

A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity

Farouk Shakib, Oliver Schulz and Herb Sewell

Several epidemiological studies indicate that the development of atopic disease is linked to circulating levels of IgE (Refs 1–5), the antibody class responsible for allergic hypersensitivity reactions⁶. IgE binds to mast cells and basophils via the high affinity Fc receptor (FcεRI), and subsequent crosslinking of FcεRI-bound IgE molecules by allergen leads to the release of pharmacologically active mediators responsible for allergic tissue damage. In addition, IgE mediates allergen presentation⁷ through binding to FcεRI on Langerhans cells and monocytes, and to a much lesser extent through binding to its low affinity receptor (FcεRII), also known as CD23 (Refs 8, 9). *In vivo* studies in mice have demonstrated that IgE also upregulates the expression of FcεRI on basophils¹⁰, thereby helping to further increase the sensitization of these cells. Therefore, it is not surprising that great efforts are being made to elucidate the molecular signals involved in IgE synthesis, as a basis for developing effective immunosuppressive strategies. Whereas considerable advances have been made in elucidating the effects of cytokines such as interleukin 4 (IL-4) and IL-13, as well as cell-surface molecules, for example CD40 and CD23, on IgE synthesis, very little attention has been given to the potential modulatory effects exerted by allergens. Recent work suggests that some allergens, such as phospholipase A₂ from bee venom¹¹ and Der p 1 from dust mite^{12–15}, in addition to acting as immunogens, have the intrinsic ability to subvert the regulatory process controlling IgE synthesis, thereby favouring an allergic outcome.

Der p 1: from allergen to enzyme

It is now recognized that an increasing number of allergens from diverse sources have enzymatic activity¹⁶. Der p 1, a 25 kDa cysteine protease, being one of the most studied^{17,18}. The Der p 1 structure has been modelled on the crystal structure of papain¹⁹, with which it shows considerable sequence similarity²⁰, most notably for residues involved in the enzyme active site. The model suggests that Der p 1 comprises two domains separated by a cleft, which contains the active site with its characteristic catalytic triad of Cys34 (containing an essential highly reactive thiol group), His170 and Asn190. The proteolytic activity of Der p 1 has been characterized using a panel of peptide substrates and shown to have profound secondary substrate specificity²¹. This, together with knowledge of cleavage sites of known

The dust mite Dermatophagoides pteronyssinus is a major cause of allergic disease in the Western world. Der p 1 is considered to be the most immunodominant allergen involved in the expression of IgE-mediated dust-mite hypersensitivity. Here, Farouk Shakib and colleagues suggest that it is the proteolytic effect of Der p 1 on CD23 and CD25 which makes it such a potent inducer of IgE synthesis.

protein substrates (Table 1), suggests that Der p 1 has a preference for small hydrophobic residues (Val/Ala) in the P2 position, charged residues (Glu/Arg) in the P1 position and small hydrophilic/hydrophobic residues (Ser/Ala) in the P1' position.

The expression pattern of Der p 1 in the mite gut suggests a digestive function²², and interestingly, in this respect, the C-terminus of the Der p 1 propeptide (Table 1) meets the strict enzymatic specificity requirements outlined above. This leads us to speculate that active Der p 1 could be generated from the inactive proenzyme in an autoproteolytic fashion, which is clearly reminiscent of digestive

enzyme activation in the mammalian gut. Inhaled Der p 1 is most likely to be active; however, the purified allergen requires reactivation by a reducing agent such as cysteine, dithiothreitol or glutathione to regenerate its thiol group which becomes oxidized during purification.

Cleavage of CD23 by Der p 1

CD23 is a calcium-dependent type II integral membrane protein that belongs to the lectin family of adhesion molecules^{23–25}; it is a 45 kDa differentiation antigen on IgM⁺/IgD⁺ B cells, but is also found on other cells, including eosinophils, follicular dendritic cells, macrophages and platelets. The extracellular portion of CD23 comprises a lectin homology domain, flanked on the C-terminal side by a short tail containing an inverse RGD sequence, and on the N-terminal side by an α-helical coiled-coil stalk mediating trimer formation^{6,26}. There is no crystal structure for CD23, but its three-dimensional structure has been modelled^{27–29} on the crystal structure of the C-type lectin domain of the rat mannose binding protein³⁰.

On B cells, membrane CD23 and its soluble fragments have been shown to be involved in the regulation of IgE synthesis^{31,32}. The binding of IgE allows membrane CD23 to deliver a negative IgE regulatory signal to the B cell^{33–35}. When CD23 is not occupied by IgE, it undergoes proteolytic cleavage releasing soluble CD23 (sCD23) fragments of different molecular size, which have been shown to either upregulate (≥25 kDa fragment) or downregulate (16 kDa fragment) IgE synthesis³⁶. Therefore, endogenous or exogenous proteases that cleave CD23 from the surface of B cells would potentially disrupt the IgE regulatory mechanism, which in some cases would lead to excessive IgE synthesis.

Table 1. Der p 1 cleavage sites of protein substrates

Protein	Position	Refs
	3 2 1 1'2'3'	
CD23	S A E S M G	27
	Q V S S G F	
α I-antitrypsin	F L E A I P	47
	K T D T S H	
	Q G D A A Q	
Insulin β-chain	L V E A L Y	53
Der p I ^a	N A E T N A	20

^aThe putative autoproteolytic cleavage site of the Der p 1 proenzyme is shown; the mature enzyme starts with threonine (T).

It has been shown that Der p 1 cleaves CD23 from the surface of cultured human B cells^{12,13}. A more detailed investigation of the CD23 cleavage by Der p 1 has used both a recombinant 40 kDa protein, representing the entire extracellular portion of human CD23 (residues Asp48–Ser321), and B-cell-surface-expressed CD23. This found that Der p 1 cleaves CD23 at two sites (Ser155–Ser156 and Glu298–Ser299) to produce a 143 residue 17 kDa fragment, containing the lectin domain and part of the C-terminal tail²⁷ (Fig. 1). This 17 kDa fragment (amino acids 156–298) contains the minimum structural requirement (amino acids 156–288) for binding both to IgE (Refs 37, 38) and to CD21 (also known as CR2)⁶, and as such should be capable of modulating IgE synthesis. However, it has not been possible to demonstrate any IgE modulatory activity for this Der p 1-derived sCD23 fragment (O. Schulz and F. Shakib, unpublished). Clearly, this would still leave open the possibility that Der p 1 upregulates IgE synthesis by disrupting the negative feedback signal, which is thought to be delivered by IgE binding to membrane CD23.



Cleavage of CD25 by Der p I

Several studies indicate that T helper 2 (Th2)-type responses are involved in the pathogenesis of allergic diseases^{39,40}, mainly through the secretion of IL-4/IL-13 and IL-5, which are responsible for stimulating IgE synthesis and eosinophil function, respectively. These observations raise the question of whether the enzymatic activity of Der p 1 biases human T cells towards the emergence of a Th2 background: this would create an allergic microenvironment. Indeed, it has been possible to demonstrate that Der p 1 cleaves CD25, the 55 kDa α subunit of the IL-2 receptor (the high affinity form of which comprises α, β and γ subunits). As a result of cleavage of surface CD25, peripheral blood T cells show markedly diminished proliferation and interferon γ (IFN-γ) secretion in response to potent stimulation by anti-CD3 antibody¹⁴ (Fig. 2).

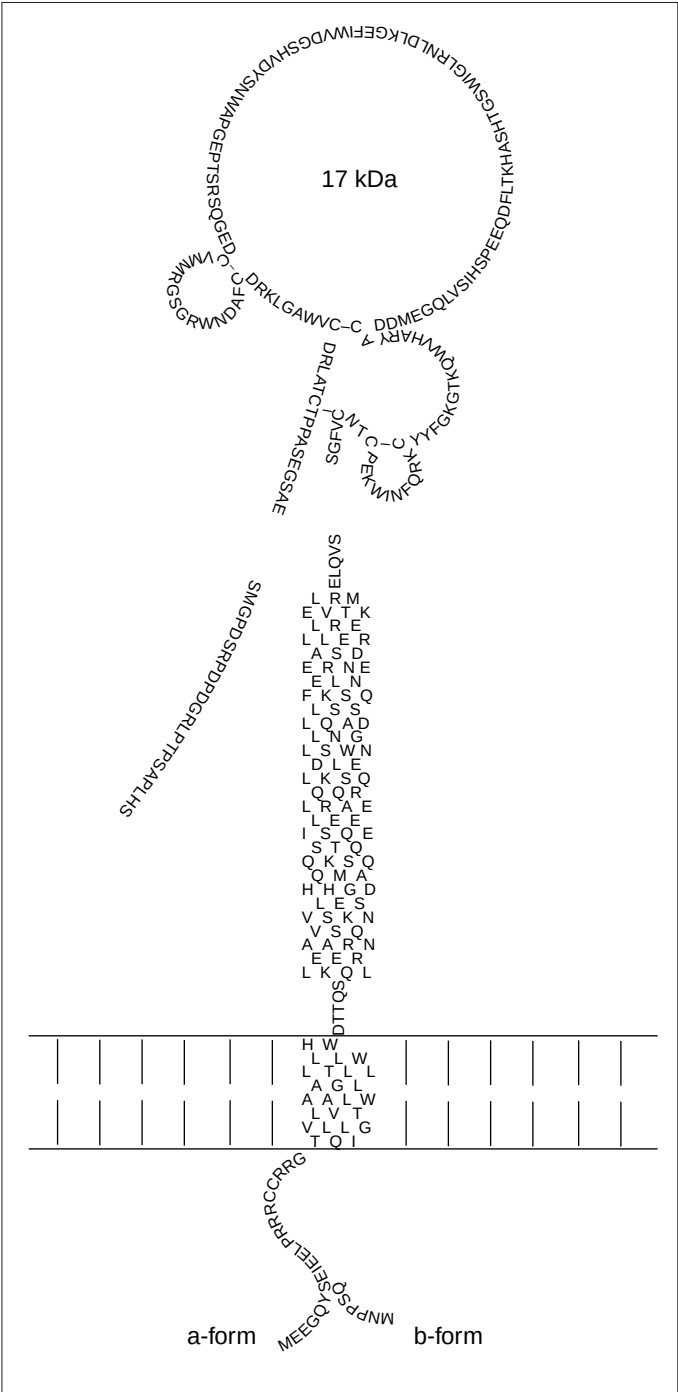


Fig. 1. Der p 1 cleaves human CD23 at two sites (Ser155–Ser156 and Glu298–Ser299) to produce a 143 residue 17 kDa fragment (amino acids 156–298), containing the lectin domain and part of the C-terminal tail²⁷. Figure adapted from an original kindly supplied by H. Gould and B. Sutton, King’s College London.

These findings clearly emphasize that Der p 1 could upset the balance of the Th1/Th2 subset distribution. It is known that each of the Th1- and Th2-cell populations promotes the development of cells within the same subset while suppressing the propagation of those of the other subset. Within this cytokine-driven process of polarization, IL-2 along with IFN-γ, and IL-4 are considered to be autocrine growth factors for Th1 and Th2 subsets, respectively⁴¹. Therefore, Der p 1-induced cleavage of CD25 is likely to lead to impaired growth of cells

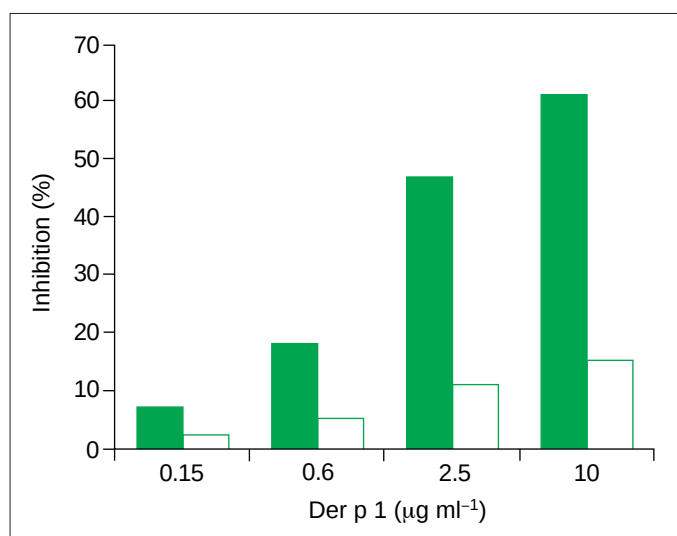


Fig. 2. Inhibition of interleukin 2-mediated human T-cell proliferation by Der p 1 requires enzymatically active protease (i.e. pre-activated with cysteine; filled bars)¹⁴.

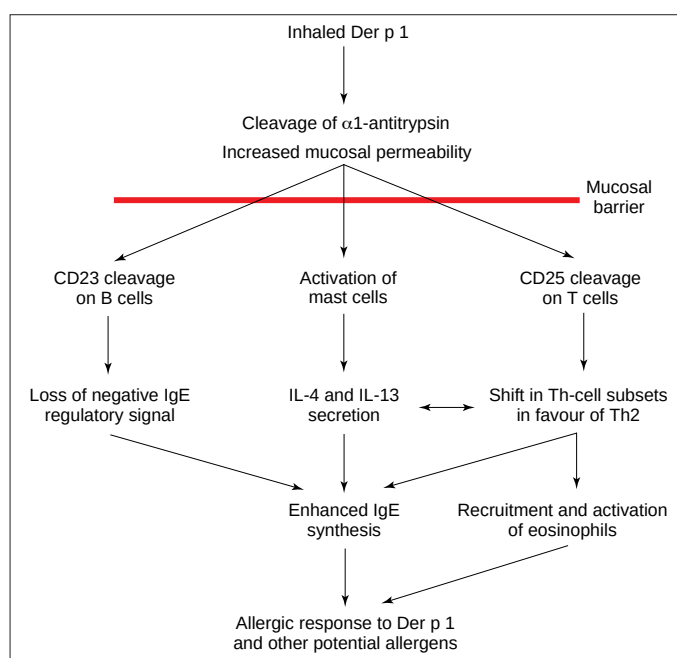


Fig. 3. Der p 1 facilitates its own passage across the mucosal barrier, gaining access to cells of the immune system and destabilizing the microenvironment within target tissues to one that is pro-allergic. Abbreviations: IL-4, interleukin 4; Th, T helper cell.

of the Th1 subset and consequently, augmentation of those of the Th2 subset. It has been shown that mite allergen-specific T-cell clones from atopic donors exhibit a Th2-type cytokine profile⁴² and require IL-4 for their optimal growth^{43,44}, which means that in an ongoing allergic response to Der p 1, such Th2 cells would be less sensitive to the enzymatic activity of Der p 1 compared with IL-2-dependent Th1 cells. To date, we have not been able to monitor the effect of Der p 1 on IL-4 receptor (IL-4R; CD124) and IL-4 secretion, owing to low levels of expression by peripheral blood T cells; therefore, it is still an open question as to whether Der p 1 has an effect on Th2-cell proliferation.

Cleavage of CD25 would be a mechanism by which Der p 1 uses its protease activity to bias the immune response in favour of IgE, this time by targeting a molecule (i.e. the IL-2R α subunit) which plays a pivotal role in the development and propagation of Th1 cells, thus favouring a pro-allergic Th2-type response⁴¹.

Passage of Der p 1 across the mucosa

The cysteine protease activity of Der p 1 not only increases the permeability of the bronchial mucosa to macromolecules, such as serum albumin, but also detaches cultured epithelial cell monolayers both from artificial and natural biopolymer substrata and causes visible disruption of epithelial architecture⁴⁵. The activation state of inhaled Der p 1 is maintained on impacting the mucosal surface by the high levels of glutathione found in airway lining fluid⁴⁶. Furthermore, Der p 1 can completely cleave α 1-antitrypsin, an antiprotease whose function is to provide innate immunity on mucosal surfaces^{12,47}. These observations provide a plausible mechanism whereby Der p 1 would facilitate its own passage (along with other allergens) across the epithelium and gain access to immune cells, such as T cells and B cells, and modulate their functions (as described above) during the process of immunological sensitization.

Proteolytic activity of Der p 1 *in vivo*

The *in vivo* proteolytic effect of Der p 1 depends on a number of factors including concentration, stability, pH optimum and susceptibility to inhibition by anti-Der p 1 antibodies during a secondary immune response. Der p 1 is found at high concentrations within faecal pellets (0.1 ng/pellet⁴⁸, which is equivalent to the tryptase content of 50 mast cells⁴⁹) and there is no doubt that it reaches cells of the immune system at sufficient concentrations (nanogram range) to elicit antibody responses. The demonstration of proteolytic activity of Der p 1 at such concentrations requires a detection method that is more sensitive than the flow cytometric procedure used for monitoring the cleavage of CD23 and CD25. In this context, we have shown that Der p 1, at a concentration as low as 10 ng ml⁻¹, cleaves the fluorogenic peptide substrate Boc-Gln-Ala-Arg-AMC (Ref. 21). Furthermore, Der p 1 is adapted to the tissue microenvironment, since it has a pH optimum of 7.0 (Ref. 21) and appears to be resistant to autoproteolysis at 37°C for several hours (O. Schulz and F. Shakib, unpublished). The presence of anti-Der p 1 antibodies in the tissue microenvironment of atopic individuals raises the possibility that such antibodies neutralize the enzymatic activity of Der p 1. We have tested the effect of three mouse monoclonal anti-Der p 1 antibodies, showing comparable affinity but different epitope specificity⁵⁰, in the CD23 cleavage assay¹² and found that at equimolar ratio they inhibit by 86% (5H8), 54% (4C1) and 39% (2C7), which suggests that the degree of inhibition is determined by the location of the epitopes recognized in relation to the enzyme active site (O. Schulz and F. Shakib, unpublished). Given that a major component of the human IgE anti-Der p 1 response has a similar epitope specificity to that of antibody 2C7 (Ref. 51), which was found to be the least inhibitory, it is likely that even during a secondary immune response Der p 1 retains a significant amount of enzymatic activity *in vivo*.

Concluding remarks

The potent allergenicity of Der p 1 may at least in part be due to its proteolytic activity, which could lead to a general increase in IgE synthesis (i.e. regardless of allergen specificity). In other words, Der p 1 may play a central role in destabilizing the microenvironment within target tissues to one that is pro-allergic, and thus aids in the initiation and propagation of the allergic cascade (Fig. 3). Naturally, the presence of other atopic risk-factors would lead to an exaggerated IgE response with consequent allergic inflammation. Indeed, recent clinical, epidemiological and serological data have indicated that specific immunotherapy against dust mite allergens in young children may prevent the development of new allergic sensitizations⁵². We therefore suggest that targeting the enzyme active site of Der p 1 with an appropriate cysteine protease inhibitor may provide a novel way of effectively controlling IgE-mediated hypersensitivity to this allergen.

O.S. is a recipient of a University of Nottingham/Peptide Therapeutics PhD studentship.

Farouk Shakib (farouk.shakib@nottingham.ac.uk), **Oliver Schulz** and **Herb Sewell** are at the Division of Molecular and Clinical Immunology, University of Nottingham Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham, UK NG7 2UH.

References

- Burrows, B., Martinez, F.D., Holonen, M., Barbee, R.A. and Cline, M.G. (1989) *New Engl. J. Med.* 320, 271–277
- Sears, M.R., Burrows, B., Flannery, E.M., Herbison, G.P., Hewitt, C.J. and Holdaway, M.D. (1991) *New Engl. J. Med.* 325, 1067–1071
- Pollart, S., Chapman, M., Fiocco, G., Rose, G. and Platts-Mills, A. (1989) *J. Allergy Clin. Immunol.* 83, 875–882
- Lichtenstein, L., Ishizaka, K., Norman, P., Sobotka, A. and Hill, B. (1973) *J. Allergy Clin. Immunol.* 52, 472–482
- Naclerio, R., Adkinson, N., Creticos, P., Baroody, F., Hamilton, R. and Norman, P. (1993) *J. Allergy Clin. Immunol.* 92, 717–721
- Sutton, B.J. and Gould, H.J. (1993) *Nature* 366, 421–428
- Mudde, G.C., Van Reijssen, F.C., Boland, G.J., De Gast, G.C., Bruijnzeel, P.L.B. and Bruijnzeel-Koomen, C.A.F.M. (1990) *Immunology* 69, 335–341
- Maurer, D., Ebner, C., Reininger, B. *et al.* (1995) *J. Immunol.* 154, 6285–6290
- Klupal, R., Osterhoff, B., Wang, B., Kinet, J-P., Maurer, D. and Stingl, G. (1997) *J. Invest. Dermatol.* 108, 336–342
- Lantz, C.S., Yamaguchi, M., Oettgen, H.C. *et al.* (1997) *J. Immunol.* 158, 2517–2521
- Dudler, T., Machado, D.C., Kolbe, L. *et al.* (1995) *J. Immunol.* 155, 2605–2613
- Schulz, O., Laing, P., Sewell, H.F. and Shakib, F. (1995) *Eur. J. Immunol.* 25, 3191–3194
- Hewitt, C.R.A., Brown, A.P., Hart, B.J. and Pritchard, D.I. (1995) *J. Exp. Med.* 182, 1537–1544
- Schulz, O., Sewell, H.F. and Shakib, F. (1998) *J. Exp. Med.* 187, 271–275
- Machado, D.C., Horton, D., Harrop, R., Peachell, P.T. and Helm, B.A. (1996) *Eur. J. Immunol.* 26, 2972–2980
- Stewart, G.A. and Thompson, P.J. (1996) *Clin. Exp. Allergy* 26, 1020–1044
- Thomas, W.R. (1993) *Clin. Exp. Allergy* 23, 350–353
- Robinson, C., Kalsheker, N.A., Srinivasan, N. *et al.* (1997) *Clin. Exp. Allergy* 27, 10–21
- Topham, C.M., Srinivasan, N., Thorpe, C.J., Overington, J.P. and Kalsheker, N.A. (1994) *Protein Eng.* 7, 869–894
- Chua, K.Y., Stewart, G.A., Thomas, W.R. *et al.* (1988) *J. Exp. Med.* 167, 175–182
- Schulz, O., Sewell, H.F. and Shakib, F. *J. Clin. Pathol.* (in press)
- Thompson, S.J. and Carswell, F. (1988) *Int. Archs. Allergy Appl. Immun.* 85, 312–315
- Ikuta, K., Takami, M., Kim, C.W. *et al.* (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 819–823
- Kikutani, H., Inui, S., Sato, R. *et al.* (1986) *Cell* 47, 657–665
- Ludin, C., Hofstetter, H., Sarfati, M. *et al.* (1987) *EMBO J.* 6, 109–114
- Beavil, R.L., Graber, P., Aubonne, N., Bonnefoy, J-Y. and Gould, H.J. (1995) *Immunology* 84, 202–206
- Schulz, O., Sutton, B.J., Beavil, R.L. *et al.* (1997) *Eur. J. Immunol.* 27, 584–588
- Padlan, E.A. and Helm, B.A. (1993) *Receptor* 3, 325–341
- Bajorath, J. and Aruffo, A. (1996) *Protein Sci.* 5, 240–247
- Weis, W.I. and Drickamer, K. (1994) *Structure* 2, 1227–1240
- Flores-Romo, L., Shields, J., Humbert, Y. *et al.* (1993) *Science* 261, 1038–1041
- Sarfati, M. and Delespesse, G. (1988) *J. Immunol.* 141, 2195–2199
- Lamers, M.C. and Yu, P. (1995) *Immunol. Rev.* 148, 71–95
- Yu, P., Kosco-Vilbois, M., Richards, M., Kohler, G. and Lamers, M.C. (1994) *Nature* 369, 753–756
- Sherr, E., Macy, E., Kimata, H., Gilly, M. and Saxon, A. (1989) *J. Immunol.* 142, 481–489
- Sarfati, M., Bettler, B., Letellier, M. *et al.* (1992) *Immunology* 76, 662–667
- Shi, J., Ghirlando, R., Beavil, R.L. *et al.* (1997) *Biochemistry* 36, 2112–2122
- Mossalayi, M.D., Arock, M., Delespesse, G. *et al.* (1992) *EMBO J.* 11, 4323–4328
- Neerven van, R.J.J., Ebner, C., Yssel, H., Kapsenberg, M.L. and Lamb, J.R. (1996) *Immunol. Today* 17, 526–532
- Romagnani, S. (1994) *Annu. Rev. Immunol.* 12, 227–257
- Abbas, A.K., Murphy, K.M. and Sher, A. (1996) *Nature* 383, 787–793
- Wierenga, E.A., Snoek, M., Bos, J.D., Jansen, H.M. and Kapsenberg, M.L. (1990) *Eur. J. Immunol.* 20, 1519–1526
- Michael, B.N. and Kalish, R.S. (1994) *Cell. Immunol.* 158, 105–115
- Van Reijssen, F.C., Wijburg, O.L.C., Gebhardt, M. *et al.* (1994) *Clin. Exp. Immunol.* 98, 151–157
- Herbert, C.A., King, C.M., Ring, P.C. *et al.* (1995) *Am. J. Respir. Cell Mol. Biol.* 12, 369–378
- Cantin, A.M., North, S.L., Hubbard, R.C. and Crystal, R.G. (1987) *J. Appl. Physiol.* 163, 152–157
- Kalsheker, N.A., Deam, S., Chambers, L., Sreedharan, S., Brocklehurst, K. and Lomas, D.A. (1996) *Biochem. Biophys. Res. Commun.* 221, 59–61
- Tovey, E.R., Chapman, M.D. and Platts-Mills, T.A.E. (1981) *Nature* 289, 592–593
- Schwartz, L.B., Irani, A.A., Roller, K., Castells, M.C. and Schechter, N.M. (1987) *J. Immunol.* 138, 2611–2615
- Platts-Mills, T.A.E. and Chapman, M.D. (1987) *J. Allergy Clin. Immunol.* 80, 755–775
- McElveen, J., Clark, M.R., Smith, S.J., Sewell, H.F. and Shakib, F. *Clin. Exp. Allergy* (in press)
- Des Roches, A., Paradis, L., Menardo, J.L., Bouges, S., Daures, J-P. and Bousquet, J. (1997) *J. Allergy Clin. Immunol.* 99, 450–453
- Hewitt, C.R.A., Horton, H., Jones, R.M. and Pritchard, D.I. (1997) *Clin. Exp. Allergy* 27, 201–207