Chapter 11
Human Chitinases: Structure, Function, and Inhibitor Discovery

Ashutosh Kumar and Kam Y. J. Zhang

Abstract Chitinases are glycosyl hydrolases that hydrolyze the β-(1-4)-linkage of N-acetyl-D-glucosamine units present in chitin polymers. Chitinases are widely distributed enzymes and are present in a wide range of organisms including insects, plants, bacteria, fungi, and mammals. These enzymes play key roles in immunity, nutrition, pathogenicity, and arthropod molting. Humans express two chitinases, chitotriosidase 1 (CHIT1) and acid mammalian chitinase (AMCase) along with several chitinase-like proteins (CLPs). Human chitinases are reported to play a protective role against chitin-containing pathogens through their capability to degrade chitin present in the cell wall of pathogens. Now, human chitinases are gaining attention as the key players in innate immune response. Although the exact mechanism of their role in immune response is not known, studies in recent years begin to relate chitin recognition and degradation with the activation of signaling pathways involved in inflammation. The roles of both CHIT1 and AMCase in the development of various diseases have been revealed and several classes of inhibitors have been developed. However, a clear understanding could not be established due to complexities in the design of the right experiment for studying the role of human chitinase in various diseases. In this chapter, we will first outline the structural features of CHIT1 and AMcase. We will then review the progress in understanding the role of human chitinases in the development of various diseases. Finally, we will summarize the inhibitor discovery efforts targeting both CHIT1 and AMCase.

Keywords Chitin · Chitinase · Chitotriosidase 1 · Acid mammalian chitinase · Inflammation · Inhibitors

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11.1 Introduction

Chitin is a polymer found in nature where β-(1-4)-linked N-acetyl-D-glucosamine (GlcNAc) units are linearly bonded together to form long chains. In chitin, N,N′-diacetylchitobiose $(GlcNAc)_2$ forms the structural subunit (Fig. 11.1). Chitin, unlike starch and glycogen that are storage polysaccharide, is a structural polysaccharide. It is the second most abundant naturally occurring polymer and is the main structural component of arthropod exoskeleton (Neville et al. 1976), crustacean shells, house dust mites (HDM), fungal cell walls (Lenardon et al. 2010) and microfilarial sheath of parasitic nematodes (Veronico et al. 2001; Foster et al. 2005). In fungal cell walls, chitin is the key structural polymer and is equivalent to peptidoglycan in bacteria. Chitin provides rigidity and structural integrity to cells, tissues and body surfaces.

X-ray diffraction analysis showed that chitin exists in three different crystalline forms, namely, α-chitin, β-chitin, and γ-chitin based on the arrangement of chitin polysaccharide chains in crystalline fibers (Rudall and Kenchington 1973; Kramer and Koga 1986; Imai et al. 2003; Rinaudo 2006) (Fig. 11.1). In addition, a non-crystalline form has also been reported in fungi (Vermeulen and Wessels 1986). Among these three forms, α-chitin is the most abundant and compact form where chitin chains are arranged in an antiparallel manner (Fig. 11.1). This type of arrangement ensures strong hydrogen bonding (Kramer and Koga 1986). The α-chitin is a major structural component of arthropod exoskeleton and fungal cell

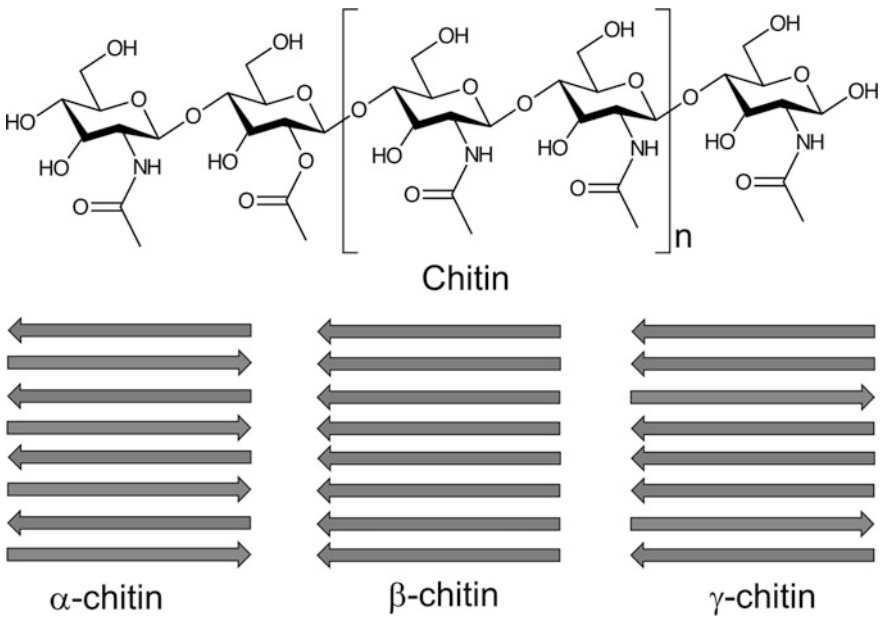


Fig. 11.1 Chitin and its crystalline forms

walls. It is also found in shells of crustaceans like shrimps and crabs. Antiparallel arrangement of chitin chains in α-chitin ensures mechanical strength and stability (Giraud-Guille and Bouligand 1986). Contrary to α-chitin, chitin chains are arranged in a parallel manner in β-chitin resulting in a less compact form due to weak intermolecular forces (Fig. 11.1). Inter-chain hydrogen bonding contacts are reduced which results in a more flexible and soft structure due to a high degree of hydration and reduced packing. β-chitin is commonly found in squid pens, peritrophic matrices and cocoons. Another chitin form, γ-chitin is the mixture of both α-chitin and β-chitin chains and is commonly found in mushrooms (Fig. 11.1). Interestingly, some animals utilize multiple forms of chitin for different biological functions, for example, squid possess α-chitin in its beak, β-chitin in its pen and γ-chitin in the stomach lining (Gooday 1990).

In chitin-containing organism, chitin is produced by a highly conserved enzyme, chitin synthase (EC 2.4.1.16). Chitin synthases belong to GT2 family of polymerizing glycosyltransferases including synthases for polymers such as cellulose, mannan, hyaluronate, etc. Chitin synthases add GlcNAc units to the nonreducing end of chitin chains. It utilizes UDP-N-acetylglucosamine (UDP-GlcNAc) as sugar donor to form a polymer of chitin (Glaser and Brown 1957). Linear polymers are first produced which are then assembled into microfibrils of various length and diameter (Imai et al. 2003; Merzendorfer 2006). These microfibrils are then extruded from cell walls in a similar manner as cellulose (Morgan et al. 2012; Gonçalves et al. 2016). Chitin biosynthesis is extensively studied; however, the detailed description is beyond the scope of this book chapter. Some reviews may be referred for chitin synthases and biosynthesis (Merzendorfer and Zimoch 2003; Merzendorfer 2006, 2011). The focus of this book chapter will be another group of enzymes which is responsible for chitin degradation.

11.2 Chitinase and Their Classification

Despite being one of the most abundant polymers, chitin doesn't accumulate in environment due to the presence of chitinolytic enzymes known as chitinases which hydrolyze β-(1-4)-linkage of GlcNAc present in chitin chains. Chitinases are widely distributed enzymes and are present in many organisms including insects, plants, bacteria, fungi, and mammals. These enzymes play roles in immunity and defense, nutrient acquisition, digestion, pathogenicity, and arthropod molting. Chitinases belong to the glycosyl hydrolase (GH) family containing a wide diversity of proteins. Based on sequence similarity, chitinases are classified into five classes (class I–V) (Hamid et al. 2013). Class I chitinases have most conserved catalytic domain with a cysteine-rich domain at N-terminus. Class II chitinases possess high similarity with catalytic domain of class I chitinases but lack cysteine-rich domain at N-terminus. Class III chitinases do not share any identity with class I and II. Class IV chitinases are similar to class I but are much smaller compared to class I

due to four deletions in catalytic domain. Class V chitinases lack sequence identity with any other class.

Based on sequence identity, structural homology and biochemical studies, chitinases are also classified into family GH18 chitinase, family GH19 chitinase (mostly plant chitinase) and family GH20 N-acetyl glucosaminidase (Fukamizo 2000). Chitinases of family GH18 and GH19 have very low sequence similarity with each other and have completely different structures. Chitinases of class III and V form family GH18 while class I, II, and IV belong to family GH19. Family GH18 chitinases hydrolyze chitin into shorter $(GlcNAc)_2$ and $(GlcNAc)_3$ fragments while β-N-acetyl-D-hexosaminidases hydrolyze these oligosaccharides into GlcNAc. Family GH18 can be further divided into chitinase and enzymatically inactive chitinase-like-lectins or chitinase-like proteins. The domain architectures and structural features of family GH18 chitinases are highly conserved (Huang et al. 2012) and consist of a catalytic domain and a cysteine-rich carbohydrate or chitin binding domain. A hinge connects the catalytic domain with carbohydrate binding domain. The catalytic domain is mostly located at the N-terminus while chitin binding domain is located at C-terminus. The catalytic domain of members of GH family is known to adopt triosephosphate isomerase (TIM) fold. TIM fold consists of a highly conserved $(\beta/\alpha)_8$-barrel structure in which β-sheets are surrounded by α-helices (Henrissat 1999; Stam et al. 2005). The active site is highly conserved and composed of aromatic amino acid residues that help in substrate binding (Perrakis et al. 1994; van Aalten et al. 2000; Boot et al. 2001; Fusetti et al. 2002; Olland et al. 2009; Fadel et al. 2015). Substrate binding occurs at a cleft in the center of $(\beta/\alpha)_8$-barrel. Substrate binding sites in GH18 chitinases employ –n to +n nomenclature where n represents GlcNAc binding subsites (Davies et al. 1997). Subsites –n are located toward the nonreducing end while +n subsites are located at the reducing end of the substrate relative to substrate cleavage site. The cleavage of chitin substrate molecule occurs between −1 and +1 GlcNAc binding subsites (Davies et al. 1997). The catalytic triad lies at the bottom of active site at −1 subsite. A conserved sequence motif in the β_4 strand (DXXDXDXE, where D stands for aspartic acid, E stands for glutamic acid, and X represents any amino acid) form the active site pocket. Glutamic acid and central aspartic acid in the conserved sequence motif are the key amino acid residues in catalysis. Glutamic acid donates a proton required for hydrolyzing the β-(1-4) glycosidic bond in chitin (Chou et al. 2006) while the second aspartate in the motif contributes to stabilization of substrate molecule during catalysis (McCarter and Stephen Withers 1994; Perrakis et al. 1994; van Aalten et al. 2000, 2001; Fusetti et al. 2002). Family GH18 chitinases make use of substrate-assisted reaction mechanism (Terwisscha van Scheltinga et al. 1995; van Aalten et al. 2001) whereas family GH19 chitinases employ fold-and-reaction mechanism (Monzingo et al. 1996) suggesting independent evolution of these two families. Family GH18 includes chitin hydrolyzing enzymes from bacteria, fungi, viruses, animals, and some chitinases from plants (class III and V) (Ohno et al. 1996). Family GH19 contains chitinases mostly from plants (class I, II, and IV) and nematodes.

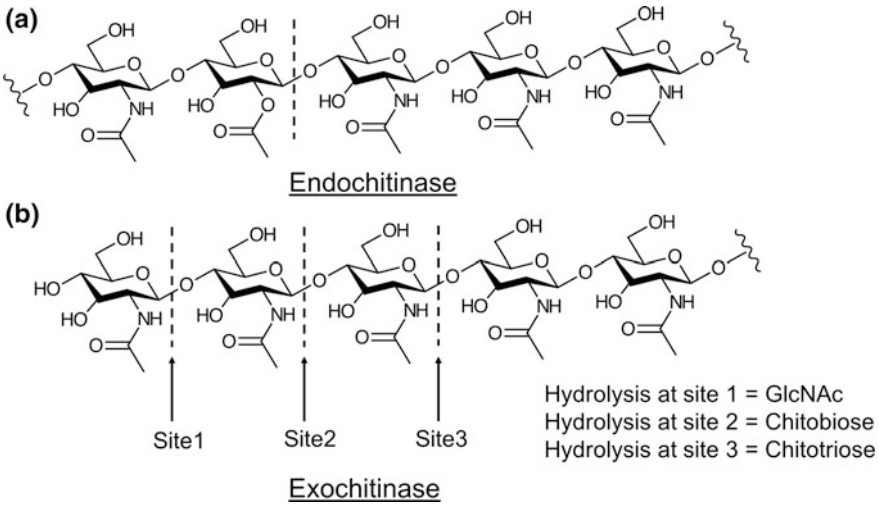


Fig. 11.2 Chitin hydrolysis by chitinase types **a** Endochitinase and **b** Exochitinase

Chitinase can be further classified based on their mode of cleavage into two classes: (a) endochitinase and (b) exochitinase. Endochitinases (EC 3.2.1.14) catalyze the hydrolysis of internal chitin chains from inner random position generating low oligomeric units of GlcNAc such as chitotriose, chitobiose, and diacetylchitobiose (Fig. 11.2a). Exochitinases (EC 3.2.1.52) cleave from the nonreducing end of chitin chains (Nikolov et al. 2010) (Fig. 11.2b). Exochitinases (EC 3.2.1.52) have been further classified into two categories, namely, chitobiosidases and β-N-acetyl-D-hexosaminidases. Chitobiosidases (EC 3.2.1.29) catalyze the release of diacetylchitobiose from the nonreducing end of chitin chains while β-N-acetyl-D-hexosaminidases cleave oligomeric GlcNAc units produced by endochitinases into GlcNAc (Fig. 11.2b).

11.3 Human Chitinases

Although chitin is neither present nor utilized as a nutrient source in humans, yet they express two chitinases: acidic mammalian chitinase (AMCase or CHIA) and chitotriosidase 1 (CHIT1) (Hollak et al. 1994; Boot et al. 1995, 2001). Both of these enzymes function as endochitinases and are members of GH18 chitinase family. These two proteins are recognized as true chitinases as they are the only ones that possess chitin hydrolyzing activity (Boot et al. 2001). In addition, structurally related chitinase-like proteins (CLPs) or chitinase-like-lectins (Chi-lectins) are expressed such as chitinase 3-like-1 (CHI3L1 or YKL-40), chitinase 3-like-2 (CHI3L2 or YKL-39), chitinase domain–containing 1 (CHID1), stabilin-1-interacting chitinase-like protein (SI-CLP) (Kzhyshkowska et al. 2006) and

oviductal glycoprotein 1 (OVGP1) (Bussink et al. 2007). Moreover, Ym1 (Chang et al. 2001) and Ym2 are only found in mouse and are produced by macrophages after fungal or parasitic infection. These proteins are catalytically inactive due to the substitutions in active site residues critical for catalysis, yet they retain the TIM-barrel structure and active site carbohydrate binding activity (Renkema et al. 1998; Houston et al. 2003). Most mammalian chitinases are members of GH18 family (Li and Greene 2010) and adopt TIM fold consisting of $(\beta/\alpha)_8$-barrel structure (Stam et al. 2005; Lombard et al. 2014).

CHIT1 is the first chitinase discovered in human (Boot et al. 1995) and is found in the genomes of all mammals (Hollak et al. 1994; Boot et al. 1995). CHIT1 is expressed in a variety of tissues such as lung, spleen, liver, thymus, and lacrimal gland (Ohno et al. 2013). Among the cells, CHIT1 is mainly expressed in innate immune cells such as activated macrophages (Hollak et al. 1994; Rao et al. 2003) and neutrophils (Malaguarnera et al. 2006). CHIT1 is expressed by macrophages and neutrophils in response to various pro-inflammatory signals. AMCase is the second chitinase identified in human that is able to degrade chitinous substrates (Boot et al. 2001, 2005). AMCase is mainly expressed in the stomach and gastrointestinal tract. AMCase has been also detected in the lung where it is secreted into the airway lumen by epithelial cells, club cells, and type 2 alveolar cells. AMCase has been reported as the major endochitinase in airway fluid (Fitz et al. 2012; Van Dyken et al. 2017). The presence of AMCase in the stomach and gastrointestinal tract suggests the role of AMCase in digestion (Boot et al. 2001, 2005; Chou et al. 2006; Ohno et al. 2012) for breaking chitin-containing food while the expression in lung implicates its role in defense against chitin-containing pathogens.

AMCase gene is located on 1q13.1-21.3 chromosome and consists of 12 exons (Boot et al. 2001). CHIT1, on the other hand, is located on 1q31-32 chromosome and also contains 12 exons. The location of both genes in chromosome 1, sequence and structural similarities and intron–exon boundary conservation suggest that these genes might have arisen due to gene duplication (Boot et al. 2001) which is believed to have taken place during the emergence of jawed and jawless fish (Hussain and Wilson 2013). Further loss of function mutations and duplications might have given rise to CLPs. Some of these CLPs are species-specific while others are found in all mammals (Hussain and Wilson 2013). Both AMCase and CHIT1 are secretory proteins with approximately 50 kD molecular weight (Boot et al. 1995, 2001). AMCase has about 51% sequence identity and 66% sequence similarity with CHIT1. AMCase like other GH18 family members contains both chitin hydrolysis and chitin binding domain. These two domains are connected to each other by a short hinge region (Renkema et al. 1997). Despite both sequence and structural similarity, these two proteins differ in their enzymatic behavior at acidic pH. AMCase is extremely stable at acidic pH and displays an optimum activity at pH 2 (Boot et al. 2001). The name AMCase is derived from this property of the enzyme. CHIT1, on the other hand, shows an optimum around pH 5 (Renkema et al. 1995; Zheng et al. 2005) and is inactive at low pH (Boot et al. 2001, 2005).

CHIT1 is found to exist in two isoforms: a 39 kDa isoform containing the catalytic domain only and a 50 kDa isoform containing the full-length CHIT1. The full-length CHIT1 is composed of a large catalytic domain connected to a comparatively small chitin binding domain (Fig. 11.3a). The two domains are linked by a proline-rich hinge. Initially, several crystal structures of the catalytic domain alone were solved both in apo form as well in complex with CHIT1 inhibitors (Fusetti et al. 2002; Rao et al. 2005a; Fadel et al. 2015) including a 1.0 Å apo structure of CHIT1 (Fadel et al. 2015). The catalytic domain structure of CHIT1 consists of a core $(\beta/\alpha)_8$-barrel domain which is similar to AMCase and other family GH18 chitinases. CHIT1 structure contains a DXXDXDXE motif at the end of fourth β sheet with Glu140 as catalytic residue. An additional α/β domain is present in between the seventh α-helix and the seventh β-strand. This additional domain consists of six β-sheets and one α-helix and gives a groove like character to the

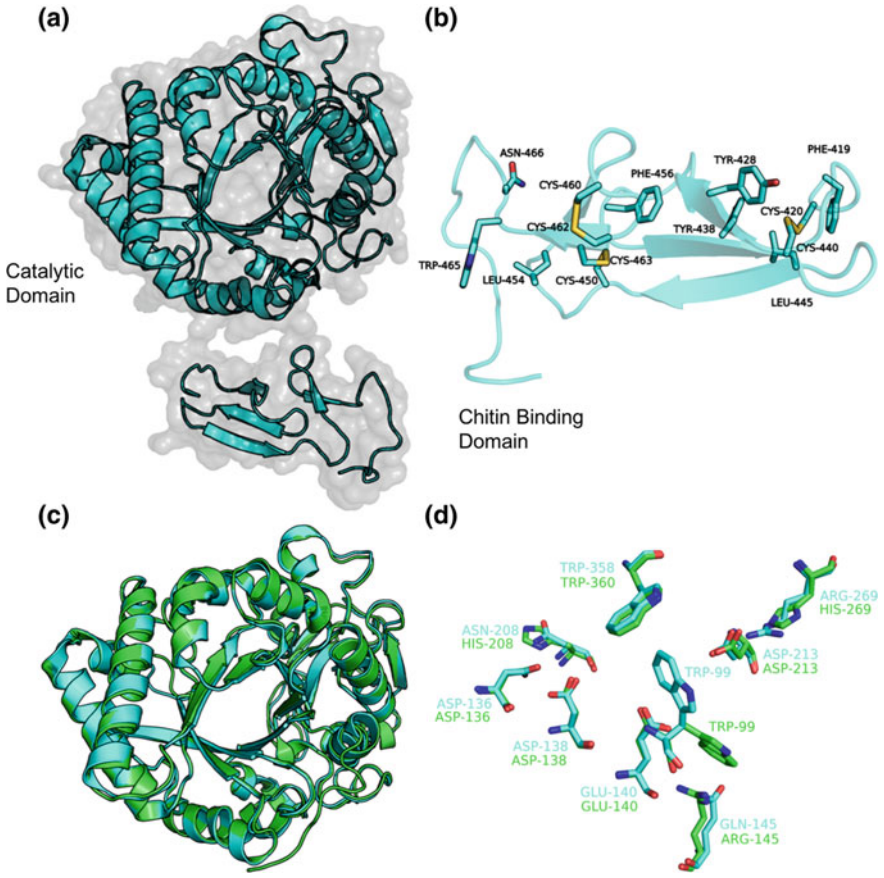


Fig. 11.3 **a** A cartoon representation of full-length CHIT1 showing catalytic and chitin binding domain. **b** CHIT1 chitin binding domain. **c** Superposition of AMCase (green) with CHIT1 (cyan). **d** Active site amino acid residues of AMCase (green) and CHIT1 (cyan)

active site (Fusetti et al. 2002). CHIT1 active site is lined by several aromatic amino acids that stack against the hydrophobic face of sugar units. The first stretch of aromatic amino acid residues lies along the −6 to −1 subsites and includes Trp31, Tyr34, Trp71 and Trp358. The second stretch of aromatic residues is located at +1 and +2 subsites and includes Trp99 and Trp218. These residues are not only identical in both CHIT1 and AMCase but also highly conserved in other family GH18 chitinases. Trp358 is especially important as it is stacked with −1 subsite GlcNAc and is responsible for accommodating −1 subsite GlcNAc in boat conformation critical for catalysis. Several studies revealed that the mutation of Trp358 abolished chitinase activity (van Aalten et al. 2000; Fusetti et al. 2002; Songsiriritthigul et al. 2008; Yang et al. 2010).

The mechanism of substrate hydrolysis in CHIT1 is similar to other GH18 family enzymes and has been recently elucidated utilizing X-ray crystallography and QM/MM studies (van Aalten et al. 2001; Fadel et al. 2015). The cleavage occurs between −1 and +1 subsites. The catalytic triad consisting of Asp136, Asp138 and Glu140 amino acid residues of DXXDXDXE motif is located at the bottom of −1 subsite. Binding of substrate causes displacement of active site water molecules and the transfer of a proton from Asp138 to Glu140. This event is followed by the distortion of pyranose ring to a boat conformation and protonation of scissile O atom of glycosidic bond by Glu140 which subsequently leads to the formation of oxazolinium ion intermediate. An aglycon sugar is next displaced allowing a water molecule to access the active site. At the same time, Asp138 receives a proton from Asp136 and turns toward Glu140 which in turn forms a hydrogen bond with N-acetyl nitrogen of −1 GlcNAc. A water molecule after being activated by Asp213 probably carries out nucleophilic attack on the anomeric carbon atom of oxazolinium ion intermediate leading to the formation of −1 GlcNAc in its original configuration (Fadel et al. 2015).

About two years ago, a full-length structure of CHIT1 was reported that shed light on the structural features of chitin-binding domain (Fadel et al. 2016) (Fig. 11.3a). The catalytic domain in the full-length CHIT1 structure is essentially the same as other CHIT1 catalytic domain structures (Fusetti et al. 2002; Rao et al. 2005a; Fadel et al. 2015). The chitin binding domain in CHIT1 belongs to CBM14 family and is structurally different from bacterial and plant chitin-binding domains (Ikegami et al. 2000; Akagi et al. 2006; Fadel et al. 2016). CHIT1 chitin binding domain is elongated in structure as compared to the globular chitin-binding domain of bacteria and plants. It is composed of a distorted β-sandwich fold containing three N-terminal antiparallel β-strands and two C-terminal antiparallel β-strands (Fadel et al. 2016). CHIT1 chitin binding domain is connected with catalytic domain by a stretch of proline-rich residues. The main difference between the full-length CHIT1 and other bacterial chitinases lies in the arrangement of chitin binding domain relative to catalytic domain. In S. marcescens chitinases, ChiA and ChiB, the chitin-binding domain is rigidly connected to the catalytic domain resulting in a completely different orientation of chitin binding domain relative to

catalytic domain (Perrakis et al. 1994; van Aalten et al. 2000). Chitin substrate interacts with chitin binding domain via a stretch of aromatic amino acid residues (Fig. 11.3b). Most of these residues are surface-exposed, especially a conserved residue Trp465 enabling strong binding with chitin substrates (Fadel et al. 2016). The active site cleft in CHIT1 contains nine GlcNAc binding sites (from −6 to +3 subsites). CHIT1 was initially believed to be an exochitinase as it has been shown to hydrolyze chitotriose, however, structural and biochemical studies demonstrated CHIT1 as an endochitinase (Fusetti et al. 2002; Kuusk et al. 2017).

The AMCase catalytic domain crystal structure was solved in apo form as well as in complex with several inhibitors including allosamidin derivative methylallosamidin (Olland et al. 2009), bisdionin C and F (Sutherland et al. 2011) and many hits from in silico, high-throughput and fragment screening (Cole et al. 2010). No structure of full-length AMCase is available till date. Catalytic domain of AMCase possesses about 57% identity with CHIT1 and it very similar to CHIT1 catalytic domain crystal structure (Fusetti et al. 2002; Rao et al. 2003, 2005b; Fadel et al. 2015, 2016) consisting of a $(\beta/\alpha)_8$-barrel core (Fig. 11.3c). The most notable difference between the structures of AMCase and CHIT1 is the three residues near the active site (Arg145, His208, and His269). Two of these residues His208 and His269 are different in CHIT1 but are conserved in human AMCase and AMcase of other species (Bussink et al. 2007) (Fig. 11.3d). These three residues make contacts with all the conserved residues within the active site and are proposed to change the pH optimum of AMCase by influencing the pK_a of the catalytic residues Asp138 and Glu140 (Olland et al. 2009). Amino acid residue His269 is Arg269 in CHIT1 and it may be responsible for higher negative charge of AMCase active site lowering down its pH optimum (Fig. 11.3d).

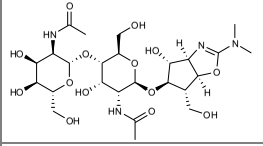
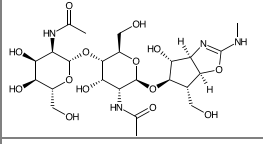
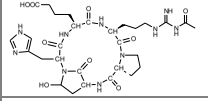
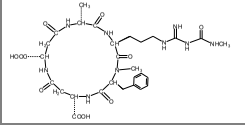
CLPs, such as YKL-40, YKL-39, CHID1, SI-CLP, OVGP1, Ym1, Ym2, are a diverse set of proteins expressed in species-specific manner. In humans and mice, they are expressed in immune and structural cells such as macrophages, neutrophils, epithelial cells, dendritic cells and chondrocytes (Sutherland 2018). CLPs are structurally related to GH18 family chitinases including both human chitinases, AMCase and CHIT1. CLPs structure consists of a 39 kDa TIM-barrel like fold similar to the catalytic domain of active chitinases (Boot et al. 2001; Fusetti et al. 2002). However, most CLPs lack chitin binding domain. Crystal structures of several human or mouse CLPs are solved (Tsai et al. 2004; Meng et al. 2010; Schimpl et al. 2012; Ranok et al. 2015) which clearly illustrates that CLPs bind to chitin oligosaccharides. CLPs retain their ability to bind chitin oligosaccharides with high affinity, however, lack chitinase activity due to the substitution of a key catalytic glutamate in DXXDXDXE motif with either leucine, isoleucine or tryptophan (Lee et al. 2011). Furthermore, it has been shown that substitution of the catalytic residue back to the one in active chitinases recovered the chitinase activity of CLPs (Schimpl et al. 2012).

11.4 Role of Human Chitinases in Diseases

Chitinases are widely distributed in many organisms and play a myriad of biological roles. In bacteria and archaea, chitinases degrade chitin for nitrogen and carbon source (Gao et al. 2003; Bhattacharya et al. 2007). In fungi, chitinases are responsible for ensuring plasticity by remodeling chitin components of cell wall (Langner and Göhre 2016). In insects, chitinases are essential for molting and the growth of insects and are required for the breakdown of chitin components in cell walls (Arakane and Muthukrishnan 2010). Plant chitinases play a major role in dealing with environmental stress such as cold, drought, or salinity (Grover 2012). Mammalian chitinases serve as a protective mechanism against chitin-containing pathogens through their capability to degrade both colloidal chitin as well as chitin present in cell walls of pathogens (Boot et al. 2001). Apart from their protective role against pathogens through chitin degradation, mammalian chitinases are now gaining attention as the key players in innate immune response against fungi, bacteria, and other pathogens.

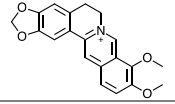
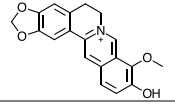
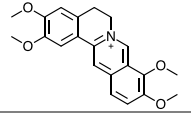
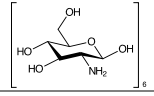
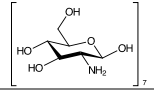
Several studies pointed out the involvement of two human chitinases, CHIT1 and AMCase, in inflammation-related diseases. Among these two active chitinases, AMCase was widely studied and reported to express at higher levels during type II inflammatory responses in several diseases in both allergic patients and murine models (Zhu et al. 2004; Shen et al. 2015). Chronic respiratory diseases such as asthma is the inflammation of airways. Asthma is characterized by the influx of eosinophils into the lung tissue, mucus metaplasia, hyper-responsiveness, and airflow obstruction. There is compelling evidence for the involvement of T-helper 2 (Th2) cells in asthma via the induction and maintenance of inflammation. Most evidences for the role of chitinases in asthma came from biomarker studies which suggested the involvement of AMCase. AMCase is highly expressed in the lung tissue of asthmatic patients and in animal models of asthma (Zhu et al. 2004; Bierbaum et al. 2005; Yang et al. 2009; Shen et al. 2015). Additionally, higher expression of AMCase has been found in the lung epithelium and alveolar macrophages of ovalbumin(OVA)-sensitized mice (Yang et al. 2009). Administration of AMCase antisera or chitinase inhibitor allosamidin reduced inflammatory cell in BAL fluid of OVA-sensitized mice and thereby alleviated asthma symptoms (Zhu et al. 2004). Moreover, it has been observed that the treatment of allergen-challenged mice with chitinase inhibitors, allosamidin or demethylallosamidin, significantly reduced eosinophilia, a hallmark of allergic inflammation (Matsumoto et al. 2009). Furthermore, the treatment of allergen-challenged mice with AMCase selective inhibitor Bisdionin F alleviated the primary features of allergic inflammation including eosinophilia (Sutherland et al. 2011). Additionally, the administration of compound **3** (Table 11.1), a highly potent AMCase inhibitor showed significant anti-inflammatory efficacy in HDM-induced allergic airway inflammation in mice (Mazur et al. 2018b). These studies suggest that inhibition of AMCase is a good strategy for the development of therapeutics against allergic airway inflammation-related diseases.

Table 11.1 A few chemical classes displaying inhibitory activities against human chitinases

Chemical class	Compound	Structure	Activity		Reference
			AMCase	CHIT1	
Allosamidine and its derivatives	Allosamidin		mAMCase IC_{50} = 400 nM	IC_{50} = 40 nM	Rao et al. (2003)
	Demethylallosamidin		NA	IC_{50} = 1.9 nM	
Cyclopentapeptides	Argadin		IC_{50} = 1.2 μM	IC_{50} = 0.013 μM	Rao et al. (2005b)
	Argifin		IC_{50} = 0.2 μM	IC_{50} = 4.5 μM	

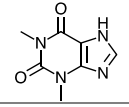
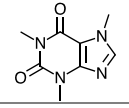
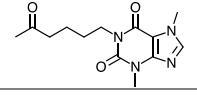
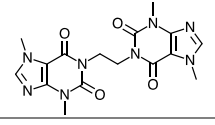
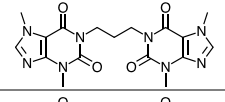
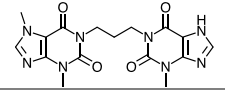
(continued)

Table 11.1 (continued)

Chemical class	Compound	Structure	Activity		Reference
			AMCase	CHIT1	
Other natural products	Berberine		K_i = 65 μM	K_i = 19 μM	Duan et al. (2018)
	Thalifendine		K_i = 55 μM	K_i = 15 μM	
	Palmatine		K_i = 70 μM	K_i = 15 μM	
Deacetylated chitooligosaccharides	$(GlcN)_6$		NA	IC_{50} = 69.5 ± 10.1 μM	Chen et al. (2014)
	$(GlcN)_7$		NA	IC_{50} = 37.8 ± 8.6 μM	

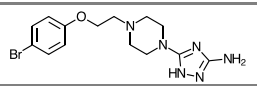
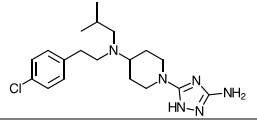
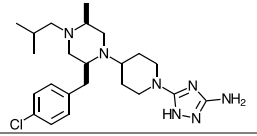
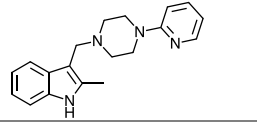
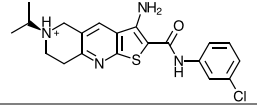
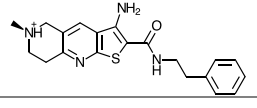
(continued)

Table 11.1 (continued)

Chemical class	Compound	Structure	Activity		Reference
			AMCase	CHIT1	
Methyl xanthine derivatives	Thiophylline		36% inhibition at 1 mM	IC_{50} = > 500 μM	Rao et al. (2005a)
	Caffeine		36% inhibition at 1 mM	IC_{50} = 257 ± 8 μM	
	Pentoxiphylline		49% inhibition at 1 mM	IC_{50} = 98 ± 8 μM	
Bisdionins	1 (Bisdionin B)		IC_{50} = 90 ± 4 μM	IC_{50} = 110 ± 10 μM	Schüttelkopf et al. (2006)
	Bisdionin C		IC_{50} = 3.4 ± 0.2 μM	IC_{50} = 8.3 ± 0.7 μM	Schüttelkopf et al. (2011)
	Bisdionin F		IC_{50} = 0.92 ± 0.04 μM	IC_{50} = 17.1 ± 1 μM	Sutherland et al. (2011)

(continued)

Table 11.1 (continued)

Chemical class	Compound	Structure	Activity		Reference
			AMCase	CHIT1	
Aminotriazoles	2		IC_{50} = 0.21 μM	IC_{50} = 4.23 μM	Cole et al. (2010)
	3		IC_{50} = 14.2 ± 1.0 nM	IC_{50} = 232 ± 48 nM	Mazur et al. (2018b)
	4		mAMCase IC_{50} = 4170 ± 42 nM	mCHIT1 IC_{50} = 29 ± 4 nM	Mazur et al. (2018a)
Other classes	5		IC_{50} = 0.7 μM	IC_{50} = 1.34 μM	Cole et al. (2010)
	6		NA	IC_{50} = 54.6 ± 7.2 μM	Jiang et al. (2016)
	7		NA	IC_{50} = 67.6 ± 8.0 μM	

However, several other studies reported contrasting results. A transgenic mice overexpressing AMCase showed normal lung function and no signs of inflammation (Reese et al. 2007). In fact, the type II inflammation resulted after the chitin challenge was ameliorated in mice with overexpressing AMCase. Moreover, AMCase deficient mice revealed no role for this enzyme in mouse model of HDM or OVA-induced allergy in the lung (Fitz et al. 2012). Another study reported that in human lungs contrary to mice lungs, the expressed AMCase is mostly inactive (Seibold et al. 2008). Furthermore, AMCase deficient mice exhibit mortality with accumulation of chitin and expression of pro-fibrotic cytokines. These mice develop pulmonary fibrosis, which was ameliorated with the restoration of AMCase activity (Van Dyken et al. 2017). These recent studies suggested that AMCase has protective role and may not be a good drug target against type II inflammation related pathologies such as asthma.

Numerous studies similarly found higher levels of another human chitinase, CHIT1 during type II inflammatory responses in several diseases in both allergic patients and murine models. Most evidences for the role of CHIT1 in various diseases came from the biomarker studies. CHIT1 has also been reported to be involved in asthma and airway hyper-responsiveness (Gavala et al. 2013). Elevated CHIT1 activity and levels were observed after allergen challenge and these levels correlated with the levels of inflammatory cells, T cell chemokines, and other pro-fibrotic factors. CHIT1 was also found to be responsible for chitinase activity in human lung (Seibold et al. 2008). Another study reported the prevalence of CHIT1 24-base pair duplication allele in patients with severe asthma (Livnat et al. 2014). This allele is reported to have reduced CHIT1 activity. However, numerous studies also showed no association between CHIT1 and airway diseases (Shuhui et al. 2009; Létuvé et al. 2010). Mice with CHIT1 null mutant showed significantly higher type II inflammatory responses to HDM or OVA challenge. Furthermore, this study suggests a protective role of CHIT1 in allergic airway responses via regulation of TGF expression (Hong et al. 2018).

As described here, various studies tried to elucidate the role of chitinases in inflammatory diseases. However, whether chitinase functions in a protective or adverse role in inflammation is not very clear. Studies in recent years begin to relate differences in inflammatory responses with chitin degradation and recognition. As chitin is not synthesized by mammals, it is considered as a target of mammalian immune system (Elieh Ali Komi et al. 2018). One study showed the direct involvement of chitin in allergic responses (Reese et al. 2007). Administration of chitin bead in the lungs of mice expressing GFP-enhanced IL-4 led to the recruitment of GFP positive basophils and eosinophils. Further, it was shown that macrophage activation was a crucial step in the recruitment of these cells. Moreover, it has been shown that intranasal administration of chitin particles activates alveolar macrophages to express cytokines including IL-12, tumor necrosis factor-α (TNF-α) and IL-18 (Shibata et al. 1997). Several receptor proteins have been identified that are believed to recognize and bind chitin. These include FIBCD1, NKR-P1, and RegIIIc (Semeňuk et al. 2001; Cash et al. 2006; Thomsen et al. 2011; Bueter et al. 2013). In addition, Toll-like receptor 2, dectin-1 and

mannose receptor are also known to be involved in immune response to chitin (Bueter et al. 2013; Elieh Ali Komi et al. 2018; Fuchs et al. 2018). These chitin receptors are present on the surface of macrophages. These receptors upon interaction with chitin stimulate the production of cytokines and mediators such as IL-17, IL-18, IL-23, and TNF-α which in turn stimulate the production of chitinases and CLPs (Zhu et al. 2004;Da Silva et al. 2008, 2009; Amarsaikhan and Templeton, 2015). It has been also reported that chitin exposure increased the expression of IL-25, IL-33, TLSP, and CCL2 in lung epithelial cells. These factors induce type II innate lymphoid cells to secrete IL-5 and IL-13 cytokines which are critical for the accumulation of eosinophils and macrophages (Van Dyken et al. 2014). Chitinases modulate the local and/or circulating concentration of chitin in the body and thereby regulating the immune response to chitin. However, the mechanism of immune response stimulation is not well understood and two alternative hypotheses have been suggested previously. According to one hypothesis, chitinases degrade exogenous chitin from sources such as fungi or HDM which consequently prevent chitin from stimulating immune responses. In the absence of chitinases, chitin may accumulate in tissues, activate innate immune cells, thereby triggering an excessive inflammatory response (Alvarez 2014). Interestingly, a contrasting hypothesis suggests that the size of chitin fragment is important, as large fragments are generally inert while smaller fragments produced due to chitinase activity trigger inflammatory response (Da Silva et al. 2009; Kogiso et al. 2011). Increasing number of evidences suggest that immune response and inflammatory cell recruitment to chitin exposure is influenced by the size of chitin particles, shape of particles, tissue of exposure, exposure duration, etc. (Alvarez 2014; Amarsaikhan and Templeton 2015). In one study, when chitin was intranasally and intraperitoneally delivered to mice, macrophage activation and eosinophil migration were observed in both routes of administration. However, a transient neutrophilic response was only observed in the case of intraperitoneal challenge (Reese et al. 2007). Similarly, highly purified chitin did not stimulate, while chitin particles of 40–70 μm in size were able to stimulate the production of TNF-α and anti-inflammatory response (Da Silva et al. 2009; Mora-Montes et al. 2011). Chitin particles of 1–10 μm in size induced both anti-inflammatory and Th1 protective responses (Da Silva et al. 2009). Chitin particles of about 0.2 μm in size were not immunogenic (Alvarez 2014). One recent study identified six GlcNAc unit long chitin chain as the smallest immunogenic unit (Fuchs et al. 2018). They further demonstrated that Toll-like receptor 2 is a primary fungal chitin sensor on human and murine immune cells and chitin oligomers bind to Toll-like receptor 2 with affinity in nanomolar range. The extent of chitin degradation is determined by chitinase activity in airways, which further influence immune responses. Differences in inflammatory responses (Roy et al. 2012) by different particle or fragment sizes might be due to the deployment of distinct chitin receptors that activate different signaling pathways. In fact, several studies have reported evidences for the stimulation of multiple signaling pathways upon chitin challenge (Reese et al. 2007; Van Dyken et al. 2014).

AMCase and CHIT1 were implicated in several other diseases such as Gaucher disease, idiopathic pulmonary fibrosis, sarcoidosis, chronic obstructive pulmonary disease (COPD), and Alzheimer's disease. However, their role is not clearly understood. Most of these evidences came from biomarker studies where either high expression or high activity of AMCase or CHIT1 was observed in patients or animal models. Gaucher disease is a lysosomal storage disorder caused by the accumulation of glucocerebroside in macrophages due to the deficiency of beta-glucosidase caused by autosomal recessive inheritance (Grabowski 2012). These affected macrophages known as Gaucher cells than displace normal cells in bone marrow and visceral organs causing organ dysfunction, skeletal manifestations, thrombopenia, etc. (Grabowski et al. 2015). Gaucher cells secrete biomarkers in the blood and CHIT1 is one such biomarkers used for the diagnosis of Gaucher disease (Hollak et al. 1994; Wajner et al. 2004; van Dussen et al. 2014), and Niemann–Pick diseases (Wajner et al. 2004). It has been observed that circulating levels of CHIT1 increased by 1000 folds in patients with Gaucher disease when compared with healthy persons. CHIT1 concentrations in serum were higher in patients with sarcoidosis than that of healthy persons (Bargagli et al. 2013). Increased CHIT1 activity in airways was observed in patients with chronic obstructive pulmonary disease (COPD) as compared to control subjects (Seibold et al. 2008; Létuvé et al. 2010; James et al. 2016). Numerous evidences suggest that inflammation is involved in the pathogenesis of Alzheimer's disease (Stefano et al. 2007; Heppner et al. 2015). CHIT1 activity was found to be significantly increased in cerebrospinal fluid of patients with Alzheimer's disease as compared to control (Mattsson et al. 2011; von Arnim et al. 2011; Watabe-Rudolph et al. 2012). However, the exact mechanism of CHIT1 involvement in Alzheimer's disease is not known. CHIT1 has been shown to play a protective role by enhancing TGFβ1 mediated clearance of amyloid β (Wang et al. 2018).

Apart from AMCase and CHIT1, increased expression of CLPs such as YKL-40 was also observed in Th2 type inflammation (Chupp et al. 2007; Komi et al. 2016). Additionally, increased levels of YKL-40 in serum and lungs of asthma patients were observed as compared to control (Chupp et al. 2007). Furthermore, it was shown that YKL-40 is the central component of Th2 inflammatory responses. In BRP-39 (homolog of YKL-40 in mice) deleted mice, type II immune response declined after OVA exposure. However, these immune responses were rescued by YKL-40 overexpression (Lee et al. 2009; Lee et al. 2011). It was further shown that YKL-40 deficiency alleviates IL-13 dependent fibrosis suggesting its critical role in Th2 inflammation (Lee et al. 2009; Kang et al. 2015). High YKL-40 levels were also found in lungs of patients with idiopathic pulmonary fibrosis (Furuhashi et al. 2010; Zhou et al. 2014). Other CLPs, Ym1, and Ym2 were also identified as allergy-associated protein in mice allergy model (Zhao et al. 2005; Song et al. 2008). Higher number of epithelial cells and macrophages expressing YKL-40 in lung samples of idiopathic pulmonary fibrosis patients was observed (Zhou et al. 2014). YKL-40 expression was also found to be elevated in various cancer cells compared with normal cells (Johansen et al. 2009; Choi et al. 2010; Lee et al. 2011). Several studies also reported the association of YKL-40 and cancer

metastasis (Jensen et al. 2003; Ma et al. 2015). Knock-down of YKL-40 resulted in the decrease of metastases in mice lung tissues and human cancer cell lines (Kim et al. 2018). Increased levels of YKL-40 in cerebrospinal fluid were also found in Alzheimer's disease patients (Hellwig et al. 2015; Janelidze et al. 2016; Zhang et al. 2018).

Although the studies described above provide clues about the role of chitin, human chitinases, and CLPs in inflammation and development of various inflammatory diseases, their specific role needs to be elucidated. Moreover, there is no clear mechanistic understanding of how they regulate inflammation and immune responses. Whether they function in a protective or adverse role in inflammation have been found to be controversial. As inflammation is a very complex process with multiple triggers, effectors, and mechanisms, and the observed outcome (pro- or anti-inflammatory) may depend on how the experiment was conducted and what "markers" were monitored. Studies have shown that size and shape of chitin particles, dose of administration, tissue of exposure and exposure duration could affect inflammatory response in different manner. Chitin particles with different sizes produced as a result of chitinase activity could interact with different cell surface receptors and may stimulate macrophages to express different effector molecules activating distinct signaling cascades. Activated macrophages may secrete pro-inflammatory cytokines, such as TNF-α, IL-12, IL-18, which recruit eosinophils, neutrophils, and basophils. These cells produce type II inflammatory response by secreting Th2 cytokines such as IL-4, IL-5, and IL-13. On the other hand, chitin can also activate macrophages to produce type I cytokines to suppress type II inflammatory response. Furthermore, studies have shown that chitinases and CLPs may not only be playing direct protective role by degrading chitin but also may be involved in augmenting the immune response against chitin and other allergens.

11.5 Current Status of CHIT1 and AMCase Inhibitor Development

Allosamidin was the first chitinase inhibitor discovered about three decades ago from the mycelia of Streptomyces species (Sakuda et al. 1986, 1987b). Allosamidin has a pseudotrisaccharide structure consisting of one allosamizoline and two N-acetyl-D-allosamine moieties (Sakuda et al. 1987a, 1988) (Table 11.1). It mimics a transition state intermediate in chitin hydrolysis (Sakuda et al. 2001). Allosamidin is a potent inhibitor against all family GH18 chitinases (Berecibar et al. 1999). However, it does not inhibit family GH19 chitinases (Sakuda et al. 1993). Family GH18 chitinases hydrolyze chitin via a substrate-assisted mechanism which involves the production of an oxazolium ion intermediate. Allosamizoline moiety in allosamidin mimics this transition state intermediate (Tews et al. 1997). Family GH19 chitinases, on the other hand, employ a fold-and-reaction mechanism (Monzingo et al. 1996) which is different from the substrate-assisted mechanism of family GH18 chitinases. Up to now, seven naturally occurring allosamidins (allosamidin,

methylallosamidin, demethylallosamidin, glucoallosamidin A, glucoallosamidin B, methyl-N-demethylallosamidin, and didemethylallosamidin) have been identified (Nishimoto et al. 1991). Allosamidin is the most widely studied among them. The inhibitory potency of allosamidin against both AMCase (Boot et al. 2001) and CHIT1 (Boot et al. 2001; Rao et al. 2003) is listed in Table 11.1. Allosamidin was utilized as a chemical probe to demonstrate the association between AMCase and asthma. It was shown that AMCase expression elevated upon exposure to allergen or IL-13 induced inflammation in lung (Zhu et al. 2004) and the administration of AMCase inhibitor allosamidin or demethylallosamidin (Table 11.1) suppressed allergen-induced eosinophilia in murine asthma model (Zhu et al. 2004; Matsumoto et al. 2009). Both allosamidin and demethylallosamidin inhibited chitinase activity in vivo, however, reduction in allergen or IL-13-induced airway hyper-responsiveness was only observed in case of demethylallosamidin.

Another class of reported family GH18 chitinase inhibitors include peptide-like compounds that can mimic protein–carbohydrate interactions. These include cyclic proline-containing dipeptides (Izumida et al. 1996; Houston et al. 2002) and cyclic pentapeptides argadin (Arai et al. 2000b) and argifin (Arai et al. 2000a; Omura et al. 2000) (Table 11.1). Cyclic proline-containing dipeptides were isolated from the broth of a marine bacterium while the cyclopeptides were isolated from Clonostachys sp. FO-7314 and Gliocladium sp. FTD-0668. Argadin and argifin were reported to inhibit both human chitinases (Rao et al. 2005b; Goedken et al. 2011). AMCase inhibitory activity of argadin and argifin were evaluated using human and murine AMCases (Goedken et al. 2011). This study reported slight differences in the AMCase inhibitory activity of argadin and argifin which might be due to differences in the purification or method of expression for human and murine AMCases (Goedken et al. 2011). In this study, Argifin was found to be much more potent than argadin for both human and murine AMCases (Table 11.1). Argadin and argifin also inhibited chitinase activity in BAL fluid obtained from OVA-challenged mice with similar potency as recombinant enzyme (Goedken et al. 2011). Although both argadin and argifin displayed inhibitory activity against human CHIT1, Argadin was found to be much more potent than Argifin (Rao et al. 2005b). Berberine and its analogs constitute another class of natural product chitinase inhibitors (Duan et al. 2018) (Table 11.1). These compounds were competitive inhibitors of family GH18 chitinases and GH20 β-*N*-acetyl-D-hexosaminidase. Barberine and two of its analogs inhibited AMCase and CHIT1 with moderate potency and are nonselective against either enzyme (Duan et al. 2018). Deacetylated chitooligosaccharides represent another class of human chitinase inhibitors. A series of fully deacetylated chitooligosaccharides $(GlcN)_{2–7}$ was reported to inhibit CHIT1 where the potency was observed to increase with the addition of GlcN unit. $(GlcN)_7$ was found to be the most potent whereas $(GlcN)_2$ was the least potent (Chen et al. 2014) (Table 11.1).

Some of the aforementioned natural products were potent AMCase and CHIT1 inhibitors, but their use as lead molecules in drug discovery was significantly impeded by their high molecular weight, presence of several stereocenters, limited availability, chemical complexity, and difficulty in synthesis. Furthermore, these

properties also hampered their use in in vivo studies to investigate the role of AMCase and CHIT1 in the development of various diseases. With a goal to develop drug-like chitinase inhibitors, several groups reported different chemical classes of drug-like compounds. Xanthine derivatives were among the first reported drug-like inhibitors of human chitinases identified by screening a commercially available library of drug molecules (Rao et al. 2005a). Screening resulted in the identification of three hits (theophylline, caffeine, and pentoxifylline) with 1,3-dimethylxanthine substructure. All three hits, however, were found to be only weak inhibitors of CHIT1 and AMCase (Rao et al. 2005a) (Table 11.1). Crystallographic analyses of theophylline, caffeine, and pentoxifylline with AfChiB1 revealed interactions mimicking allosamidin (Rao et al. 2005a). Although the inhibitory values of these methylxanthine derivatives were not very high, they represent a chemical class which is easily available, low molecular weight, low cost and generally considered safe. Additionally, their binding mode with AfChiB1 stimulated the identification of other drug-like inhibitors. In one such study, a library of 5.1 million commercially available compounds was filtered for 3-methylxanthine substructure (Schüttelkopf et al. 2006). Further prioritization of hits utilizing molecular docking and visual inspection followed by evaluation of inhibitory activity against AfChiB1 resulted in the identification of compound 1 (1-(2-(theobromine-1-yl)ethyl)-theobromine) which is actually two linked caffeine molecules. Compound 1 showed improvement of two orders of magnitude when compared with caffeine, its parent compound (Schüttelkopf et al. 2006). Compound 1 also displayed moderate inhibition of CHIT1 and murine AMCase. Crystal structure of Compound 1 in complex with AfChiB1 revealed strained geometry of this compound within the binding pocket (Schüttelkopf et al. 2006) when compared to its parent compound (Rao et al. 2005a). Syntheses of several dicaffeine scaffold derivatives (named as Bisdionins) with variable linker length to alleviate ligand strain resulted in compounds with improved human chitinase inhibitory potency (Table 11.1) (Schüttelkopf et al. 2011). Especially, Bisdionin C displayed low micromolar inhibition of both AMCase and CHIT1. Although Bisdionin C displayed reasonable potency with excellent drug-like properties, it is nonselective and inhibits both human chitinases with more or less equal potency. As selective inhibitors are important to understand functional differences in AMCase and CHIT1, Bisdionin F was synthesized taking hints from the co-crystal structure of Bisdionin C in complex with AMCase (Sutherland et al. 2011). Crystal structure of Bisdionin C in complex with AMCase revealed that Bisdionin C occupies −1, −2 and −3 GlcNAc binding subsites of AMCase and a methyl group at N7 position of xanthine ring imposes an unfavorable conformation of AMCase pocket residue Asp138 (Fig. 11.4a). Bisdionin F was synthesized by removing this methyl group from the xanthine scaffold. This structure-guided optimization resulted in the improvement of AMCase inhibitory activity by one order of magnitude for Bisdionin F when compared with Bisdionin C (Sutherland et al. 2011) (Table 11.1). Co-crystal structure of Bisdionin F with AMCase revealed that Asp138 adopted a favorable conformation interacting with the compound via an additional hydrogen bond at N7 position (Sutherland et al. 2011) (Fig. 11.4b). Furthermore, as improvement in potency was

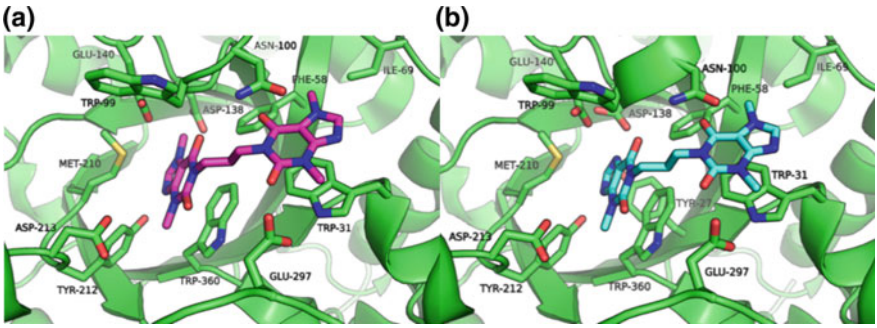


Fig. 11.4 Co-crystal structures of CHIT1 with **a** Bisdionin C and **b** Bisdionin F

observed only for AMCase but not for CHIT1, a 20-fold selectivity for AMCase was obtained (Sutherland et al. 2011). Bisdionin F also displayed similar effects when evaluated in vivo and its treatment attenuated chitinase activity while alleviating some hallmarks of allergic inflammation including eosinophilia.

Another study employed a combination of high-throughput screening, fragment-based drug design and in silico screening to identify several drug-like inhibitors of AMCase and CHIT1 (Cole et al. 2010). Although some of these reported compounds were highly potent AMCase and reasonably active CHIT1 inhibitors (compound 2 and 5), most of them lack selectivity toward either enzyme (Table 11.1). The most potent of the reported AMCase inhibitors (compound 2) was orally active and reduced the chitinase activity in BAL fluid of mice challenged with the combination of HDM and cockroach allergens (Cole et al. 2010). Co-crystal structures of many of the reported compounds were obtained that stimulated research toward the development of more potent and selective human chitinase inhibitors. Another class of drug-like inhibitors were derived from the natural product chitinase inhibitor argifin (Arai et al. 2000a; Omura et al. 2000). The amino-(3-methylureido)-methaniminium functional group of argifin was used as a query to identify structurally similar compounds. Resulting hits were further prioritized with molecular docking and selected compounds were evaluated for inhibitory activities against AMCase. Seven of the tested compounds showed IC_{50} values of ≤ 100 μM (Wakasugi et al. 2013). No CHIT1 inhibitory activities were reported for these compounds. In pursuit of the development of agrochemicals, our group has also identified two new chemical classes of compounds demonstrating inhibitory activity against chitinase of destructive crop pest Asian corn borer, *Ostrinia furnacalis*. These two chemical scaffolds were identified following a hierarchical virtual screening approach (Kumar and Zhang 2015) where a combination of shape similarity and molecular docking was employed to filter a library of about four million commercially available compounds. One of these classes (compound 6 and 7) exhibited broad-spectrum activity against various chitinases with moderate inhibitory activity against CHIT1 (Jiang et al. 2016).

As previously described in this manuscript, contrasting observations were obtained in different experiments meant to study the role of AMCase/CHIT1 in

disease development. These contrasting observations prompted researchers to develop AMCase and/or CHIT1 inhibitors that are much more potent, highly selective for one of the enzyme and possessed a good pharmacokinetic profile that is suitable for animal studies. In one such study, the previously reported compound 2 (Cole et al. 2010) was selected as a starting point for the development of more potent and selective AMCase inhibitors (Mazur et al. 2018b). Although wyeth1 was relatively less potent and nonselective for AMCase (Table 11.1), it was a suitable starting point due to its drug-like profile and the availability of co-crystal structure (PDB code 3RM4). Chemical synthesis guided by structure-based design and chitinase activity evaluation against human and murine AMCase and CHIT1 resulted in a series of aminotriazoles with high AMCase and CHIT1 inhibitory activity (Mazur et al. 2018b). Several compounds were found to be potent against AMCase with IC_{50} in low nanomolar range and reasonable selectivity against CHIT1. Compound 3 was reported to be especially interesting as AMCase inhibitor due to its high potency, specificity and good pharmacokinetic properties (Table 11.1). Compound 3 also demonstrated significant reduction in inflammation in HDM-induced allergic airway inflammation model where reduction in AMCase activity highly correlated with inflammatory cell influx in BAL fluid (Mazur et al. 2018b). However, compound 3 was not pursued further due to dopamine receptor off-target activity and potential concerns about safety. Utilizing compound 2 scaffold as a starting point the same research group also reported compound 4 that was highly potent and possessed 143-fold selectivity for mouse CHIT1 over mouse AMCase (Mazur et al. 2018a) (Table 11.1).

11.6 Conclusion

Humans express two chitinases, AMCase and CHIT1 to degrade chitin from environmental sources. Recent evidences suggest that chitinases may also play a key role in innate immune response against pathogens. Several studies have shown the involvement of both AMCase and CHIT1 in the development of various diseases such as asthma, COPD, idiopathic pulmonary fibrosis, Alzheimer disease, and cancer. However, the mechanism of their involvement is not well understood. Most of the information about their role came from biomarker studies, where either human chitinases were highly expressed or elevated chitinase activity was observed. Moreover, their role in disease development is not devoid of controversies. There is no clear understanding about the mechanism of their immune regulation and whether they play a protective or adverse role in inflammation is still controversial. As inflammation is a very complex process with multiple triggers, effectors, and mechanisms, and the observed outcome (pro- or anti-inflammatory) may depend on how the experiment was conducted, what inducer was used, dose and method of administration, exposure duration and what "markers" were monitored. Recent studies also pointed out the role of chitin recognition and degradation in the activation of pro- and anti-inflammatory responses. Chitin fragments of

different sizes may interact with different receptors on the surface of macrophages triggering a distinct immune response. These studies suggest that chitinases may play a direct protective role by degrading chitin as well as may be involved in augmenting the immune response against chitin and other allergens. However, further characterization of interactions between chitin and chitin receptors and associated pathways will be required to understand the biological role of chitinases in humans. Recent reports also highlighted the involvement of various CLPs and some of the CLPs have been shown to play a central role in human responses to pathogens or disease conditions. Further work is required to study their role in inflammation. Lack of understanding of chitinase role in disease development did not prevent researchers from developing inhibitors of AMCase and CHIT1. Though initial efforts were focused toward natural product inhibitors, drug-like inhibitors with high potency and better pharmacokinetic properties were later developed. These inhibitors played a significant role in studying both AMCase and CHIT1 and implications of inhibiting these proteins. However, studying the role of these proteins is much more challenging and requires the specific inhibition of one of these proteins. Identifying selective inhibitors against these proteins is challenging due to the similar fold, functions and very conserved active site. This may be the reason why almost all of the inhibitors developed for these two proteins are nonselective or only weakly selective. The level of selectivity of current generation of inhibitors may not be adequate for in vivo studies as much higher dose is utilized and with that dose it is very difficult to avoid inhibition of both proteins. Therefore, the discovery of highly selective inhibitors for AMCase and CHIT1 is necessary in order to better understand the roles of chitin and chitinases in inflammation and development of inflammatory diseases.

References

Akagi K-I, Watanabe J, Hara M, Kezuka Y, Chikaishi E, Yamaguchi T, Akutsu H, Nonaka T, Watanabe T, Ikegami T (2006) Identification of the substrate interaction region of the chitin-binding domain of streptomyces griseus chitinase C. J Biochem 139:483–493

Alvarez F (2014) The effect of chitin size, shape, source and purification method on immune recognition. Molecules 19:4433

Amarsaikhan N, Templeton SP (2015) Co-recognition of β-glucan and chitin and programming of adaptive immunity to Aspergillus fumigatus. Front Microbiol 6:344–344

Arai N, Shiomi K, Iwai Y, Omura S (2000a) Argifin, a new chitinase inhibitor, produced by Gliocladium sp. FTD-0668. II. Isolation, physico-chemical properties, and structure elucidation. J Antibiot (Tokyo) 53:609–614

Arai N, Shiomi K, Yamaguchi Y, Masuma R, Iwai Y, Turberg A, Kolbl H, Omura S (2000b) Argadin, a new chitinase inhibitor, produced by clonostachys sp.FO-7314. Chem Pharm Bull 48:1442–1446

Arakane Y, Muthukrishnan S (2010) Insect chitinase and chitinase-like proteins. Cell Mol Life Sci 67:201–216

Bargagli E, Bennett D, Maggiorelli C, Di Sipio P, Margollicci M, Bianchi N, Rottoli P (2013) Human chitotriosidase: a sensitive biomarker of sarcoidosis. J Clin Immunol 33:264–270

Berecibar A, Grandjean C, Siriwardena A (1999) Synthesis and biological activity of natural aminocyclopentitol glycosidase inhibitors: mannostatins, trehazolin, allosamidins, and their analogues. Chem Rev 99:779–844

Bhattacharya D, Nagpure A, Gupta RK (2007) Bacterial chitinases: properties and potential. Crit Rev Biotechnol 27:21–28

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

Boot RG, Blommaart EFC, Swart E, Ghauharali-Van Der Vlugt K, Bijl N, Moe C, Place A, Aerts JMFG (2001) Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem 276:6770–6778

Boot RG, Bussink AP, Verhoek M, de Boer PA, Moorman AF, Aerts JMFG (2005) Marked differences in tissue-specific expression of chitinases in mouse and man. J Histochem Cytochem 53:1283–1292

Boot RG, Renkema GH, Strijland A, Van Zonneveld AJ, Aerts JMFG (1995) Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem 270:26252–26256

Bueter CL, Specht CA, Levitz SM (2013) Innate sensing of chitin and chitosan. PLoS Pathog 9: e1003080

Bussink AP, Speijer D, Aerts JMFG, Boot RG (2007) Evolution of mammalian chitinase(-Like) members of family 18 glycosyl hydrolases. Genetics 177:959–970

Cash HL, Whitham CV, Behrendt CL, Hooper LV (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science 313:1126–1130

Chang N-CA, Hung S-I, Hwa K-Y, Kato I, Chen J-E, Liu C-H, Chang AC (2001) A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. J Biol Chem 276:17497–17506

Chen L, Zhou Y, Qu M, Zhao Y, Yang Q (2014) Fully deacetylated chitooligosaccharides act as efficient glycoside hydrolase family 18 chitinase inhibitors. J Biol Chem 289:17932–17940

Choi IK, Kim YH, Kim JS, Seo JH (2010) High serum YKL-40 is a poor prognostic marker in patients with advanced non-small cell lung cancer. Acta Oncol 49:861–864

Chou Y-T, Yao S, Czerwinski R, Fleming M, Krykbaev R, Xuan D, Zhou H, Brooks J, Fitz L, Strand J, Presman E, Lin L, Aulabaugh A, Huang X (2006) Kinetic characterization of recombinant human acidic mammalian chitinase. Biochemistry 45:4444–4454

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

Cole DC, Olland AM, Jacob J, Brooks J, Bursavich MG, Czerwinski R, Declercq C, Johnson M, Joseph-Mccarthy D, Ellingboe JW, Lin L, Nowak P, Presman E, Strand J, Tam A, Williams CMM, Yao S, Tsao DHH, Fitz LJ (2010) Identification and characterization of acidic mammalian chitinase inhibitors. J Med Chem 53:6122–6128

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

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

Davies GJ, Wilson KS, Henrissat B (1997) Nomenclature for sugar-binding subsites in glycosyl hydrolases. Biochem J 321:557–559

Duan Y, Liu T, Zhou Y, Dou T, Yang Q (2018) Glycoside hydrolase family 18 and 20 enzymes are novel targets of the traditional medicine berberine. J Biol Chem

Elieh Ali Komi D, Sharma L, Dela Cruz CS (2018) Chitin and its effects on inflammatory and immune responses. Clin Rev Allergy Immunol 54:213–223

Fadel F, Zhao Y, Cachau R, Cousido-Siah A, Ruiz FX, Harlos K, Howard E, Mitschler A, Podjarny A (2015) New insights into the enzymatic mechanism of human chitotriosidase (CHIT1) catalytic domain by atomic resolution X-ray diffraction and hybrid QM/MM. Acta Crystallograph Sect D 71:1455–1470

Fadel F, Zhao Y, Cousido-Siah A, Ruiz FX, Mitschler A, Podjarny A (2016) X-Ray crystal structure of the full length human chitotriosidase (CHIT1) reveals features of its chitin binding domain. PLoS One 11:e0154190

Fitz LJ, Declercq C, Brooks J, Kuang W, Bates B, Demers D, Winkler A, Nocka K, Jiao A, Greco RM, Mason LE, Fleming M, Quazi A, Wright J, Goldman S, Hubeau C, Williams CMM (2012) Acidic mammalian chitinase is not a critical target for allergic airway disease. Am J Respir Cell Mol Biol 46:71–79

Foster JM, Zhang Y, Kumar S, Carlow CKS (2005) Parasitic nematodes have two distinct chitin synthases. Mol Biochem Parasitol 142:126–132

Fuchs K, Gloria YC, Wolz OO, Herster F, Sharma L, Dillen CA, Täumer C, Dickhöfer S, Bittner Z, Dang TM, Singh A, Haischer D, Schlöffel MA, Koymans KJ, Sanmuganantham T, Krach M, Roger T, Le Roy D, Schilling NA, Frauhammer F, Miller LS, Nürnberger T, Leibundgut-Landmann S, Gust AA, Macek B, Frank M, Gouttefangeas C, Dela Cruz CS, Hartl D, Weber AN (2018) The fungal ligand chitin directly binds TLR2 and triggers inflammation dependent on oligomer size. EMBO Rep

Fukamizo F (2000) Chitinolytic enzymes: catalysis, substrate binding, and their application. Curr Protein Pept Sci 1:105–124

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

Fusetti F, Von Moeller H, Houston D, Rozeboom HJ, Dijkstra BW, Boot RG, Aerts JMFG, Van Aalten DMF (2002) Structure of human chitotriosidase: implications for specific inhibitor design and function of mammalian chitinase-like lectins. J Biol Chem 277:25537–25544

Gao J, Bauer MW, Shockley KR, Pysz MA, Kelly RM (2003) Growth of hyperthermophilic archaeon *Pyrococcus furiosus* on chitin involves two family 18 chitinases. Appl Environ Microbiol 69:3119–3128

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

Giraud-Guille MM, Bouligand Y (1986) Chitin-protein molecular organization in arthropod. In: Muzzarelli R, Jeuniaux C, Gooday GW (eds.) Chitin in nature and technology, Boston, MA, Springer US, pp 29–35

Glaser L, Brown DH (1957) The synthesis of chitin in cell-free extracts of neurospora crassa. J Biol Chem 228:729–742

Goedken ER, O'Brien RF, Xiang T, Banach DL, Marchie SC, Barlow EH, Hubbard S, Mankovich JA, Jiang J, Richardson PL, Cuff CA, Cherniack AD (2011) Functional comparison of recombinant acidic mammalian chitinase with enzyme from murine bronchoalveolar lavage. Protein Expr Purificat 75:55–62

Gonçalves IR, Brouillet S, Soulié M-C, Gribaldo S, Sirven C, Charron N, Boccara M, Choquer M (2016) Genome-wide analyses of chitin synthases identify horizontal gene transfers towards bacteria and allow a robust and unifying classification into fungi. BMC Evol Biol 16:252

Gooday GW (1990) Physiology of microbial degradation of chitin and chitosan. Biodegradation 1:177–190

Grabowski GA (2012) Gaucher disease and other storage disorders. ASH Educat Prog Book 2012:13–18

Grabowski GA, Zimran A, Ida H (2015) Gaucher disease types 1 and 3: Phenotypic characterization of large populations from the ICGG gaucher registry. Am J Hematol 90: S12–S18

Grover A (2012) Plant chitinases: genetic diversity and physiological roles. Crit Rev Plant Sci 31:57–73

Hamid R, Khan MA, Ahmad M, Ahmad MM, Abdin MZ, Musarrat J, Javed S (2013) Chitinases: an update. J Pharm Bioallied Sci 5:21–29

Hellwig K, Kvartsberg H, Portelius E, Andreasson U, Oberstein TJ, Lewczuk P, Blennow K, Kornhuber J, Maler JM, Zetterberg H, Spitzer P (2015) Neurogranin and YKL-40: independent markers of synaptic degeneration and neuroinflammation in Alzheimer's disease. Alzheimers Res Ther 7:74–74

Henrissat B (1999) Classification of chitinases modules

Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci 16:358

Hollak CE, Van Weely S, Van Oers MH, Aerts JM (1994) Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Investig 93:1288–1292

Hong JY, Kim M, Sol IS, Kim KW, Lee C-M, Elias JA, Sohn MH, Lee CG (2018) Chitotriosidase inhibits allergic asthmatic airways via regulation of TGF-β expression and Foxp3 + Treg cells. Allergy 73:1686–1699

Houston DR, Eggleston I, Synstad BR, Eijsink VGH, Aalten DMFV (2002) The cyclic dipeptide CI-4 [cyclo-(l-Arg-d-Pro)] inhibits family 18 chitinases by structural mimicry of a reaction intermediate. Biochem J 368:23–27

Houston DR, Recklies AD, Krupa JC, Van Aalten DMF (2003) Structure and ligand-induced conformational change of the 39-kDa glycoprotein from human articular chondrocytes. J Biol Chem 278:30206–30212

Huang Q-S, Xie X-L, Liang G, Gong F, Wang Y, Wei X-Q, Wang Q, Ji Z-L, Chen Q-X (2012) The GH18 family of chitinases: Their domain architectures, functions and evolutions. Glycobiology 22:23–34

Hussain M, Wilson JB (2013) New paralogues and revised time line in the expansion of the vertebrate GH18 family. J Mol Evol 76:240–260

Ikegami T, Okada T, Hashimoto M, Seino S, Watanabe T, Shirakawa M (2000) Solution structure of the chitin-binding domain of bacillus circulans WL-12 chitinase A1. J Biol Chem 275:13654–13661

Imai T, Watanabe T, Yui T, Sugiyama J (2003) The directionality of chitin biosynthesis: a revisit. Biochem J 374:755–760

Izumida H, Imamura N, Sano H (1996) A novel chitinase inhibitor from a marine bacterium, Pseudomonas sp. J Antibiot (Tokyo) 49:76–80

James AJ, Reinius LE, Verhoek M, Gomes A, Kupczyk M, Hammar U, Ono J, Ohta S, Izuhara K, Bel E, Kere J, Söderhäll C, Dahlén B, Boot RG, Dahlén S-E (2016) Increased YKL-40 and chitotriosidase in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 193:131–142

Janelidze S, Hertze J, Zetterberg H, Landqvist Waldö M, Santillo A, Blennow K, Hansson O (2016) Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. Ann Clinic Transl Neurol 3:12–20

Jensen BV, Johansen JS, Price PA (2003) High levels of serum HER-2/neu and YKL-40 independently reflect aggressiveness of metastatic breast cancer. Clin Cancer Res 9:4423–4434

Jiang X, Kumar A, Liu T, Zhang KYJ, Yang Q (2016) A novel scaffold for developing specific or broad-spectrum chitinase inhibitors. J Chem Inf Model 56:2413–2420

Johansen JS, Schultz NA, Jensen BV (2009) Plasma YKL-40: a potential new cancer biomarker? Fut Oncol 5:1065–1082

Kang M-J, Yoon CM, Nam M, Kim D-H, Choi J-M, Lee CG, Elias JA (2015) Role of chitinase 3–Like-1 in interleukin-18–induced pulmonary type 1, type 2, and type 17 inflammation; alveolar destruction; and airway fibrosis in the murine lung. Am J Respir Cell Mol Biol 53:863–871

Kim KC, Yun J, Son DJ, Kim JY, Jung JK, Choi JS, Kim YR, Song JK, Kim SY, Kang SK, Shin DH, Roh YS, Han SB, Hong JT (2018) Suppression of metastasis through inhibition of chitinase 3-like 1 expression by miR-125a-3p-mediated up-regulation of USF1. Theranostics 8:4409–4428

Kogiso M, Nishiyama A, Shinohara T, Nakamura M, Mizoguchi E, Misawa Y, Guinet E, Nouri-Shirazi M, Dorey CK, Henriksen RA, Shibata Y (2011) Chitin particles induce size-dependent but carbohydrate-independent innate eosinophilia. J Leukoc Biol 90:167–176

Komi DEA, Kazemi T, Bussink AP (2016) New insights into the relationship between chitinase-3-like-1 and asthma. Curr Allerg Asthma Rep 16:57

Kramer KJ, Koga D (1986) Insect chitin: Physical state, synthesis, degradation and metabolic regulation. Ins Biochem 16:851–877

Kumar A, Zhang KYJ (2015) Hierarchical virtual screening approaches in small molecule drug discovery. Methods 71:26–37

Kuusk S, Sørlie M, Väljamäe P (2017) Human chitotriosidase is an endo-processive enzyme. PLoS One 12:e0171042–e0171042

Kzhyshkowska J, Mamidi S, Gratchev A, Kremmer E, Schmuttermaier C, Krusell L, Haus G, Utikal J, Schledzewski K, Scholtze J, Goerdt S (2006) Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. Blood 107:3221–3228

Langner T, Göhre V (2016) Fungal chitinases: function, regulation, and potential roles in plant/pathogen interactions. Curr Genet 62:243–254

Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, Humbles A, Kearley J, Coyle A, Chupp G, Reed J, Flavell RA, Elias JA (2009) Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13–induced tissue responses and apoptosis. J Exp Med 206:1149–1166

Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA (2011) Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol 73:479–501

Lenardon MD, Munro CA, Gow NAR (2010) Chitin synthesis and fungal pathogenesis. Curr Opin Microbiol 13:416–423

Létuvé S, Kozhich A, Humbles A, Brewah Y, Dombret M-C, Grandsaigne M, Adle H, Kolbeck R, Aubier M, Coyle AJ, Pretolani M (2010) Lung chitinolytic activity and chitotriosidase are elevated in chronic obstructive pulmonary disease and contribute to lung inflammation. Am J Pathol 176:638–649

Li H, Greene LH (2010) Sequence and structural analysis of the chitinase insertion domain reveals two conserved motifs involved in chitin-binding. PLoS ONE 5:e8654

Livnat G, Bar-Yoseph R, Mory A, Dagan E, Elias N, Gershoni R, Bentur L (2014) Duplication in CHIT1 gene and the risk for aspergillus lung disease in CF patients. Pediatr Pulmonol 49:21–27

Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495

Ma B, Herzog EL, Lee CG, Peng X, Lee C-M, Chen X, Rockwell S, Koo JS, Kluger H, Herbst RS, Sznol M, Elias JA (2015) Role of chitinase 3–like-1 and semaphorin 7a in pulmonary melanoma metastasis. Can Res 75:487–496

Malaguarnera L, Di Rosa M, Zambito AM, Dell'ombra N, Nicoletti F, Malaguarnera M (2006) Chitotriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. Gut 55:1313–1320

Matsumoto T, Inoue H, Sato Y, Kita Y, Nakano T, Noda N, Eguchi-Tsuda M, Moriwaki A, Kan-O K, Matsumoto K, Shimizu T, Nagasawa H, Sakuda S, Nakanishi Y (2009) Demethylallosamidin, a chitinase inhibitor, suppresses airway inflammation and hyperresponsiveness. Biochem Biophys Res Commun 390:103–108

Mattsson N, Tabatabaei S, Johansson P, Hansson O, Andreasson U, Månsson J-E, Johansson J-O, Olsson B, Wallin A, Svensson J, Blennow K, Zetterberg H (2011) Cerebrospinal fluid microglial markers in Alzheimer's disease: elevated chitotriosidase activity but lack of diagnostic utility. NeuroMol Med 13:151–159

Mazur M, Bartoszewicz A, Dymek B, Salamon M, Andryianau G, Kowalski M, Olejniczak S, Matyszewski K, Pluta E, Borek B, Stefaniak F, Zagozdzon A, Mazurkiewicz M, Koralewski R, Czestkowski W, Piotrowicz M, Niedziejko P, Gruza MM, Dzwonek K, Golebiowski A,

Golab J, Olczak J (2018a) Discovery of selective, orally bioavailable inhibitor of mouse chitotriosidase. Bioorg Med Chem Lett 28:310–314

Mazur M, Olczak J, Olejniczak S, Koralewski R, Czestkowski W, Jedrzejczak A, Golab J, Dzwonek K, Dymek B, Sklepkiewicz PL, Zagozdzon A, Noonan T, Mahboubi K, Conway B, Sheeler R, Beckett P, Hungerford WM, Podjarny A, Mitschler A, Cousido-Siah A, Fadel F, Golebiowski A (2018b) Targeting acidic mammalian chitinase is effective in animal model of asthma. J Med Chem 61:695–710

Mccarter JD, Stephen Withers G (1994) Mechanisms of enzymatic glycoside hydrolysis. Curr Opin Struct Biol 4:885–892

Meng G, Zhao Y, Bai X, Liu Y, Green TJ, Luo M, Zheng X (2010) Structure of human Stabilin-1 interacting chitinase-like protein (SI-CLP) reveals a saccharide-binding cleft with lower sugar-binding selectivity. J Biol Chem 285:39898–39904

Merzendorfer H (2006) Insect chitin synthases: a review. J Comp Physiol B 176:1–15

Merzendorfer H (2011) The cellular basis of chitin synthesis in fungi and insects: Common principles and differences. Eur J Cell Biol 90:759–769

Merzendorfer H, Zimoch L (2003) Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. J Exp Biol 206:4393–4412

Monzingo AF, Marcotte EM, Hart PJ, Robertas JD (1996) Chitinases, chitosanases, and lysozymes can be divided into procaryotic and eucaryotic families sharing a conserved core. Nat Struct Biol 3:133

Mora-Montes HM, Netea MG, Ferwerda G, Lenardon MD, Brown GD, Mistry AR, Kullberg BJ, O'Callaghan CA, Sheth CC, Odds FC, Brown AJP, Munro CA, Gow NaR (2011) Recognition and blocking of innate immunity cells by candida albicans chitin. Infect Immun 79:1961–1970

Morgan JLW, Strumillo J, Zimmer J (2012) Crystallographic snapshot of cellulose synthesis and membrane translocation. Nature 493:181

Neville AC, Parry DA, Woodhead-Galloway J (1976) The chitin crystallite in arthropod cuticle. J Cell Sci 21:73

Nikolov S, Petrov M, Lymperakis L, Friák M, Sachs C, Fabritius H-O, Raabe D, Neugebauer J (2010) Revealing the design principles of high-performance biological composites Using Ab initio and multiscale simulations: the example of lobster cuticle. Adv Mater 22:519–526

Nishimoto Y, Sakuda S, Takayama S, Yamada Y (1991) Isolation and characterization of new allosamidins. J Antibiot (Tokyo) 44:716–722

Ohno M, Togashi Y, Tsuda K, Okawa K, Kamaya M, Sakaguchi M, Sugahara Y, Oyama F (2013) Quantification of chitinase mRNA levels in human and mouse tissues by real-time PCR: species-specific expression of acidic mammalian chitinase in stomach tissues. PLoS ONE 8: e67399

Ohno M, Tsuda K, Sakaguchi M, Sugahara Y, Oyama F (2012) Chitinase mRNA levels by quantitative PCR using the single standard DNA: acidic mammalian chitinase is a major transcript in the mouse stomach. PLoS ONE 7:e50381

Ohno T, Armand S, Hata T, Nikaidou N, Henrissat B, Mitsutomi M, Watanabe T (1996) A modular family 19 chitinase found in the prokaryotic organism Streptomyces griseus HUT 6037. J Bacteriol 178:5065–5070

Olland AM, Strand J, Presman E, Czerwinski R, Joseph-Mccarthy D, Krykbaev R, Schlingmann G, Chopra R, Lin L, Fleming M, Kriz R, Stahl M, Somers W, Fitz L, Mosyak L (2009) Triad of polar residues implicated in pH specificity of acidic mammalian chitinase. Protein Sci 18:569–578

Omura S, Arai N, Yamaguchi Y, Masuma R, Iwai Y, Namikoshi M, Turberg A, Kolbl H, Shiomi K (2000) Argifin, a new chitinase inhibitor, produced by Gliocladium sp. FTD-0668. I. Taxonomy, fermentation, and biological activities. J Antibiot (Tokyo) 53:603–608

Perrakis A, Tews I, Dauter Z, Oppenheim AB, Chet I, Wilson KS, Vorgias CE (1994) Crystal structure of a bacterial chitinase at 2.3 Å resolution. Structure 2:1169–1180

Ranok A, Wongsantichon J, Robinson RC, Suginta W (2015) Structural and thermodynamic insights into chitooligosaccharide binding to human cartilage chitinase 3-like protein 2 (CHI3L2 or YKL-39). J Biol Chem 290:2617–2629

Rao FV, Andersen OA, Vora KA, Demartino JA, Van Aalten DMF (2005a) Methylxanthine drugs are chitinase inhibitors: investigation of inhibition and binding modes. Chem Biol 12:973–980

Rao FV, Houston DR, Boot RG, Aerts JM, Hodkinson M, Adams DJ, Shiomi K, van Aalten DMF (2005b) Specificity and affinity of natural product cyclopentapeptide inhibitors against A. fumigatus, human, and bacterial chitinases. Chem Biol 12:65–76

Rao FV, Houston DR, Boot RG, Aerts JMFG, Sakuda S, Van Aalten DMF (2003) Crystal structures of allosamidin derivatives in complex with human macrophage chitinase. J Biol Chem 278:20110–20116

Reese TA, Liang H-E, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM (2007) Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature 447:92

Renkema GH, Boot RG, Au FL, Donker-Koopman WE, Strijland A, Muijsers AO, Hrebicek M, Aerts JMFG (1998) Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. Eur J Biochem 251:504–509

Renkema GH, Boot RG, Muijsers AO, Donker-Koopman WE, Aerts JMFG (1995) Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. J Biol Chem 270:2198–2202

Renkema GH, Boot RG, Strijland A, Donker-Koopman WE, Berg M, Muijsers AO, Aerts JMFG (1997) Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase. Eur J Biochem 244:279–285

Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31:603–632

Roy RM, Wüthrich M, Klein BS (2012) Chitin elicits CCL2 from airway epithelial cells and induces CCR112-dependent innate allergic inflammation in the lung. J Immunol 189:2545–2552

Rudall KM, Kenchington W (1973) The chitin system. Biol Rev 48:597–633

Sakuda S, Isogai A, Makita T, Matsumoto S, Koseki K, Kodama H, Suzuki A (1987a) Structures of allosamidins, novel insect chitinase inhibitors, produced by actinomycetes. Agric Biol Chem 51:3251–3259

Sakuda S, Isogai A, Matsumoto S, Suzuki A (1987b) Search for microbial insect growth-regulators. 2. Allosamidin, a novel insect chitinase inhibitor. J Antibiot 40:296–300

Sakuda S, Isogai A, Matsumoto S, Suzuki A, Koseki K (1986) The structure of allosamidin, a novel insect chitinase inhibitor, produced by streptomyces Sp. Tetrahedron Lett 27:2475–2478

Sakuda S, Isogai A, Matsumoto S, Suzuki A, Koseki K, Kodama H, Yamada Y (1988) Absolute configuration of allosamizoline, an aminocyclitol derivative of the chitinase inhibitor allosamidin. Agric Biol Chem 52:1615–1617

Sakuda S, Isogai A, Suzuki A, Yamada Y (1993) Chemistry and biochemistry of the chitinase inhibitors, allosamidins. Actinomycetologica 7:50–57

Sakuda S, Sugiyama Y, Zhou Z-Y, Takao H, Ikeda H, Kakinuma K, Yamada Y, Nagasawa H (2001) biosynthetic studies on the cyclopentane ring formation of allosamizoline, an aminocyclitol component of the chitinase inhibitor allosamidin. J Organ Chem 66:3356–3361

Schimpl M, Rush CL, Betou M, Eggleston IM, Recklies AD, Van Aalten DM (2012) Human YKL-39 is a pseudo-chitinase with retained chitooligosaccharide-binding properties. Biochem J 446:149–157

Schüttelkopf AW, Andersen OA, Rao FV, Allwood M, Lloyd C, Eggleston IM, Van Aalten DMF (2006) Screening-based discovery and structural dissection of a novel family 18 chitinase inhibitor. J Biol Chem 281:27278–27285

Schüttelkopf AW, Andersen OA, Rao FV, Allwood M, Rush CL, Eggleston IM, Van Aalten DMF (2011) Bisdionin C—A rationally designed, submicromolar inhibitor of family 18 chitinases. ACS Med Chem Lett 2:428–432

Seibold MA, Donnelly S, Solon M, Innes A, Woodruff PG, Boot RG, Burchard EG, Fahy JV (2008) Chitotriosidase is the primary active chitinase in the human lung and is modulated by genotype and smoking habit. J Allergy Clin Immunol 122:944–950.e943

Semeňuk T, Krist P, Pavlíček J, Bezouška K, Kuzma M, Novák P, Křen V (2001) Synthesis of chitooligomer-based glycoconjugates and their binding to the rat natural killer cell activation receptor NKR-P1. Glycoconj J 18:817–826

Shen C-R, Juang H-H, Chen H-S, Yang C-J, Wu C-J, Lee M-H, Hwang Y-S, Kuo M-L, Chen Y-S, Chen J-K, Liu C-L (2015) The correlation between chitin and acidic mammalian chitinase in animal models of allergic asthma. Int J Mol Sci 16:27371–27377

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

Shuhui L, Mok YK, Wong WSF (2009) Role of mammalian chitinases in asthma. Int Arch Allergy Immunol 149:369–377

Song H-M, Jang A-S, Ahn M-H, Takizawa H, Lee S-H, Kwon J-H, Lee Y-M, Rhim T, Park C-S (2008) Ym1 and Ym2 expression in a mouse model exposed to diesel exhaust particles. Environ Toxicol 23:110–116

Songsiriritthigul C, Pantoom S, Aguda AH, Robinson RC, Suginta W (2008) Crystal structures of Vibrio harveyi chitinase A complexed with chitooligosaccharides: Implications for the catalytic mechanism. J Struct Biol 162:491–499

Stam MR, Blanc E, Coutinho PM, Henrissat B (2005) Evolutionary and mechanistic relationships between glycosidases acting on α- and β-bonds. Carbohyd Res 340:2728–2734

Stefano S, Piras MR, Rita B, Giannina A, Fois ML, Giulio R, Salvatore M (2007) Chitotriosidase and Alzheimers disease. Curr Alzheimer Res 4:295–296

Sutherland TE (2018) Chitinase-like proteins as regulators of innate immunity and tissue repair: helpful lessons for asthma? Biochem Soc Trans 46:141–151

Sutherland TE, Andersen OA, Betou M, Eggleston IM, Maizels RM, van Aalten D, Allen JE (2011) Analyzing airway inflammation with chemical biology: dissection of acidic mammalian chitinase function with a selective drug-like inhibitor. Chem Biol 18:569–579

Terwisscha Van Scheltinga AC, Armand S, Kalk KH, Isogai A, Henrissat B, Dijkstra BW (1995) Stereochemistry of chitin hydrolysis by a plant chitinase/lysozyme and x-ray structure of a complex with allosamidin evidence for substrate assisted catalysis. Biochemistry 34:15619–15623

Tews I, Terwisscha Van Scheltinga AC, Perrakis A, Wilson KS, Dijkstra BW (1997) Substrate-assisted catalysis unifies two families of chitinolytic enzymes. J Am Chem Soc 119:7954–7959

Thomsen T, Schlosser A, Holmskov U, Sorensen GL (2011) Ficolins and FIBCD1: Soluble and membrane bound pattern recognition molecules with acetyl group selectivity. Mol Immunol 48:369–381

Tsai M-L, Liaw S-H, Chang N-C (2004) The crystal structure of Ym1 at 1.31Å resolution. J Struct Biol 148:290–296

Van Aalten DMF, Komander D, Synstad B, Gåseidnes S, Peter MG, Eijsink VGH (2001) Structural insights into the catalytic mechanism of a family 18 exo-chitinase. Proc Natl Acad Sci 98:8979–8984

Van Aalten DMF, Synstad B, Brurberg MB, Hough E, Riise BW, Eijsink VGH, Wierenga RK (2000) Structure of a two-domain chitotriosidase from Serratia marcescens at 1.9-Å resolution. Proc Natl Acad Sci 97:5842–5847

Van Dussen L, Hendriks EJ, Groener JEM, Boot RG, Hollak CEM, Aerts JMFG (2014) Value of plasma chitotriosidase to assess non-neuronopathic Gaucher disease severity and progression in the era of enzyme replacement therapy. J Inherit Metab Dis 37:991–1001

Van Dyken SJ, Liang H-E, Naikawadi RP, Woodruff PG, Wolters PJ, Erle DJ, Locksley RM (2017) Spontaneous chitin accumulation in airways and age-related fibrotic lung disease. Cell 169:497–509.e413

Van Dyken SJ, Mohapatra A, Nussbaum JC, Molofsky AB, Thornton EE, Ziegler SF, McKenzie AN, Krummel MF, Liang HE, Locksley RM (2014) Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and γδ T cells. Immunity 40:414–424

Vermeulen CA, Wessels JGH (1986) Chitin biosynthesis by a fungal membrane preparation. Eur J Biochem 158:411–415

Veronico P, Gray L, Jones J, Bazzicalupo P, Arbucci S, Cortese M, Di Vito M, De Giorgi C (2001) Nematode chitin synthases: gene structure, expression and function in Caenorhabditis elegans and the plant parasitic nematode Meloidogyne artiellia. Mol Genet Genom 266:28–34

Von Arnim C, Watabe-Rudolph M, Song Z, Schnack C, Begus-Nahrmann Y, Rolyan H, Otto M, Tumani H, Thal D, Attems J, Jellinger K, Kestler H, Rudolph K (2011) Chitinase enzyme activity in cerebral spinal fluid is strongly associated with Alzheimer's Disease. Alzheimer's Dementia 7:S288

Wajner A, Michelin K, Burin MG, Pires RF, Pereira MLS, Giugliani R, Coelho JC (2004) Biochemical characterization of chitotriosidase enzyme: comparison between normal individuals and patients with Gaucher and with Niemann-Pick diseases. Clin Biochem 37:893–897

Wakasugi M, Gouda H, Hirose T, Sugawara A, Yamamoto T, Shiomi K, Sunazuka T, Ōmura S, Hirono S (2013) Human acidic mammalian chitinase as a novel target for anti-asthma drug design using in silico screening. Bioorg Med Chem 21:3214–3220

Wang X, Yu W, Fu X, Ke M, Xiao Q, Lü Y (2018) Chitotriosidase enhances TGFβ-Smad signaling and uptake of β-amyloid in N9 microglia. Neurosci Lett 687:99–103

Watabe-Rudolph M, Song Z, Lausser L, Schnack C, Begus-Nahrmann Y, Scheithauer M-O, Rettinger G, Otto M, Tumani H, Thal DR, Attems J, Jellinger KA, Kestler HA, Von Arnim CaF, Rudolph KL (2012) Chitinase enzyme activity in CSF is a powerful biomarker of Alzheimer disease. Neurology 78:569–577

Yang C-J, Liu Y-K, Liu C-L, Shen C-N, Kuo M-L, Su C-C, Tseng C-P, Yen T-C, Shen C-R (2009) Inhibition of acidic mammalian chitinase by RNA interference suppresses ovalbumin-sensitized allergic asthma. Hum Gene Ther 20:1597–1606

Yang J, Gan Z, Lou Z, Tao N, Mi Q, Liang L, Sun Y, Guo Y, Huang X, Zou C, Rao Z, Meng Z, Zhang K-Q (2010) Crystal structure and mutagenesis analysis of chitinase CrChi1 from the nematophagous fungus Clonostachys rosea in complex with the inhibitor caffeine. Microbiology 156:3566–3574

Zhang H, Ng KP, Therriault J, Kang MS, Pascoal TA, Rosa-Neto P, Gauthier S, Initiative TaSDN, Aging NIO, Imaging NIOB, Bioengineering, Abbvie ASA, Foundation ASDD, Biotech A, Bioclinica I, Biogen Company B-MS, Cerespir I, Inc., CE Elan Pharmaceuticals, I Lilly, E, Company, Euroimmun, Hoffmann-La, F., Ltd., R., Its Affiliated Company Genentech, I., Fujirebio, Healthcare G, Ltd., I, Research, JaI Development, L, Johnson, Research JP, Llc., D, Lumosity Lundbeck Merck Co., I, Meso Scale Diagnostics L, Research N, Technologies N, Corporation, NP, Inc. P, Imaging P, Servier, Company TP, Therapeutics T, Research, CIOH, Health, FFTNIO (2018) Cerebrospinal fluid phosphorylated tau, visinin-like protein-1, and chitinase-3-like protein 1 in mild cognitive impairment and Alzheimer's disease. Transl Neurodegenerat 7, 23

Zhao J, Zhu H, Wong CH, Leung KY, Wong WSF (2005) Increased lungkine and chitinase levels in allergic airway inflammation: a proteomics approach. Proteomics 5:2799–2807

Zheng T, Rabach M, Chen NY, Rabach L, Hu X, Elias JA, Zhu Z (2005) Molecular cloning and functional characterization of mouse chitotriosidase. Gene 357:37–46

Zhou Y, Peng H, Sun H, Peng X, Tang C, Gan Y, Chen X, Mathur A, Hu B, Slade MD, Montgomery RR, Shaw AC, Homer RJ, White ES, Lee C-M, Moore MW, Gulati M, Geun Lee C, Elias JA, Herzog EL (2014) Chitinase 3–Like 1 suppresses injury and promotes fibroproliferative responses in mammalian lung fibrosis. Sci Transl Med 6:240ra276-240ra276

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