

# A Major House Dust Mite Allergen Disrupts the Immunoglobulin E Network by Selectively Cleaving CD23: Innate Protection by Antiproteases

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## Summary

Asthma is a chronic life-threatening disease of worldwide importance. Although allergic asthma and related atopic conditions correlate strongly with immune sensitization to house dust mites, it is unclear why antigens from mites provoke such powerful allergic immune responses. We have characterized the protease activity of *Der p* I, the group I protease allergen of the house dust mite *Dermatophagoides pteronyssinus*, and here report that it cleaves the low-affinity immunoglobulin (Ig) E Fc receptor (CD23) from the surface of human B lymphocytes. *Der p* I selectively cleaves CD23 and has no effect on the expression of any other B cell surface molecules tested. We speculate that this loss of cell surface CD23 from IgE-secreting B cells may promote and enhance IgE immune responses by ablating an important feedback inhibitory mechanism that normally limits IgE synthesis. Furthermore, since soluble CD23 is reported to promote IgE production, fragments of CD23 released by *Der p* I may directly enhance the synthesis of IgE.  $\alpha_1$ -Antiprotease, a pulmonary antiprotease, is also shown to inhibit the cleavage of CD23 by *Der p* I. This may be significant in the etiopathogenesis of asthma, because other indoor pollutants associated with asthma are known to potentially inhibit this antiprotease. These data suggest that the proteolytic activity of *Der p* I, the group I allergen of the house dust mite *D. pteronyssinus*, is mechanistically linked to the potent allergenicity of house dust mites. Furthermore, inhibition of *Der p* I by  $\alpha_1$ -antiprotease suggests a mechanism by which confounding factors, such as tobacco smoke, may act as a risk factor for allergic asthma.

Allergic asthma is a worldwide problem of considerable clinical importance. Links between asthma and exposure to house dust and its microfauna have been recognized for >300 yr (1). More recently, causal links have been proven between immunity to house dust mites and allergic disorders, including asthma, rhinitis, dermatitis, and conjunctivitis (2–5).

House dust mites of the family *Pyroglyphidae* infest most homes in temperate climates. In the United Kingdom, three species, *Dermatophagoides pteronyssinus*, *D. farinae*, and *Euroglyphus maynei*, commonly flourish in the indoor environment (6). This is due, in part, to fuel-saving measures that reduce ventilation and create an ideal mite habitat of high humidity and temperature. Early exposure to the high levels of house dust mites encouraged by these conditions and the confounding influence of indoor pollution are widely blamed for recent rises in the incidence of allergic asthma (7). Despite this, it remains unclear why mites provoke such powerful allergic immune responses. It has been suggested that certain biochemical properties of an antigen

may determine allergenicity (8). Enzymes in particular appear to be potentially allergenic (9–15), and several of the well-characterized house dust mite allergens are known to be, or suspected of being, enzymes (16–20). It is not understood, however, how and why this category of protein antigens consistently provokes allergic immune responses.

Most individuals allergic to house dust mites possess IgE antibodies to the group I allergens of mites. The group I allergen of *D. pteronyssinus* (*Der p* I)<sup>1</sup> is a 30-kD protein containing sequences conserved in the active sites of cysteine proteases (16). It is produced in the midgut of the mite, where its role is probably related to the digestion of food substrates in, or associated with, human skin (21). Up to 0.2 ng of proteolytically active *Der p* I is incorporated into the peritrophic membrane of each mite fecal pellet, 40 of

<sup>1</sup>Abbreviations used in this paper: APMSF, 4-[amidinophenyl] methanesulfonyl fluoride; *Der p* I, the group I allergen of *Dermatophagoides pteronyssinus*; E64, 1-trans-epoxysuccinyl-leucylamide-[4-guanidino]-butane; sCD23, soluble CD23.

which may be produced by a single mite in a day (4). The pellets vary in size between 10 and 40  $\mu\text{m}$  and are therefore ideal respirable delivery vehicles for *Der p* I to the respiratory tract (4).

Events leading to the development of allergic immune responses are controversial. In atopic individuals, allergen-activated CD4<sup>+</sup> Th2-like T lymphocytes are thought to secrete high levels of cytokines such as IL-4, which promote IgE synthesis by B cells (22–25). In nonallergic individuals, the immune response to the same antigens is suspected additionally to include Th1 cytokines such as IFN- $\gamma$ . These may modulate the immune response by inhibiting development of Th2-like T cells and thus balance the cytokine profile of the response to prevent allergy. Most important in the context of this study, however, is that IgE synthesis may also be controlled by a feedback inhibition mechanism that is mediated by IgE-containing immune complexes binding to the low-affinity receptor for IgE, CD23 on B cells.

Several cell types in the normal individual, including eosinophils, monocytes, B cells, and T cells, express constitutively low levels of CD23. Activation of these cell types by inflammatory and infectious diseases or neoplastic transformation induces high-level CD23 expression (26, 27). In patients with asthma, the macrophages, eosinophils, and epithelial cells of the respiratory tract are also reported to express high levels of CD23 constitutively (26–28).

Unlike the other Fc receptors, CD23 is not a member of the Ig superfamily. Instead, the molecule contains a calcium-dependent lectin domain and heptad hydrophobic repeats, suggesting an  $\alpha$ -helical coiled structure more typical of the selectin and asialoglycoprotein receptor family members (29). CD23 is a type II integral membrane protein, the cytoplasmic NH<sub>2</sub> terminus of which, in humans, is encoded by one of two alternate exons to give the CD23A and CD23B isoforms (30). CD23A is developmentally regulated and expressed only by antigen-activated human B cells, whereas CD23B is expressed on many cell types in both humans and mice and is inducible with IL-4 (30). Monomeric 45-kD CD23 monomers associate to form homotrimers at the cell surface (29, 31). The  $\alpha$ -helical coils of monomeric CD23 are predicted to form a coiled-coil stalk containing the transmembrane region. At the COOH terminus, the lectin heads, which are exposed to the extracellular environment, act as an Fc receptor by binding the C $\epsilon$ 3 domains of IgE (29, 32). In addition to binding to IgE, CD23 also interacts with CD21, the CR2 (33). This interaction may be important in homotypic adhesion between B cells, the enhancement of IgE synthesis, and the rescue of germinal center B cells from apoptosis (29).

CD23 exerts its regulatory influence on the IgE network by binding IgE and IgE-containing immune complexes. High levels of IgE-containing immune complexes that bind to CD23 on the surface of IgE-secreting B cells transduce a feedback-inhibitory signal that prevents further IgE synthesis (27, 34, 35). In the absence of CD23, such as in CD23 knockout mice (35), this mechanism of feedback in-

hibition is not present, causing IgE levels to be increased and sustained in response to T cell-dependent antigens.

Immunoregulatory CD23 is also cleaved from the cell surface by a mechanism that is not yet fully understood but that may involve proteolysis (36, 37). Cleavage occurs in the coiled stalk, giving rise to trimeric fragments that resolve at molecular weights of 37, 33, 29, 25, and 16 kD (36, 37). These soluble CD23 (sCD23) molecules retain the IgE-binding lectin heads and thus bind to IgE. The increased expression of cell surface CD23 observed in inflammatory, infectious, and neoplastic conditions is often mirrored by an increased level of plasma sCD23 and IgE (38). This is most probably related to the activity of sCD23 as a pleiotropic cytokine. In particular, soluble fragments of CD23 >29 kD are reported to enhance IgE synthesis by B cells (39–41). The mechanism of sCD23-enhanced IgE synthesis probably involves sCD23 binding to surface membrane IgE and CD21 (29), but because sCD23-mediated enhancement of IgE synthesis is T cell-independent, this mechanism only operates once T cell dependent Ig isotype switching to IgE has taken place.

Loss of CD23 from the cell surface or an increase in the level of plasma sCD23 is thus highly proallergic. Any extraneous agent changing the expression of cell surface CD23 or the level of sCD23 may affect the regulation of IgE synthesis and hence the severity of allergic symptoms.

To prevent proteolytic damage by infectious agents and activated inflammatory cells, the normal respiratory tract is protected by antiproteases. An antiprotease of the serpin family,  $\alpha_1$ -antiprotease, is largely responsible for inactivating the endogenously produced neutrophil serine protease elastase and thus maintaining the protease-antiprotease balance of the lung (42). Hereditary abnormalities or xenobiotic-induced damage that reduces the efficiency of this antiprotease is known to tip this balance and thus constitutes a powerful risk factor for lung disease, including asthma, by allowing the proteolytic products of macrophages and neutrophils to predominate (42, 43). Similarly, inhaled proteolytically active *Der p* I in mite fecal particles may also place the respiratory tract at risk of focal proteolytic attack.

Reports on the increase in the incidence of asthma have focused on the influence of cofactors that increase susceptibility to allergic asthma (44, 45). Of these, the indoor pollutants of tobacco smoke have received particular attention (46–48). Passive or intentional exposure to such pollutants may inactivate  $\alpha_1$ -antiprotease either directly, by oxidation of an active center methionine residue at position 358, or indirectly, by eliciting and activating pulmonary phagocytes to produce oxidants with the same effects (42).

Here we report that *Der p* I, a protease allergen of the house dust mite *D. pteronyssinus*, cleaves the low-affinity IgE Fc receptor (CD23) from B lymphocytes. This may deprive the B cell of an important mechanism to limit IgE synthesis and generate fragments of CD23 that directly enhance IgE synthesis. Cleavage of CD23 is inhibited by  $\alpha_1$ -antiprotease, suggesting that environmental circumstances that reduce the efficiency of innate antiprotease defenses

may allow *Der p* I to access the immune system. Here, by cleaving CD23, the allergen may dysregulate the IgE network, possibly contributing to the development of allergic asthma. Based on these data, we speculate that the biological activity of *Der p* I may account for its potent allergenicity and suggest a mechanism by which indoor pollutants could act as a confounding influence on the development of allergic asthma caused by house dust mites.

## Materials and Methods

**Purification Of *Der p* I.** *Der p* I was purified from laboratory cultures of *D. pteronyssinus* (49) by affinity chromatography using the anti-group I mite allergen mAb, 4C1 (50) (generously donated by Dr. Martin Chapman, University of Virginia Health Sciences Center, Charlottesville, VA) coupled to cyanogen bromide-activated Sepharose 4B. Whole-mite culture (15 g) was stirred for 16 h at 4°C in 200 ml PBS, pH 8.0. Debris and insoluble material were removed by centrifugation at 13,000 g and 0.2- $\mu$ m filtration. The extract was applied to the affinity column, which was washed extensively with PBS, pH 8.0. *Der p* I was eluted with 50 mM glycine, 50% ethylene glycol, pH 10.0. After dialysis against PBS, pH 8.0, *Der p* I was concentrated to 1 mg/ml<sup>-1</sup> using an ultrafiltration unit (Centriprep 10; Amicon, Beverly, MA) and stored at -80°C. Purity was assessed by silver-stained 15% SDS-PAGE according to standard methods (51). Zymograms were run as for SDS-PAGE, with 1% evaporated milk included in the resolving gel (52). Once run, the zymograms were incubated for 16 h at 37°C in PBS, pH 8.0, followed by staining with Coomassie blue.

**Fluorogenic Substrate and Use of Enzyme Inhibitors.** The fluorogenic substrate FITC-casein was prepared according to standard methods (53) and used as follows: *Der p* I (7.5  $\mu$ g/ml<sup>-1</sup>) was incubated for 1 h at 37°C with FITC-casein (250  $\mu$ g/ml<sup>-1</sup>) in 50 mM 2-amino-2-methyl-1,3-propanediol-HCl, pH 8.0 (the pH optimum for *Der p* I; data not shown). The reaction was stopped with TCA, incubated at room temperature for 1 h, and centrifuged at 13,000 g for 30 min at 4°C. The fluorescence of triplicate samples of supernatant was then recorded at an excitation wavelength of 490 nm and an emission detection wavelength of 525 nm. 1  $\mu$ g of *Der p* I typically exhibited 0.06 tryptic U of activity, equivalent to 300 fluorescent U.

In experiments using protease inhibitors, proteases were preincubated at 37°C with the inhibitor for 30 min before the addition of substrate. The protease inhibitors L-trans-epoxysuccinyl-leucylamide-[4-guanidino]-butane (E64) (Novabiochem, Nottingham, UK), 4-[amidinophenyl] methanesulphonyl fluoride (APMSF) (Novabiochem), pepstatin-A (Novabiochem), EDTA (Sigma

Chemical Co., Poole, Dorset, UK) and 1,10-phenanthroline (Novabiochem) were reconstituted as recommended by the manufacturers and used over a range of concentrations either side of those recommended (full data not shown). Recombinant human  $\alpha_1$ -antiprotease was generously donated by Drs. Rob Stockley and Carol Llewellyn-Jones (University of Birmingham, Birmingham, UK).

**Purification of Immunoaffinity-purified *Der p* I on Thiol-Sepharose.** Activated thiol-Sepharose 4B (Pharmacia Biotech, Milton Keynes, UK) was swollen and equilibrated in 100 mM phosphate buffer, pH 7.0. *Der p* I (150  $\mu$ g) was applied to the affinity column, which was washed in 500 mM sodium chloride, 100 mM phosphate buffer, pH 7.0, and eluted with 500 mM sodium chloride and 100 mM phosphate buffer, pH 8.0, containing 5 mM cysteine. Protein elution was monitored by UV absorbance at 280 nm, and protease activity was assessed using FITC-casein as a substrate.

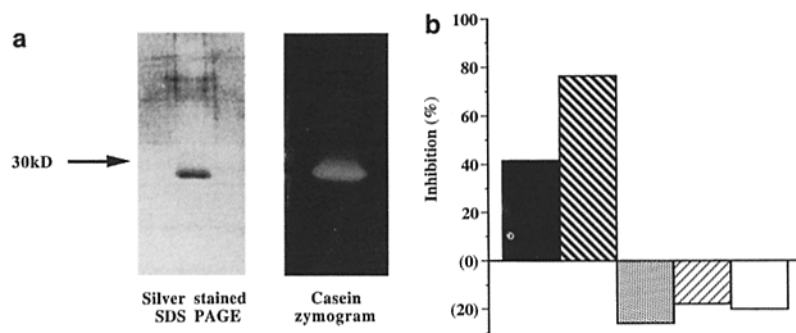
***Der p* I-mediated Cleavage of CD23 from EBV-transformed B Cells and Immunofluorescence.** The EBV-transformed human B cell line BC5 was prepared from the PBMC of a normal blood donor using the EBV-containing supernatant from the B958 cell line. BC5 cells were washed three times in serum-free PBS, pH 8.0. The washed cells (10<sup>6</sup>) were incubated with 5  $\mu$ g/ml<sup>-1</sup> of *Der p* I for 1 h at 37°C. Inhibitors were used as already described. Direct immunofluorescence was performed by standard methods using FITC-conjugated anti-CD23 mAb (BU38, generously donated by Roger Drew, The Binding Site Ltd., Birmingham, UK), FITC-conjugated anti-CD20 (Dako Ltd., High Wycombe, UK), or FITC-conjugated anti-human Ig (Dako UK).

Indirect immunofluorescence was performed by standard methods using mAbs specific for monomorphic determinants of HLA-DR (L243, ATCC HB55), HLA-DP (B721.2), and HLA-DQ (SPLV.3, generously donated by Dr. Hergen Spits of the Netherlands Cancer Institute, Amsterdam, Netherlands), followed by FITC-conjugated goat anti-mouse Ig. Cells were analyzed using a flow cytometer (on FACScan<sup>®</sup>; Beckton Dickinson & Co., Mountain View, CA).

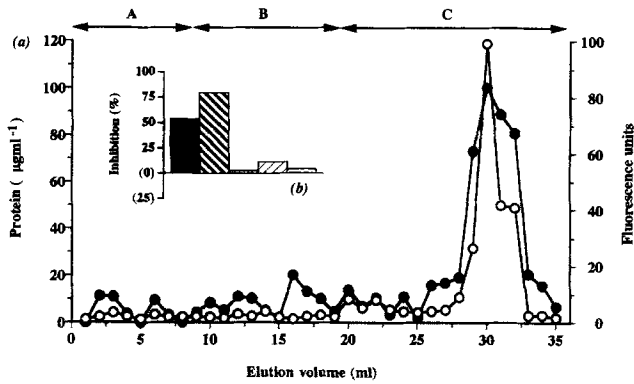
## Results and Discussion

**Purification of *Der p* I.** *Der p* I was immunoaffinity purified using the anti-mite group I allergen-specific mAb 4C1. The purified enzyme allergen resolved as a single 25–27-kD band on SDS-polyacrylamide gels. Casein zymograms of the same material showed a single proteolytic species coincident with the 25–27-kD protein (Fig. 1 a).

**Characterization of the Mechanistic Class of *Der p* I.** Based on sequence analysis, *Der p* I is often described as a cysteine



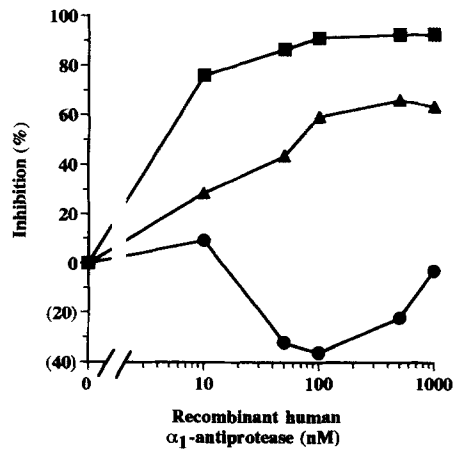
**Figure 1.** Purification of *Der p* I and characterization of protease activity. (a) Affinity-purified *Der p* I resolved as a 25–27-kD proteolytic species on SDS-PAGE and casein zymograms. (b) Pure *Der p* I was sensitive to the cysteine and serine protease inhibitors, 5  $\mu$ M E64 (■) and 50  $\mu$ M APMSF (▨), but not to inhibitors of metallo- or aspartic proteases, 1 mM 1,10-phenanthroline (▩), 10 mM EDTA (▤), and 1  $\mu$ M pepstatin-A (□).



**Figure 2.** Cysteine and serine protease activities of *Der p* I purified by immuno- and active site affinity chromatography. (a) After immunoaffinity purification, further active site affinity chromatography of *Der p* I confirmed that cysteine and serine protease activities were properties of the same protein. All *Der p* I loaded bound to thiol-Sepharose 4B in 100 mM phosphate buffer, pH 7.0 (A), none of the protein (○) was desorbed by washing with 100 mM phosphate buffer, pH 7.0, and 500 mM sodium chloride (B). All the protein applied bound to the column and was eluted with 100 mM phosphate buffer, pH 8.0, and 500 mM sodium chloride containing 5 mM cysteine (C). The single peak of eluted protein resolved at 25–27 kD, and using a FITC-casein substrate (●), contained mixed cysteine–serine protease activity, (b) as judged by sensitivity to E64 (5 μM, ■) and APMSF (50 μM, ▨) and insensitivity to 1 mM 1,10-phenanthroline (▩), 10 mM EDTA (▧) and 1 μM pepstatin-A (□).

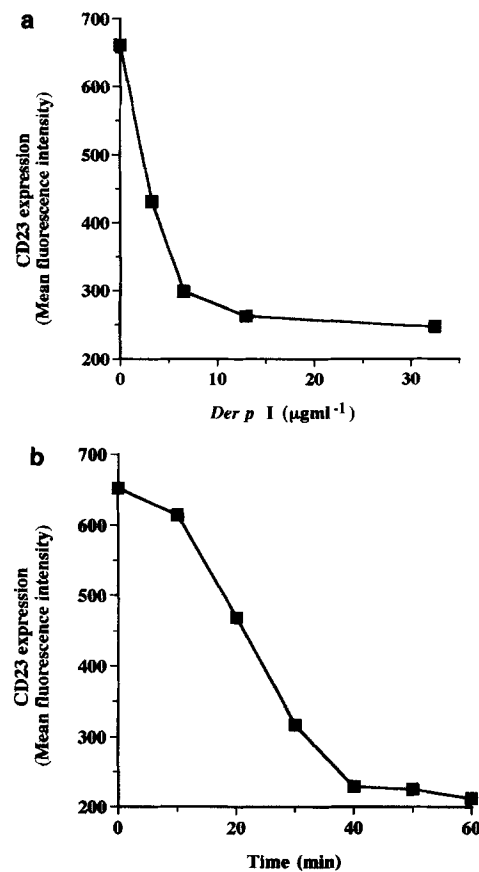
protease (16). The mechanistic class of *Der p* I, however, has not been rigorously evaluated experimentally. Using the fluorogenic substrate FITC-casein and irreversible class-specific protease inhibitors, we determined the class of protease to which *Der p* I belongs. The activity of *Der p* I was inhibited by the cysteine protease inhibitor E64, the serine protease inhibitor APMSF, but not by the aspartic protease inhibitor pepstatin A or the metalloprotease inhibitors EDTA and 1,10-phenanthroline (Fig. 1 b); suggesting that *Der p* I exhibits an unusual mixed cysteine–serine protease activity. To determine whether the cysteine and serine activities of immunoaffinity-purified *Der p* I could be separated, we further purified *Der p* I by active site affinity chromatography using activated thiol-Sepharose 4B. All the *Der p* I loaded onto the column was absorbed by the thiol-Sepharose 4B, and no protein was eluted during the washing steps. Bound material was eluted with 5 mM cysteine and characterized using class-specific protease inhibitors. As before, the activity of *Der p* I was inhibited by the cysteine protease inhibitor E64 and the serine protease inhibitor APMSF, but not by the aspartic protease inhibitor pepstatin A or the metalloprotease inhibitors EDTA and 1,10-phenanthroline (Fig. 2). This confirms that even after sequential purification by two different criteria, *Der p* I maintains a characteristic mixed cysteine–serine protease activity.

Our finding that *Der p* I exhibits a mixed mechanistic class based on inhibitor sensitivity is not unique; similar dual activities have been reported for a fungal protease (54) and a proteasome (55). Unlike the multisubunit proteases, however, a structural model of *Der p* I based on the cysteine proteases papain, actinidin, and papaya protease Ω

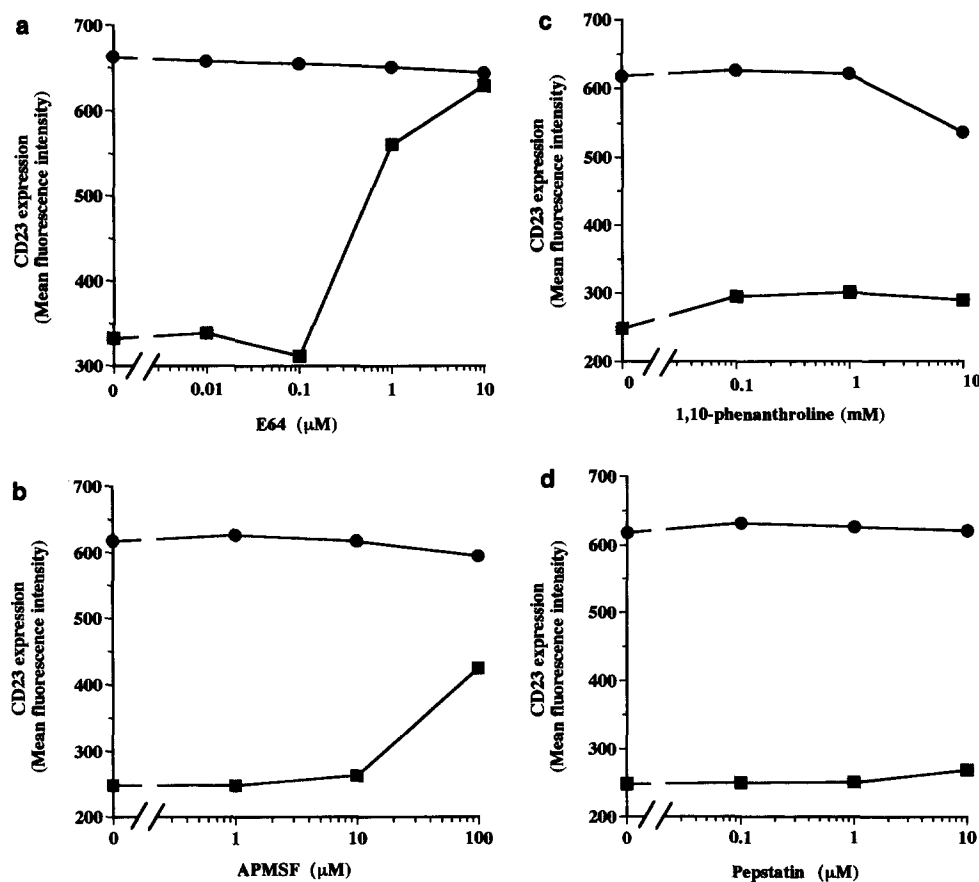


**Figure 3.** *Der p* I is inhibited by endogenous antiprotease  $\alpha_1$ -antiprotease. Recombinant human  $\alpha_1$ -antiprotease inhibited *Der p* I (▲) and the serine protease trypsin (■) but had no effect on the cysteine protease papain (●).

shows that the enzyme has only a single active site (56). It is, therefore, probable that other characteristics of the enzyme or inhibitors are responsible for the apparently mixed



**Figure 4.** *Der p* I cleaves CD23 from the surface of EBV-transformed human B cells. (a) Cleavage of CD23 was dependent on the concentration of *Der p* I and (b) the period of incubation.



**Figure 5.** The mixed cysteine-serine protease activity of *Der p I* cleaves CD23. In the presence of *Der p I* (■), cleavage of CD23 was inhibited by E64 (a) and APMSF (b) but not by 1,10-phenanthroline (c) or pepstatin-A (d). None of the inhibitors themselves, in the absence of *Der p I*, had any effect on the expression of CD23 (●).

mechanistic class of the enzyme when determined with class-specific inhibitors.

Most cysteine proteases are optimally active at a slightly acidic or neutral pH (57). In this respect, *Der p I* is unusual because optimal activity is seen at pH 8.0 (data not shown), in keeping with the estimated pH of the posterior midgut of the mite (58). At pH 8.0, it is possible that the properties of the inhibitors and characteristics of the active site of the protease are different from those at the lower pH at which other cysteine proteases are assayed, perhaps accounting for the apparent mixed cysteine-serine protease activity observed with *Der p I*.

*Innate Antiproteases May Protect Against Sensitization to Der p I.* Since *Der p I* exhibits serine protease activity, we examined whether  $\alpha_1$ -antiprotease, an important inhibitor of serine proteases in the respiratory tract, constitutes an innate form of defense to damage and allergic sensitization by proteolytically active *Der p I*. We have, therefore, compared the ability of recombinant human  $\alpha_1$ -antiprotease to inhibit the activity of *Der p I* and the archetypal cysteine and serine proteases papain and trypsin.

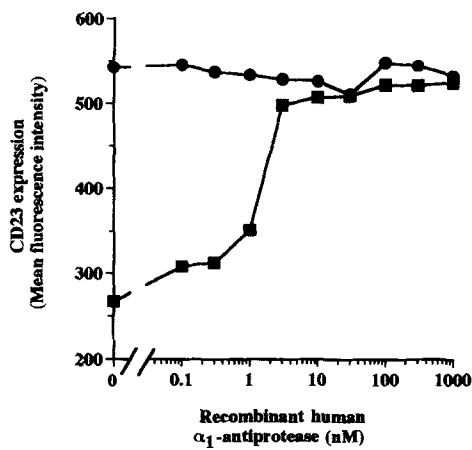
Using FITC-casein as a substrate, recombinant human  $\alpha_1$ -antiprotease was found to inhibit both *Der p I* and the serine protease trypsin but had no effect on the cysteine protease papain (Fig. 3).

Under circumstances in which antiprotease defenses are reduced, proteolytically active *Der p I* in inhaled mite feces

may cause focal injury to the ciliated epithelium. This may not only delay clearance of the allergen and induce chemotactic cytokine and chemokine secretion by epithelial cells (59), but it may also allow *Der p I* and other allergens in the fecal pellet access to the underlying immune system. Efficient inhibition of *Der p I* by  $\alpha_1$ -antiprotease and other respiratory tract antiproteases (42) may thus be important in preventing allergic sensitization.

This series of events may contribute to immune sensitization, but it does not explain why *Der p I* so frequently causes allergic immunity. Allergic immune responses may be regulated by changes in Th1 and Th2 subsets or by mechanisms within the IgE network. Because the release of CD23 from the cell surface and its appearance in the plasma are regulated by a process that may involve proteolysis (37), we examined whether the proteolytic activity of *Der p I* interferes with the expression of CD23, a critical regulator of the IgE network.

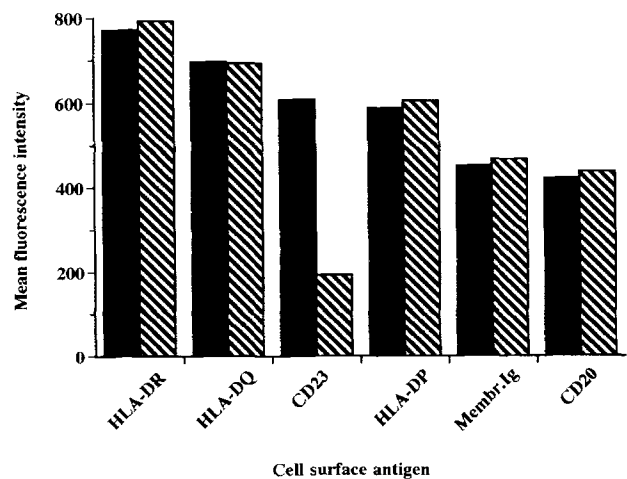
*Der p I Cleaves CD23, a Critical Regulator of the IgE Network.* EBV transformed human B cells express high levels of CD23; this provides a convenient source of native, cell-associated CD23 for use as a substrate. When incubated with *Der p I*, CD23 was removed from the surface of the EBV B cells in a time- and concentration-dependent manner (Fig. 4). In accordance with our data showing the mixed cysteine-serine activity of *Der p I*, the cleavage of CD23 was inhibited by the irreversible class-specific protease inhibi-



**Figure 6.** Cleavage of CD23 is inhibited by  $\alpha_1$ -antiprotease. Cleavage of CD23 from the surface of EBV-transformed human B cells by *Der p* I was inhibited by the respiratory tract antiprotease  $\alpha_1$ -antiprotease (■). The  $\alpha_1$ -antiprotease alone in the absence of *Der p* I had no effect on the expression of CD23 (●).

tors E64 and APMSF (Fig. 5, *a* and *b*), but not by 1,10-phenanthroline or pepstatin A (Fig. 5, *c* and *d*), thus suggesting that the unusual mixed cysteine-serine activity characteristic of *Der p* I was responsible for cleavage of CD23 from the B cell surface. Interestingly,  $\alpha_1$ -antiprotease also inhibited cleavage of CD23 by *Der p* I, suggesting that disruption of the IgE network may be prevented by an efficient pulmonary antiprotease defence system (Fig. 6). It is interesting to speculate that the unique structure of CD23 among the Ig Fc receptors presents an accessible cleavage site for *Der p* I that is not present in other Fc receptors that belong to the Ig superfamily.

The reproducible induction of allergic immune responses by *Der p* I suggests that cleavage of CD23 may be selective, as indiscriminate cleavage of all cell surface proteins would have more severe consequences for the immune system. We therefore determined whether *Der p* I preferentially cleaved CD23 from the cell surface by examining the expression of several other B cell surface molecules on EBV-transformed B cells after incubation with *Der p* I. The expression levels of CD20, surface Ig, HLA-DR, HLA-DP, and HLA-DQ were unchanged by incubation



**Figure 7.** *Der p* I cleaves CD23 selectively. The expression of five cell surface molecules, in addition to CD23, were examined after incubation at 37°C for 1 h in the presence (■) and absence (▨) of *Der p* I. The failure of *Der p* I to cleave any cell surface molecule apart from CD23 demonstrated the selectivity of cleavage.

with *Der p* I, confirming that *Der p* I selectively cleaves CD23 (Fig. 7). This result is particularly interesting because HLA-DR is reported to be noncovalently linked to CD23 (60).

This study suggests a mechanism by which house dust mites may provoke powerful allergic immune responses. Reduced antiprotease defence caused by pollution or inflammation may allow *Der p* I to injure the respiratory epithelium and access the immune system. Here, *Der p* I may selectively alter the level of expression of CD23, a critical regulator of the IgE network, by causing the loss of an IgE immune complex-mediated feedback inhibition pathway and the enhancement of a sCD23-mediated IgE promoting pathway, which may result in high IgE responses. Several parasites that provoke high protective IgE responses (61) are also known to expel or secrete proteolytic enzymes (52), and it has already been suggested that in such cases, a similar mechanism involving CD23 cleavage by these exogenous proteases may be responsible for inducing protective allergic rather than harmful allergic immune responses (62).

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