

Review article

Serine proteases, their inhibitors and allergy

This paper reviews four serine protease inhibitors and three protease gene defects that are associated with allergic conditions, suggesting an important role for these genes and their products in the development of allergy. Serine protease inhibitors may have a therapeutic potential in the treatment of allergy.

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There are several recognized mutations and defects in genes controlling serine protease inhibitors and proteases associated with allergic conditions published in recent years (1–11). Serine proteases and serine/cysteine protease inhibitors (serpins) are involved in the maintenance of the epithelial barrier in the skin and the airways. One proposed mechanism is that a defective barrier allows easier penetration of allergens. An imbalance in proteases and antiproteases can result in lysis of the epithelial barrier because of exogenous proteases from allergens or bacteria and endogenous proteases, such as trypsin disrupting cellular adhesion molecules.

Serpins are a superfamily of proteins that are produced across a wide range of species including animals, viruses and plants. Serpins have 30% sequence homology with the archetype serpin (α -1-antitrypsin) and a characteristic structure of 3 β -sheets (A–C) and an exposed reactive loop which functions as a pseudo-substrate for the target protease. After binding to the reactive loop, the protease undergoes conformational change and is subsequently inactivated.

Collectively the genetic defects in proteases and their inhibitors represent a mechanism by which allergic diatheses might arise. It is the purpose of this review to summarize these defects, discuss mechanisms by which

they are known to affect the epithelial structure and immune responses, and review therapeutic use of anti-proteases in allergic conditions. The defects of proteases and serpins are summarized in Table 1 and are described in more detail below.

Specific defects

Proteases

Chromosome 2q14: dipeptidyl peptidase 10. Allen et al. identified linkage between a microsatellite D2S308 on chromosome 2q14 and asthma (1). Subsequent analysis showed an association with single nucleotide polymorphisms and linkage disequilibrium mapping to the initial exons of the serine proteases family dipeptidyl peptidase (DPP) 10. The authors found some mutations to be associated with atopy and skin prick test reactivity. Two murine studies have also linked a homologous region to asthma (12, 13). Structural analysis of this protease substrate suggested DPP10 normally functions to cleave key pro-allergic cytokines including eotaxin and RANTES. Dysfunction of the DPP10 gene could increase the amounts of these allergic mediators. Zagha et al. (14) reported that DPP10 also modulates Kv4-mediated A-type potassium channels, and suggested dysfunction of this gene would result in increased excitability of the cell membrane. Zagha et al. pointed out that DPP10 is expressed in the trachea at moderate levels (14) and A-type currents are present in many smooth muscle types (15), so the role of this gene in asthma could extend beyond chemokine homeostasis in the innate immune response.

Chromosome 14q11.2: mast cell chymase promoter region. Mutations in the chymase promoter region have

Abbreviations: ACT, α 1-antichymotrypsin; Der p1, *Dermatophagoides pteronyssinus* 1 allergen; Der f1, *Dermatophagoides farinae* 1 allergen; DPP10, dipeptidyl peptidase 10; HAE, hereditary angioedema; LETKI, lymphoepithelial Kazal-type-related inhibitor; PAI-1, plasminogen activator inhibitor-1; PAR, protease-activated receptor; MCC, mast cell chymase; SCCA, squamous cell carcinoma antigen; SCCE, stratum corneum chymotryptic enzyme; serpin, serine (or cysteine) protease inhibitor; SLPI, secretory leucocyte protease inhibitor.

Table 1. Serine proteases and their inhibitors (serpins) associated with allergic conditions

	Chromosome	Association
Protease		
DPP10	2q14	Asthma (1)
MCC	14q11.2	Atopic dermatitis and elevated IgE (2–4)
SCCE	19q13.41	Atopic dermatitis (5)
Serpins		
LETKI	5p32	Netherton's syndrome (6; elevated IgE, severe dermatitis, food and inhalant allergy) Atopic dermatitis (7)
PAI-1	7q21.3–q22	Asthma and elevated IgE (8, 9)
C1 EI	11q12–31.1	Hereditary angioedema (10)
ACT	14q32.1	Asthma, elevated IgE, positive skin prick test and bronchial hyper-reactivity (11)

IgE, immunoglobulin E; DPP10, dipeptidyl peptidase 10; MCC, mast cell chymase; SCCE, stratum corneum chymotryptic enzyme; LETKI, lymphoepithelial Kazal-type-related inhibitor; PAI-1, plasminogen activator inhibitor-1; ACT, α 1-antichymotrypsin.

been associated with atopic dermatitis in Japanese patients and with UK adults with self-reported eczema and elevated immunoglobulin (Ig)E (2–4). The gene defect associated with atopic dermatitis is a BstX1 (11903G/A) polymorphism. In all three studies, defects in the chymase promoter region have not been associated with asthma. The number of chymase-positive mast cells is increased in patients with atopic dermatitis (16) and chymase-specific inhibitors have been used therapeutically in animal models of atopic dermatitis (17–19).

Chromosome 19q13.41: stratum corneum chymotryptic enzyme. An association has been reported between a 4 bp (AACC) insertion in the stratum corneum chymotryptic enzyme (SCCE) gene and atopic dermatitis (5). This enzyme is reported to have a role in the homeostasis of the outer layer of the skin by cleaving intracellular proteins in the outer epidermis, resulting in desquamation. The mechanism whereby SCCE leads to increased proteolysis and more inflammation remains undetermined; however, it is possible it may have greater proteolytic capacity in its mutant form or in mutant form it may avoid degradation by serpins.

Serine protease inhibitors

Chromosome 5q32: LETKI-1

A mutation in SPINK5 on chromosome 5q32, encoding a serine protease inhibitor, lymphoepithelial Kazal-type-related inhibitor (LETKI), has been linked to Netherton's syndrome (6). Netherton's syndrome is a rare, severe autosomal recessive condition characterized by atopic manifestations with high serum IgE levels, allergy, often with angioedema, and hyper-eosinophilia. Subsequent mutation analysis of SPINK5 found an association between mutations of Glu420-Lys with atopy and atopic

dermatitis in two independent panels of families, indicating the relevance of this serine protease inhibitor in more common allergic conditions (7). While LETKI is a serine protease inhibitor, it has <30% homology to α -1-antitrypsin. It does not undergo conformational transition-like classical serpins and the proteinase activity is reversible.

Chromosome 7q21.3–q22: plasminogen activator inhibitor 1

Polymorphisms of 4G/5G in the serpin plasminogen activator inhibitor-1 (PAI-1) have been associated with asthma and increased IgE in UK and Czech populations (8, 9). In contrast to other serpin defects, individuals homozygous for the 4G allele have higher levels of the serpin PAI-1 and this trait is associated with increased asthma risk (8, 9, 20). The gene for PAI is induced within the airways of patients with asthma (21).

Chromosome 11q12–13.1: C1 esterase inhibitor

Hereditary angioedema (HAE) is caused by defects in the serpin C1 esterase inhibitor and has been associated with at least 97 different mutations (10). While HAE is not associated with elevated IgE, it is included in this review because the clinical phenotype is one of a severe allergic reaction with angioedema and anaphylaxis. HAE also demonstrates the clinical efficacy of serpin replacement therapy, and C1 esterase inhibitor has an emerging role as an anti-inflammatory agent (22).

Chromosome 14q32.1: serpin cluster/ α 1-antichymotrypsin

Allergic asthma in Italy has been associated defects in α 1-antichymotrypsin (ACT) on chromosome 14 (11). This population was associated with transmission of the allele 15Ala-Act and the phenotypes of elevated IgE, asthma, positive skin prick test result and bronchial hyper-reactivity.

The role of proteases and serine protease inhibitors in inflammation

Serine proteases and their inhibitors have multiple functions. They are involved in immune development, inflammation and tissue repair. In their simplest and most obvious role, protease inhibitors can degrade some allergens, as many of these are serine or cysteine proteases. The environmental levels of serine proteases have been linked to asthma disease (23). Protease activity of class 1 allergens can cleave complement factor C5 to its active components, which is reported to have a key role in airway hyper-responsiveness (24, 25).

Serine proteinase inhibitors are likely to be crucial in the maintenance of epithelial barriers. Netherton's syndrome is characterized by a reduction in epithelial

integrity (6) and loss of the epidermal barrier protein. The recent report of defects of filaggrin protein; an epithelial protein essential for barrier function, reinforces the critical role of epithelial integrity in the development of eczema and some forms of asthma (26). Filaggrin gene mutations occurred in 9% of the populations studied and was associated with atopic dermatitis and asthma occurring in the context of atopic dermatitis (26).

Proteases from bacterial and dust mite sources can cause epithelial disruption and proteolysis (27–29). The serpin squamous cell carcinoma antigen (SCCA)2 can inhibit the proteolytic activity of the dust mite proteases Der p1 and Der f1 on respiratory epithelial cells (30). In addition to exogenous proteolysis from proteases, mutations in the endogenous proteases mast cell chymase (MCC) and SSCE could contribute to defects in the epithelial barrier. The third component of this balance, the serine protease inhibitors, is important in the homeostasis of endogenous and exogenous proteases, and defects of serine protease inhibitors are associated with epithelial dysregulation. If the epithelium becomes disrupted, then there is an increase in trans-epidermal water loss and also the subepithelium is more exposed to bacteria and allergens (summarized in Fig. 1).

Endogenous proteases, like mast cell tryptase are important in the acute allergic response and via proteolysis they can cause epithelial lysis. Tryptase is also

involved at many other levels of the allergic inflammatory response. Tryptase activation of protease-activated receptor 2 (PAR-2) on mast cells increases the human airway smooth muscle contractile response (31). Tryptase also stimulates myocyte proliferation (32), cytokine synthesis by human airways smooth muscle cells (33), mast cell chemotaxis and bronchial hyper-responsiveness (34). Inhibition of tryptase has been shown to modulate the late phase of response to allergen challenge (35). Tryptase appears to have a central role in mast cell myositis – a new feature of allergic asthma (36), and this topic has been reviewed recently by Berger et al. (37).

To understand how defects in protease homeostasis might specifically stimulate allergic responses, such as a TH2 cytokine shift and IgE stimulation, the immunological responses of dust mite serine and cysteine protease allergens provides some mechanistic clues and are illustrated in Fig. 2. The house dust mite cysteine proteases Der p1 and Der f1 influences TH2 and IgE regulation via several mechanisms. Der p1 cleaves surface CD23 from B cells and enhances IgE production by ablating an inhibitory feedback mechanism that normally limits IgE synthesis (38). Cleaved CD23 results in increased soluble CD23 that increases interleukin (IL)-4-mediated IgE production (39). The pulmonary serpin α -1-antitrypsin has been shown to inhibit the cleavage of CD23 by Der p1 (38).

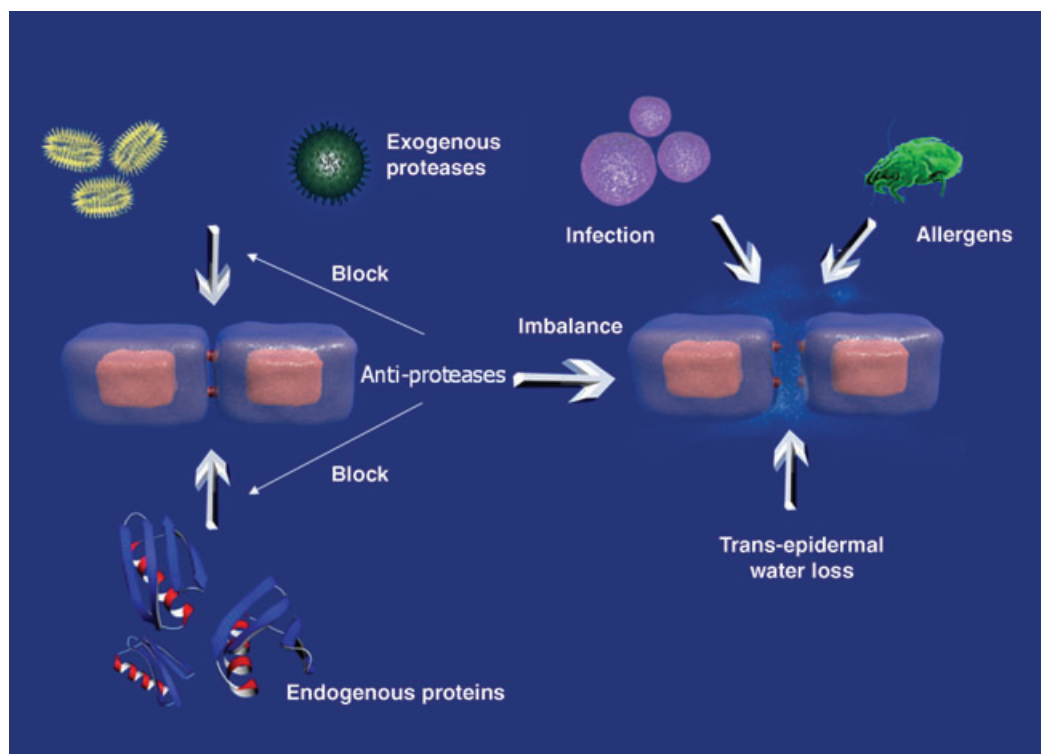


Figure 1. Endogenous and exogenous proteases can disrupt epithelium; however, this is normally counteracted by protease inhibitors. An imbalance of proteases and their inhibitors can result in epithelial disruption, trans-epidermal water loss in the skin and entry of allergens and infection beneath the epithelium.

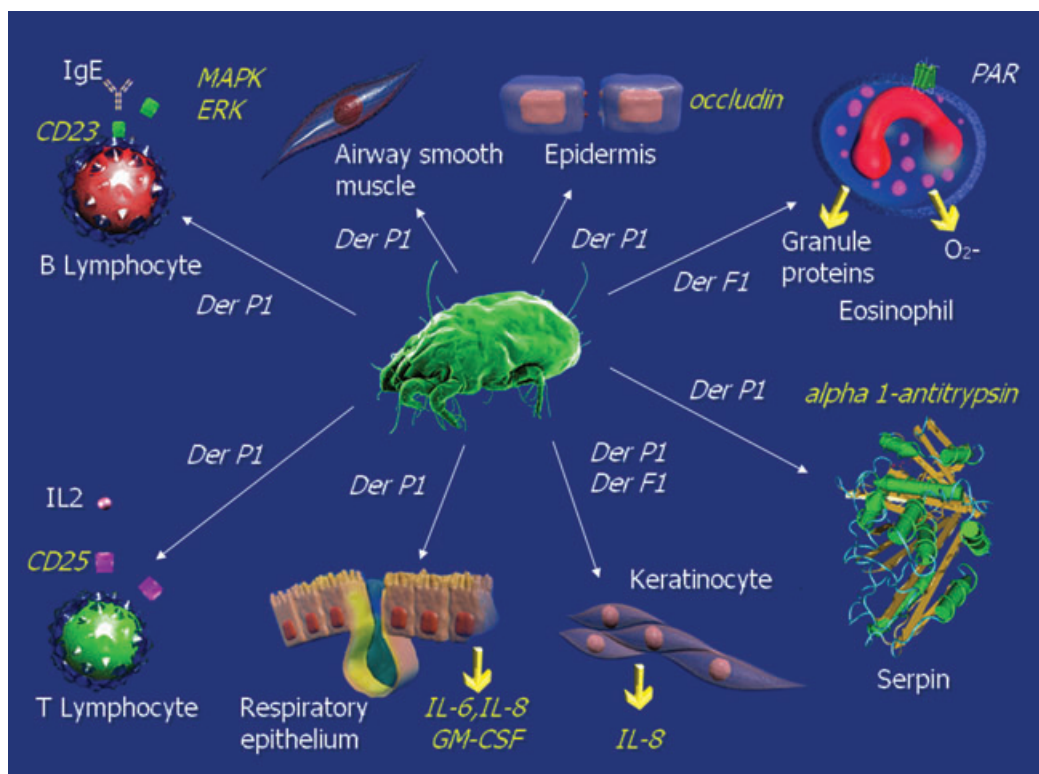


Figure 2. Potential mechanisms by which dust mite proteases might promote an allergic response.

Functionally, house dust mite stimulates IgE production, and the importance of proteases as having an orchestrating role in the development of atopy is underlined by the finding that house dust mite increases IgE production to bystander allergens (40, 41). House dust mite protease is also capable of cleaving the IL-2 receptor (CD25) from T cells, blocking a major TH1 cytokine, resulting in increased IL-4 and less IFN- γ from T lymphocytes (42). In addition to these allergy-promoting effects, Der p1 catalytically inactivates the serpin α -1-antitrypsin by a thiol-dependent mechanism involving specific cleavage of the reactive centre loop, reducing the level of protection against destructive proteases (43). The cysteine protease Der f1 proteolytically activates PAR receptors on eosinophils, inducing activation, degranulation and super oxide production similar to that of an allergic response (44). Protease activity of Der p1 enhances constriction and delays relaxation responses in airway epithelial cultures via mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways (45). Dust mite allergens are also reported to stimulate keratinocytes and airway epithelial cells to release cytokines and chemokines that may be involved in the development of allergy (46, 47). Use of the antiprotease Cystatin A has been shown to limit the IL-8 response in keratinocytes, providing an immunological barrier to mite proteases (48). Murine studies

have demonstrated that administration of proteolytically active Der p1 to mice through the intranasal route leads to significant inflammatory cellular infiltration of the lungs and systemic production of IgE (49).

The knowledge of mite proteases and some limited work with antiproteases helps to provide some clues as to how defects in proteases and their inhibitors could result in an increased susceptibility to allergy; however, these mechanisms require specific investigation in relation to their respective genetic defects.

Therapeutic serine protease inhibitors

Replacement of serine protease inhibitors in known deficient states is recognized therapeutically in HAE and α -1-antitrypsin deficiency. The serpin C1 esterase inhibitor is also being used effectively in a wide range of inflammatory conditions including bacterial sepsis, cytokine-induced vascular leak, pancreatitis and transplant rejection (50, 51). *In vitro* studies demonstrate that C1 esterase inhibitor can block T-lymphocyte activation, proliferation and generation of cytotoxic T lymphocytes (52, 53). The serine protease inhibitor aprotinin has been found to have some efficacy in the treatment of trypsin-induced shock (54, 55). Serine protease inhibitors have also been used to treat atopic dermatitis using α -1-antitrypsin inhibitor, with the authors at the time

fortuitously interpreting that 'atopic dermatitis may be one example where inflammation is due to an imbalance of serine proteases and their naturally occurring inhibitors' (56). Specific chymase inhibitors SUN-C8257, Y-40613 (2-[5-amino-2-(4-fluorophenyl)-1,6-dihydro-6-oxo-1-pyrimidinyl]-N-[1-[(5-methoxycarbonyl-2-benzoxazolyl)carbonyl]-2-phenylethyl]acetamide) and SUN-8077 [3-(3-aminophenylsulfonyl)-7-chloroquinazoline 2,4(1H, 3H)-dione] have been shown to reduce dermatitis in animal models (17–19). SUN-C8257 was shown to reduce accumulation of inflammatory cells including mast cells and eosinophils in the skin (18). *In vitro* IgE production from mouse B cells was increased by purified rat chymase and suppressed by Y-40613 (17). SUN-C8077 inhibited an allergen-induced biphasic skin response, suggesting the role of chymase in chemotaxis and late phase responses (19). Animal studies of topically applied secretory leucocyte protease inhibitor (SLPI) reduces allergic conjunctivitis via inhibition of eosinophil migration and degranulation with antigen challenge (57).

Our current treatment of allergic conditions relies heavily on antihistamines and anti-inflammatory agents. The use of serine protease inhibitors in allergic and inflammatory conditions could provide a mode of ther-

apy more aligned to the mechanisms of the disease and subsequent tissue injury.

Further questions

Despite there being a family of defects associated with serine protease inhibitors and protease defects, there is still relatively little known about the respective genes and their products. Collectively serine protease inhibitors are potential targets for investigating the genetic basis of allergic conditions. Knowledge of interactions, such as infections, diet, environmental factors and current anti-inflammatory therapies with key serine protease inhibitors is required. The anti-inflammatory potential of serine protease inhibitors requires further investigation in allergic conditions.

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