

REVIEW

The interaction between the dust mite antigen Der p 1 and cell-signalling molecules in amplifying allergic disease

O. SCHULZ, H. F. SEWELL and F. SHAKIB

Division of Molecular and Clinical Immunology, University of Nottingham, Queen's Medical Centre, Nottingham, UK

Introduction

It has recently been suggested that nonimmunological properties of some allergens, such as enzymatic activity, might contribute to their allergenicity [1]. Within this context, a number of studies have focused on the proteolytic activity of the dust mite cysteine protease Der p 1, the most significant domestic allergen. In this article, we review the proteolytic activity of Der p 1 and its effects on cells of the innate (mast cells) and adaptive (lymphocytes) immune systems, with a view to demonstrating a link between enzymatic activity and allergenicity. Collectively, these findings reveal previously unrecognized mechanisms by which the proteolytic activity of Der p 1 may perturb immunological signalling in favour of an allergic outcome [2].

Enzymatic properties of Der p 1

We have recently established the first continuous rate (kinetic) assay for measuring the proteolytic activity of Der p 1, using the fluorogenic peptide substrate Boc-Gln-Ala-Arg-AMC [3]. Hydrolysis of this substrate requires preactivation of the purified Der p 1 preparation with a reducing agent (DTT) [3], and is inhibited by the class-specific cysteine protease inhibitor E-64 [4]. These results provide compelling evidence that this allergen is a cysteine protease, thereby complementing and confirming previous data that were based on sequence homology with papain [5].

The results also reveal that Der p 1 has unusual proteolytic specificity [3]. For instance, Der p 1 exhibits preference for small aliphatic residues in the P₂ position (Ala/Val), and therefore has a different secondary (P₂) substrate specificity compared with other papain-related cysteine proteases which tend to favour aromatic or basic residues (Phe/Arg) in this position [6,7]. Such preference has also been confirmed by analysing Der p 1 cleavage sites of protein substrates [2], which show that the nature of the amino acid

residues in the P₂ (Ala/Val) and the P₁' (Ser/Ala) positions determine the overall affinity and reactivity of Der p 1. However, like papain and cathepsin B, Der p 1 shows little specificity for the P₁ position (Arg/Glu/Ser), which may explain why these cysteine proteases produce similar cleavage patterns with respect to peptide and protein substrates, such as the oxidized β -chain of insulin [8,9], α 1-antitrypsin [10–12] and CD23 [13,14]. The definition of the proteolytic specificity of Der p 1 can now be used to predict other potential molecular targets within the immune system.

On the whole, the behaviour of Der p 1 towards naturally occurring protease inhibitors is consistent with the notion that this enzyme is a member of the papain family of cysteine proteases [4]. Thus, the proteolytic activity of Der p 1 is not inhibited by class-specific inactivators/inhibitors of serine proteases (SBTI, aprotinin, and α 1-antitrypsin), aspartic proteases (pepstatin A) and metalloproteases (ethylenediaminetetraacetic acid). Der p 1 is strongly inhibited only by antipain ($K_i = 190$ nM), a tight-binding, reversible inhibitor of serine and cysteine proteases [15]. Der p 1 is also inhibited by E-64 ($k_{app} = 1180$ M⁻¹ s⁻¹), an irreversible inhibitor of cysteine proteases, but the rate of inhibition was found to be considerably lower compared with that seen with papain ($k_{app} = 638\,000$ M⁻¹ s⁻¹), cathepsin B ($k_{app} = 89\,400$ M⁻¹ s⁻¹), cathepsin L ($k_{app} = 96\,250$ M⁻¹ s⁻¹), glycyl endopeptidase ($k_{app} = 58\,000$ M⁻¹ s⁻¹) and ananain ($k_{app} = 302\,297$ M⁻¹ s⁻¹) [16–18]. Der p 1 resists inhibition by hen egg white cystatin (HEWC) [4], a property shared by some cysteine proteases from papaya (glycyl endopeptidase) [17], pineapple (stem bromelain) [18], and beans [19], all of which are also thought to be allergenic [20].

Passage of Der p 1 across the mucosa

Der p 1 has been shown to detach cultured epithelial cell monolayers from both artificial and natural biopolymer substrata and cause visible disruption of epithelial architecture, thereby increasing the permeability of the bronchial mucosa to macromolecules, such as serum albumin [21]. Furthermore, we [11] and others [12] have shown that Der p 1 completely cleaves human α 1-antitrypsin, an

Correspondence: F. Shakib, Division of Molecular and Clinical Immunology, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

antiprotease whose function is to protect the respiratory mucosa against serine proteases. Therefore, cleavage of α 1-antitrypsin by Der p 1 might potentially upset the local protease-antiprotease balance and enhance the activity of endogenous serine proteases, especially those from neutrophils (elastase, cathepsin G), thereby leading to further tissue damage. These observations provide a plausible mechanism whereby Der p 1 would facilitate its own passage (along with other allergens) across the epithelium and gains access to cells of the immune system.

Der p 1 in the microenvironment

The proteolytic effect of Der p 1 *in vivo* is likely to be influenced by a number of factors including concentration, pH optimum, stability and susceptibility to inhibition by anti-Der p 1 antibodies.

Der p 1 is sequestered in mite faecal pellets, where it accounts for 15 to 20% of the total protein content and amounts to a concentration of 0.1 ng/pellet [22]. It is therefore not surprising that Der p 1 is so abundant in the domestic environment (house dust), reaching concentrations of up to 30 μ g/g. Because of their small size (10–20 μ m), faecal pellets become airborne and therefore act as a vehicle for delivering Der p 1 to the respiratory mucosa. Indeed, nanogram quantities of Der p 1 have been detected in BAL fluid from dust mite-allergic individuals [23], which suggests that prior to dilution the concentration of Der p 1 within the mucosa might be in the microgram range. Elution of Der p 1 from faecal pellets occurs instantaneously and is complete in less than 1 min [24]. The activation state of inhaled Der p 1 is likely to be maintained by the high levels of glutathione found in airway lining fluid [25]. Der p 1 is also optimally active at neutral pH [3] and is fairly resistant to autoproteolysis at 25–37°C (O. Schulz, unpublished observations). Taken together, these data suggest that the respiratory mucosa is exposed to high concentrations of Der p 1 whose cysteine protease activity is well adapted to the local tissue microenvironment.

The presence of anti-Der p 1 antibodies in the tissue microenvironment of atopic individuals raises the possibility that such antibodies might neutralize the enzymatic activity of Der p 1. Three mouse monoclonal anti-Der p 1 antibodies were tested in the kinetic and CD23 cleavage assays and the extent to which they inhibited the proteolytic activity of Der p 1 was found to be dependent on the location of their epitopes in relation to the enzyme active site [2]. Given that antibody 2C7, the least inhibitory, has a similar epitope specificity to that of human immunoglobulin E (IgE) [26], it is likely that Der p 1 retains a significant amount (60%) of enzymatic activity even when complexed with antibody (1:1 ratio). In fact, IgE antibodies could

potentially protect Der p 1 from endogenous proteases and help to target Der p 1 to cell surface receptors.

Effect of Der p 1 on innate immunity

Recent work has shown that Der p 1 induces mast cell and basophil degranulation and stimulates interleukin (IL)-4 synthesis and secretion independently of IgE [27]. These actions of Der p 1, which were due to its proteolytic activity, could significantly influence IgE production, since mast cells and basophils support IgE synthesis by secreting IL-4 and IL-13 and provide contact-mediated help for B cells through CD40 L [28,29]. IgE-independent mast cell and basophil activation has also been linked to the induction of bee venom phospholipase A2-specific IgE in mice given intraperitoneal injections of enzymatically active phospholipase A2 [30]. These observations suggest that mast cell degranulation by enzymatically active allergens may help trigger an IgE response at the onset of specific immunity. In other words, enzymatically active allergens could potentially influence the innate immune system to bias the adaptive immune response in favour of TH2.

Effect of Der p 1 on adaptive immunity

Shedding of lymphocyte surface molecules occurs through proteolytic cleavage by endogenous proteases, a process that is thought to regulate lymphocyte functions such as adhesion [31] and cytokine-mediated activation [32]. Among the physiologically cleaved surface molecules are Fc receptors (e.g. Fc ϵ R2, also called CD23) [33] and cytokine receptors (e.g. IL-2R, also called CD25) [34]. Proteolysis of CD23 and CD25 by an exogenous protease, such as Der p 1, could therefore potentially compromise the physiological functions of B and T cells, respectively.

Proteolysis of CD23 by Der p 1

On B cells, membrane CD23 and its soluble fragments have been shown to be involved in the regulation of IgE synthesis [35,36]. The binding of IgE allows membrane CD23 to deliver a negative IgE regulatory signal to the B cell [37–39]. When CD23 is not occupied by IgE, it undergoes proteolytic cleavage releasing soluble CD23 (sCD23) fragments of different molecular sizes, which have been shown to either up-regulate (25 kDa) or down-regulate (16 kDa) IgE synthesis [40,41]. Therefore, exogenous proteases that cleave CD23 from the surface of B cells could potentially disrupt an important IgE regulatory mechanism.

Der p 1 was shown to cleave CD23 from the human B cell line RPMI 8866 and IL-4-treated tonsillar B cells. This action of Der p 1 appeared to be selective because other B cell surface markers were not affected [11,42]. Detailed

investigation of CD23 cleavage by Der p 1 was carried out using B cell-derived CD23 and a recombinant 40-kDa CD23, representing the entire extracellular portion of human CD23 (residues Asp48–Ser321). This revealed that Der p 1 cleaves CD23 at two sites (Ser155–Ser156 and Glu298–Ser299) to produce a 143-residue 17-kDa fragment (a.a.156–298), containing the lectin domain and part of the C-terminal tail [14]. Der p 1 cleaves CD23 probably in a sequential manner, starting with the site in the C-terminal tail (Glu298–Ser299), which is then followed by cleavage of the newly accessible site in the neck region (Ser155–Ser156). Interestingly, the generation of 15–17-kDa IgE-binding sCD23 fragments was also observed with papain and lysosomal cysteine proteases [13,43,44], therefore suggesting that cysteine proteases in general induce limited proteolysis of CD23.

The Der p 1-derived 17-kDa sCD23 fragment contains the minimum structural requirements for binding to both IgE [45] and CD21 [41], and as such should be capable of modulating IgE synthesis [40]. However, it has not been possible to demonstrate any *in vitro* IgE modulatory activity for the Der p 1-derived native and recombinant sCD23 fragments [4]. Therefore, it seems unlikely that Der p 1 exerts an IgE modulatory effect via sCD23. On the other hand, Der p 1-treated B cells lose their ability to bind IgE as a consequence of CD23 cleavage [14]. This would therefore still leave open the possibility that Der p 1 may up-regulate polyclonal IgE synthesis by disrupting the negative feedback signal, which is thought to be delivered by IgE binding to membrane CD23. It could be argued that cleavage of CD23 by Der p 1 would interfere with IgE-mediated antigen presentation by B cells [46,47]. This would have a down-regulatory effect on IgE synthesis, since B cells are thought to be good at presenting antigen to TH2 cells [48]. However, the predominant IgE binding receptor in peripheral tissues is FcεRI [49], which is also thought to be the main receptor involved in IgE-mediated antigen presentation by Langerhan's cells and monocytes [50].

Der p 1 would also be expected to cleave CD23 from monocytes/macrophages. CD23 on monocytes/macrophages is dramatically up-regulated in patients with atopic asthma [51] and has been implicated in the regulation of inflammatory processes in patients with rheumatoid arthritis [52]. Activation of monocytes/macrophages through binding of membrane CD23 to IgE and sCD23 to the integrin receptors, CD11b/CD18 and CD11c/CD18, leads to the production of pro-inflammatory cytokines including TNF-α and interferon (INF)-γ [53–55]. Cytokines produced by macrophages induce TH1, but inhibit TH2 responses [56,57]. Therefore, Der p 1-induced cleavage of CD23 could potentially deprive macrophages of an important activation receptor, thereby interfering with macrophage-mediated TH1 cell differentiation.

Proteolysis of CD25 by Der p 1

Atopic diseases are thought to be T cell-mediated disorders, resulting from a dysfunctional control of TH2 responses, leading to increased IgE synthesis and eosinophil activation [58,59]. In this connection, it has been suggested that allergens have the intrinsic ability to influence the TH1/TH2 subset balance [60,61]. Therefore, this raises the question of whether the proteolytic activity of Der p 1 could bias human T cells towards the emergence of a TH2 response.

We have recently shown for the first time that Der p 1 cleaves human CD25, the 55-kDa α subunit of the receptor for the TH1 cytokine IL-2. As a result of cleavage of surface CD25, peripheral blood T cells show markedly diminished proliferation and INF-γ secretion in response to stimulation by anti-CD3 antibody [62]. These findings suggest a role for the enzymatic activity of Der p 1 in perturbing the homeostatic control of the TH1/TH2 subset distribution. It is known that each of the TH1 and TH2 cell populations promote the development of cells of the same subset while suppressing the propagation of those of the other subset [63]. Within this cytokine-driven process of polarization, IL-2 along with INF-γ, and IL-4 are considered to be autocrine growth factors for the TH1 and TH2 subsets, respectively. Therefore, Der p 1-induced cleavage of CD25 is likely to lead to impaired growth of cells of the TH1 subset and consequent augmentation of those of the TH2 subset. Mite allergen-specific T-cell clones from atopic donors exhibit a TH2 cytokine profile [64] and require IL-4 for their optimal growth [65,66]. This means that in an ongoing allergic response such TH2 cells would be less sensitive to the enzymatic activity of Der p 1 compared with IL-2/INF-γ-dependent TH1 cells. Indeed, it has recently been shown that the enzymatic activity of Der p 1 does not affect the proliferative response of peripheral blood T cells to exogenously added IL-4 [4], and that the administration of Der p 1 to mice promotes a TH2 response by modulating the balance between IL-4 and INF-γ [67].

An intriguing question is the interrelationship between the immunogenic (proliferative) and the proteolytic (anti-proliferative) effects of Der p 1 on the growth of Der p 1-specific T cells. Previous reports using Der p 1-specific human T cells have demonstrated that purified Der p 1 is likely to induce T-cell proliferation at concentrations lower (<1 μg/mL) [68,69] than those shown to have an inhibitory effect on T cells. However, such direct comparison is not possible since these studies have used Der p 1 preparations which were almost certainly enzymatically inactive. Obviously, T-cell activation (and consequent CD25 expression) is required before Der p 1 can exert its antiproliferative effect. This has important implications for the activity of Der p 1 *in vivo*. Der p 1 would be expected to act on

antigen-stimulated T cells (including those activated by Der p 1) and therefore influence immune responses in an oligoclonal, antigen non-specific fashion.

Recent evidence suggest that CD8⁺ T cells suppress IgE production [70]. For example, in animal models allergen exposure results usually in a transient IgE response, the termination of which is accompanied by the appearance of INF- γ -secreting allergen-specific CD8⁺ T cells [71]. Thus, CD8⁺ T cells have been proposed to limit sensitization to inhaled aeroallergens by inducing immune deviation towards TH1 in the upper respiratory tract, a process believed to occur very early in life [72]. In this connection, it is interesting to note that Der p 1-induced CD25 cleavage has a stronger antiproliferative effect on CD8⁺ (85% inhibition) than on CD4⁺ (50% inhibition) T cells [4]. These findings suggest yet another potential mechanism by which Der p 1 uses its protease activity to bias the immune response in favour of IgE, this time by abrogating the IgE suppressive effect of CD8⁺ T cells.

Conclusions

We have presented evidence suggesting that the potent allergenicity of Der p 1 may at least in part be due to its proteolytic activity. Der p 1 biases the immune response in favour of TH2 by targeting three cell types involved in the homeostatic control of IgE synthesis, namely mast cells, B cells and T cells. Thus, Der p 1 may play a central role in destabilizing the microenvironment within target tissues to one that is pro-allergic, and hence aids in the initiation and propagation of the allergic cascade [2].

References

- Robinson C, Kalsheker NA, Srinivasan N et al. On the significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterization. *Clin Exp Allergy* 1997; 27:10–21.
- Shakib F, Schulz O, Sewell HF. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. *Immunol Today* 1998; 19:313–6.
- Schulz O, Sewell HF, Shakib F. A sensitive fluorescent assay for measuring the cysteine protease activity of Der p 1, a major allergen from the dust mite *Dermatophagoides pteronyssinus*. *J Clin Pathol: Mol Pathol* 1998;51:222–4.
- Schulz O. The pro-allergic potential of the cysteine protease activity of Der p 1. PhD Thesis, University of Nottingham 1998.
- Chua KY, Stewart GA, Thomas WR et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J Exp Med* 1988; 167:175–82.
- Schlechter I, Berger A. On the active site of proteases. III. Mapping the active site of papain: specific peptide inhibitors of papain. *Biochem Biophys Res Comm* 1968; 32:898–902.
- Rawlings ND, Barrett AJ. Families of cysteine peptidase. *Methods Enzymol* 1994;244: 461–86.
- Dunn BM. Determination of protease mechanism. In: Beynon RJ, Bond JS, eds. *Proteolytic Enzymes: a Practical Approach*. IRL Press: Oxford, 1994: 57–81.
- Hewitt CRA, Horton H, Jones RM, Pritchard DI. Heterogeneous proteolytic specificity and activity of the house dust proteinase allergen Der p 1. *Clin Exp Allergy* 1997; 27:201–7.
- Johnson D, Travis J. Inactivation of human α 1 proteinase inhibitor by thiol proteinases. *Biochem J* 1977; 163:639–41.
- Schulz O, Laing P, Sewell HF, Shakib F. Der p 1, a major allergen of the house dust mite, proteolytically cleaves the low-affinity receptor for human IgE (CD23). *Eur J Immunol* 1995; 25:3191–4.
- Kalsheker NA, Deam S, Chambers L et al. The house dust mite allergen Der, p. 1 catalytically inactivates α 1-antitrypsin by specific centre loop cleavage: a mechanism that promotes airway inflammation and asthma. *Biochem Biophys Res Com* 1996; 221:59–61.
- Peterson LH, Conrad DH. Fine specificity, structure and proteolytic susceptibility of the human lymphocyte receptor for IgE. *J Immunol* 1985; 135:2654–60.
- Schulz O, Sutton BJ, Beavil RL et al. Cleavage of the Low-Affinity Receptor for Human Ige (CD23) by a mite cysteine protease: nature of the cleaved fragment in relation to the structure and function of CD23. *Eur J Immunol* 1997; 27:584–8.
- Umezawa H. Structures and activities of protease inhibitors of microbial origin. *Meth Enzymol* 1976; 45:678–95.
- Barrett AJ, Kembhavi AA, Brown MA et al. L-trans-Epoxysuccinyl-leucylamido (4-guanidino) butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H and L. *Biochem J* 1982; 201:189–98.
- Buttle DJ. Glycyl endopeptidase. *Meth Enzymol* 1994; 24:539–55.
- Rowan AD, Buttle DJ. Pineapple cysteine endopeptidases. *Meth Enzymol* 1994; 244:555–68.
- Csoma C, Polgar L. Proteinase from germinating bean cotyledons: evidence for involvement of a thiol group in catalysis. *Biochem J* 1984; 222:769–76.
- Travis J, Potempa J, Maeda H. Are bacterial proteinases pathogenic factors? *Trends Microbiol* 1995; 3:405–7.
- Herbert CA, King CM, Ring PC et al. Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p 1. *Am J Respir Cell Mol Biol* 1995; 12:369–78.
- Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592–3.
- Ferguson P, Broide DH. Environmental and bronchoalveolar lavage *Dermatophagoides pteronyssinus* antigen levels in atopic asthmatics. *Am J Respir Crit Care Med* 1995; 151:71–4.
- Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124:630–5.
- Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J Appl Physiol* 1987; 163:152–7.

- 26 McElveen JE, Clark MR, Smith SJ, Sewell HF, Shakib F. Primary sequence and molecular model of the variable region of a mouse monoclonal anti-Der p I antibody showing a similar epitope specificity as human IgE. *Clin Exp Allergy* 1998; 28:1427–34.
- 27 Machado DC, Horton D, Harrop R et al. Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol* 1996; 26:2972–80.
- 28 Gauchat J-F, Henchoz S, Mazzei G et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993; 365:340–3.
- 29 Pawankar R, Okuda M, Yssel H et al. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997; 99:1492–9.
- 30 Dudler T, Machado DC, Kolbe L et al. A link between catalytic activity, IgE-independent mast cell activation, and allergenicity of bee venom phospholipase A2. *J Immunol* 1995; 155:2605–13.
- 31 Bazil V. Physiological enzymatic cleavage of leukocyte membrane molecules. *Immunol Today* 1995; 16:135–40.
- 32 Fernandez-Botran R, Chilton PM, Ma Y. Soluble cytokine receptors: their roles in immunoregulation, disease and therapy. *Adv Immunol* 1996; 63:269–336.
- 33 Letellier M, Sarfati M, Delespesse G. Mechanisms of formation of IgE-BFs (soluble CD23). I. FcεRI bearing B cells generate IgE-binding factors of different molecular weights. *Mol Immunol* 1989; 26:1105–12.
- 34 Rubin L, Jay G, Nelson DL. The released interleukin 2 receptor binds interleukin 2 efficiently. *J Immunol* 1986; 137:3841–4.
- 35 Delespesse G, Sarfati M, Wu C et al. The low-affinity receptor for IgE. *Immunol Rev* 1992; 125:77–97.
- 36 Flores-Romo L, Shields J, Humbert Y et al. Inhibition of an in vivo antigen-specific IgE response by antibodies to CD23. *Science* 1993; 261:1038–41.
- 37 Luo H, Hofstetter H, Banchereau J, Delespesse G. Cross-linking of CD23 antigen by its natural ligand (IgE) or by anti-CD23 antibody prevents B lymphocyte proliferation and differentiation. *J Immunol* 1991; 146:2122–9.
- 38 Yu P, Kosco-Vilbois M, Richards M et al. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 1994; 369:753–6.
- 39 Lamers MC, Yu P. Regulation of IgE, synthesis. Lessons from the study of IgE transgenic and CD23-deficient mice. *Immunol Rev* 1995; 148:71–95.
- 40 Sarfati M, Bettler B, Letellier M et al. Native and recombinant soluble CD23 fragments with IgE suppressive activity. *Immunology* 1992; 76:662–7.
- 41 Mossalayi MD, Arock M, Delespesse G et al. Cytokine effects of CD23 are mediated by an epitope distinct from the IgE binding site. *EMBO J* 1992; 11:4323–8.
- 42 Hewitt CRA, Brown AP, Hart BJ, Pritchard DI. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 1995; 182:1537–44.
- 43 Sarfati M, Nakajima T, Frost H et al. Purification and partial characterization of IgE-binding factors secreted by a human B lymphoblastoid cell line. *Immunology* 1987; 60:539–45.
- 44 Grenier-Brosette N, Bourget I, Akoundi C et al. Spontaneous and ligand-induced endocytosis of CD23 (Fcε receptor II) from the surface of B lymphocytes generates a 16-kDa intracellular fragment. *Eur J Immunol* 1992; 22:1573–7.
- 45 Shi J, Ghirlando R, Beavil RL et al. Interaction of the Low-Affinity Receptor CD23/FcεRII lectin domain with the Fcε3–4 fragment of human immunoglobulin E. *Biochemistry* 1997; 36:2112–22.
- 46 Pirron U, Schlunck T, Prinz JC, Rieber EP. IgE-dependent antigen focusing by B lymphocytes is mediated by the low-affinity receptor for IgE. *Eur J Immunol* 1990; 20:1547–51.
- 47 Heyman B, Tianmin L, Gustavsson S. In vivo enhancement of the specific antibody response via the low-affinity receptor for IgE. *Eur J Immunol* 1993; 23:1739–42.
- 48 Gajewski TF, Pinnas M, Wong T, Fitch FW. Murine Th1 and Th2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J Immunol* 1991; 146:1750–8.
- 49 Klubal R, Osterhoff B, Wang B et al. The high-affinity receptor for IgE is the predominant IgE-binding structure in lesional skin of atopic dermatitis patients. *J Invest Dermatol* 1997; 108:336–42.
- 50 Maurer D, Ebner C, Reininger B et al. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. *J Immunol* 1995; 154:6285–90.
- 51 Williams J, Johnson S, Mascali JJ et al. Regulation of low affinity IgE receptor (CD23) expression on mononuclear phagocytes in normal and asthmatic subjects. *J Immunol* 1992; 149:2823–9.
- 52 Bonnefoy J-Y, Plater-Zyberk C, Lecoanet-Henchoz S et al. A new role for CD23 in inflammation. *Immunol Today* 1996; 17:418–20.
- 53 Armant M, Ishihara H, Rubio M et al. Regulation of cytokine production by soluble CD23: costimulation of interferon γ secretion and triggering of tumor necrosis factor α release. *J Exp Med* 1994; 180:1005–11.
- 54 Dugas B, Mossalayi MD, Damais C, Kolb J-P. Nitric oxide production by human monocytes: evidence for a role of CD23. *Immunol Today* 1995; 16:574–80.
- 55 Lecoanet-Henchoz S, Gauchat J-F, Aubry J-P et al. CD23 regulates monocyte activation through a novel interaction with the adhesion molecules CD11b-CD18 and CD11c-CD18. *Immunity* 1995; 3:119–25.
- 56 Hsieh C-S, Macatonia SE, Tripp CS et al. Development of Th1 CD4+ T cells through IL–12 produced by *Listeria*-induced macrophages. *Science* 1993; 260:547–9.
- 57 Manetti R, Parronchi P, Giudizi M-G et al. Natural killer cell stimulatory factor (interleukin 12) induces T helper type 1 (Th1) -specific immune responses and inhibits the development of IL-4 producing Th cells. *J Exp Med* 1993; 177:1199–204.
- 58 Romagnani S. Lymphokine production by human T cells in disease states. *Ann Rev Immunol* 1994; 12:227–53.
- 59 Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. *Immunol Today* 1992; 13:501–7.
- 60 Romagnani S. Regulation of the development of type 2 T-helper cells in allergy. *Curr Opin Immunol* 1994; 6:838–46.

- 61 Aebischer I, Stadler BM. TH1-TH2 cells in allergic responses: at the limits of a concept. *Adv Immunol* 1996; 61:341–403.
- 62 Schulz O, Sewell HF, Shakib F. Proteolytic cleavage of CD25, the α subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J Exp Med* 1998; 187:271–5.
- 63 Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; 383:787–93.
- 64 Wierenga EA, Snoek M, Bos JD et al. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *Eur J Immunol* 1990; 20:1519–26.
- 65 Michael BN, Kalish RS. House dust mite-responsive human T-cells require both interleukin 2 (IL2) and interleukin 4 for optimal proliferation, whereas IL2 alone is sufficient for proliferation of tetanus toxoid-responsive T cells. *Cell Immunol* 1994; 158:105–15.
- 66 Reijssen van FC, Wijburg OLC, Gebhardt M et al. Different growth factor requirements for human Th2 cells may reflect in vivo induced anergy. *Clin Exp Immunol* 1994; 98:151–7.
- 67 Comoy EE, Pestel J, Duez C et al. The house dust mite allergen, *Dermatophagoides pteronyssinus*, promotes type 2 responses by modulating the balance between IL-4 and IFN- γ . *J Immunol* 1998; 160:2456–62.
- 68 Rawle FC, Mitchell EB, Platts-Mills TAE. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P1: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984; 133:195–201.
- 69 Bheekha Eskura R, Wasserbauer E, Hammerschmid F et al. Regulation and targeting of T-cell immune responses by IgE and IgG antibodies. *Immunology* 1995; 86:343–50.
- 70 Kemeny DM, Noble A, Holmes BJ, Diaz-Sanchez D. Immune regulation: a new role for the CD8⁺ T cell. *Immunol Today* 1994; 15:107–10.
- 71 McMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993; 178:889–99.
- 72 Holt PG, Macaubas C. Development of long term tolerance versus sensitisation to environmental allergens during the perinatal period. *Curr Opin Immunol* 1997; 9:782–7.