

Chitinases and chitinase-like proteins in T_H2 inflammation and asthma

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Chitin is the second most abundant biopolymer in nature, where it protects crustaceans, parasites, fungi, and other pathogens from the adverse effects of their environments, hosts, or both. Because chitin does not exist in mammals, it had been assumed that the chitinases that degrade it are also restricted to lower life forms. However, chitinases and chitinase-like proteins have recently been noted in mice and human subjects. The prototypic chitinase, acidic mammalian chitinase, was also noted to be induced during T_H2 inflammation through an IL-13-dependent mechanism. It was also shown to play an important role in the pathogenesis of T_H2 inflammation and IL-13 effector pathway activation and demonstrated to be expressed in an exaggerated fashion in human asthmatic tissues. The finding that chitinases contribute to host antiparasite responses and asthmatic T_H2 inflammation support the concept that asthma might be a parasite-independent antiparasite response. (*J Allergy Clin Immunol* 2005;116:497-500.)

Key words: IL-13, acidic mammalian chitinase, chitinase, breast regression protein 39, T_H2 inflammation

Asthmatic airways dysfunction used to be considered largely in terms of the contraction of airway smooth muscle (bronchospasm). However, it is now believed that asthma is a chronic inflammatory disease and that dysregulated T_H2 inflammation plays a major role in the pathogenesis of this disorder. Studies of this response have demonstrated that IL-4 plays a major role in the initiation of T_H2 inflammation. In contrast, IL-13 is now believed to

Abbreviations used

AMCase: Acidic mammalian chitinase
BRP-39: Breast regression protein 39
C-CL: Chitinase and chitinase-like
HcGP-39: Human cartilage glycoprotein-39 kd

be a major effector molecule at sites of T_H2 pathology.^{1,2} In keeping with this important role of IL-13, investigators have worked diligently to define the mechanisms that IL-13 uses to induce its asthma-like tissue effects, including eosinophilic inflammation, mucus metaplasia, airway fibrosis, and airways hyperresponsiveness on methacholine challenge. These studies demonstrated that IL-13 mediates these responses by activating and/or regulating the expression of a complex cascade of downstream genes, including chemokines, matrix metalloproteinases, cathepsins, antiproteases, adenosine deaminase, adenosine receptors, vascular endothelial cell growth factor, and TGF- β s. Recently, IL-13 has also been shown to regulate the production of chitinases and chitinase-like proteins.³ Although the roles of these molecules in biology are poorly understood, these observations provide potential insights into the pathways of T_H2 inflammation and the nature of the asthmatic response.

BIOLOGY OF CHITIN AND CHITINASES

Chitin is a polymer of N-acetylglucosamine that, after cellulose, is the second most abundant polysaccharide in nature. Although it does not have a mammalian counterpart, chitin is found in the walls of fungi; exoskeleton of crabs, shrimp, and insects; the microfilarial sheath of parasitic nematodes; and the lining of the digestive tracts of many insects.⁴⁻¹¹ These pathogens use chitin to protect the invader from the harsh conditions inside the animal or plant host. In these circumstances, an absence of chitin can lead to the death of the pathogen.

Chitin deposition is regulated by biosynthesis and degradation. Chitinases are endo- β -1,4-N-acetylgluco-

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samidases. They have been studied most intensely in lower life forms, where they are produced in significant quantities by hosts defending against infections with chitin-containing organisms. This can be appropriately considered part of the innate immune response against a chitin-containing pathogen. Chitinase production can also play a key role in the life cycle of chitin-containing fungi and parasites, where they control growth and molting, and can be used by pathogens to invade or exploit chitin-containing structures to complete their life cycle in the host.¹¹⁻¹³ As a result of the importance of chitin in the protection of pathogens and the importance of appropriately regulated chitinase production in the life cycle of many pathogens, chitin synthesis inhibitors and chitinase inhibitors have received significant attention in the context of pharmaceutical approaches designed to develop new biopesticides and microbial biocontrol agents to eradicate insects, fungal pathogens, and helminthic parasites. Of the chitinase blockers, allosamidin and its derivatives are the most prominent and best characterized inhibitors described to date.

MAMMALIAN CHITINASE AND CHITINASE-LIKE GENES

Until a few years ago, it was generally assumed that mammals lacked chitinases. Recent studies in human subjects and rodents, however, have identified a family of chitinase and chitinase-like (C-CL) genes in both species. Acidic mammalian chitinase (AMCase), chitotriosidase, oviductin, human cartilage glycoprotein-39 kd (HcGP-39)/YKL-40, and YKL-39 have been described in human subjects, whereas YM-1, YM-2, AMCase, oviductin, and breast regression protein 39 (BRP-39) have been described in mice.^{5,6,14-16} To date, AMCase and BRP-39 have been most intently studied and are reviewed below.

AMCase

AMCase was the second mammalian chitinase to be cloned. It is a 50-kd product of genes on mouse chromosome 3 and human chromosome 1 at p13.1-p21.3.^{5,17} The enzyme is acid stable, with a pH optimum of 2.0, and has true chitinase bioactivity when tested with crab, fungal, and synthetic substrates. This enzyme might also have non-chitin-related biologic effector properties because an AMCase-like protein has been reported to possess fibroblast growth-promoting activity,¹⁸ and AMCase has been implicated in host defenses and food processing.⁵ Our studies demonstrate, for the first time, that AMCase plays a key role in T_H2 inflammation (see below).

BRP-39

BRP-39 was discovered in mouse breast cancer cells,¹⁹ and a variety of homologues (with different names) have been described in different species. They include human

cartilage glycoprotein-39 kd (HcGP-39), human YKL-40, porcine 38-kd heparin-binding glycoprotein (GP38K), and bovine 39-kd whey protein. BRP-39 and HcGP-39/YKL-40 are 39-kd products of genes on chromosome 1 in the mouse and human subject, respectively, that do not have chitinase activity. A variety of lines of evidence, however, have implicated BRP-39-like molecules in the pathogenesis of tissue remodeling because they can regulate stromal cell proliferation, differentiation, and survival. Interestingly, HcGP-39/YKL-40 might also play an important role in disease pathogenesis because circulating levels of this moiety are increased in rheumatoid arthritis, osteoarthritis, metastatic breast cancer, hepatic fibrosis, severe purulent meningitis, and severe community-acquired pneumonia.²⁰⁻²⁵

FUNCTIONS OF MAMMALIAN C-CL PROTEINS

One of the most pressing issues in chitinase biology relates to our almost complete lack of understanding of the functions of these strongly conserved (and therefore presumably biologically important) enzymes in human subjects.¹⁴ We are also lacking in our knowledge of the importance of chitinase degradation of chitin, glycosaminoglycans, and/or glycoproteins¹⁷ and the ability of some C-CL proteins to act as lectins and bind to heparin and heparan sulfate moieties²⁶ in mediating their biologic properties. Mammalian chitinase-like proteins are induced at sites of inflammation (eg, parasitic infections)¹⁵ and remodeling.²² This raises the possibility that these molecules play active roles in human antiparasite and anti-infective defense and repair responses. When viewed in combination, it is reasonable to believe that mammalian enzymes with true chitinase activity (eg, AMCase and chitotriosidase) can play a direct role in host responses to chitin-containing pathogens. It is also reasonable to postulate that these or other chitinase proteins can also (1) play a role as sentinels that trigger responses to parasites, infections, and/or antigen challenge; (2) function directly as chemotactic agents or indirectly by inducing other chemokines that attract eosinophils and T cells to sites of parasitic infection; and/or (3) modulate tissue inflammation, immunity, and/or remodeling.

C-CL PROTEINS IN T_H2- AND IL-13-INDUCED INFLAMMATION

Host immune responses to parasites include an early innate component designed to control or eliminate parasitic infestation and, over time, an adaptive response that controls the parasite in an antigen-specific fashion. The immune response to parasites is frequently T_H2 dominated and is known to share key features with tissue allergy, including the increased production of IL-4, IL-5, IL-13, and IgE and prominent tissue eosinophilia. As a result, it is believed that T_H2 inflammatory responses originally

evolved to deal with parasites and other pathogens and that allergy, atopic asthma, and the inflammatory and remodeling responses that are associated with these disorders occur when these T_H2 responses are poorly controlled, are elicited in a parasite pathogen-independent fashion, or both.^{27,28}

As noted earlier, chitinases are believed to play a key role in the innate immunity to parasites and other infectious agents. It is thus reasonable to hypothesize that, when produced in a dysregulated fashion, they also play an important role in the pathogenesis of allergy and/or asthma. This concept is supported by the observation in some,²⁹ but not all,¹⁵ studies that YM-1 protein is a potent chemotactic agent for eosinophils and $CD4^+$ T cells. Additional support can be derived from studies that demonstrate that chitin is a potent T- and B-cell adjuvant that can shift developing immune responses in a T_H1 direction⁴ and that some microbial and plant chitinases are potent allergens in the pathogenesis of avocado, latex, and house dust mite allergy.^{14,30,31}

To test the hypothesis that chitinases are induced during and play essential roles in T_H2 tissue responses, mRNA, protein, and bioassay evaluation techniques have been used to assess the expression of AMCase in lungs from mice with antigen-induced T_H2 inflammatory responses and transgenic mice in which IL-13 was overexpressed in a lung-specific fashion. These studies demonstrated that AMCase mRNA and protein are prominently induced in epithelial cells and macrophages in these murine modeling systems.³ They also demonstrated that this induction was T_H2 specific and that IL-13 was necessary and sufficient for AMCase induction in the mouse lung.³ This inductive event appeared to play an important role in T_H2 inflammation because AMCase-based interventions with anti-AMCase antiserum or allosamidin diminished bronchoalveolar lavage and tissue inflammation and airways hyperresponsiveness.³ Interestingly, this inhibition was not associated with a decrease in T_H2 cytokine induction, IL-4, or IL-13 receptor component expression of signal transducer and activator of transcription 6 phosphorylation.³ It was, however, associated with a decrease in the ability of IL-13 to induce many of the chemokines that are involved in the generation of its tissue responses.³ These observations suggest that AMCase plays a critical role in IL-13 effector response activation.

AMCase IN HUMAN ASTHMA

The studies noted earlier demonstrate that AMCase is prominently expressed in lungs from antigen-sensitized and challenged and IL-13 transgenic mice. To begin to define the relevance of these findings to human T_H2 inflammatory responses, *in situ* hybridization has been used to compare the expression of AMCase in lung biopsy samples from healthy subjects and patients with asthma. AMCase mRNA was not readily detected in lung tissues from the patients without known lung disease. In contrast, AMCase was readily appreciated, histologically and

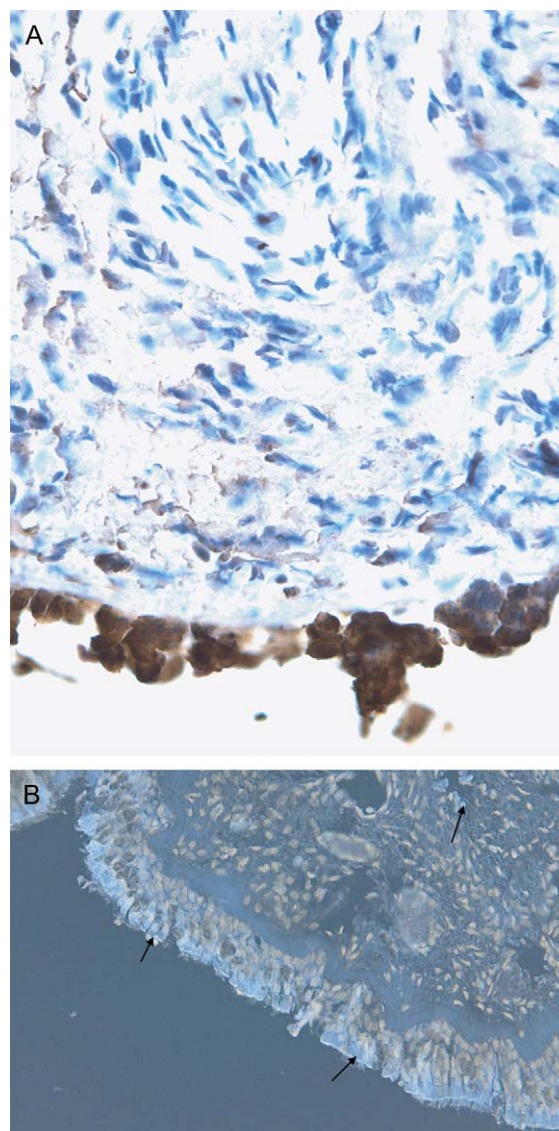


FIG 1. **A,** *In situ* hybridization of a frozen section from an asthmatic individual (moderately severe) showing positive signal in the epithelium and inflammatory cells using cRNA Dig-labeled probe, 400 $\times$ magnification. **B,** Fluorescent *in situ* hybridization of a frozen section from an asthmatic patient showing positive signal in the epithelium and inflammatory cells using cRNA probe coding for AMCase mRNA.

morphometrically, in the epithelial cells and subepithelial cells in tissues from patients with asthma³ (Fig 1). These studies demonstrate that, in accordance with the T_H2 inflammation and IL-13 transgenic models, AMCase is expressed in an exaggerated fashion in lung tissues from patients with asthma. The recent description of a polymorphism in AMCase that is associated with lower bronchodilator responsiveness in Puerto Rican asthmatic subjects³² further supports the contention that AMCase plays an important role in asthma pathogenesis.

THE TIP OF THE ICEBERG

There is mounting evidence that C-CL proteins are highly regulated and can contribute to tissue inflammatory and remodeling responses. This can be readily appreciated in studies of the novel chitinase AMCase that is stimulated in an IL-13-dependent, T_H2 -specific fashion in the murine lung where it plays an important role in T_H2 inflammation and IL-13 effector pathway activation. In accordance with these findings, AMCase is also overexpressed in human asthma. These studies highlight the tip of the iceberg in terms of the definition of the processes that regulate and the biologic roles of C-CL proteins in biology. It is difficult to know whether the roles of AMCase in these modeling systems reflect its roles in other situations. It is particularly tempting to speculate that C-CL proteins might have different effects in situations in which chitin is present or absent. Thus additional investigations are needed to define the roles of AMCase and other C-CL proteins in asthma, parasite infection, and other T_H2 - and T_H1 -mediated responses.

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