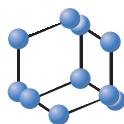
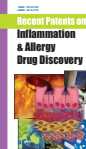


REVIEW ARTICLE

BENTHAM
SCIENCE

Chitinases: Therapeutic Scaffolds for Allergy and Inflammation

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Abstract: Background: Chitinases are the evolutionary conserved glycosidic enzymes that are characterized by their ability to cleave the naturally abundant polysaccharide chitin. The potential role of chitinases has been identified in the manifestation of various allergies and inflammatory diseases. In recent years, chitinases inhibitors are emerging as an alluring area of interest for the researchers and scientists and there is a dire need for the development of potential and safe chitinase antagonists for the prophylaxis and treatment of several diseases.

Objective: The present review expedites the role of chitinases and their inhibitors in inflammation and related disorders.

Methods: At first, an exhaustive survey of literature and various patents available related to chitinases were carried out. Useful information on chitinases and their inhibitor was gathered from the authentic scientific databases namely SCOPUS, EMBASE, PUBMED, GOOGLE SCHOLAR, MEDLINE, EMBASE, EBSCO, WEB OF SCIENCE, etc. This information was further analyzed and compiled up to prepare the framework of the review article. The search strategy was conducted by using queries with key terms “chitin”, “chitinase”, “chitotriosidase”, “acidic mammalian chitinase”, “chitinase inhibitors”, “asthma” and “chitinases associated inflammatory disorders”, etc. The patents were searched using the key terms “chitinases and uses thereof”, “chitinase inhibitors”, “chitin-chitinase associated pathological disorders” etc. from www.google.com/patents, www.freepatentsonline.com, and www.scopus.com.

Results: The present review provides a vision for apprehending human chitinases and their participation in several diseases. The patents available also signify the extended role and effectiveness of chitinase inhibitors in the prevention and treatment of various diseases viz. asthma, acute and chronic inflammatory diseases, autoimmune diseases, dental diseases, neurologic diseases, metabolic diseases, liver diseases, polycystic ovary syndrome, endometriosis, and cancer. In this regard, extensive pre-clinical and clinical investigations are required to develop some novel, potent and selective drug molecules for the treatment of various inflammatory diseases, allergies and cancers in the foreseeable future.

Conclusion: In conclusion, chitinases can be used as potential biomarkers in prognosis and diagnosis of several inflammatory diseases and allergies and the design of novel chitinase inhibitors may act as key and rational scaffolds in designing some novel therapeutic agents in the treatment of variety of inflammatory diseases.

Keywords: Acidic Mammalian Chitinases (AMCase), allergy, allosamidin (Allo), chitinases, chitotriosidase (CHIT1), inflammatory disorders.

1. INTRODUCTION

Chitinases (EC 3.2.1.14) are the enzymes which belong to the 18 glycosyl hydroxylase (GH18) family, a class of ancient, conserved evolutionary enzymes [1]. They are distributed in a variety of organisms that include bacteria, viruses, fungi, parasites, insects, plants and mammals. They

undergo the cleavage of glycosidic bond by the hydrolysis of chitin via a substrate assisted mechanism [2].

Chitin is a linear, insoluble polysaccharide of β -1,4-linked polymer containing *N*-acetyl glucosamine as the monosaccharide units [3-5]. Although humans do not synthesize chitin of their own, yet their genome expresses certain chitinases which can specifically degrade chitin upon confrontation during inhalation or ingestion, thereby exhibiting an anti-pathogenic function [6, 7].

Recently, chitinases have emerged as an area of interest owing to their significant role in human health care [8, 9]. In

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the recent times, researchers have come across with various evidence suggesting the potential role of chitinases not only in the human defense system against parasites but also act as biomarkers for inflammatory diseases, asthma, acute and chronic inflammatory diseases, autoimmune diseases, dental diseases, neurologic diseases, metabolic diseases, liver diseases, polycystic ovary syndrome, endometriosis and cancer [10].

In this review, we intend to illuminate several chitinases and their inhibitors along with the related recent patents for the development of specific therapeutic approaches in the management of various diseases in particular, allergy and inflammatory disorders [11].

2. CHITINASES

Chitinases are the glycosidases which have been isolated from the stomachs of certain mammals, including humans [12]. Chitinase activity can also be detected in human blood [13, 14] and possibly cartilage [15]. Their sizes range from 20kDa to about 90kDa [2].

In humans, chitinases or chitin degrading enzymes consists of both enzymatically active chitinases and chitinase-like proteins also referred to as chi-lectins which are devoid of enzymatic activity [16]. The chitinases can be further classified as endochitinases and exochitinases based upon their mechanism of action i.e. whether it is endolytic or exolytic [17]. The endochitinases include Chitotriosidase or Chitinase 1(CHIT1) [18, 19] and Acidic Mammalian Chitinase (AMCase) [20, 21]. They cleave the chitin polymer randomly at internal sites [22]. The exochitinases referred to as Chitobiase (CTBS), catalyze the hydrolysis of chitin polymer in a progressive manner. Also identified are a set of enzymes that lack the catalytic site partially or completely [8]. However, they have retained carbohydrate binding pockets [23, 24]. They are collectively termed as Chitinase-Like Proteins (CLPs) or Chi- Lectins (ChiLs) [25]. The ChiLs so far identified in humans include Chitinase-3-Like 1 (CHI3L1), CHI3L2, Oviductin (OVGP1) and Stablin-1 interacting Chitinase-Like Protein (SI-CLP) or CHID [26]. The phylogenetic studies have revealed that the active chitinases resulted from an early gene duplication event and

the later duplication events followed by gene mutations resulted in the evolution of CLPs or ChiLs [27].

Chitinases exhibit their roles in both innate and adaptive immunity [28]. They are essential for the following three different functions: Chitinases are expressed in certain organisms during development to aid in the remodeling of their biological matrices to accommodate changes in body size and shape [29]. Secondly, chitinases help some organisms digest chitin containing food. Chitinases are also expressed in mammals, including humans and mice, that are prone to cause some reactions with chitin-containing pathogens (e.g. house dust mites, fungi, parasites) to degrade the chitin protective covering on the infectious microorganisms. This way the inner core of chitin is susceptible to attacks by chitinases that ultimately lead to death and expulsion from the body of the chitin-containing organisms [30].

The mechanism of action of family 18 chitinases is an unusual substrate assisted reaction mechanism, in which oxazolinium ion intermediate is formed as described in Fig. (1). Conserved glutamate protonates the glycosidic bond and the oxygen of the *N*-acetyl group on the -1 sugar (the sugar on the non-reducing end of the glycosidic bond) [31].

3. CHITOTRIOSIDASES

Chitotriosidase (CHIT1) is the first mammalian chitinase to be discovered. The gene encoding this is located on chromosome 1q31-q32. Boot *et al.* showed that CHIT1 has 12 exons [32]. It is synthesized as a 50kDa protein containing a 39kDa N-terminal catalytic triose-phosphate isomerase fold, which is characterized by the $(\beta/\alpha)_8$ barrel. It is a true chitinase having trans-glycosylation activity towards chitin [2].

Hollak *et al.* showed that it is both a diagnostic hallmark of Gaucher's Disease (GD) and a marker for monitoring the efficacy of various therapeutic approaches for the treatment of GD [33]. Weisner *et al.* demonstrated that recombinant CHIT1 plays a physiological role in the host defense mechanism by inhibiting fungal hyphal growth [34]. Di Rosa *et al.* suggested that chitinases regulate the immune response to chitin [35]. Boot *et al.* depicted that its activity is very low in

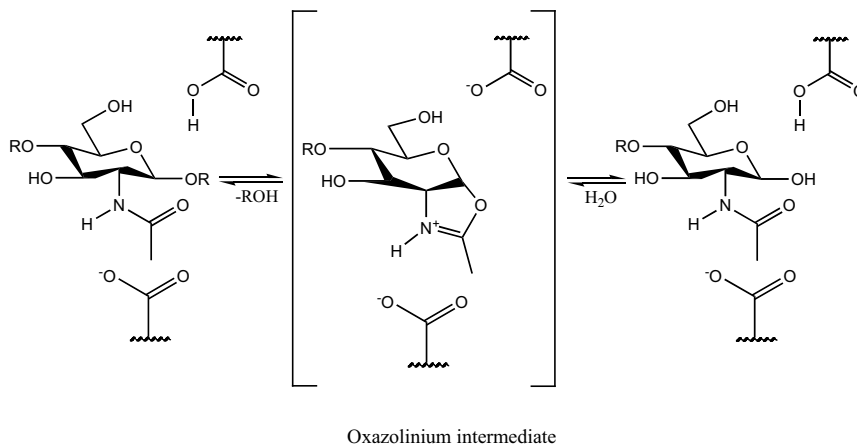


Fig. (1). Mechanism of action of chitinases undergoing chitin hydrolysis. Here, R is β -1,-linked *N*-acetylglucosamine residue. Chitinases hydrolyze the glycosidic linkage of the repeating β -1,-linked *N*-acetylglucosamine-containing polymer chitin. The hydrolysis is accompanied with the formation of an oxazolinium intermediate followed by the degradation of chitin in simpler monosaccharides.

healthy population [32]. On the other hand, Malaguarnera *et al.* reported that its activity increases a great deal during acute and chronic inflammatory disorders [36]. Van Ejjik *et al.* unveiled the fact that it is induced in neutrophils by TLR signaling and in macrophages by NOD-2 signaling. They also depicted that the people having the mutant allele exhibited an increased susceptibility to various infections like *Candida albicans*, *Wuchereria bancrofti*, *Cryptococcus neoformans* and *Plasmodium falciparum* [37].

Malaguarnera *et al.* confirmed that CHIT1 provides innate protection against malaria. They also stressed on the fact that in fibroblastic hepatic tissue, tissue remodeling is modulated by CHIT1 over expression in Kupffer cells [38]. Kim *et al.* proved that CHIT1 A442G polymorphism is linked to increased childhood atopy [39]. Conversely, Di Rosa *et al.* demonstrated that a functional CHIT1 gene polymorphism retards progression of NAFLD [40]. Artieda *et al.* reported that atherosclerotic plaque formation and thrombosis are enhanced by chitotriosidases produced by macrophages [41]. Bargagli *et al.* [42] and Iyer *et al.* [43] proposed that CHIT1 is elevated specifically in the later inflammatory conditions like tuberculosis and leprosy. Therefore, they are implicated in the progression of many such diseases as depicted in Fig. (2).

Sotgiu *et al.* showed the correlation between chitotriosidase activity and stroke severity [44]. Di Rosa *et al.* depicted that chitotriosidase mRNA expression was elevated in Alzheimer's disease [45]. Ries *et al.* showed that evaluation of CHIT1 activity helps in guiding further confirmatory assays for NPC (Niemann Pick disease type- C) and SMD (Sphingomyelinase deficiency) [46]. As far as male Fabry disease patients are concerned, the only marker discovered till date

for monitoring the efficacy of enzyme replacement therapy in them is CHIT1. Barone *et al.* pointed to the increased secretion of CHIT1 in response to the lysosomal system damage mediated by iron [47].

Di Rosa *et al.* depicted the increased mRNA CHIT1 expression in monocytes on treatment with IL-4. The Th2 cytokine IL-4 promotes immune responses to parasites. This finding helps us to envision clearly the association of increased CHIT1 secretion with the pathophysiological conditions mediated by Th2 cells [35]. Gavala *et al.* proposed the role of CHIT1 in asthma and airway hyper responsiveness [48]. Lee *et al.* demonstrated that CHIT1 play a significant role in IL-13 driven alveolar fibrosis by accentuating MAPK and TGF β signaling in mice [49].

Overall it can be confirmed that CHIT1 play a role in both innate and adaptive immune responses to inflammatory stimuli and that their inhibition would definitely have beneficial effects in various diseased states.

4. ACIDIC MAMMALIAN CHITINASES

Acidic Mammalian Chitinases (AMCase) are true chitinases which are located on chromosome 1 (1q13.1e21.3) [50]. They contain a 30-kDa N-terminal catalytic domain just like CHIT1 and are expressed mainly in lungs (airway epithelial cells) and gastrointestinal system of humans and mice [51]. They also contain a C-terminal chitinase binding domain (CBM). The enzymes are acid stable and have an optimum pH of 2.0; therefore, they are able to function well in the harsh environment of the stomach [50, 52].

Zhu *et al.* [51] and Zimmermann *et al.* [53] showed that AMCases are specifically up regulated in response to Th2

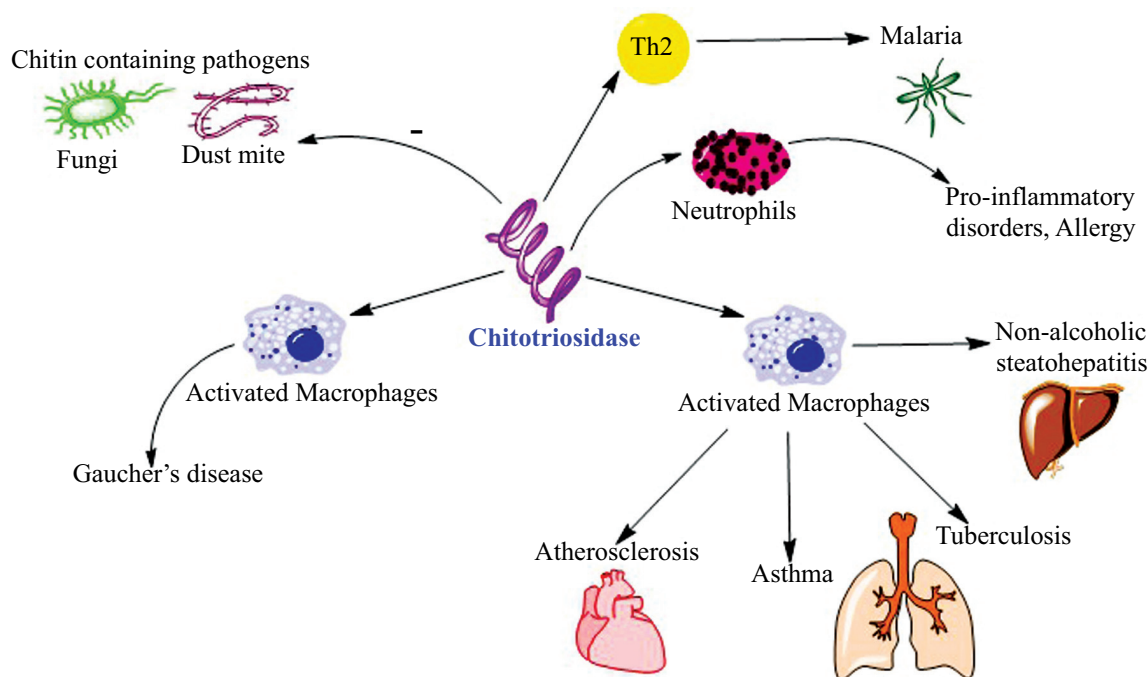


Fig. (2). Chitotriosidase (CHIT1) and cell type specificity and its role in associated pathological disorders. CHIT1 is the first mammalian chitinase to be discovered and characterized. It is a potential biomarker of activated macrophages in tissues hence exhibits a significant role in the manifestation of both innate and adaptive immunity. It exhibits hydrolysis as well as trans-glycosylation as its chitinolytic activity.

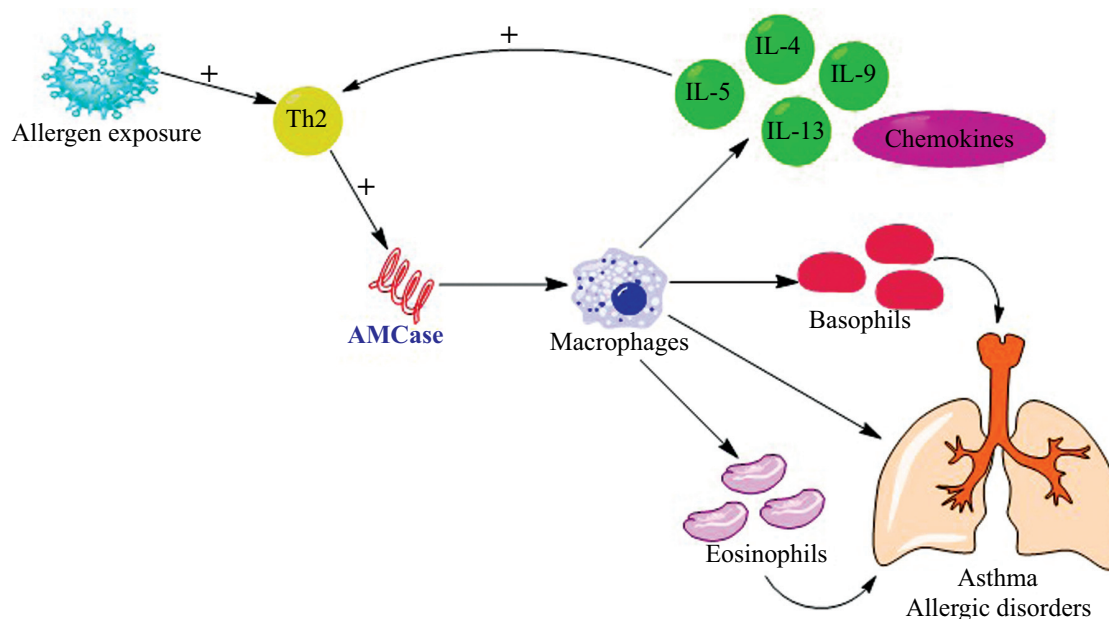


Fig. (3). Acidic mammalian chitinase (AMCase), cell type specificity and immune response mechanism. AMCase exhibits its true chitinase activity via hydrolysis of chitin polymer. It manifests its potential role in Th2 mediated pathophysiological conditions such as asthma and allergies. Higher expression of AMCases is detected in the lung tissues of asthmatic patients.

inflammation in the lungs (Fig. 3). Di Rosa *et al.* demonstrated that AMCases modulate tissue inflammation and immunity by either directly acting as chemotactic agents or by indirectly inducing other chemokines that attract T cells and eosinophils to the sites of parasitic infection [54]. AMCase is closely associated with most of the pathophysiological conditions that are mediated by Th2 cells. Now-a-days, the use of antimicrobials and antibiotics has increased a lot, especially in industrialized countries [55]. They suppress bacterial populations which produce chitinases therefore the levels of chitins are going up. In such settings, production of a hyper functional AMCase enzyme would prove beneficial to the subjects [7]. Matsumoto *et al.* showed that the prevalence of new onset asthma is high in individuals employed in industries, in which the exposure to chitin is anticipated to be high, like in shellfish processors [56]. Seibold *et al.* reported that pre-treatment of chitin with recombinant AMCase decreased chitin's inflammatory effects to a great deal [57].

They have also identified an AMCase isoform which shows significant activity at almost all pH values; the haplotype encoding it is found to be protective against asthma in various ethnic populations of the United States. Bierbaum *et al.* suggested that AMCases help airway epithelial cells in evading apoptosis by AKT signaling and PI3K stimulation [58].

5. CHITINASE INHIBITORS

Zhu *et al.* demonstrated chitinase inhibitors to have anti-inflammatory potential against asthma and allergic diseases, including atopic dermatitis and allergic rhinitis [51].

With an intent of identifying a useful chitinase inhibitor, Sunazuka *et al.* have developed a potent 1,5- Disubstituted triazole inhibitor of *Serratia marcescens* chitinase (*SmChi*) B from an azide-bearing arginin fragment and application of

the chitinase template utilizing the *in situ* click chemistry approach [59]. The new inhibitor expressed a 300 fold increase of inhibition against *SmChiB* compared to that of arginin [60]. Allosamidin, a pseudo trisaccharide first isolated from the mycelium of *Streptomyces* sp., is the most extensively studied chitinase inhibitor [61]. Various biological properties have been reported associated with its activity as a chitinase inhibitor, including inhibition of cell separation in fungi, toxicity towards insect larvae and blocking of malaria parasite penetration into the mosquito mid gut [62, 63]. Zhu *et al.* additionally reported that this compound was shown to decrease the number of inflammatory cells in a mouse model of asthma by targeting the Acidic Mammalian Chitinase (AMCase) [51]. Although total synthesis of allosamidin has been reported previously in Berecibar *et al.*, 1999 [64], the length and complexity of the synthetic routes involved severely limit both the availability of this compound and the scope for structure-based design of novel allosamidin-derived inhibitors [65]. A similar type of problem appears with argifin because its molecular weight and number of hydrogen-bond donors/ acceptors well exceed the Lipinski criteria for drug-likeness [66].

Matsumoto *et al.* reported that treatment with chitinase inhibitor- Demethylallosamidin (DMA) attenuated IL-13-induced Airway Hyper Responsiveness (AHR) [67] even at a lower dose than that of Allosamidin (Allo), a metabolite of *Streptomyces*. It also suppressed allergen-induced AHR. These findings suggest that DMA is a more effective inhibitor of asthmatic responses than Allo and could be a potential therapeutic target for asthma [68].

Andersen *et al.* underwent the progressive dissection of argifin, a natural product cyclopentapeptide. The peptide fragments inhibited chitinase B1 from *Aspergillus fumigatus* (AfChiB1), the human chitotriosidase and chitinase activity

in lung homogenates from a murine model of chronic asthma, with potencies ranging from high nano molar to high micro molar inhibition [66]. Withers *et al.* described the proficient and readily scalable synthesis of novel, potent chitobiose and chitotriose thiazolines, especially in their thioamide forms possessing broad spectrum inhibitory activities, which could be used as probes for the modulation of chitinase activity in the biological systems [69].

van Aalten *et al.* identified methyl xanthine derivatives such as caffeine, theophylline and pentoxifylline to be the possible human chitinase inhibitor leads after screening a drug library of marketed drug molecules and devised their therapeutic role as anti-inflammatory agents, with pleiotropic mechanism of action thereby providing synthetically accessible scaffolds for further optimization [70]. van Aalten *et al.*

also demonstrated the inhibitory effects of four new cyclic peptides (CI-4) derivatives by undertaking their structural optimization and hence providing further structural insights and new scaffolds to understand the mechanism of inhibition of chitinases [71]. In another study, they described the enzymological and structural analysis of the interaction between the enzyme chitinase ChiB from *S. marcescens* and the previously designed and synthesized *N-N*-diacetyl-chitobionoxime-*N*-phenylcarbamate (HM508), a GlcNAc2-derivative and identified their efficient inhibitory role [72].

6. RECENT PATENTS

In the past two decades, several other chitinase inhibitors have been identified and patented as shown in Table 1.

Table 1. Patents of Novel Inventions in the Treatment of Various Chitinase Associated Disorders and Inhibitors Thereof.

S. No.	Disease to be Treated	Patent Title	Patent Number	Inventors	Date of Publication	Legal Status	References
1.	Asthma, acute and chronic inflammatory diseases, autoimmune diseases, dental diseases, neurologic diseases, metabolic diseases, liver diseases, polycystic ovary syndrome, endometriosis, and cancer	Substituted amino triazoles useful as human chitinase inhibitors	US10208020	Mazur, M., Koralewski, R., Borek, B., Olezniczak, S., Czestkowski, W., Piotrowicz, M.C., Olczak, J.P., Golebiowski, A.A., Bartoszewicz, A. Maziarecz, E., Kowalski, M.I.	19.02.2019	Granted	[73]
2.	Renal injury and renal failure	Methods for evaluating renal injury and renal failure using urine levels of chitinase - 3 - like protein 1	US20190120858	Anderberg, J., Gray, J., McPherson, P., Nakamura, K., Kampf, J.P.	25.04.2019	Granted	[74]
3.	Epithelial and cutaneous disorders	Cosmetic use of chitinase - type proteins	US9926587	Bernard, D., Berville, M.D.	27.03.2018	Granted	[75]
4.	Inflammation and age-related pulmonary fibrosis	Chitinase administration to the airway to treat inflammation and age-related pulmonary fibrosis	WO2018191379	Richard, L., Steven, V.D.	18.10.2018	Granted	[76]
5.	Fungal infection	New macrocyclic amidinourea derivatives, methods of preparation and uses thereof as chitinase inhibitors	US20160137617	Sanfilippo, S., Posteraro, B., Sanguinetti, M., Botta, M., Maccari, G., De Luca, F., Docquier, J., Deodato, D.	19.05.2016	Granted	[77]
6.	Asthma and allergic responses	Substituted amino triazoles useful as human chitinase inhibitors	US2016176843	Golebiowski, A.A., Koralewski, R., Czestkowski, W., Matyszewski, K., Olejniczak, S., Olczak, J., Beckett, P.	23.06. 2016	Granted	[78]

Table (1) contd....

S. No.	Disease to be Treated	Patent Title	Patent Number	Inventors	Date of Publication	Legal Status	References
7.	Asthma and allergic responses	Substituted amino triazoles useful as acidic mammalian chitinase inhibitors	US20160368894	Golebiowski, A.A., Koralewski, R., Czeszkowski, W., Matyszewski, K., Olejniczak, S., Olczak, J., Beckett, P.	22.12.2016	Granted	[79]
8.	Asthma	Substituted amino triazoles, and methods using same	WO2015095701	Corman, M.M., Gollebiowski, A., Beckett, R.P., Mazur, M., Olejniczak, S.	25.06.2015	Granted	[80]
9.	IL-13 mediated inflammation	C/CLP antagonists and methods of use thereof	US20110059100	Reed, J.L., White, W., Coyle, A., Kozhich, A., Elias, J., Doncki, N., Gao, C., Wu, H.	10.03.2011	Granted	[81]
10.	Inflammatory disorders	Novel human acidic mammalian chitinase and use thereof.	US20090191552	Vora, K.A., Demartino, J.A., Mudgett, J.S., Poster, J., Wolfe, G.	30.07.2009	Granted	[82]
11.	Th2-driven and/or IL-mediated inflammatory diseases	C/CLP antagonists and methods of use thereof	WO2007027748	Reed, J.L., White, W., Coyle, A., Kozhich, A., Elias, J.	08.03.2007	Granted	[83]
12.	Various degenerative diseases involving inflammation, tissue repair and tissue remodeling	Use of chito oligomer formulations to modify abnormal activity of chitinase like proteins	WO2006054319	Olafur, B.E., Jon, M.E., Johannes, G., Finnbogi, T.	26.05.2006	Granted	[84]
13.	Asthma	Inhibitors of Acidic Mammalian Chitinase as asthma therapeutics	WO2004092404	Maximillian, T.F.	28.10.2004	Granted	[85]
14.	Inflammatory disease	Methods, compositions and kits relating to chitinases and chitin like molecules and inflammatory disease	WO03009808	Elias, J., Zhu, Z.	06.02.2003	Granted	[86]
15.	Fungal infection	Purified chitinases and use thereof	US6251390	Harman, G.E., Broadway, R.M., Tronsmo, A., Lorito, M., Hayes, C.K., Pietro, A.D.	26.06.2001	Granted	[87]

Mazur *et al.* disclosed the unexpected discovery owing to the chemical modification of amino-triazole-4-amino piperidine small compounds with a heterocyclic ring substitution (morpholine or piperazine) leading to the better fixation of their molecular geometry and increasing the molecular rigidity that resulted in an unexpected enhancement in their inhibitory efficacy towards acidic mammalian chitinase. Hence, the substituted amino -triazole compounds can be used in the treatment of various disorders associated with the up regulation or dysregulation of AMCCase activity and CHIT1 activity such as asthma reactions caused by allergens as well as acute and chronic inflammatory diseases, autoimmune, metabolic, neurologic diseases and cancer US10208020 (2019) [73].

Anderberg *et al.* disclosed that chitinase-3-like protein 1 can be used as a biomarker in a subject with renal injury and hence the present invention described different assays, meth-

ods and compositions to measure the chitinase-3-like protein 1 for the prognosis, diagnosis, risk-stratification, stage determination, monitoring and providing effective therapeutic regimen to a subject suffering from or suspected of a renal injury US20190120858 (2019) [74].

Bernard *et al.* in their invention disclosed the cosmetic and therapeutic use of YKL-40 protein that belongs to the family of chitinase- type proteins or of peptides derived from this protein or of analogs thereof, of a nucleic acid sequences encoding such a polypeptide or an agent that modulates the expression of such a polypeptide especially for the stimulation of terminal epithelial differentiation US9926587 (2018) [75].

Richard *et al.* disclosed the novel therapeutic delivery of chitinases in the aerosolized form promoting the clearance of environmentally derived chitins and hence exerting a benefi-

cial role in the prevention and treatment of pulmonary fibrosis. Also, the invention encompassed novel diagnostic methods for the detection of chitin and chitinase activity WO2018191379 (2018) [76].

Sanfilippo *et al.* described the synthesis of a new series of amidinourea derivatives for the treatment of fungal infections of candida species, IL-13 and Th2 mediated inflammation, allergic airway diseases and asthma. The disclosure also provided pharmaceutical compositions and formulations for the treatment of fungal infections to be used as chitinase inhibitors US20160137617 (2016) [77].

Golebiowski *et al.* in their patents described the synthesis of a novel series of amino triazole compounds substituted with carboxylate group or bioisosteric polar functional group as therapeutically potent Acidic mammalian chitinase inhibitors for the treatment of asthma caused by different allergens. They also disclosed methods for the treatment of fungal or parasitic infections that comprises of administration of therapeutically effective amount of synthesized compounds under consideration US20160368894, US2016176843 (2016) [78, 79].

Corman *et al.* disclosed the novel substituted amino triazoles and their respective pharmaceutically acceptable salts, hydrates and solvates as potential inhibitors of Acidic mammalian chitinases. The invention also provided different methods for the treatment of asthma and allergies and to monitor the asthma treatment as well WO2015095701 (2015) [80].

Reed *et al.* in their disclosure described the novel molecules, compounds and methods of use of the same as C/CLP antagonists for the treatment of disorders associated with the up regulation of one or more C/CLPs, Th-2 driven inflammatory disorders of the lungs, inflammatory arthritis and IL-13 mediated inflammation. These antagonists include any suitable molecules that disrupt one or more activities of C/CLPs including antibodies, antibody fragments, having specificity for C/CLP ad their fragments. The patent also revealed isolated antagonists identified via the disclosed methods and medicaments and therapeutic compositions comprising the same US20110059100 (2011) [81].

Vora *et al.* disclosed in their patent the novel variant acidic mammalian chitinase with improved stability and an isolated nucleic acid sequence that is responsible for encoding the variant enzyme. The invention further provided the nucleic acid constructs, vectors and host cells comprising of nucleic acid sequences and methods for the production of recombinant variant polypeptide. Also, the potential role of AMCCase in high throughput screening assays to identify whether a test compound is capable of modulating its chitinolytic activity was described US20090191552 (2009) [82].

Reed *et al.* in their patent disclosed the novel C/CLP antagonists, their compositions and various methods for the treatment or amelioration and management of Th-2 driven and IL-13 mediated inflammatory disorders of lungs and inflammatory arthritis associated with the up regulation of C/CLPs WO2007027748 (2007) [83].

Olafur *et al.* described the use of novel chiti oligomer formulations for the modulation of inflammation in patients suffering from connective tissue, cardiovascular tissue asso-

ciated diseases, central nervous system or degenerative disorders thereby reducing the abnormally high expression of chitinase-like proteins. It was also revealed that chitoligomeric formulations promote tissue regeneration instead of tissue repair and do not allow the fibroblasts to invade the tissue sites WO2006054319 (2006) [84].

Maximillian *et al.* described different methods for screening of agents involved in the exhibition of certain symptoms and pathological complications involved in asthma. The administration of therapeutic amount of these agents which include AMCCase inhibitors, IL-13 antagonists and also the soluble antagonists of IL-13 receptors led to the decrease in the expression or production of AMCCase protein that can be useful in treating asthma. The disclosure also included a method for monitoring the efficacy of treatment of asthma WO2004092404 (2004) [85].

Elias *et al.* stated in their invention that administration of an effective amount of chitinase-like molecule inhibitor to a mammal resulted in the treatment of inflammatory diseases. The chitinase-like molecule inhibitor is selected from a group consisting of a chemical compound, an antibody, a nucleic acid, a ribozyme and an anti-sense nucleic acid molecule. The disclosure also provided methods to identify new chemical compounds for the treatment of asthma, COPD, allergies and inflammatory diseases as for the first time the expression of IL-13 and chitinase-like molecule was found to be associated with inflammatory diseases WO03009808 (2003) [86].

Harman *et al.* in their patent provided the biologically pure compositions of chitinases containing an endochitinase and an exochitinase that can be used for the treatment of fungal infections and various insect related infections by inhibiting chitin containing fungi and insects US6251390 (2001) [87].

7. CHITIN AND CHITINASE EXPOSURE - ASSOCIATED PATHOGENIC MECHANISMS

Human chitinases play a significant role in mediating different allergic reactions and auto-immune diseases. They cause several allergic reactions in atopic individuals or in individuals with chitin exposure in high doses or for longer duration of time [88]. The females are at higher risk of attaining the chitinase allergies since they possess the hormone estrogen [89]. Cytokines or chemokines are secreted upon the recognition of chitins via pattern recognition receptors (pprs) [90]. Bacterial clearance is then promoted by CXCL8 which is a neutrophil attractant at the site of infection. Glycosylation of important host proteins takes place rendering them either active or inactive. The glycol moieties are then cleaved from these glycol proteins by the pathogenic or allergen acquires chitinases as they are glycosidic in nature, leading the alteration of their functions and hence resulting in flustered protein cascade precipitating various diseases like auto-immune diseases, asthma, cystic fibrosis and cancer [91- 94].

The chitin binding domains are present in lethal viruses such as Ebola and hepatic C virus and Zika virus, which are used by them to manipulate their hosts and carrier organisms [95]. In addition to this, the mosquito vector *Aedes aegypti*

that is held responsible for transmitting dengue and yellow fever in humans [96].

The pathogenic bacterium, *Vibrio cholera* also expresses chitinases that causes gut inflammation by triggering the immune responses [97].

The chitin-chitinase allergies could be referred to as occupational allergies since they exhibit a major dependency on the occupation of individuals. For instance, prohevein, a cysteine rich protein present in rubber (*Hevea brasiliensis*), bears high homology with human chitin-binding proteins, causes IgE-associated allergy in workers wearing gloves in laboratories or healthcare practices [98]. The carpenters who work with woods like chestnut are more prone to asthma and rhinitis since they are exposed to the tree chitinases for longer durations [99, 100]. Similarly, people working in seafood processing industries are susceptible to allergies associated with chitinases of fish and crustaceans [101-103] (Fig. 4).

The potential immune modulatory role of chitinases has been studied in house-dust mite allergic patients where they induce basophil activation in IgE dependent manner [104]. The findings of a murine model administered with chitin has demonstrated the ability of chitinases to induce the infiltration of tissues of IL-4 expressing innate immune responses with Interlukin (IL)-4- and IL-13- expressing cells, including basophils, eosinophils and T-helper cells thereby expressing the significant role of chitinases in the modulation of allergic and parasitic worm immunity [105, 106].

CONCLUSION

In conclusion, chitinases can be used as significant biomarkers in prognosis and diagnosis of several inflammatory diseases and allergies. Therefore, by utilizing the findings of experiments performed with knockout animal models and interpretation of different mechanisms involved in their physiological as well as pathological roles, they could serve as potential candidates in the development of novel, potent and selective drug molecules. Hence, further extensive pre-clinical and clinical investigations are required to develop some novel chitinase inhibitors to treat various inflammatory diseases, allergies and cancers in the foreseeable future.

CURRENT & FUTURE DEVELOPMENTS

The present review article summarized the applications of chitinases in biomedical and environmental field. Chitinases are glycosyl hydrolases that are widely expressed in mammals. Their diversity is not only restricted to GH 18 and GH 19 family of enzymes as previously anticipated. In the recent years, chitinase research has attained unparalleled growth at the molecular levels. Current studies have revealed their effective role in both adaptive and innate immunity and therefore, they are being used as biomarkers in several inflammatory and malignant disorders. Promising results have been achieved in the direct application of parenteral chitinase preparations to treat systemic fungal infections. Chitinases have attained prominence as bio control agents for insect vectors of certain human diseases. Also, the production of

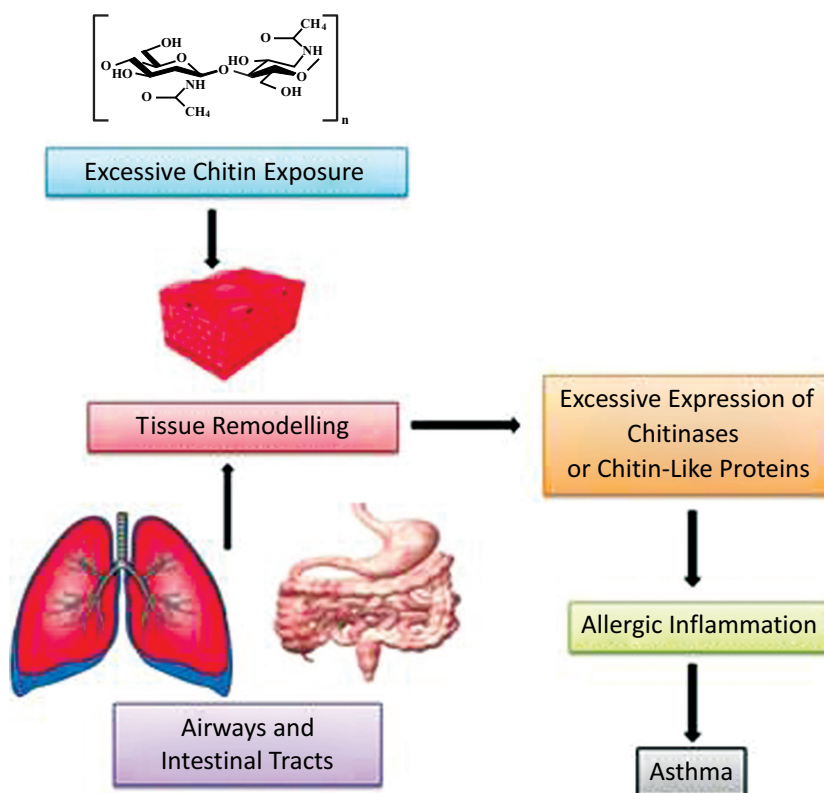


Fig. (4). Chitin-chitinase- associated occupational allergies. Chitinase associated allergies are often associated with the population of workers working for an occupation that involves consistent exposure with chitins and homologous human chitin binding proteins.

synthetic polysaccharides as prophylactic and therapeutic agents and neoglycoproteins as significant tools for structure function analysis with the aid of chitinases has drawn special attention in the recent times.

In the future, additional studies and experiments are required to explore the role of specific cell types involved in the secretion of chitinases via protein engineering and to better apprehend the biology and immune biology of chitinases along with the identification of their active sites for the enhancement of human immunity. Also, there stands a possibility of utilizing chitinases as food preservatives to increase the shelf life of certain food products. At last, chitinases can be studied further for their environmental applications as they serve as anti-pollutants to control the water pollution since they convert the chitinous waste of marine organisms into simpler compounds. Therefore, they can act as environment conservers by remediation of various organic contaminants. Also, it is hoped that this review will encourage the researchers to explore chitinases at a broad scale in order to develop new methodologies and therapeutic approaches for the betterment of mankind

LIST OF ABBREVIATIONS

GD	=	Gaucher's Disease
TLR	=	Toll Like Receptor
NOD-2	=	Nucleotide-binding Oligomerization Domain-containing protein 2
CHIT1	=	Chitotriosidase
NAFLD	=	Non-Alcoholic Fatty Liver Disease
CBM	=	C-Terminal Chitinase Binding Domain
AHR	=	Airway Hyperresponsiveness
NPC	=	Niemann Pick Disease Type-C
SMD	=	Sphingomyelinase Deficiency
IL-13	=	Interleukin-13
MAPK	=	Mitogen Activated Protein Kinase
TGF- β	=	Transforming Growth Factor Beta

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this review.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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