Research Focus

The potential role of chitin in allergic reactions

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Allergy, a potentially life-threatening condition, has at its heart an overly zealous T-helper type 2 response to environmental antigens. We are constantly flooded by potential allergens, both airborne and ingested. Although innocuous to some, common antigens can cause serious allergic reactions in susceptible individuals. Chitin, a polymerized sugar and fundamental component of arthropods and fungi, is not commonly deemed a potential allergen but can cause sensitization through frequent exposure. A recent intriguing study has highlighted the role of chitin in allergic reactions.

Introduction

Chitin is a polysaccharide of β-D-glucose containing amino groups, and is a key structural component of helminths, arthropods and fungi (Figure 1). Chitin is second only to cellulose as the most abundant biological polymer on the planet. Despite its ubiquity, chitin does not accumulate in the environment because bacteria and saprophytes can catabolize it. Mammals, which cannot synthesize or metabolize chitin, are equipped with a wide range of enzymes to detect and dispose of chitin and/or chitin-containing organisms. Examples of these enzymes include acidic mammalian chitinase (AMCase), chitotriosidase (CHIT1) and chitinase-3-like proteins 3 and 4 (Ym-1 and Ym-2, respectively, in mice).

Recent genomic studies analyzing chitinase genes have found high conservation in CHIT1 across mammals and have identified an inactivating 24-base-pair duplication in some alleles of the human gene [1]. Chitinases such as CHIT1 might be important factors in immune responses against chitin-containing pathogens, and similar mutations in humans could decrease chitinolytic activity and increase chitin accumulation. Furthermore, other studies have shown increased expression of AMCase in the lungs of individuals affected with asthma and have linked polymorphisms in AMCase to asthma susceptibility in children (Figure 1) [2,3], raising the possibility that inherent deficits in human chitin degradation could underlie airway inflammation and favour allergic reactions.

Epithelial cells and antigen-presenting cells (APCs) in the airways or the gut are often exposed to chitin by inhalation or ingestion and respond by increasing the expression of chitinases and chitin-binding proteins. An immune response to chitin can develop into T-helper type 1 (Th1) or Th2 responses depending on the presence of other microbial components and the APCs involved in the processing [4,5]. As we review here, Reese *et al.* [4] have now proposed and demonstrated a role for chitin in Th2 inflammatory responses during helminth infection that might be responsible for allergic reactions.

Helminth infection and Th2 recruitment to the site of inflammation

Following on from previous studies of *Nippostrongylus brasiliensis* infection in animal models [6], Reese *et al.* [4] proposed that chitin could be a key allergen in some helminth infections and, indeed, demonstrated that it can recruit eosinophils and basophils to tissues. Th2 immune responses are typically induced by parasitic worm infection and, in some cases, can cause unwanted allergic reactions. After exposure to *N. brasiliensis*, the lungs of infected mice showed induction of interleukin-4 (IL-4) and IL-13. This in turn triggered an increase in chitinase (AMCase and Ym2), signalling not only the presence of chitin at the site, but also the need to degrade it.

Reese *et al.* [4] lent further support to their hypothesis by administering chitin directly to the lungs of mice expressing a green fluorescent protein (GFP)-enhanced transcript of IL-4 (*4get* mice). Within hours of chitin exposure, IL-4–GFP-positive cells (in particular eosinophils and basophils) were recruited to the lungs of these mice. Moreover, chitin-induced eosinophil and basophil recruitment is independent of Toll-like receptors (TLRs), because administration of chitin to mice deficient in TLR-4 or myeloid differentiation factor 88 was found to elicit allergic inflammatory responses. This important finding by Reese *et al.* [4] compels definition of the receptors involved in chitin detection by the cells of the innate immune system.

Alternatively activated macrophages mediate eosinophil recruitment in response to chitin

Tissue-specific macrophages closely resemble an alternative phenotype of activated macrophage [7]. They can be separated from classically activated macrophages by expression of the mannose receptor Ym-1 and the enzyme arginase-1. Previous work has shown that the chitinase-like proteins Ym-1 and Ym-2, signature markers of alternatively activated macrophages (AAMs), can be expressed in a manner dependent on the Th2-associated signalling molecule Stat6 [8].

To explore the role of AAMs in the immunological response to chitin, Reese *et al.* [4] generated a strain of mice, termed YARG mice, that coexpress enhanced yellow fluorescent protein (eYFP) and arginase-1, which effectively causes AAMs to fluoresce yellow. Administration of chitin to the lungs or peritoneal cavity of YARG mice elicited the accumulation of AAMs, followed by the

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Available online 7 September 2007.

 1471-4906/$ – see front matter doi:10.1016/j.it.2007.08.005

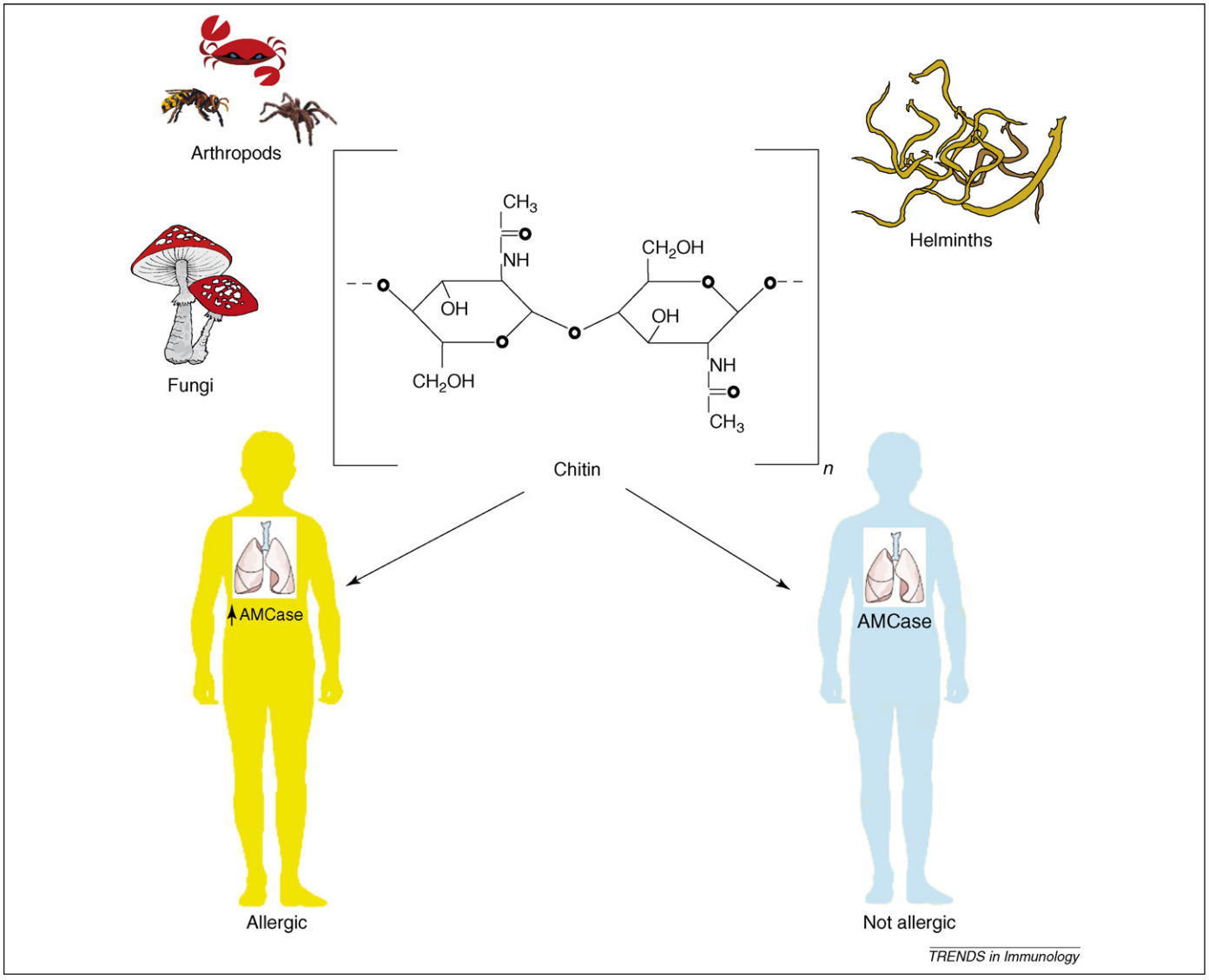

Figure 1. Environmental exposure to chitin can provoke allergy or asthma in genetically susceptible individuals. Chitin is a ubiquitous environmental compound and can be encountered daily from a multitude of common sources; differences in chitinase (AMCase) expression or activity might interfere with an appropriate immune response to the compound.

recruitment of eosinophils (Figure 2). Depletion of peritoneal macrophages by clodronate liposome treatment prevented the recruitment of eosinophils. Trafficking of eosinophils to the site of chitin exposure was also shown to be dependent on leukotriene B_4. Indeed, mice deficient in the high-affinity leukotriene B_4 receptor (BLT1) did not effectively recruit eosinophils in response to chitin. Pretreatment of chitin with enzymatically active AMCase precluded the accumulation of innate effector cells. Furthermore, by exposing transgenic mice that constitutively overexpress AMCase in the lungs to chitin, Reese *et al.* [4] confirmed the importance of the defensive enzymatic degradation of chitin to limit a prolonged Th2 inflammatory response. With these experiments, the authors have elegantly demonstrated the importance of the AMCase both *in vitro* and *in vivo*.

Controversial and unresolved issues

As Reese *et al.* [4] correctly acknowledge, other studies in the field suggest that chitin has a rather different role in immune responses. Oral administration of chitin, for example, has been shown to downmodulate a murine model of allergic airway inflammation [5]. In this study, Shibata *et al.* [5] not only demonstrate the capacity of chitin to induce the secretion of Th1 cytokines, but also propose that chitin preparations could be an attractive therapy in allergic human disease. Another study by Strong *et al.* [9] demonstrates that direct application of chitin microparticles to the respiratory tract can alleviate allergic symptoms in a mouse model of ragweed allergy and might be a useful treatment for respiratory allergy and allergic asthma. In both studies, the importance of the route of administration (oral and nasal, respectively) seems to be a key factor for the ability of chitin to induce a Th1 (protective) anti-allergic effect.

Several other factors, aside from the administration route, might account for the Th1 versus Th2 response to chitin. Particle size can influence the Th1–Th2 polarization. A Th1 response is elicited when macrophages phagocytose microparticles of chitin, but not when soluble chitin or particles too large to engulf are used [10]. Interestingly, administration of unphagocytosable Sephadex

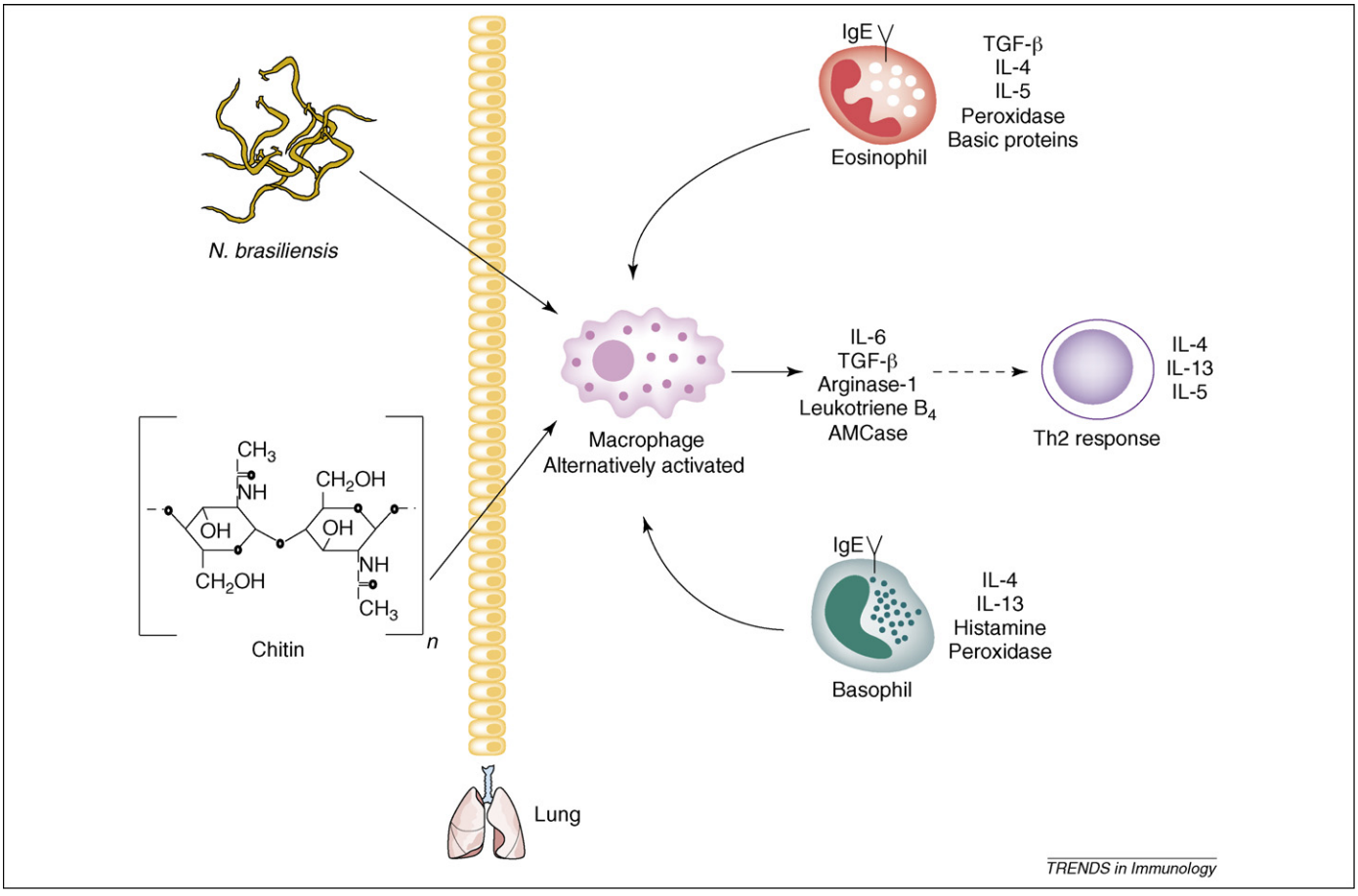

Figure 2. Allergic immune response to chitin or the helminth *N. brasiliensis* in the lung. Infection with helminths or chitin alternatively activates macrophages, causing upregulation of chitinolytic and chitin-binding proteins. Secretion of leukotriene B_4 recruits innate immune cells such as eosinophils and basophils, and might initiate a Th2 response.

(cross-linked dextran) beads can also induce a local eosinophilia in the lung and peritoneal cavity that shows a similar dependence on leukotriene B_4 [11,12]. Contamination with microbial components (e.g. lipopolysaccharide) in chitin preparations might also engage different pattern recognition receptors (PRRs), and additional studies are required to identify specific PRRs in chitin recognition. Furthermore, a comparison between different epithelia and different tissue-resident APCs is needed to understand the diverse T-cell response to chitin. Clarification of the ability of chitin to polarize T-helper responses (Th1, Th2 or even Th17) will be a great help in understanding its eventual role in inciting allergy.

Concluding remarks

Allergy is a complex immunological process, arising from an unknown combination of environmental and genetic factors. Exposure to chitin (from dust mites, mould, shellfish or insects) might be the primary external determinant in allergy development. Intermittent low-level exposure could induce allergy in genetically predisposed individuals. Understanding the potential allergenic role of chitin is important, not only because of its abundance in the environment but also because of its commonplace use in dental and surgical appliances, biomedical materials and cosmetic products [13]. Indeed, various studies indicate that chitin and related molecules can accelerate wound healing, and remedies based on these molecules are widely used today [13].

On the one hand, the ability of chitin to alternatively activate macrophages could be beneficial for stimulating tissue repair [14]; on the other hand, chitin might recruit polymorphonuclear leukocytes and trigger allergic reactions. Most toxicological tests are performed on animals, which might have more efficient chitinase activity than humans, and more studies will be required to address the genetic basis of differences in chitinase functions and allergy.

Acknowledgements

We thank Prof. Anne Cooke and Dr. Jenny Phillips for helpful discussion. P.Z. is funded by the Biotechnology and Biological Sciences Research Council, UK and O.B. is supported by a Herchel Smith Fellowship from Williams College, MA, USA.

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