

# Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies

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## Clinical & Experimental Allergy

### Summary

Mammalian chitinase and chitinase-like proteins (CLPs) are a family of mediators increasingly associated with infection, T cell-mediated inflammation, wound healing, allergy and asthma. Although our current knowledge of the function of mammalian chitinases and CLPs is very limited, important information can be deduced from research carried out in lower organisms, and in different immunopathological conditions. Enzymatically active mammalian chitinase proteins may have evolved to degrade the copious amounts of chitin mammals are exposed to on a daily basis, and to form an innate barrier to chitin-containing organisms. CLPs are homologous to chitinases but lack the ability to degrade chitin. It is most striking that both chitinases and CLPs are up-regulated in T-helper type 2 (Th2)-driven conditions, and the first evidence is now emerging that these proteins may accentuate Th2 reactivity, and possibly contribute to the repair process that follows inflammation. Following studies demonstrating that chitinase inhibition leads to an attenuated allergic response, several strategies are being used to develop enzyme inhibitors for therapeutic use in human diseases. In this review, we will summarize recent insights into the effects of chitinases and CLPs in the context of Th2-dominated pathology with particular focus on allergy and asthma, discussing whether chitinase enzyme inhibitors may be of therapeutic value.

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### Introduction

Chitin (Fig. 1) is an essential structural component of the fungal cell wall and is present in the exoskeleton of arthropods and the microfilarial sheath of nematodes [1], acting as a protective layer against the harsh conditions that may be endured by the pathogen or arthropod. Mammals do not synthesize chitin, yet it is the second most abundant glycopolymer on earth, with an estimated  $10^{10}$  tonnes of chitin produced each year [2]. It was generally assumed that mammals lacked the ability to produce chitinase proteins, the enzymes responsible for chitin degradation. However, recent findings have not only demonstrated that mammals produce chitinases, but also that increased secretion of chitinases is closely associated with T-helper type 2 (Th2)-dominated pathophysiological conditions including infection, fibrosis, allergy and asthma [3–5].

Chitinases belong to the glycoside hydrolase family 18, which also encompasses enzymatically inactive chitinase-like proteins (CLPs). Only the true chitinases have a functioning catalytic domain, which facilitates the hydro-

lysis of glycosidic bonds [6], resulting in chitin degradation. While the role of chitinases and CLPs in settings of human allergic inflammation and other pathologies have only recently been highlighted, more information is available on the function and evolution of chitinases in other organisms. This review gives a broad perspective of the effects and functions of chitinases and CLPs in the context of their association with allergy and asthma.

### Why are chitinases and chitinase-like proteins produced in mammals in the absence of chitin synthesis?

Early research into mammalian chitinases questioned why mammals express both true chitinase proteins and CLPs in the absence of endogenous chitin synthesis. This question continues to be at the forefront of chitinase and CLP research and still remains unanswered. Investigating the evolutionary history of the gene family throughout the animal kingdom reveals a relatively late expansion and diversification within mammalian groups [7, 8]. In particular, the inactive CLPs appear to result from more recent evolutionary events [8], such as the mutation of crucial

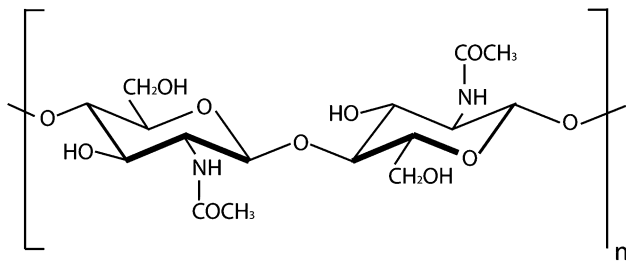


Fig. 1. Structure of chitin showing two repeating *N*-acetylglucosamine units that form long-chain polymers through covalent  $\beta$ -1,4 linkages.

residues within the catalytic domain of chitinase, including aspartic acid 136 and glutamic acid 140 (Fig. 2), to form CLPs such as murine Ym1 and Ym2 [7, 9].

Studies on chitinases in lower organisms may provide some interesting parallels for understanding the function of the mammalian chitinases and CLPs. The action and pattern of chitinase expression in plants, bacteria, viruses and fungi illustrate a number of diverse roles in morphogenesis, nutrition and stress [10]. However, a more common feature of chitinase activity in all organisms appears to be a host defence mechanism against chitin-containing pathogens. In plants, the expression and activity of chitinases are induced or up-regulated upon fungal attack [11, 12] and result in the degradation of chitin-containing fungal walls and hence inhibition of fungal growth [13]. These findings in plants have led to an exploration for utilizing chitinases in agricultural applications, such as anti-fungal reagents [14] and have no doubt aided attempts at designing chitinase inhibitors for use in mammals.

### Chitinases and chitinase-like proteins in mammalian infection

In mammals, expression of chitinases and CLPs is greatly amplified during many infections, highlighting their potential contribution to host defences. Indeed, the chitinase-like molecule, Ym1, was first reported as a prominent novel product in mice infected with the helminths *Trichinella spiralis* [15], *Brugia malayi* [16] and *Schistosoma mansoni* [17]. Ym1 production is very strikingly associated with a distinct cell phenotype termed the alternatively activated macrophage (AAM $\phi$ ). High levels of IL-4 and IL-13 in Th2-driven inflammatory settings of infection and allergy stimulate abundant numbers of AAM $\phi$ s [18], which also up-regulate resistin-like molecule- $\alpha$ , arginase-1 and the mannose receptor [5, 16, 19–21]. Hence, while fungal infections with *Cryptococcus neoformans* are normally limited by a Th1 response, in IFN- $\gamma$ -deficient and IL-13 overexpressing mice there is a dominant Th2 response accompanied by alternative activation of macrophages and production of Ym1 that is associated with more severe disease [22, 23].

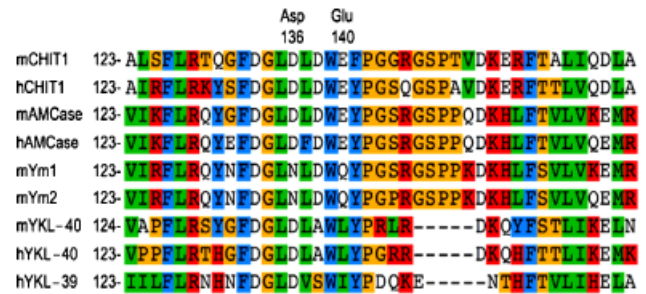


Fig. 2. Multiple sequence alignment of key residues within the catalytic domain of chitotriosidase with various human and murine chitinases and chitinase-like proteins. Aspartic acid and glutamic acid residues that are important for chitin degradation are indicated. CHIT1, chitotriosidase; AMCase, acidic mammalian chitinase; m, murine; h, human.

AAM $\phi$ s in helminth infections are thought to facilitate the anti-parasitic inflammatory response and promote tissue repair caused by large migrating metazoan parasites [24–26]. The abundant secretion of Ym1 by murine AAM $\phi$ s and alveolar macrophages suggests that it is required in some quantity to fulfil an as yet undefined role in inflammation and repair/remodelling. This is supported by the finding that Ym1 can interact and bind components of the extracellular matrix (ECM) [27]. It is thus tempting to speculate that chitinases and CLPs, through interactions with ECM and host sugars, provide a physical basis for tissue repair and remodelling, appropriately during helminth infection and inappropriately during asthma.

The presence and function of AAM $\phi$ s in humans has been more difficult to define than with mouse models. However, chitinases and CLPs are certainly prominent in the human response to infection, and human alveolar macrophages from allergic/asthmatic patients express the active chitinase, AMCase [4], which is also seen in mouse models of helminth infection and allergy [5, 28]. Elevated chitinase activity and/or protein levels in humans have implicated AMCase, chitotriosidase and CLP YKL-40 in severe infections (see Table 1). Human plasma levels of chitotriosidase activity are increased upon infection with fungal pathogens [29] and malaria parasites [30], consistent with an involvement in host defence. Additionally, *in vitro* cell culture experiments demonstrate an increase in chitotriosidase expression or chitinase activity when human macrophages or neutrophils are stimulated with IFN- $\gamma$ , TNF- $\alpha$  or Toll-like receptor (TLR) ligands [31–33], suggesting that chitotriosidase forms part of the innate response that combats infection. However, chitinase activity may not always promote host defence. Chitinase produced by malaria parasites such as *Plasmodium falciparum* degrade the chitinous peritrophic membrane in order to facilitate crossing into the insect midgut [34]. Interestingly, insects fed on the blood from malaria patients, with higher plasma levels of chitotriosidase, had

Table 1. The proposed role of chitinases and chitinase-like proteins (CLPs) in pathological conditions

Disease	Implicated chitinase and/or CLP	Findings	References
Asthma/Allergy	AMCase (human/murine)	Polymorphisms associated with asthma; increased activity/protein in allergic airway inflammation; increased expression in lung tissue of asthmatics; increased expression in ocular allergies	[4, 36–38]
	Ym1/Ym2 (murine)	Increased protein in models of allergic airway inflammation	[38–41]
	YKL-40 (human)	Increased expression in serum and lungs of asthmatics	[42, 43]
Infection	BRP-39 (murine)	Increased protein in models of allergic airway inflammation	[44, 45]
	Chitotriosidase (human)	Increased activity upon fungal infection; polymorphisms associated with susceptibility to filariasis; increased serum activity in malaria patients	[29, 30, 46]
	YKL-40 (human)	Increased serum levels during endotoxaemia and streptococcus pneumoniae	[47, 48]
	Ym1/2 (murine)	Increased during pulmonary infection with <i>Cryptococcus neoformans</i> ; increased during nematode infection; increased during chronic <i>Trypanosoma brucei</i> infection	[5, 16, 19, 23, 49; reviewed in 99]
Arthritis	BRP-39 (murine)	Pathogenic role in colitis, exacerbating intestinal inflammation	[50, 51]
	YKL-39 (human)	Increased in osteoarthritic cartilage; induces arthritis in mice	[52–54]
	YKL-40 (human)	Decreased in osteoarthritic cartilage; increased in serum and synovial fluid of osteoarthritis patients; detectable expression in synovial joint and cartilage of rheumatoid arthritis patients; induces proliferation of chondrocytes and synoviocytes	[55–57]
Fibrotic-Related Diseases	Chitotriosidase (human)	Increased expression in Kupffer cells from patients with non-alcoholic steatohepatitis; increased serum activity in patients with sarcoidosis; increased activity in BAL fluid of patients with idiopathic pulmonary fibrosis and sarcoidosis	[3, 58–60]
	YKL-40 (human)	Serum levels increased with hepatic fibrosis; increased serum levels in patients with hepatic fibrosis due to schistosomiasis	[61–63]
	Ym1/Ym2 (murine)	Increased expression in silica-induced pulmonary fibrosis; increased expression in liver fibrosis induced by <i>Schistosoma mansoni</i> infection; increased expression in herpesvirus-induced lung fibrosis	[64–66]
Injury	Ym1/Ym2 (murine)	Increased expression following incisional wound and olfactory injury; induction of protein following brain stab-wound	[26, 27, 67]

BRP-39, breast regression protein-39.

visibly damaged peritrophic membranes following 20 h of feeding [35]. Therefore, the high levels of chitotriosidase activity in malaria patients may actually aid malaria transmission from the mosquito vector.

Some confusion over the role of chitotriosidase in human parasite infection comes from studies of a genetic chitotriosidase variation, deemed allele H. In this variant, a 24-bp duplication alters mRNA splicing so that the mature protein no longer contains residues essential for enzymatic activity [46]. Hence, patients homozygous for allele H have no chitinase activity. Recent studies have explored the distribution of this allele. Some studies have shown that in areas with current or recent malaria endemicity, there is a very low frequency of the mutation associated with high serum chitinase activity, whereas in areas with no malaria and improved hygiene there is a significantly higher proportion of the population with the deletion allele [68, 69]. Other studies, however, suggest a high frequency of mutation in areas of Sardinia, where there is a high incidence of malaria [70] and in Taiwan, where malaria has only been recently eradicated [71]. Similarly conflicting results occur with regard to lymphatic

filariasis, a disease in which the transmissible microfilarial stage contains chitin. In South India, allele H carriers are more susceptible to infection [46], while a similar study in Papua New Guinea showed no such association [72]. Nonetheless, the apparent loss of the wild-type allele in some areas, suggests a selective advantage of the mutation. High levels of serum chitinase activity may help protect against malaria and possibly nematode infection but perhaps at a cost of higher pathology in other settings. This is supported by a strict correlation between chitotriosidase levels and the severity of multiple sclerosis [68] as well as the pathology of Gaucher's disease, in which plasma chitotriosidase levels are elevated several hundred-fold in symptomatic patients [73]. The specific functions of chitotriosidase need to be more thoroughly explored before any conclusions can be drawn regarding the evolution and development of chitotriosidase allele H. It also remains to be seen whether other chitinase proteins can compensate for a loss of chitotriosidase activity.

The roles of chitinases and CLPs in human host defence thus remain unresolved. From current knowledge it would

appear that both chitinases and CLPs are strongly associated with both innate and adaptive immune responses, but direct evidence is still lacking that they play an effector role in anti-parasite immunity. Whether their primary function is in the arena of protective immunity, or tissue repair, it is clear that in different contexts these proteins may be both beneficial and detrimental for the host.

### Chitinases and chitinase-like proteins in inflammatory diseases

In addition to their expression during host responses to pathogen exposure, chitinases and CLPs have a prominent place in various patho-physiological conditions, as listed in Table 1. It is quite striking to find that chitinases and/or CLPs are induced under conditions where the failure of immune regulation is key to inflammation and pathogenesis. This is evident in both acute disease settings, such as some allergies, and more chronic settings such fibrotic-related diseases, and in both experimental models and human patients.

A good example of such studies are those on Ym proteins and AMCase, in allergic airway-related pathology, as both proteins are up-regulated in the murine lung in response to Th2 cytokines IL-13 and IL-4 [4, 39], as shown in Fig. 3. In these models, both Ym1 and the closely related Ym2 are consistently up-regulated in the lung [23, 39]. Ym1 and Ym2 are highly homologous CLPs that represent a recent gene duplication event [74], and as yet there is little evidence that they fulfil distinct functions. Ym protein expression is dependent on Th2-cytokines IL-4 and IL-13, as shown by reduced expression in allergic

mice deficient in CD4<sup>+</sup> T cells or in which IL-4 and IL-13 signalling through the common IL-4R $\alpha$  subunit was disrupted [39]. Moreover, mice deficient in downstream components of the Th2 response, such as STAT6 [75] or the IL-21 receptor [64] also show greatly reduced chitinase and CLP responses.

Despite observing a dramatic increase in chitinases and CLPs in the lungs of allergic animals and human patients, the specific function(s) of these proteins are largely speculative. In the case of human patients, chitinases and CLPs have been identified as potential biomarkers of disease, making these proteins clinically relevant, whether from the approach of developing new therapeutics or as tools for disease prognosis and screening. While some propose that AMCase promotes inflammatory cell influx into the lung, and induces chemokines and Th2 cytokines [76], generation of transgenic mice that overexpress AMCase constitutively in the lungs led to no apparent changes to lung histology [77]. This suggests that overexpression of chitinase alone is not a sufficient stimulus to drive disease and that increased chitinases and CLP production may be a consequence of disease and the reactions involved in disease progression rather than a direct cause.

The following sections discuss recent insights into the function of chitinases and CLPs in the context of allergic airway pathology and whether these proteins are good targets for therapeutic intervention.

### The allergic immune response

The allergic immune response is a complex interplay between T helper and T regulatory cells, the mechanisms

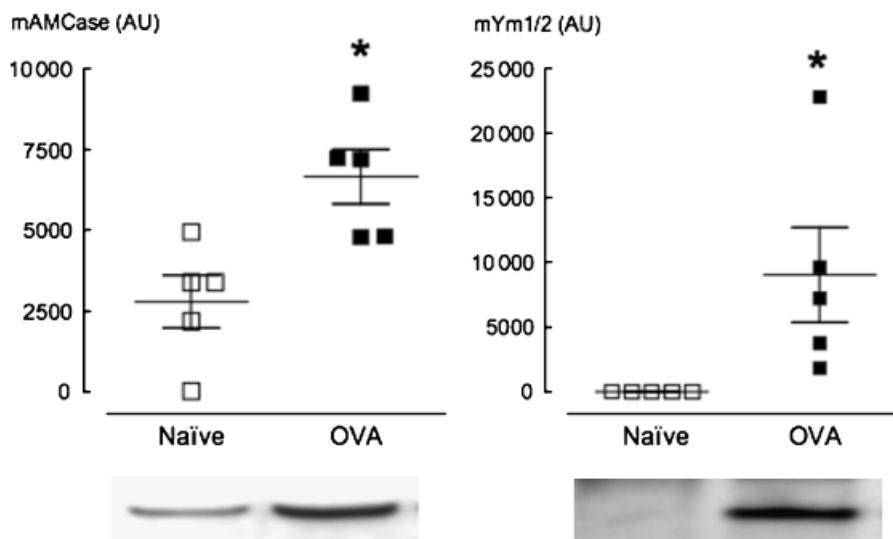


Fig. 3. Western blot with anti-AMCase and anti-Ym1/2 peptide. Female Balb/c mice were sensitized on day 0 and 14 with 20  $\mu$ g ovalbumin (OVA), *i.p.*, before being challenged with 50  $\mu$ g OVA or phosphate-buffered saline (PBS), *i.t.*, on days 28 and 30. Lung tissue was harvested 24 h after the last challenge and homogenized. Densitometry values and Western blot - are shown for mAMCase and mYm1/2 in OVA-challenged mice compared with PBS-challenged naïve mice. Upon allergic OVA challenge, the protein levels of both AMCase and Ym1/2 are significantly increased over PBS challenged mice. AMCase, acidic mammalian chitinase.

of which have been extensively reviewed previously [78, 79]. In brief, acute allergic sensitization in individuals involves Th2 cell expansion following allergen exposure. Th2 cells secrete a multitude of cytokines, including IL-4, IL-5, IL-9 and IL-13, as well as chemokines such as thymus and activation-regulated chemokine and macrophage-derived chemokine, leading to further Th2 cell recruitment and activation of B cells. Under the influence of IL-4, B cells produce allergen-specific IgE that circulates and binds surface Fcε-receptors on mast cells and basophils. Further exposure to allergen results in cross-linking of IgE on mast cells and basophils causing cell degranulation releasing histamine, proteases, chemokines, prostaglandins, leukotrienes and a host of other mediators resulting in bronchoconstriction and recruitment of activated eosinophils, neutrophils, lymphocytes and macrophages. In some individuals, chronic allergic reactivity manifests as asthma due to airway wall remodelling, airways hyperresponsiveness (AHR) and chronic inflammation.

Current trends suggest that the western world is in the clutches of an asthma and allergy epidemic, attributed in no small part to the diminished exposure to both pathogenic and non-pathogenic organisms [80–82]. This trend gave rise to the ‘hygiene hypothesis’, first described of as an imbalance of Th1 vs. Th2 responses [83] caused by a lack of microbial exposure, potentiating Th2 cell responsiveness. However, this view was challenged when it became clear that helminth infections, which primarily evoke a Th2 response, could also protect against allergy [84]. The current view suggests an imbalance between regulating and effector responses with regulatory T cells (Tregs) playing a key role in suppressing both Th1- and Th2-dominated responses through the production of suppressive cytokines such as IL-10 and TGF-β [84–86].

Children with asthma have a decreased number of Tregs in the bronchial alveolar lavage fluid accompanied by enhanced Th2 responsiveness [87]. Furthermore, the ability of Tregs to restrain Th2 reactivity is compromised in allergic patients [88], suggesting that Tregs play a pivotal role in keeping the allergic immune response in check [89]. Support for this regulatory-orientated approach to the hygiene hypothesis comes from murine studies in which helminth infection protected against allergic airway inflammation and this effect was dependent on Treg cells [86]. With relevance to the potential role of CLPs in promoting/mediating allergic airway inflammation, Ym1 is inhibited in ovalbumin (OVA)-challenged airways that have a pre-existing helminth infection (Fig. 4).

#### Chitinase-like proteins: lectins and ligands

Although CLPs lack enzymatic activity, most contain a chitin-binding domain, similar to chitinase proteins. Indeed the human CLPs (YKL-39 and YKL-40) have been

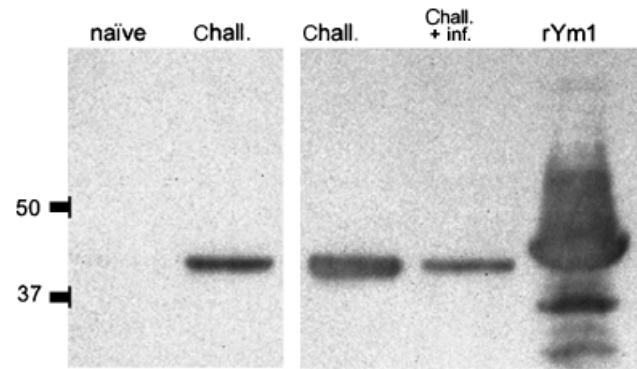


Fig. 4. Western blot with anti-Ym1 peptide antibody. Ym1 expression is reduced in allergic animals with concurrent helminth infection. Data are representative of four to five mice per group. (a) Ym1 is secreted in the BALF in mice sensitized and challenged with Der p 1 in a model acute allergic airway inflammation. (b) Infection with the intestinal nematode *Heligmosomoides polygyrus* reduces Ym1 secretion in Der p 1-challenged mice. rYM1 is recombinant Ym1 protein expressed in *Escherichia coli* (see [86] for methods).

demonstrated to tightly bind chitin particles within their inactive catalytic domain [90]. Surprisingly, no experimental studies have tested the chitin-binding ability of Ym1. Early computational modelling of the Ym1 crystal structure proposed that a monoglucosamine was bound within the inactive catalytic domain [91]. However, generation of a refined high-resolution crystal structure of Ym1 suggests that the site, thought to be a saccharide-binding site, may have been mistaken for an electron density cloud generated by a water cluster [92]. In addition, monitoring tryptophan fluorescence following incubation of Ym1 with glucosamine or *N*-acetylglucosaminidase oligomers revealed no significant structural changes to the protein. Based on these studies and in the absence of any experimental data, it is unlikely that Ym1 binds chitin within the inactive catalytic domain. Regardless of computational modelling, purified Ym1 protein has been demonstrated to bind heparin and heparan sulphates [15]. Heparin and heparan sulphate proteoglycans (HSPGs) are abundant on the cell surface. While HSPGs have long been thought of as non-specific protein ‘carriers’, it is now clear that HSPGs specifically interact and mediate protein–protein interactions. HSPGs, such as syndecan, play diverse roles serving to facilitate intracellular signalling, bind ECM proteins and can control cell proliferation, differentiation, adhesion and migration [93, 94] and are hence important regulators of wound repair. The best-studied example of HSPG interactions comes from the fibroblast growth factor family (FGFs). HSPGs are required for high-affinity binding of FGF to its receptor FGF-R1 and subsequent downstream signalling [95, 96]. Aside from experimental evidence demonstrating Ym1 binds heparin and HSPGs [15], nothing is known regarding their interaction. Advancement in the

knowledge of Ym1 function, particularly in conditions where ECM protein turnover and wound healing are prominent, will likely involve understanding the Ym1–HSPG interaction and whether HSPG facilitates downstream effects of Ym1 and possibly other CLPs.

Saccharide binding may be limited to a sub-class of CLPs and indeed other chitinase family members, such as human CLP 40-kDa mammary gland protein (MGP-40), do not appear able to bind saccharides due to conformational changes preventing the MGP-40 $\beta$ -barrel from accommodating carbohydrates [97]. Whether the inactive CLPs act to recognize and bind chitin containing pathogens, or whether they bind particles that contain a chitin motif, are questions yet to be resolved. What is apparent is that mammals do express abundant amounts of carbohydrate polymers that may bind to CLPs. Thus, it seems likely that although the historical function of these proteins was to bind chitin, they have now evolved to interact with host sugars.

It will be crucial to understanding the function of Ym1/2 and indeed other CLPs to determine the ligands to which they bind. It is not yet known whether the actions of Ym1/2 are facilitated through receptor binding. However, a receptor, stabilin-1 has been identified for human SI-CLP, for which stabilin-1 acts as an intracellular sorting receptor, delivering SI-CLP to CD63<sup>+</sup> lysosomes [98], a sub-population of secretory committed lysosomes, in human macrophages. While it would be exciting to speculate that stabilin-1 acts a receptor for all CLPs, there is no evidence of any other interactions, human or otherwise.

### Function of chitinase-like proteins

While the functions of Ym1/Ym2 and other CLPs remain unknown, several important findings point to a possible function in an immunological context, most strikingly the expression of these proteins during Th2-biased inflammation and repair. As mentioned above, Ym1/2 are greatly up-regulated in the lungs of allergic animals [23, 39], and are produced by the AAM $\phi$ s induced in allergic pulmonary inflammation. In lung infection models AAM $\phi$  are believed to resolve inflammation and induce wound healing by promoting the production of fibronectin and TGF- $\beta$ , ECM deposition, cell proliferation and angiogenesis [99]. The abundance of Ym1 produced by AAM $\phi$ s may reflect a requirement to bind widely expressed carbohydrate components of the ECM, but as yet this notion has not been experimentally tested.

The possibility that Ym1 is instrumental in the genesis of the Th2 response, rather than being a later-stage corollary of a Th2-dominated environment, is supported by two sets of data. Firstly, Ym1 is rapidly expressed after trauma, even in immune-deficient SCID or RAG<sup>-/-</sup> mice [26, 28]. Secondly, when Ym1 is induced in dendritic cells (DCs) by statin treatment, the ability of these DCs

to drive Th2 responses is abolished by anti-Ym1 antibody [100].

Recent reports have suggested that another murine CLP, breast regression protein-39 (BRP-39), a direct homologue of human YKL-40, is also increased upon allergic challenge in murine lungs [44, 45]. Importantly, these data parallel findings that YKL-40 is increased in both the serum and the lungs of asthmatic patients and its levels reflect disease severity [42, 101]. Moreover, a single-nucleotide polymorphism within the chitinase-3-like-1 gene (which encodes the protein YKL-40) is significantly associated both with serum YKL-40 levels and with overt asthma and AHR [43]. While some may raise questions over the relevance of studying Ym1 and Ym2 for human pathology, as the gene for Ym1/2 does not exist in humans, the totality of data on CLPs in allergic inflammation does support the proposition that CLPs in general, and quite possibly Ym1 and Ym2 in particular, play an important role in regulating disease. Considering the homology between all members of the CLP family, information learned about the function of Ym1/2 will aid research into understanding not only the role of CLPs in allergic inflammation and other diseases, but also general function of these proteins in the broader context.

To date, the most direct studies examining CLP function have explored the cellular effects of soluble YKL-40 protein *in vitro*. In this setting, YKL-40 is mitogenic, stimulating the proliferation of synovial fibroblast-like cells at sub-nanomolar concentrations [55], effects that are partially dependent on phosphorylation of mitogen-activated protein kinase (MAPK) and phospho-inositol-3 kinase (PI3K) pathways [102]. Additionally, YKL-40 attenuates IL-1 $\beta$ - and TNF- $\alpha$ -induced matrix metalloproteinase secretion and IL-8 secretion in skin fibroblast cells [103]. Fibroblasts are key cells at the forefront of regulating tissue remodelling events and the control of ECM turnover. Indications that YKL-40 acts directly on fibroblast cells to stimulate mitogenesis and induce cellular responses are suggestive of an active role in remodelling pathologies. Whether these cellular effects of YKL-40 are common to all CLPs is not known. However, the direct regulation of signalling cascade pathways and the rapid nature of MAPK and PI3K induction is suggestive of receptor-mediated actions, raising the question again as to whether CLPs, like SI-CLP, bind an as yet unknown receptor. Increased secretion of YKL-40 in patients with increasing asthma severity [42] indicates that the protein may drive matrix remodelling, a very important pathological feature of more chronic and severe asthma [104]. Considering the fibroblast mitogenic actions of YKL-40, it would be interesting to examine whether patients with increased YKL-40 have significant fibrosis in the airways as observed in chronic asthma patients. Similarly in a murine setting where airway wall remodelling can be monitored in models of chronic allergen exposure [105],

it will be important to examine whether the levels of Ym1/2 and BRP-39, and other chitinases and CLPs, remain up-regulated, and whether Ym1 influences the fibrotic process. It seems likely that Ym1 will contribute to fibrogenesis in chronic allergy and asthma considering its ability to bind HSPG [27] and that Ym1 is up-regulated in both silica- [65] and herpes virus-induced pulmonary fibrosis [66, 106].

While CLPs in mammals are currently associated with Th2 inflammation, their spectrum of activity is clearly linked to remodelling/repair and ECM turnover. Determining the differences between murine and human CLPs and discovering strategies that interfere with their function and/or production will lead the way for a better understanding of the pathology of Th2-driven conditions.

### Chitinase proteins in allergy

Zhu et al. [4] were the first to implicate an active chitinase protein, AMCCase, in allergic inflammation, making way for a new area of chitinase and CLP research. An acute murine model of OVA allergen challenge demonstrated that AMCCase was induced in the lungs, both in BAL fluid and lung tissue, under conditions of Th2 but not Th1 polarization. Further experiments with IL-4- and IL-13-deficient mice revealed that induction of AMCCase was dependent on these cytokines [4]. Interestingly, treatment of IL-13 overexpressing mice with anti-AMCCase serum resulted in diminished expression of chemokines normally induced by IL-13, suggesting that AMCCase may be an intermediary in the chemokine induction pathway. Consistent with this, epithelial cell transfection with recombinant AMCCase induced eotaxin and monocyte chemoattractant protein-1 to a similar extent as seen with IL-13 treatment, further supporting the notion that chemokine genes are a key target of AMCCase [4].

AMCCase is expressed by epithelial cells and alveolar macrophages, both key cell types in regulating allergen-induced inflammation and asthma in the lungs [107, 108]. Common genetic variants of human AMCCase have been identified and include a polymorphism encoding the region preceding the catalytic domain, potentially influencing chitinase activity [36]. This particular AMCCase variant showed a possible association with asthma in a cohort of paediatric patients. It will be of considerable importance to understand if such mutations confer a functional phenotype on AMCCase, in addition to clarifying its role in allergy and asthma.

Chitotriosidase, in contrast to AMCCase, is expressed exclusively in phagocytes [107]. While there is a basal expression level of chitotriosidase in the lung, neither protein nor mRNA expression is altered upon allergic challenge, making chitotriosidase unlikely to play an exacerbating role in allergy and asthma [109]. Furthermore, genetic mutations of chitotriosidase have been

investigated in a cohort of asthmatic patients, but no causal relationship was identified [110]. If chitotriosidase is not involved in allergies, it may nevertheless be important in other Th2 dominated pathologies and in host defence as described in the previous section. Further instances of chitinase expression in allergy have been shown in ocular allergies, which are generally considered Th2 manifestations [111], although Th1 cytokines may also contribute to the allergic pathology [112]. Patients with allergic ocular pathologies such as conjunctivitis have an increased level of chitinase activity in tear fluid and increased epithelial expression of AMCCase [37]. The increase of AMCCase expression and activity is also observed in a rabbit model of uveitis [113]. While expression of AMCCase has only been demonstrated in epithelial cells from ocular allergic patients, it will be interesting to determine whether resident macrophages similarly have up-regulated levels of AMCCase, or indeed other chitinases and CLPs.

### Implications for targeting chitinases in the treatment of allergy and asthma

The increase in AMCCase expression and chitinase activity in Th2 allergies may indicate that AMCCase is acting to exacerbate allergic reactions, possibly through chemokine induction. It is therefore not surprising that AMCCase has been the target for developing allergy and asthma therapeutics. Chitinase inhibitors are not a new class of drug, having been exploited in the agricultural and food industry for many decades. The use of chitinase inhibitors as mammalian therapeutic drugs is, however, more recent, following on from the studies implicating chitinases in infection and allergy. One natural inhibitor of chitinase enzymatic activity is allosamidin, a pseudo-trisaccharide from *Streptomyces* now being developed as a therapeutic agent [114]. Allosamidin, and other chitinase inhibitors, were initially proposed as potential bio-pesticides, as such agents inhibited growth of mites and housefly larva [114]. Allosamidin had earlier been used in animals as an anti-malarial drug, displaying an inhibitory effect on both human and avian malaria parasite transmission from the mosquito midgut [115]. However, these results are not supported by another study, where allosamidin failed to inhibit chitinase activity from the rodent parasite *Plasmodium berghei* [116]. This lack of effect was thought to reflect divergence among malaria parasite chitinases, suggesting allosamidin does not inhibit every chitinase enzyme. Allosamidin has now gained further interest as a potential treatment for allergy and asthma with promising initial studies driving the development of novel chitinase inhibitors [117].

Allosamidin, when administered to allergen-challenged mice, resulted in a dose-dependent decrease of eosinophils and lymphocytes recruited into the BALF and of BALF

chitinase activity [4]. Use of AMCase anti-sera in the same study had more extensive effects: in addition to reduced cell recruitment and chitinase activity, antibody neutralization lowered AHR and decreased expression of IL-13-induced chemotactic factors. Thus, if the effects of allosamidin are solely due to a loss of enzymatic activity, it would appear that much of the inflammatory properties of AMCase reside outside the catalytic site. Additionally, as the parameters of AHR and IL-13-induced chemotactic factors were not reported in allosamidin-treated animals, it is not clear whether inhibition of chitinase activity is sufficient to impact on airway physiology, airway histology and Th2 cytokine induction.

Overall such studies highlight AMCase as a primary target for the treatment of allergies and asthma, and have led to a search for novel chitinase inhibitors that are less expensive and easier to synthesize than allosamidin [117–119]. Nevertheless, more extensive research into chitinase enzymes needs to take place before we can truly define AMCase as a therapeutic target. When considering the actions of AMCase and other enzymatically active chitinases in mammalian biology it is likely that complete inhibition of chitinase enzymatic activity with a non-specific chitinase inhibitor, will result in adverse actions. Thus the therapeutic value of inhibitors needs to be considered in the context of potential disadvantages of preventing chitin breakdown, as discussed below.

### Considerations for the therapeutic inhibition of chitinase activity

Chitinase inhibitors, such as allosamidin, are thought to inhibit enzymatic activity by mimicking the enzyme reaction intermediate and hence bind tightly in the catalytic domain [118]. Allosamidin binds to a broad range of chitinase proteins from varying species, inhibiting human chitotriosidase and murine AMCase with IC<sub>50</sub> values 40 and 400 nM, respectively, [120, 121]. The broad spectrum of activity of allosamidin, and indeed other peptide-based chitinase inhibitors [119], do raise some concerns over potential therapeutic use. Aside from the fact that chitinases are induced under Th2-biased pathological conditions, chitinases also form an important host defence mechanism against chitin-containing pathogens and chitin inhalation by mammals, as discussed earlier in this review. While the use of allosamidin has demonstrated benefits at reducing inflammatory cell influx in a murine model of allergic inflammation, non-specific inhibition of chitinase activity may result in greater susceptibility to fungal pathogens, chitin-containing parasites and allergic reactions against inhaled chitin. These concerns have been heightened with recent studies that showed chitin administration results in the accumulation of inflammatory cells to the lung [77, 122].

However, there does seem to be a divergence of opinion concerning the effects of chitin as an allergen, with some groups suggesting chitin alleviates allergic inflammation in mouse models of allergy [123]. Understanding more fully the interaction of chitin with the mammalian immune system will be key to driving therapeutic development of chitinase inhibitors.

### Is chitin a mammalian allergen?

An obvious and important question regarding the therapeutic use of generic chitinase inhibitors in allergy and asthma is whether chitinases are deleterious or advantageous in the setting of allergic inflammation. This critical issue remains controversial, with limited experimental evidence to support either notion. In the classical model of OVA-induced allergic inflammation, the induction of chitinase AMCase was found to potentiate the disease process, as inhibition of chitinase activity or protein levels attenuates inflammation and AHR [4]. To the contrary, a beneficial effect of chitinase expression has been suggested by data showing that AMCase treatment of chitin ablates the ability of chitin to recruit eosinophils and basophils into the lungs [77]. Thus, within the overall equation of the role of chitin, we must consider whether chitin can act directly as an allergen.

Recent results do suggest that chitin is indeed a mammalian allergen. Administration of chitin into the lungs resulted in eosinophil, basophil and neutrophil accumulation in the BALF and lung, and induced alternative activation of murine macrophages [77, 122, 124]. Chitin also stimulated the production of IL-17 and IL-17A receptor expression through a MyD88- and TLR2-dependent pathway [122], with IL-17 a leading mechanistic explanation for the acute inflammation observed in the lungs of mice following chitin administration. Again, however, there is controversy because earlier studies had demonstrated that chitin was anti-allergenic, reducing allergen-induced increases in BALF eosinophils and lymphocytes, IgE levels, Th2 cytokines IL-4 and IL-5, goblet cell hyperplasia and subepithelial fibrosis [123, 125, 126]. The down-regulatory effects of chitin on the allergic response may be partially due to the induction of Th1 cytokines [125, 127]. The stark differences in the effects of chitin may reflect dose regime and chitin preparation, but importantly, the studies examining chitin in allergen-induced models [125, 127] did not examine the effects of chitin in naïve mice, in which chitin has been demonstrated to cause Th2 polarization [77]. Chitin may induce a Th1 response in allergic animals to antagonize an already primed Th2 response. It is thus possible that an increase in chitin due to chitinase inhibition may not result in adverse effects in individuals already allergic. There are currently no clear answers to the questions raised. Therefore, it is essential that studies be designed to mimic the potential

allergic-induction by chitin following chitinase inhibition.

An approach to reducing the impact of chitinases and CLPs on the development of allergy and asthma while maintaining chitin degradation, has yet to be addressed, but may involve targeting the function or expression of the non-enzymatic CLP molecules. CLPs are up-regulated in both human patients and murine models of allergy and asthma and may be more important in allergy and asthma than active chitinases as mice constitutively overexpressing AMCcase in the lungs displayed no apparent histological changes [77]. Therefore, designing inhibitors, blocking agents or antagonists of CLP function may be an exciting approach that would essentially leave chitinase activity intact, enabling mammals to maintain the host defence mechanisms to degrade chitin. The design of CLP inhibitors will require extensive research into protein function and the mechanisms by which these proteins act, but it is an approach that should be considered.

## Conclusions

Enzymatically active chitinase proteins and inactive CLPs are highly prominent in mammalian allergy, asthma and other Th2 driven pathologies, and their recent evolutionary diversification indicates that they play crucial functional roles in the pathogenesis and resolution. The critical questions for the immediate future will be whether the expression of these proteins are a cause, or a consequence, of inflammation and the restorative response to injury. Clearly, we have just skimmed the surface of chitinase and CLP research. As new CLPs and chitinases are being identified, such as murine BYm – a protein homologous to Ym1 and Ym2 [8], and new genetic constructs of gene deletion and forced expression become available, our understanding will be enhanced. To date, inhibition of chitinase enzymatic activity has proved to be successful in alleviating the responses to aeroallergens. However, chitinase inhibition raises concerns over the effects of eliminating the host defence mechanisms available to degrade chitin, particularly in light of studies suggesting chitin can induce the accumulation of innate cells in the lung. Other approaches of developing specific inhibitors of AMCcase enzymatic activity, leaving chitotriosidase activity intact, or developing therapeutic agents that inhibit the function of CLPs, should be addressed in the future.

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