources of Ym1. The field urgently awaits genetic tools to delete either *Chil3* or *Chil4*, but creation of such tools is hampered by the fact that the chromosome 3 locus has a gene duplication.

Evolutionary biology of the chitinase and CLP family

It is intriguing that the genes coding for chitin synthase have been lost in mammals, while several genes encoding chitinases have been preserved. Not only have chitinase genes persisted, but multiple gene duplication events and loss-of-function mutations have led to emergence and diversification of CLPs with species- and tissue-specific expression patterns (9, 11, 132, 133). Especially CLPs have evolved at a speed that points towards positive selection rather than natural genetic drift (8, 9, 11, 132, 133), indicating distinct evolutionary benefits. One possible explanation is offered by the ‘innovation, amplification, and divergence’ model of gene evolution (133, 134). This model infers that active chitinases were no longer needed, which enabled (or facilitated) mutations in critical regions such as the catalytic active site in duplicated genes, giving rise to new, non-enzymatic proteins. However, these “dead enzymes” were still selectively maintained for

631 their beneficial and novel regulatory functions (133, 134). Specific sites within CLP
632 proteins are under positive selective pressure that drives CLP functional diversity across
633 species - yet, the sugar-binding barrel structure is highly conserved (8). As many authors
634 have remarked, it is tempting to look at CLPs as examples of recent adaptations and gene
635 evolution 'happening right now' (11, 133). In addition, despite the shared chromosomal
636 location and high homology, individual genes have evolved independently and can have
637 distinct functions within and across species (2, 8). This also raises concerns as to whether
638 chitinase and CLP research using mouse models of allergic asthma is relevant to human
639 pathology.

640 So the question remains: Why are chitinases and CLPs evolutionary hot spots?
641 One possibility is that they might still be taking part in the defence against chitin-containing
642 pathogens. However, in higher organisms, their chitin-independent and regulatory
643 functions seem to be dominant. This is best illustrated by the human *CHIT1* gene, for
644 which the 24-bp duplication polymorphism leaves around 5% of the Caucasian population
645 without an active enzyme, with no obvious effects for the host's defense system (51, 52).
646 Nevertheless, the polymorphism is strongly associated with an increased rate of lung
647 function decline in COPD, pointing to a protective role in pulmonary tissue homeostasis
648 (133). Similarly, AMCase shows regulatory and Th2-promoting functions that are
649 completely independent of its enzymatic activity (77). In case of CLPs, regulatory functions
650 have been discussed in the context of type-2 related tissue inflammation and remodeling
651 through binding to extracellular matrix components. While Ym1 has specific binding
652 capacity for GlcNAc oligomers and heparin/heparan sulfate proteoglycans, YKL-40
653 interacts with type I collagen. Whether CLPs could perform their regulatory functions also
654 through protein–protein interactions, i.e, by binding to specific surface receptors on
655 immune cells or by altering the substrate specificity of the true chitinases, remains to be
656 established. The mining of multi-omics data may help modelling interactomes to identify
657 new interaction partners and biological processes associated with the non-chitin binding,
658 regulatory function of chitinases and CLPs.

659

660 **Conclusions and future perspective**

Despite extensive research into the role of chitinases and CLPs in allergic airway disease, there is still no agreement on whether they are mere biomarkers of disease or actual disease drivers. However, their ubiquitous presence and evolutionary conservation suggest they have functions that are far more complex and diverse than previously realized.

Functions ascribed to chitinases and CLPs include but are not limited to: (1) a role in host response against chitin-containing pathogens, by either degrading or binding to chitin, which triggers an innate immune response; (2) directly promoting chemotaxis or production of pro-inflammatory cytokines; (3) modulating tissue remodeling and fibrosis, possibly through interactions with components of the host extracellular matrix. These functions are likely beneficial to combat chitin-bearing parasites, a process in which these molecules are required for expulsion and wound healing. However, in pulmonary diseases, these same properties may cause pathology (117).

In the future, it will be crucial to identify yet unknown receptors and/or ligands of chitinases and CLPs to better understand their type-2 immunity-triggering properties. One limitation of mouse models in human CLP research is assigning orthology and/or functional homology to human genes and proteins. Although BRP-39 is the predicted genetic ortholog of YKL-40, Ym1/Ym2 proteins, which are often disregarded as being restricted to the mouse system, are found among the most upregulated genes after allergen exposure, similar to YKL-40 in humans. As such, considering CLPs in their entirety and understanding their functional redundancies could help to understand their potential and value for human CLP research in allergic airway diseases. Lastly, as there have been quite opposing findings between different chitinase and CLP studies reported in the literature, a number of considerations with major potential impact on research outcomes should be taken into account: (1) differences in allergen models and routes of exposure; (2) genetic background of the mouse strains as well as genetic strategies to create knockout strains; (3) differences in the housing and husbandry conditions (i.e., diet, commensals).

In conclusion, chitin is a size-dependent, pathogen-associated molecular pattern that is recognized by the human immune system through TLR2 and possibly other

receptors. Acute chitin challenges in the lungs result in the activation of complex pathways inducing a mixed neutrophilic-eosinophilic infiltrate, which can be reduced by active chitinases. However, chitinases might have functions beyond their chitinolytic activity. This is suggested by the evolution driving CLP duplication and studies that find roles for active chitinases in the absence of chitin.

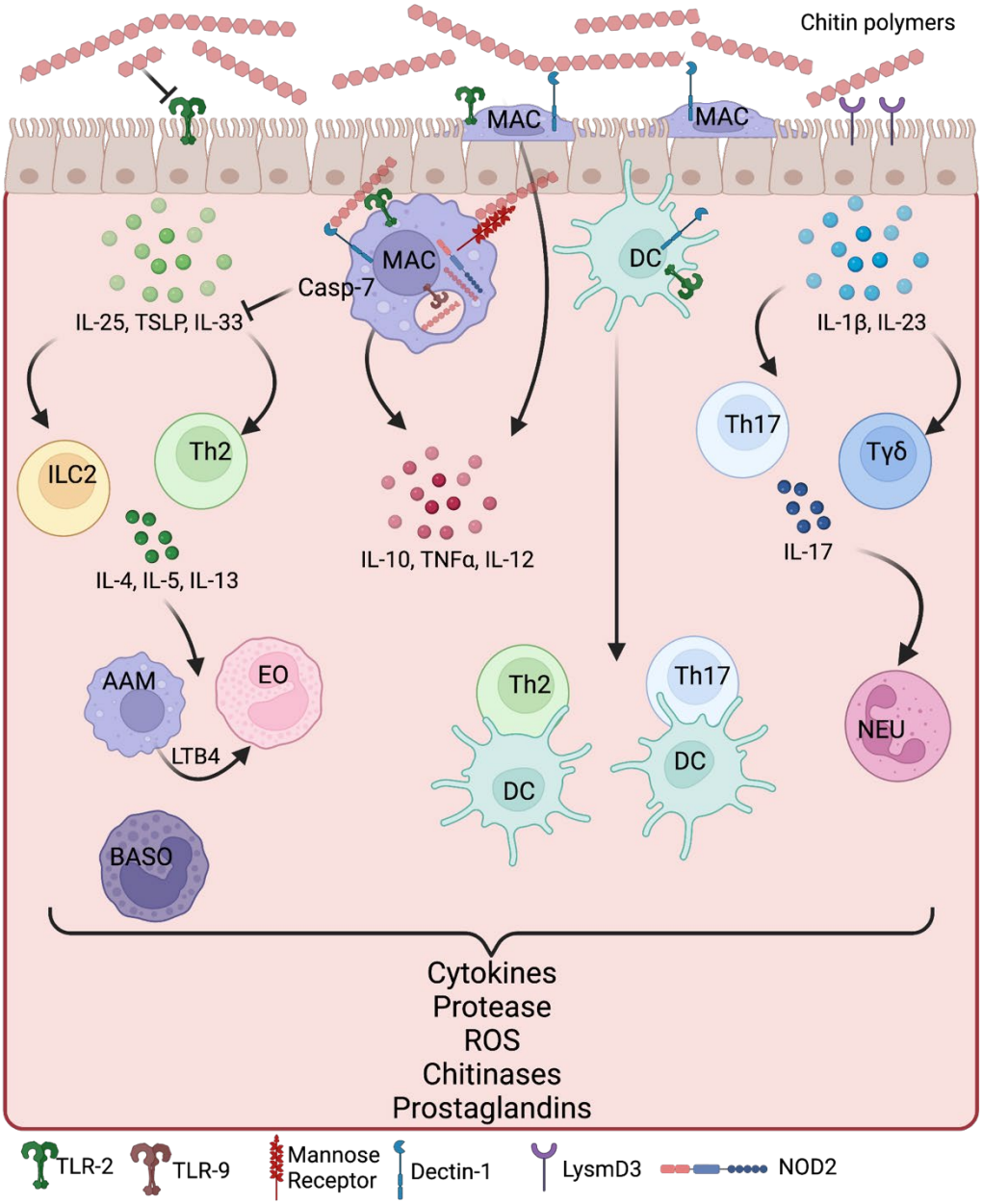
Table and Figures

True chitinases					
Gene	**Protein**	**Human**	**Mouse**	**Tissue/cellular expression**	**Reference**
CHIA	AMCase	Chr1	Chr3	Macropahges, lung epithelial cells	**(42, 45, 48)**
CHIT1	CHIT1	Chr1	Chr1	macrophages, neutrophils, microglia	**(5, 43, 45)**
Chitinase-like proteins (CLPs) = chitolectins = chilectins					
Gene	**Protein**	**Human**	**Mouse**		
CHIL1/Chil1	YKL40/BRP39 (CHI3L1, HC-gp39, GP39, cartilageglycoprotein1)	Chr1	Chr1	chondrocytes, sinovial cells, macrophages, fibroblasts, neutrophils, epithelial cells, osteoclasts, astrocytes	**(85)**
CHIL2	YKL39 (CHI3L2,chondrocyteprotein 39)	Chr1		cartilage chondrocytes	**(135, 136)**
Chil3	Ym1 (Chi3l3, ECF-L)		Chr3	macrophages, dendritic ells, monocytes, neutrophils	**(107, 112, 113, 117-120)**
Chil4	Ym2 (CHI3L4)		Chr3	bronchial epithelial cells, gut	**(107, 112, 113)**
Chil5/Chil6/Gm6522	Ym3/Ym4		Chr3	pseudogene	**(39)**

OVGP1	OVGP1 (oviductin,mucin 9)	Chr1	Chr3	oviductal epithelial cells	**(137, 138)**
SI-CLP	SI-CLP (CHID1)	Chr11	Chr7	monocytes, macrophages, neutrophils	**(139-141)**

700 Table 1: Summary of chitinases and CLPs genes found in humans and mice

701

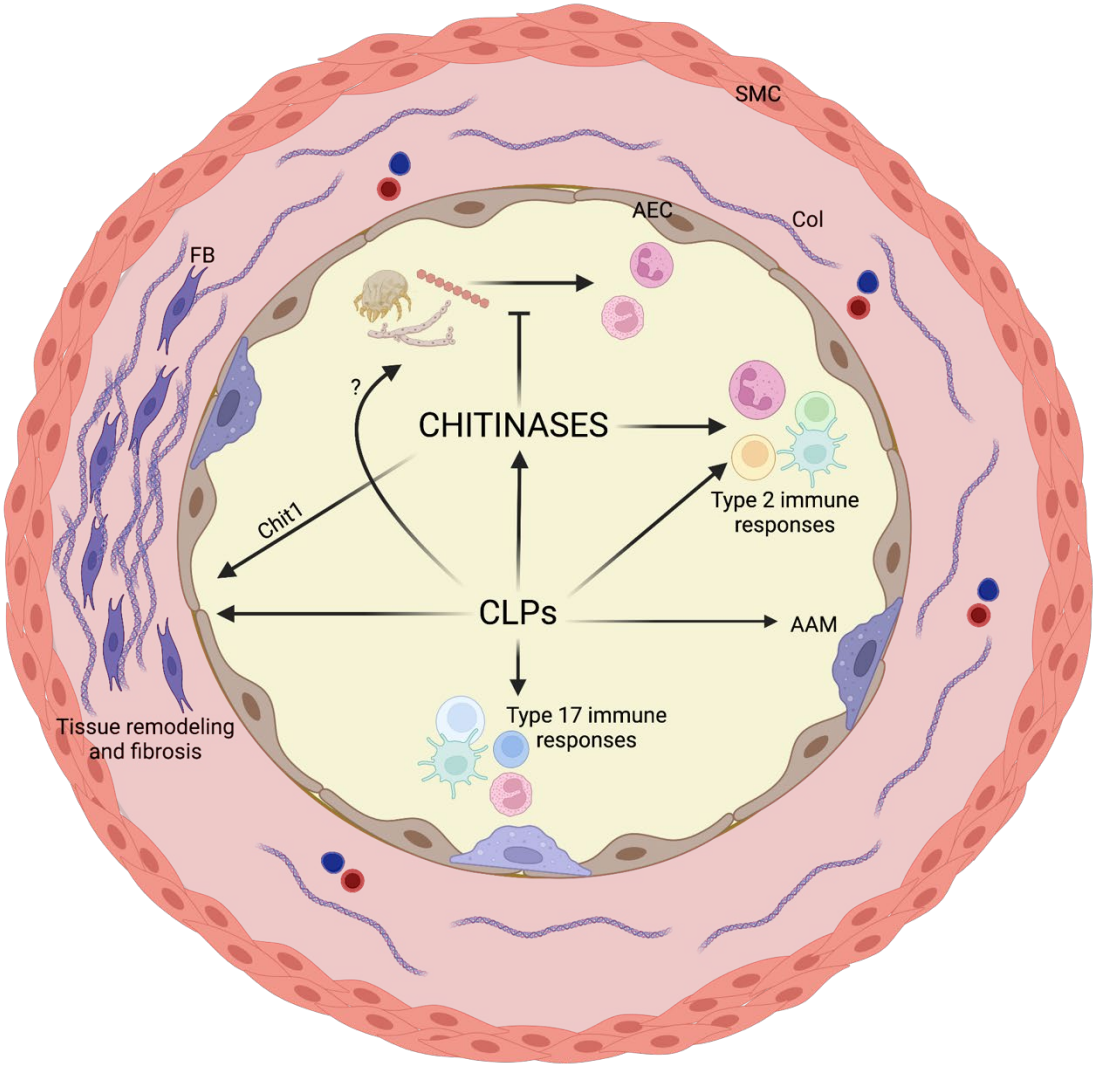


702

703 **Figure 1: Overview of chitin induced immune responses in the airways.**

Depending on its size, chitin can stimulate epithelial and immune cells in the airways by triggering pattern recognition receptors (PRR). TLR-2, TLR-9, mannose receptor, dectin-1, LysMD3, and NOD2 have been implicated in chitin sensing. Activation of epithelial cells by chitin leads to the production of type-2 cytokines (such as IL-25, TLSLP, and IL-33), but also production of IL-1ß and IL-23. These epithelial cytokines can stimulate adaptive and innate type-2 cells (Th2 cells, ILC2, basophils, eosinophils, and AAM) and type-17 cells (Th17 cells, γδ T cells, and neutrophils), which produce many effector cytokines and mediators that contribute to inflammation, damage, and tissue remodelling. Moreover, the epithelial cytokine response can stimulate DCs to activate adaptive immune responses. DCs express PRR implicated in chitin sensing, but direct effects of chitin on DCs have not been investigated. Chitin can directly stimulate macrophages to secrete a variety of cytokines such as IL-10, TNFα, and IL-12. In addition, appropriately sized chitin can be phagocytosed by macrophages, also leading to cytokine secretion. Chitin phagocytosis by macrophages leads to the induction of casp-7, which can inactivate IL-33, leading to inhibition of type 2 immune responses.

Abbreviations: AAM: alternatively activated macrophage; BASO: basophil; Casp: caspase; DC: dendritic cell; EO: eosinophil; IL: interleukin; ILC: innate lymphoid cell; LTB4: leukotriene-B4; MAC: macrophage; NEU: neutrophil; ROS: reactive oxygen species; T gd: γδ T cell; Th: T helper cell; TNFa: tumor necrosis factor α; TSLP: thymic stromal lymphopoietin.



728
729 **Figure 2: Overview of functions of chitinases and CLPs in the airways.**

730 When environmental chitin, i.e. the one contained in house dust mites and fungi, is
731 inhaled, it is degraded by active chitinases (AMCase and Chit1), thereby limiting chitin-
732 induced airway inflammation. At the same time, AMCase might also contribute to type-2
733 immune responses in the airways. In addition, Chit1 secreted by activated macrophages
734 might contribute to tissue remodeling and fibrosis through yet unknown mechanisms.
735 CLPs do not cleave chitin but might control the substrate specificity of real chitinolytic
736 enzymes by competitive or synergistic binding. In addition, CLPs might be implicated in
737 chitin sensing by binding to chitin polymers. Moreover, CLPs contribute to type-2 immune
738 responses and alternative activation of macrophages but have also been implicated in
739 Th17 skewing. Like Chit1, CLPs are implicated in tissue remodeling and repair by

interaction with extracellular matrix components and consequently facilitating matrix deposition and tissue repair.

Abbreviations: AAM: alternatively activated macrophage; AEC: alveolar epithelial cell; Chit1: chitotriosidase; CLP: chitinase-like protein; Col: collagen; FB: fibroblast; SMC: smooth muscle cell.

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References

1. Koch BE, Stougaard J, Spaink HP. Keeping track of the growing number of biological functions of chitin and its interaction partners in biomedical research. Glycobiology. 2015;25(5):469-82.
2. Di Rosa M, Distefano G, Zorena K, Malaguarnera L. Chitinases and immunity: Ancestral molecules with new functions. Immunobiology. 2016;221(3):399-411.
3. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011;73:479-501.
4. Shuhui L, Mok YK, Wong WS. Role of mammalian chitinases in asthma. Int Arch Allergy Immunol. 2009;149(4):369-77.
5. van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, et al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol. 2005;17(11):1505-12.
6. Sutherland TE, Maizels RM, Allen JE. Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies. Clin Exp Allergy. 2009;39(7):943-55.
7. Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021;184(6):1469-85.
8. Sutherland TE. Chitinase-like proteins as regulators of innate immunity and tissue repair: helpful lessons for asthma? Biochem Soc Trans. 2018;46(1):141-51.
9. Ziatabar S, Zepf J, Rich S, Danielson BT, Bollyky PI, Stern R. Chitin, chitinases, and chitin lectins: Emerging roles in human pathophysiology. Pathophysiology. 2018;25(4):253-62.
10. Weigel PH. Hyaluronan Synthase: The Mechanism of Initiation at the Reducing End and a Pendulum Model for Polysaccharide Translocation to the Cell Exterior. Int J Cell Biol. 2015;2015:367579.
11. Stern R. Go Fly a Chitin: The Mystery of Chitin and Chitinases in Vertebrate Tissues. Front Biosci (Landmark Ed). 2017;22(4):580-95.
12. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, et al. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med. 2005;11(11):1173-9.
13. 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(6):4279-86.
14. 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(6):3573-82.

15. Fuchs K, Cardona Gloria Y, Wolz OO, Herster F, Sharma L, Dillen CA, et al. The fungal ligand chitin directly binds TLR2 and triggers inflammation dependent on oligomer size. EMBO Rep. 2018;19(12).
16. Wagener J, Malireddi RK, Lenardon MD, Koberle M, Vautier S, MacCallum DM, et al. Fungal chitin dampens inflammation through IL-10 induction mediated by NOD2 and TLR9 activation. PLoS Pathog. 2014;10(4):e1004050.
17. 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(5):2462-7.
18. Mora-Montes HM, Netea MG, Ferwerda G, Lenardon MD, Brown GD, Mistry AR, et al. Recognition and blocking of innate immunity cells by Candida albicans chitin. Infect Immun. 2011;79(5):1961-70.
19. Schlosser A, Thomsen T, Moeller JB, Nielsen O, Tornoe I, Mollenhauer J, et al. Characterization of FIBCD1 as an acetyl group-binding receptor that binds chitin. J Immunol. 2009;183(6):3800-9.
20. He X, Howard BA, Liu Y, Neumann AK, Li L, Menon N, et al. LYSMD3: A mammalian pattern recognition receptor for chitin. Cell Rep. 2021;36(3):109392.
21. Roy RM, Paes HC, Nanjappa SG, Sorkness R, Gasper D, Sterkel A, et al. 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. 2013;4(2).
22. Shibata Y, Foster LA, Metzger WJ, Myrvik QN. Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. Infect Immun. 1997;65(5):1734-41.
23. Kim LK, Morita R, Kobayashi Y, Eisenbarth SC, Lee CG, Elias J, et al. AMCase is a crucial regulator of type 2 immune responses to inhaled house dust mites. Proc Natl Acad Sci U S A. 2015;112(22):E2891-9.
24. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 2007;447(7140):92-6.
25. Roy RM, Wuthrich M, Klein BS. Chitin elicits CCL2 from airway epithelial cells and induces CCR2-dependent innate allergic inflammation in the lung. J Immunol. 2012;189(5):2545-52.

26. Van Dyken SJ, Mohapatra A, Nussbaum JC, Molofsky AB, Thornton EE, Ziegler SF, et al. Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and gammadelta T cells. Immunity. 2014;40(3):414-24.
27. Arae K, Ikutani M, Horiguchi K, Yamaguchi S, Okada Y, Sugiyama H, et al. Interleukin-33 and thymic stromal lymphopoietin, but not interleukin-25, are crucial for development of airway eosinophilia induced by chitin. Sci Rep. 2021;11(1):5913.
28. Cartier A, Lehrer SB, Horth-Susin L, Swanson M, Neis B, Howse D, et al. Prevalence of crab asthma in crab plant workers in Newfoundland and Labrador. Int J Circumpolar Health. 2004;63 Suppl 2:333-6.
29. Thomassen MR, Kamath SD, Bang BE, Nugraha R, Nie S, Williamson NA, et al. Occupational Allergic Sensitization Among Workers Processing King Crab (Paralithodes camtschaticus) and Edible Crab (Cancer pagurus) in Norway and Identification of Novel Putative Allergenic Proteins. Front Allergy. 2021;2:718824.
30. Kin NW, Stefanov EK, Dizon BL, Kearney JF. Antibodies generated against conserved antigens expressed by bacteria and allergen-bearing fungi suppress airway disease. J Immunol. 2012;189(5):2246-56.
31. Arae K, Morita H, Unno H, Motomura K, Toyama S, Okada N, et al. Chitin promotes antigen-specific Th2 cell-mediated murine asthma through induction of IL-33-mediated IL-1beta production by DCs. Sci Rep. 2018;8(1):11721.
32. Choi JP, Lee SM, Choi HI, Kim MH, Jeon SG, Jang MH, et al. House Dust Mite-Derived Chitin Enhances Th2 Cell Response to Inhaled Allergens, Mainly via a TNF-alpha-Dependent Pathway. Allergy Asthma Immunol Res. 2016;8(4):362-74.
33. Diamantstein T, Klos M, Osawa H, Chen ZC. Chitin: an immunological adjuvant and a polyclonal B-lymphocyte activator. Int Arch Allergy Appl Immunol. 1982;68(4):377-81.
34. Ozdemir C, Yazi D, Aydogan M, Akkoc T, Bahceciler NN, Strong P, et al. Treatment with chitin microparticles is protective against lung histopathology in a murine asthma model. Clin Exp Allergy. 2006;36(7):960-8.
35. 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(12):1794-800.
36. Bae MJ, Shin HS, Kim EK, Kim J, Shon DH. Oral administration of chitin and chitosan prevents peanut-induced anaphylaxis in a murine food allergy model. Int J Biol Macromol. 2013;61:164-8.

37. 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(3):1314-21.
38. Chen W, Jiang X, Yang Q. Glycoside hydrolase family 18 chitinases: The known and the unknown. Biotechnol Adv. 2020;43:107553.
39. Hussain M, Wilson JB. New paralogues and revised time line in the expansion of the vertebrate GH18 family. J Mol Evol. 2013;76(4):240-60.
40. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, et al. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem. 2001;276(9):6770-8.
41. Zimmermann N, Mishra A, King NE, Fulkerson PC, Doepker MP, Nikolaidis NM, et al. Transcript signatures in experimental asthma: identification of STAT6-dependent and -independent pathways. J Immunol. 2004;172(3):1815-24.
42. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science. 2004;304(5677):1678-82.
43. Seibold MA, Donnelly S, Solon M, Innes A, Woodruff PG, Boot RG, et al. Chitotriosidase is the primary active chitinase in the human lung and is modulated by genotype and smoking habit. J Allergy Clin Immunol. 2008;122(5):944-50 e3.
44. Boot RG, Renkema GH, Strijland A, van Zonneveld AJ, Aerts JM. Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem. 1995;270(44):26252-6.
45. Boot RG, Bussink AP, Verhoek M, de Boer PA, Moorman AF, Aerts JM. Marked differences in tissue-specific expression of chitinases in mouse and man. J Histochem Cytochem. 2005;53(10):1283-92.
46. Vannella KM, Ramalingam TR, Hart KM, de Queiroz Prado R, Sciurba J, Barron L, et al. Acidic chitinase primes the protective immune response to gastrointestinal nematodes. Nat Immunol. 2016;17(5):538-44.
47. Fitz LJ, DeClercq C, Brooks J, Kuang W, Bates B, Demers D, et al. Acidic mammalian chitinase is not a critical target for allergic airway disease. Am J Respir Cell Mol Biol. 2012;46(1):71-9.
48. Van Dyken SJ, Liang HE, Naikawadi RP, Woodruff PG, Wolters PJ, Erle DJ, et al. Spontaneous Chitin Accumulation in Airways and Age-Related Fibrotic Lung Disease. Cell. 2017;169(3):497-509 e13.

885 49. Wiesner DL, Specht CA, Lee CK, Smith KD, Mukaremera L, Lee ST, et al. Chitin
886 recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal
887 infection. PLoS Pathog. 2015;11(3):e1004701.
888 50. Schmitz NA, Thakare RP, Chung CS, Lee CM, Elias JA, Lee CG, et al. Chitotriosidase
889 Activity Is Counterproductive in a Mouse Model of Systemic Candidiasis. Front Immunol.
890 2021;12:626798.
891 51. Boot RG, Renkema GH, Verhoek M, Strijland A, Bliek J, de Meulemeester TM, et al. The
892 human chitotriosidase gene. Nature of inherited enzyme deficiency. J Biol Chem.
893 1998;273(40):25680-5.
894 52. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and
895 functional studies in asthma and immune-mediated diseases. Curr Opin Allergy Clin Immunol.
896 2009;9(5):401-8.
897 53. Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB, et al. Genetic
898 polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria
899 bancrofti in South India. Genes Immun. 2001;2(5):248-53.
900 54. Malaguarnera L, Simpore J, Prodi DA, Angius A, Sassu A, Persico I, et al. A 24-bp
901 duplication in exon 10 of human chitotriosidase gene from the sub-Saharan to the Mediterranean
902 area: role of parasitic diseases and environmental conditions. Genes Immun. 2003;4(8):570-4.
903 55. Hise AG, Hazlett FE, Bockarie MJ, Zimmerman PA, Tisch DJ, Kazura JW. Polymorphisms
904 of innate immunity genes and susceptibility to lymphatic filariasis. Genes Immun. 2003;4(7):524-
905 7.
906 56. Cho SJ, Weiden MD, Lee CG. Chitotriosidase in the Pathogenesis of Inflammation,
907 Interstitial Lung Diseases and COPD. Allergy Asthma Immunol Res. 2015;7(1):14-21.
908 57. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, et al.
909 Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of
910 Pulmonary Fibrosis. Am J Respir Crit Care Med. 2019;199(12):1517-36.
911 58. Bosteels C, Maes B, Van Damme K, De Leeuw E, Declercq J, Delporte A, et al.
912 Sargramostim to treat patients with acute hypoxic respiratory failure due to COVID-19 (SARPAC):
913 A structured summary of a study protocol for a randomised controlled trial. Trials. 2020;21(1):491.
914 59. Lee CG, Herzog EL, Ahangari F, Zhou Y, Gulati M, Lee CM, et al. Chitinase 1 is a
915 biomarker for and therapeutic target in scleroderma-associated interstitial lung disease that
916 augments TGF-beta1 signaling. J Immunol. 2012;189(5):2635-44.

60. Letuve S, Kozhich A, Humbles A, Brewah Y, Dombret MC, Grandsaigne M, et al. Lung chitinolytic activity and chitotriosidase are elevated in chronic obstructive pulmonary disease and contribute to lung inflammation. Am J Pathol. 2010;176(2):638-49.
61. James AJ, Reinius LE, Verhoek M, Gomes A, Kupczyk M, Hammar U, et al. Increased YKL-40 and Chitotriosidase in Asthma and Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2016;193(2):131-42.
62. Gavala ML, Kelly EA, Esnault S, Kukreja S, Evans MD, Bertics PJ, et al. Segmental allergen challenge enhances chitinase activity and levels of CCL18 in mild atopic asthma. Clin Exp Allergy. 2013;43(2):187-97.
63. Lee P, Waalen J, Crain K, Smargon A, Beutler E. Human chitotriosidase polymorphisms G354R and A442V associated with reduced enzyme activity. Blood Cells Mol Dis. 2007;39(3):353-60.
64. Bierbaum S, Superti-Furga A, Heinzmann A. Genetic polymorphisms of chitotriosidase in Caucasian children with bronchial asthma. Int J Immunogenet. 2006;33(3):201-4.
65. Wu AC, Lasky-Su J, Rogers CA, Klanderman BJ, Litonjua A. Polymorphisms of chitinases are not associated with asthma. J Allergy Clin Immunol. 2010;125(3):754-7, 7 e1-7 e2.
66. Kim KW, Park J, Lee JH, Lee HS, Lee J, Lee KH, et al. Association of genetic variation in chitotriosidase with atopy in Korean children. Ann Allergy Asthma Immunol. 2013;110(6):444-9 e1.
67. Hong JY, Kim M, Sol IS, Kim KW, Lee CM, Elias JA, et al. Chitotriosidase inhibits allergic asthmatic airways via regulation of TGF-beta expression and Foxp3(+) Treg cells. Allergy. 2018;73(8):1686-99.
68. Cho WS, Kim TH, Lee HM, Lee SH, Lee SH, Yoo JH, et al. Increased expression of acidic mammalian chitinase and chitotriosidase in the nasal mucosa of patients with allergic rhinitis. Laryngoscope. 2010;120(5):870-5.
69. Ramanathan M, Jr., Lee WK, Lane AP. Increased expression of acidic mammalian chitinase in chronic rhinosinusitis with nasal polyps. Am J Rhinol. 2006;20(3):330-5.
70. Musumeci M, Bellin M, Maltese A, Aragona P, Bucolo C, Musumeci S. Chitinase levels in the tears of subjects with ocular allergies. Cornea. 2008;27(2):168-73.
71. Bierbaum S, Nickel R, Koch A, Lau S, Deichmann KA, Wahn U, et al. Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. Am J Respir Crit Care Med. 2005;172(12):1505-9.

72. Seibold MA, Reese TA, Choudhry S, Salam MT, Beckman K, Eng C, et al. Differential enzymatic activity of common haplotypic versions of the human acidic Mammalian chitinase protein. J Biol Chem. 2009;284(29):19650-8.
73. Matsumoto T, Inoue H, Sato Y, Kita Y, Nakano T, Noda N, et al. Demethylallosamidin, a chitinase inhibitor, suppresses airway inflammation and hyperresponsiveness. Biochem Biophys Res Commun. 2009;390(1):103-8.
74. Sutherland TE, Andersen OA, Betou M, Eggleston IM, Maizels RM, van Aalten D, et al. Analyzing airway inflammation with chemical biology: dissection of acidic mammalian chitinase function with a selective drug-like inhibitor. Chem Biol. 2011;18(5):569-79.
75. Yang CJ, Liu YK, Liu CL, Shen CN, Kuo ML, Su CC, et al. Inhibition of acidic mammalian chitinase by RNA interference suppresses ovalbumin-sensitized allergic asthma. Hum Gene Ther. 2009;20(12):1597-606.
76. Mazur M, Olczak J, Olejniczak S, Koralewski R, Czestkowski W, Jedrzejczak A, et al. Targeting Acidic Mammalian chitinase Is Effective in Animal Model of Asthma. J Med Chem. 2018;61(3):695-710.
77. Hartl D, He CH, Koller B, Da Silva CA, Kobayashi Y, Lee CG, et al. Acidic mammalian chitinase regulates epithelial cell apoptosis via a chitinolytic-independent mechanism. J Immunol. 2009;182(8):5098-106.
78. O'Dea EM, Amarsaikhan N, Li H, Downey J, Steele E, Van Dyken SJ, et al. Eosinophils are recruited in response to chitin exposure and enhance Th2-mediated immune pathology in Aspergillus fumigatus infection. Infect Immun. 2014;82(8):3199-205.
79. Van Dyken SJ, Garcia D, Porter P, Huang X, Quinlan PJ, Blanc PD, et al. Fungal chitin from asthma-associated home environments induces eosinophilic lung infiltration. J Immunol. 2011;187(5):2261-7.
80. Chang NC, Hung SI, Hwa KY, Kato I, Chen JE, Liu CH, et al. A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. J Biol Chem. 2001;276(20):17497-506.
81. George L, Mitra A, Thimraj TA, Irmler M, Vishweswaraiah S, Lunding L, et al. Transcriptomic analysis comparing mouse strains with extreme total lung capacities identifies novel candidate genes for pulmonary function. Respir Res. 2017;18(1):152.
82. Tang H, Fang Z, Sun Y, Li B, Shi Z, Chen J, et al. YKL-40 in asthmatic patients, and its correlations with exacerbation, eosinophils and immunoglobulin E. Eur Respir J. 2010;35(4):757-60.

982 83. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in
983 the lung and circulation of patients with severe asthma. N Engl J Med. 2007;357(20):2016-27.
984 84. Kuepper M, Bratke K, Virchow JC. Chitinase-like protein and asthma. N Engl J Med.
985 2008;358(10):1073-5; author reply 5.
986 85. Zhao T, Su Z, Li Y, Zhang X, You Q. Chitinase-3 like-protein-1 function and its role in
987 diseases. Signal Transduct Target Ther. 2020;5(1):201.
988 86. Rathcke CN, Vestergaard H. YKL-40--an emerging biomarker in cardiovascular disease
989 and diabetes. Cardiovasc Diabetol. 2009;8:61.
990 87. Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, et al.
991 Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation.
992 J Biol Chem. 2003;278(45):44058-67.
993 88. Furuhashi K, Suda T, Nakamura Y, Inui N, Hashimoto D, Miwa S, et al. Increased
994 expression of YKL-40, a chitinase-like protein, in serum and lung of patients with idiopathic
995 pulmonary fibrosis. Respir Med. 2010;104(8):1204-10.
996 89. Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, et al. Role of YKL-40 in
997 bronchial smooth muscle remodeling in asthma. Am J Respir Crit Care Med. 2012;185(7):715-22.
998 90. Tang H, Sun Y, Shi Z, Huang H, Fang Z, Chen J, et al. YKL-40 induces IL-8 expression
999 from bronchial epithelium via MAPK (JNK and ERK) and NF-kappaB pathways, causing bronchial
1000 smooth muscle proliferation and migration. J Immunol. 2013;190(1):438-46.
1001 91. Ling H, Recklies AD. The chitinase 3-like protein human cartilage glycoprotein 39 inhibits
1002 cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha.
1003 Biochem J. 2004;380(Pt 3):651-9.
1004 92. Bigg HF, Wait R, Rowan AD, Cawston TE. The mammalian chitinase-like lectin, YKL-40,
1005 binds specifically to type I collagen and modulates the rate of type I collagen fibril formation. J Biol
1006 Chem. 2006;281(30):21082-95.
1007 93. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, et al. Effect of variation in CHI3L1
1008 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med. 2008;358(16):1682-91.
1009 94. Zhu Y, Yan X, Zhai C, Yang L, Li M. Association between risk of asthma and gene
1010 polymorphisms in CHI3L1 and CHIA: a systematic meta-analysis. BMC Pulm Med.
1011 2017;17(1):193.
1012 95. Gomez JL, Crisafi GM, Holm CT, Meyers DA, Hawkins GA, Bleecker ER, et al. Genetic
1013 variation in chitinase 3-like 1 (CHI3L1) contributes to asthma severity and airway expression of
1014 YKL-40. J Allergy Clin Immunol. 2015;136(1):51-8 e10.

96. Abe K, Nakamura Y, Yamauchi K, Maemondo M. Role of genetic variations of chitinase 3-like 1 in bronchial asthmatic patients. Clin Mol Allergy. 2018;16:9.
97. Rathcke CN, Holmkvist J, Husmoen LL, Hansen T, Pedersen O, Vestergaard H, et al. Association of polymorphisms of the CHI3L1 gene with asthma and atopy: a populations-based study of 6514 Danish adults. PLoS One. 2009;4(7):e6106.
98. Jin Y, Song J, Xu F, Zhang D, He J, Zheng J, et al. Association between YKL-40 and asthma: a systematic meta-analysis. Sleep Breath. 2022;26(3):1011-22.
99. Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clin Rev Allergy Immunol. 2019;56(2):219-33.
100. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, et al. 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. 2009;206(5):1149-66.
101. Liu L, Zhang X, Liu Y, Zhang L, Zheng J, Wang J, et al. Chitinase-like protein YKL-40 correlates with inflammatory phenotypes, anti-asthma responsiveness and future exacerbations. Respir Res. 2019;20(1):95.
102. Gomez JL, Yan X, Holm CT, Grant N, Liu Q, Cohn L, et al. Characterisation of asthma subgroups associated with circulating YKL-40 levels. Eur Respir J. 2017;50(4).
103. Hinks TSC, Brown T, Lau LCK, Rupani H, Barber C, Elliott S, et al. Multidimensional endotyping in patients with severe asthma reveals inflammatory heterogeneity in matrix metalloproteinases and chitinase 3-like protein 1. J Allergy Clin Immunol. 2016;138(1):61-75.
104. Tong X, Wang D, Liu S, Ma Y, Fan H. Can YKL-40 be used as a biomarker and therapeutic target for adult asthma? Eur Respir J. 2018;51(1).
105. Konradsen JR, James A, Nordlund B, Reinius LE, Soderhall C, Melen E, et al. The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. J Allergy Clin Immunol. 2013;132(2):328-35 e5.
106. Santos CB, Davidson J, Covar RA, Spahn JD. The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children. Ann Allergy Asthma Immunol. 2014;113(3):263-6.
107. Parkinson JE, Pearson S, Ruckerl D, Allen JE, Sutherland TE. The magnitude of airway remodeling is not altered by distinct allergic inflammatory responses in BALB/c versus C57BL/6 mice but matrix composition differs. Immunol Cell Biol. 2021;99(6):640-55.
108. Sutherland TE, Logan N, Ruckerl D, Humbles AA, Allan SM, Papayannopoulos V, et al. Chitinase-like proteins promote IL-17-mediated neutrophilia in a tradeoff between nematode killing and host damage. Nat Immunol. 2014;15(12):1116-25.

109. He CH, Lee CG, Dela Cruz CS, Lee CM, Zhou Y, Ahangari F, et al. Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor alpha2. Cell Rep. 2013;4(4):830-41.
110. Nikota JK, Botelho FM, Bauer CM, Jordana M, Coyle AJ, Humbles AA, et al. Differential expression and function of breast regression protein 39 (BRP-39) in murine models of subacute cigarette smoke exposure and allergic airway inflammation. Respir Res. 2011;12:39.
111. Mackel JJ, Garth JM, Jones M, Ellis DA, Blackburn JP, Yu Z, et al. Chitinase 3-like-1 protects airway function despite promoting type 2 inflammation during fungal-associated allergic airway inflammation. Am J Physiol Lung Cell Mol Physiol. 2021;320(4):L615-L26.
112. Zhu W, Lonnblom E, Forster M, Johannesson M, Tao P, Meng L, et al. Natural polymorphism of Ym1 regulates pneumonitis through alternative activation of macrophages. Sci Adv. 2020;6(43).
113. Ohno M, Kida Y, Sakaguchi M, Sugahara Y, Oyama F. Establishment of a quantitative PCR system for discriminating chitinase-like proteins: catalytically inactive breast regression protein-39 and Ym1 are constitutive genes in mouse lung. BMC Mol Biol. 2014;15:23.
114. Webb DC, McKenzie AN, Foster PS. Expression of the Ym2 lectin-binding protein is dependent on interleukin (IL)-4 and IL-13 signal transduction: identification of a novel allergy-associated protein. J Biol Chem. 2001;276(45):41969-76.
115. Song HM, Jang AS, Ahn MH, Takizawa H, Lee SH, Kwon JH, et al. Ym1 and Ym2 expression in a mouse model exposed to diesel exhaust particles. Environ Toxicol. 2008;23(1):110-6.
116. Qureshi AM, Hannigan A, Campbell D, Nixon C, Wilson JB. Chitinase-like proteins are autoantigens in a model of inflammation-promoted incipient neoplasia. Genes Cancer. 2011;2(1):74-87.
117. Nair MG, Gallagher IJ, Taylor MD, Loke P, Coulson PS, Wilson RA, et al. Chitinase and Fizz family members are a generalized feature of nematode infection with selective upregulation of Ym1 and Fizz1 by antigen-presenting cells. Infect Immun. 2005;73(1):385-94.
118. Arora M, Chen L, Paglia M, Gallagher I, Allen JE, Vyas YM, et al. Simvastatin promotes Th2-type responses through the induction of the chitinase family member Ym1 in dendritic cells. Proc Natl Acad Sci U S A. 2006;103(20):7777-82.
119. Goren I, Pfeilschifter J, Frank S. Uptake of neutrophil-derived Ym1 protein distinguishes wound macrophages in the absence of interleukin-4 signaling in murine wound healing. Am J Pathol. 2014;184(12):3249-61.

120. Ikeda N, Asano K, Kikuchi K, Uchida Y, Ikegami H, Takagi R, et al. Emergence of immunoregulatory Ym1(+)Ly6C(hi) monocytes during recovery phase of tissue injury. Sci Immunol. 2018;3(28).
121. Guo L, Johnson RS, Schuh JC. Biochemical characterization of endogenously formed eosinophilic crystals in the lungs of mice. J Biol Chem. 2000;275(11):8032-7.
122. Roediger B, Kyle R, Tay SS, Mitchell AJ, Bolton HA, Guy TV, et al. IL-2 is a critical regulator of group 2 innate lymphoid cell function during pulmonary inflammation. J Allergy Clin Immunol. 2015;136(6):1653-63 e7.
123. Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. Science. 2019;364(6442).
124. Cai Y, Kumar RK, Zhou J, Foster PS, Webb DC. Ym1/2 promotes Th2 cytokine expression by inhibiting 12/15(S)-lipoxygenase: identification of a novel pathway for regulating allergic inflammation. J Immunol. 2009;182(9):5393-9.
125. Penas F, Mirkin GA, Vera M, Cevey A, Gonzalez CD, Gomez MI, et al. Treatment in vitro with PPARalpha and PPARgamma ligands drives M1-to-M2 polarization of macrophages from T. cruzi-infected mice. Biochim Biophys Acta. 2015;1852(5):893-904.
126. Owhashi M, Arita H, Hayai N. Identification of a novel eosinophil chemotactic cytokine (ECF-L) as a chitinase family protein. J Biol Chem. 2000;275(2):1279-86.
127. Choi M, Jeong H, Kim S, Kim M, Lee M, Rhim T. Targeted delivery of Chil3/Chil4 siRNA to alveolar macrophages using ternary complexes composed of HMG and oligoarginine micelles. Nanoscale. 2020;12(2):933-43.
128. Gu J, Choi M, Ban T, Kim S, Kim Y, Lee M, et al. Therapeutic response to HMGB1-R3V6-conjugated Ym1/Ym2 siRNA complex in ovalbumin-induced murine asthma. J Control Release. 2015;213:e102.
129. Sutherland TE, Ruckerl D, Logan N, Duncan S, Wynn TA, Allen JE. Ym1 induces RELMalpha and rescues IL-4Ralpha deficiency in lung repair during nematode infection. PLoS Pathog. 2018;14(11):e1007423.
130. Draijer C, Robbe P, Boorsma CE, Hylkema MN, Melgert BN. Dual role of YM1+ M2 macrophages in allergic lung inflammation. Sci Rep. 2018;8(1):5105.
131. Sun YJ, Chang NC, Hung SI, Chang AC, Chou CC, Hsiao CD. The crystal structure of a novel mammalian lectin, Ym1, suggests a saccharide binding site. J Biol Chem. 2001;276(20):17507-14.

132. Bussink AP, Speijer D, Aerts JM, Boot RG. Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. Genetics. 2007;177(2):959-70.
133. Adrangi S, Faramarzi MA. From bacteria to human: a journey into the world of chitinases. Biotechnol Adv. 2013;31(8):1786-95.
134. Bergthorsson U, Andersson DI, Roth JR. Ohno's dilemma: evolution of new genes under continuous selection. Proc Natl Acad Sci U S A. 2007;104(43):17004-9.
135. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem. 1993;268(34):25803-10.
136. Kirkpatrick RB, Emery JG, Connor JR, Dodds R, Lysko PG, Rosenberg M. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. Exp Cell Res. 1997;237(1):46-54.
137. Laheri S, Ashary N, Bhatt P, Modi D. Oviductal glycoprotein 1 (OVGP1) is expressed by endometrial epithelium that regulates receptivity and trophoblast adhesion. J Assist Reprod Genet. 2018;35(8):1419-29.
138. Arias EB, Verhage HG, Jaffe RC. Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein. Biol Reprod. 1994;51(4):685-94.
139. Meng G, Zhao Y, Bai X, Liu Y, Green TJ, Luo M, et al. Structure of human stabilin-1 interacting chitinase-like protein (SI-CLP) reveals a saccharide-binding cleft with lower sugar-binding selectivity. J Biol Chem. 2010;285(51):39898-904.
140. Kzhyshkowska J, Mamidi S, Gratchev A, Kremmer E, Schmuttermaier C, Krusell L, et al. Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. Blood. 2006;107(8):3221-8.
141. Xiao W, Meng G, Zhao Y, Yuan H, Li T, Peng Y, et al. Human secreted stabilin-1-interacting chitinase-like protein aggravates the inflammation associated with rheumatoid arthritis and is a potential macrophage inflammatory regulator in rodents. Arthritis Rheumatol. 2014;66(5):1141-52.