

Missions of Protease Allergens in the Epithelium

Toshiro Takai

Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Sources of allergens, such as mites, cockroaches, fungi and pollen, produce or contain proteases [1–3]. Proteolytic activity has been proposed to be involved in the pathogenesis of allergies by facilitating the passage of allergens across tissue barriers, cleaving molecules and affecting the functions of various cells and immune responses [1, 2, 4–17]. The proteases involved are frequently themselves allergens, recognized by IgE in sensitized patients. For example, house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, produce at least 4 groups of protease allergens: major group 1 allergens with cysteine protease activity and group 3, 6 and 9 allergens with serine protease activity.

Tai et al. [18] report a novel human endogenous substrate cleaved by a protease allergen. Pen ch 13 is a major allergen from the airborne fungus *Penicillium chrysogenum* and characterized as a serine protease. The protease activity of Pen ch 13 damages the epithelial barrier by cleaving a tight junction protein [19] and induces a pro-inflammatory response in epithelial cells by possibly activating protease-activated receptor 1 and 2 similarly to Pen c 13, a protease allergen from another fungal species, *Penicillium citrinum* [20]. Tai et al. [18] demonstrated that treatment of human bronchial epithelial cells with Pen ch 13 caused a decrease in the cell surface expression of CD44 and an increase in the amount of soluble CD44 released into the culture supernatant. The decrease in

CD44 expression was inhibited with a serine protease-specific inhibitor. Their finding is important because some reports have suggested that CD44 contributes to the repair of epithelial damage [21, 22]. Although various molecules have been reported to be cleaved by protease allergens [1], until this study by Tai et al. [18], no adhesion molecules contributing to tissue repair have been reported as substrates for protease allergens.

Tai et al. [18] discussed that the loss of CD44 expression and/or the increased shedding of soluble CD44 in the bronchial epithelium might cause persistent inflammation and unsuccessful repair in asthma patients. CD44 [23] has been implicated in various functions, many of which depend on its interaction with hyaluronan (HA) [22]. HA, the molecular mass of which normally ranges from 10^6 to 10^7 Da, is an extracellular matrix glycosaminoglycan composed of repeating disaccharide units. The CD44-HA interaction regulates cell growth, survival, activation and chemotaxis [22, 24–26]. During tissue injury, HA accumulates and is degraded to lower-molecular-weight fragments, which stimulate the expression of inflammatory genes [27, 28]. In bleomycin-induced acute pulmonary inflammation, clearance of the HA fragments is crucial to resolving lung inflammation and is dependent on CD44 expressed on hematopoietic cells (possibly, alveolar macrophages) [21]. Phagocytosis of apoptotic eosinophils by bronchial epithelial cells can

be enhanced by 60–70% by epithelial cell CD44 ligation [29]. It is tempting to speculate that cleavage of CD44 by protease allergens causes unregulated inflammation and prolongs the repair of damaged epithelia in microenvironments where allergen source-containing particles, such as fungal spores, body fragments of mites and cockroaches and their feces, and pollen grains, exist.

The role of CD44 *in vivo* has been examined using CD44-deficient mice and anti-CD44 monoclonal antibodies (mAbs) in various models of inflammation. Both anti- and proinflammatory roles of CD44 have been reported [22]. In a model of asthma, the intraperitoneal administration of anti-CD44 mAbs reduced the recruitment of lymphocytes and eosinophils [25], and in a model of allergic dermatitis, CD44-deficient mice showed reduced recruitment of leukocytes [26], suggesting a proinflammatory role for CD44 and not supporting the proposal by Tai et al. [18]. However, some reports suggest that CD44 helps to resolve inflammation of the lung and repair epithelial damage, for example, in bleomycin-induced acute pulmonary inflammation [21, 22]. As CD44 is expressed in various types of cells, it is perhaps not surprising that models of different types of inflammation with various insults using mice with a systemic deletion of CD44 or mice administered systemically with mAbs to

inhibit the HA-binding function of CD44 have suggested different, even paradoxical, roles of CD44. Airway epithelium-specific loss of CD44 expression and/or airway epithelium-specific increase in soluble CD44 in modified models of asthma with different phenotypic or temporal aspects should be examined to reveal the true role of CD44 in the epithelium including bronchial epithelial cells, alveolar macrophages and other types of cells.

In conclusion, Tai et al. [18] proposed a new role for protease allergens – disturbance of the repair of epithelial damage, in addition to the other roles suggested so far (disruption of epithelial barriers, cleavage of endogenous protease inhibitors, induction of proinflammatory responses and promotion of Th2 skewing and IgE production). However, whether the epithelium-specific loss of CD44 and epithelium-specific increase in soluble CD44 can result in unsuccessful repair in allergies is yet to be investigated. Protease allergens are not always similar in substrate specificity, target cell types, signaling pathways (or pathways inhibited by protease allergens), cellular response (or response inhibited) and sensitivity to endogenous protease inhibitors. Whether protease allergens and proteases contained in other sources, such as mites, cockroaches and pollen, can cleave CD44 should be addressed in future studies.

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