

Expression of *Dermatophagoides pteronyssinus* Allergen, *Der p* II, in *Escherichia coli* and the Binding Studies with Human IgE¹

K.Y. Chua, R.J. Dilworth, W.R. Thomas

Clinical Immunology Research Unit, Princess Margaret Hospital, Subiaco, Australia

Abstract. Lambda gt11 clones expressing the major house dust mite allergen, *Der p* II, have been reported to react with IgE in the serum of a high proportion of allergic patients. The clones described, however, only produced small quantities of protein which was not fused to the β -galactosidase of the vector. A construct of the *Der p* II is described which produces a fusion of *Der p* II, minus its leader sequence, with the glutathione-S transferase in the pGEX vector. This could be readily isolated and was shown to react with IgE in 22 of 24 patient sera showing reactivity to native *Der p* II, the sera not reacting having low reactivity to native protein. Absorption analysis showed that the recombinant material removed most of the IgE reactivity of patients to native *Der p* II. The construct described, therefore, should be valuable for quantitative studies of a pure mite allergen.

Introduction

Antigens from mites of the genus *Dermatophagoides* are an important source of allergen in house dust [1]. Although IgE reactivity is often directed to several allergens [2-4], reactivity to 2 groups of allergen is particularly frequent [5, 6]. The major faecal or class-I antigens which are *Der p* I, *Der f* I and *Der m* I from *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Dermatophagoides microcerus*, respectively, react with at least 80% of sera. This 25-kD allergen has been studied by several groups and a cDNA clone has been isolated and used to show, by amino acid sequence homology, that it is a thiol protease similar to papain [7].

Another group of allergens with an estimated molecular weight of 15 kD has been shown to react with IgE from a similar number of patients. This group of

allergens initially purified from *D. pteronyssinus* as DpX [8] is represented by *Der p* II and *Der f* II from *D. pteronyssinus* and *D. farinae* and has now been defined by reactivity with monoclonal antibodies and N-terminal amino acid sequences [5, 6]. A lambda gt11 cDNA encoding *Der p* II has also been isolated in our laboratory by IgE plaque immunoscreening with serum for allergic people [9]. In initial tests this cDNA expressed reactivity with 14 of 17 allergic sera showing the importance of the allergen. Sequence data of the first 40 residues showed its identity to *Der p* II and that it coded for a polypeptide with a calculated molecular weight of 14,131 daltons. However, because the cDNA coding for *Der p* II was not in the reading frame [10] of the lac Z gene of lambda gt11, it was produced in an unfused and unstable form which, despite its antigenicity, was not produced in quantity and was difficult to purify. Herein we describe the production and isolation of a stable *Der p* II fusion construct. Serological tests showed that it retained most but not all of the reactivity of its native counterpart with IgE.

¹ This is publication No. 327 of the Clinical Immunology Research Unit of the Princess Margaret Children's Medical Research Foundation.

Materials and Methods

Antisera

Allergic sera were collected from house dust mite-sensitive patients with rhinitis and/or asthma. These included sera from 12 adults and 12 children with positive *D. pteronyssinus* skin tests and high RAST scores. A hyper-IgE serum, 100,000 U IgE/ml, was a gift from Dr. N.R. Lynch, Institute of Biomedicine, Caracas, Venezuela. It was shown not to contain IgE reactive with major mite allergens and was used as a control. Anti-*Der p II* monoclonal antibody was produced by Dr. R.C. Aalberse [5] and was supplied by Dr. M.D. Chapman, Virginia, USA. Anti-mouse immunoglobulin was purchased from Behring Diagnostics, La Jolla, Calif., USA.

All sera were absorbed by mixing with an equal volume of *Escherichia coli* lysate [11] overnight at 4°C and cleared by centrifugation at 1,500 g, 20 min. Non-fat milk powder and sodium azide were added to 5 and 0.02%, respectively. Human allergic sera and monoclonal anti-*Der p II* were used at 1:2 and 1:1,000 dilutions, respectively. Both hyper-IgE and anti-mouse immunoglobulins were used at a 1:200 dilution. Antisera used in absorption studies were absorbed with an equal volume of bacterial lysates containing approximately 10 mg/ml of recombinant *Der p II* antigen.

Preparation of Mite Allergens

D. pteronyssinus and spent mite media were gifts from Commonwealth Serum Laboratories, Melbourne, Australia. Native (non-recombinant) *Der p I* and *Der p II* were isolated from *D. pteronyssinus*-spent growth medium using monoclonal antibody affinity chromatography. The monoclonal antibody used to affinity purify *Der p I* [12] and *Der p II* [5] were kindly provided by M. Chapman, Virginia, USA. For preparation of the affinity columns, approximately 10 mg of immunoglobulin precipitated from Mab-bearing ascites by 50% ammonium sulphate was coupled to 3 ml of swollen CNBr-activated sepharose 4B (Pharmacia, Piscataway, N.J., USA) by the standard method [11]. An extract of spent mite medium was prepared by mixing 500 g of medium with 0.1 M Tris-HCl (pH 7.4) overnight with stirring. The extract was clarified by centrifugation at 10,000 g for 30 min. To isolate allergens, 200 ml of extract were passed over a 3-ml column containing the immunosorbent. Bound allergens after washing with PBS and borate-NaCl-Tween buffer [11] were eluted using 0.1 M glycine buffer (pH 2.6) or water titrated to pH 11 with ammonium hydroxide. Only single bands were detected when monitored by SDS-PAGE and Coomassie blue staining.

Molecular Cloning

The vector used for cloning in these experiments was pGEX-1, one of a series [13] which expresses cloned sequences as a fusion to the carboxy terminus of the glutathione transferase from *Schistosoma japonicum*. This was used because other fusions with this protein have tended to be soluble and can be isolated by affinity chromatography on glutathione-agarose.

For cloning the pGEX-1 vector was digested with appropriate restriction enzymes (described below) and dephosphorylated by treatment with bacterial alkaline phosphatase. The 0.6-kb cDNA insert that codes for *Der p II* was isolated from lambda gt11 pII clone C1 by a polyethylene glycol precipitation procedure [7] or from pUC9 pII (C1) by standard methods. The insert or fragments from restriction enzyme digests of the fragments were ligated to

pGEX-1 and transformed into *E. coli* strain HB101. The above manipulations were carried out according to Maniatis et al. [14]. Transformants were selected on Luria-glucose agar containing 100 µg/ml of ampicillin. Recombinant clones were identified by restriction enzyme analyses. Screening of allergenic expression by the recombinant clones was carried out by colony radioimmunoassay using serum from allergic humans to detect for IgE binding.

Preparation of Bacterial Lysates

E. coli were grown overnight in Luria broth containing 100 µg/ml ampicillin at 37°C then diluted 1:10 in fresh medium and incubated at 37°C for 3 h with rigorous shaking. Isopropylthiogalactopyranoside (Sigma, St. Louis, Mo., USA) was then added to 0.1 mM and incubated for a further 3 h. Cell pellets were washed by centrifugation at 10,000 g for 30 min at room temperature and resuspended to 0.01 of the culture volume in 10 mM Tris-HCl, 150 mM NaCl (TBS, pH 7.5) containing 5 mM EDTA, 100 U/ml of Trasylol (Bayer, Leverkusen, FRG). Lysozyme (Boehringer, Mannheim, FRG) was added to 0.25 mg/ml and incubated at room temperature for 30 min. MgCl₂ and DNase (Boehringer, Mannheim, FRG) were added to 5 mM and 20 µg/ml, respectively, and incubated for further 15 min. The mixture was then subjected to 3 freeze-thaw treatments, followed by three 30-sec sonications with a Branson 150-W sonifier and a 0.25-inch tapered microtip.

Glutathione-Agarose Affinity Chromatography

A 2-ml column was prepared by hydrating glutathione agarose (Sigma, St. Louis, Mo., USA) in water. Triton X-100 was added to bacterial lysate to 1% and precleared by centrifugation (100,000 g for 20 min) and the solution passed through the column at room temperature. The column was washed with TBS buffer, then the glutathione transferase-fusion protein was eluted with 5 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0).

Sodium Dodecylsulphate-Polyacrylamide Electrophoresis (SDS-PAGE) and Immunoblotting

SDS-PAGE was performed by the technique of Laemmli [15] and either stained with Coomassie blue 250 or transferred to nitrocellulose for Western blotting [16] to be developed by radioimmunoassay.

Dot-Blot

Dot blots were performed by spotting 2 µl of protein to nitrocellulose. This was air-dried and washed in TNT buffer for radioimmunoassay.

Radioimmunoassay

Nitrocellulose filters with bound antigens were washed with three 20-min changes of 10 mM Tris-HCl with 0.05% Tween-20 (TNT buffer, pH 7.4) with rocking. Filters were then incubated for 2 h at room temperature with rocking with antiserum absorbed with *E. coli* extract [11]. Skim milk was added to 5% to the antiserum. Filters were washed three times in TNT then incubated with either ¹²⁵I-staphylococcus protein A (10⁶ cpm/ml; 5 × 10⁷ cpm/µg protein A) [11] or with ¹²⁵I-monoclonal anti-IgE (2 × 10⁶ cpm/ml; 10⁸ cpm/µg) [10, 17] for 2 h in TNT at room temperature with rocking. Filters were autoradiographed after 3 washes of 15 min in TNT. For IgE serum was typically used neat or at a 1/2 dilution in *E. coli* extract. When monoclonal anti-*Der p II* was used, an additional incubation with rabbit antimouse immunoglobulin was performed before de-

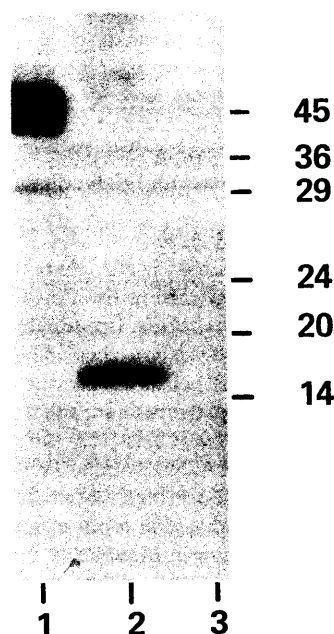


Fig. 1. Reactivity of monoclonal anti-*Der p II* with lysates of *E. coli* expressing *Der p II* cDNA constructs. 1 = pGEW-lpII (S2R1) showing Mr 41-kD fusion with *gst* protein *gst-Der p II*; 2 = pUC9pII (C1) showing Mr 15-kD reactivity; 3 = pUC9 vector control lysate.

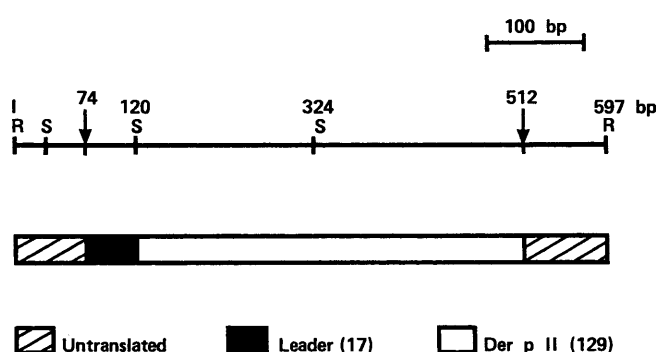


Fig. 2. Construction of pGEXpII (S2R1) to express *Der p II*-*gst* fusion. The mature *Der p II* begins with an Asp residue at nucleotide 120. The *Der p II* cDNA from clone C1 was digested at the Sau 3A site between nucleotide 119 and 120 and the fragment S2R1 from nucleotide 120–597 ligated in pGEX-1 vector digested with Bam HI and EcoRI: this was designed to express a fusion with the glutathione-S transferase (*gst-Der p II*). S = Sau 3A site; E = EcoRI site.

veloping with protein A. Autoradiography was carried out with intensifying screens at -70°C .

Colony Radioimmunoassay

To screen for antigenic *E. coli* transformants, bacterial colonies were replica plated onto nitrocellulose filters and incubated at 37°C for 2–3 h. Colonies were then induced by transferring the filter to an agar plate with a soft agar overlay containing 10 mM isopropylthiogalactopyranoside. Induction was carried out for 2 h at 37°C . Induced cells were lysed by treatment with 1% SDS solution in the presence of an atmosphere of chloroform for 15–30 min. Filters were washed three times in TNT buffer (three 20-min washes) for immunoassay as described above.

Results

Construction of High Expression Vector for *Der p II*

The cDNA clone C1 [10] encoding *Der p II* cloned in lambda gt11 and pUC9 expresses a product reactive with IgE in mite allergic serum despite the fact that its nucleotide sequence is in a different reading frame to the β -galactosidase fusion partners in these vectors. Previously this has been shown by IgE plaque radioimmunoassay with lambda gt11 pII (C1) [10]. Figure 1 (track 3) shows a Western blot of a lysate of *E. coli* JM83 transformed with pUC9 pII (C1) producing a band of Mr 15 kD reacting with monoclonal anti-*Der p II*. However, although this expression was detectable by radioimmunoassay, typical large fusion protein bands could not be detected by SDS-PAGE and the recombinant allergen could not be isolated. To produce a protein that could be isolated and used quantitatively, a fusion construct with glutathione transferase in the pGEX-1 vector was made. As illustrated in figure 2 the pII (C1) cDNA encodes the 129 residue mature *Der p II* with a 17-residue hydrophobic leader sequence [10]. Fortuitously, the junction between the leader peptide and mature *Der p II* codes for a Sau 3A restriction endonuclease site which would permit the cloning of Sau 3A fragment of pII (C1) into the Bam HI site of pGEX-1 in such a way that the N-terminal Asp of *Der p II* would be expressed contiguous with the glutathione transferase of pGEX-1. Therefore, pII (C1) DNA was partially digested with Sau 3A and the fragment with a size corresponding to that from the Sau 3A site at 120 (fig. 2) to the 3' EcoRI site (477 bp) isolated by agar electrophoretic techniques and cloned in pGEX-1 digested with Bam HI and EcoRI. After transformation, colonies reactive with IgE in allergic serum or monoclonal antibody were isolated and the identity of their

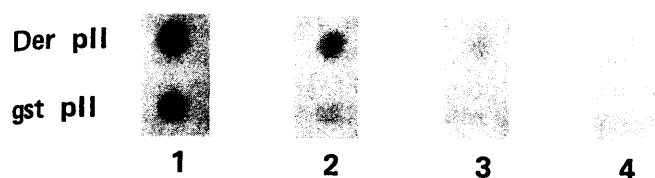


Fig. 3. IgE reactivity of native *Der p II* and the *Der p II* cDNA expressed as the *gst-Der p II* fusion by pGEXpII (S2R1). Dot blots with 0.15 and 0.25 μ g of *Der p II* and *gst Der p II* were spotted. Panel 1 shows an example of serum reacting strongly with *Der p II* and *gst-pII*; panel 2, an example of weaker reactivity to both, and panel 3, weak reactivity to *Der p II* only. Panel 4 shows the lack of detectable reaction with 500 U/ml IgE in a hyper-IgE serum. The results summarising the reactivity of 24 sera are shown in table 1.

Table 1. Reaction of *Der p II* and recombinant *Der p II* with IgE in allergic serum

	Native <i>Der p II</i>	<i>gst-pII</i> (S2R1) ^a
Adult (n = 12)	12	12
Children (n = 12)	12	10 ^b

^a Recombinant *Der p II* glutathione-S-transferase fusion.

^b The children showing no reactivity with recombinant *Der p II* gave weak reactions with native *Der p II*.

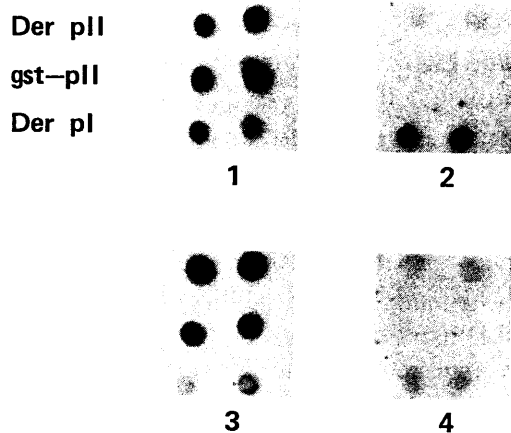


Fig. 4. Absorption of anti *Der p II* IgE reactivity from allergic serum with *gst-Der p II* - pGEX-pII (S2R1) lysates. Sera were absorbed with *gst-pII* lysate (panels 2, 4) or vector control (panels 1, 3) and tested by IgE dot blot with 0.15 μ g *Der p II*, 0.25 μ g *gst-pII* or 0.15 μ g *Der p I*. The 2 sera illustrated here (1, 2) and (3, 4) show that *gst-Der p II* absorbs out all reactivity to itself and most reactivity to native *Der p II*. Similar results were obtained with 7 other sera.

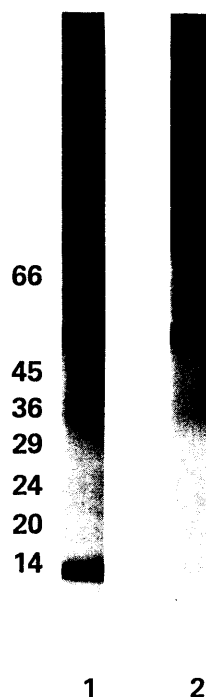


Fig. 5. Western blot of house dust mite extract with allergic serum absorbed with *gst-Der p II* - pGEXpII (S2R1) lysate. House dust mite extract 1.5 mg/track was electrophoresed on 8–18% SDS-PAGE gels and blotted onto nitrocellulose and reacted with allergic serum absorbed with *E. coli* lysate (1) or *E. coli* lysate expressing *gst-Der p II* (2) and IgE reactivity was measured. There was a selective reduction in the intensity of a Mr 15-kD band (bottom band) corresponding to *Der p II*.

insert verified by restriction mapping and by the size of the fusion protein produced. Figure 1 (track 1) shows the lysate of pGEX-1 pII (S2R1) has a protein of Mr 42 K reactive with monoclonal anti-*Der p II* corresponding to a fusion of the 27-kD glutathione transferase and the 14-kD *Der p II*. This produced a large band in SDS-PAGE and could be readily isolated by glutathione-agarose affinity chromatography. The yield was typically 10 mg/l of culture.

Reaction of *Der p II* Fusions with Allergic Serum

To evaluate the antigenicity of the recombinant *Der p II*, the fusion was isolated by affinity chromatography and used for dot-blot analysis with allergic serum from 12 mite-allergic children and 12 adults. 0.15 and 0.25 μ g of native *Der p II* and recombinant *Der p II*, from pGEX pII (S2R1), was dotted and

tested for reactivity with IgE. Figure 3 shows representative results, including panel 4 which shows that non-allergic hyper-IgE serum at 500 U/ml does not give reactions. All sera tested showed reactivity to native *Der p* II. 6 of 12 children and 1 of 12 adults gave strong reactivity to *Der p* II (e.g., fig. 3, panel 1). All the adults gave reactivity with recombinant *Der p* II fusion while 2 of 12 children gave no reactivity (e.g. fig. 3, panel 3). These were retested with 2.5- μ g spots, but were still negative. These results are summarised in table 1.

Absorption of Der p II IgE Reactivity with Recombinant Der p II

Nine sera from the panel above was absorbed by mixing with an equal volume of pGEX pII(S2R1) lysate containing about 1 mg recombinant *Der p* II and then tested for reactivity to the native and recombinant proteins by dot-blot assay. All reactivity to recombinant *Der p* II was removed, but in 7 of 9 cases significant reactivity to the native *Der p* II remained. This, however, was considerably reduced (fig. 4). The absorption could also be demonstrated by Western blotting where the reactivity of IgE in allergic serum to the Mr 15-K band in house dust mite was considerably reduced but not eliminated (fig. 5).

Discussion

Der p I and *Der p* II are the two major house dust mite allergens documented to react with IgE in the serum of 80% of patients [2-4, 6]. Their importance has recently been highlighted by the finding that paediatric patients, as judged by Western blotting, tend to have strong reactions to these antigens and few reactions to other antigens. Thus in a recent report by Thompson et al. [18], 10 of 16 children showed responses only to *Der p* I and *Der p* II. Our own unpublished work has also had similar results. Since it is clear that many adults respond to a larger number of components, the response of children to the major allergens and its apparent progression to other components needs further study.

The availability of *Der p* I and *Der p* II expressed from the cDNA clone should greatly facilitate this and similar studies by providing detailed primary structure and clonally purified allergens. These can be used to study epitopes and T-cell responses where the supply of sufficient pure allergen can be difficult. Our

initial observations with *Der p* I produced from lambda gt11 as a fusion with β -galactosidase showed that this was poorly soluble and would not react with IgE [19]. The *Der p* II expressed from a gt11-derived clone, however, was isolated by IgE immunoscreening and reacted with IgE from a high proportion of IgE allergic sera. Sequence analysis showed that it was not in the reading frame of the β -galactosidase and this agreed with the finding that it produced a band of Mr about 15 kD detectable by Western blotting rather than large amounts of fusion protein. This is illustrated by the band in figure 1 which demonstrates that *Der p* II can be expressed from pUC9 which is also out of the reading frame. The mechanism of how the unfused protein is expressed is unknown although there are other examples in the literature [20, 21]. It is, however, known that overlapping stop and start codons as found in *Der p* II cDNA [10] allow ribosomes to reposition themselves on polycistronic mRNA [22].

Using a fortuitous restriction site a construct which produces a fusion of the mature *Der p* II (minus its leader sequence) as a fusion with the glutathione-S transferase of pGEX-1 has been made (*gst-Der p* II). This produces a fusion protein in large quantity (10-20% of cell protein as judged by SDS-PAGE) which can be isolated by glutathione agarose affinity chromatography. The studies here show that 22 of 24 sera with IgE reactivity to *Der p* II also reacted with the *gst-Der p* II fusion. The 2 non-reactors only had weak reactivity to the native protein. The 100% frequency of reactivity of the serum with native *Der p* II and 92% with *gst-Der p* II shows the importance of the allergen although the sera used in this study were preselected for high mite RAST scores. In keeping with these results, it was possible to absorb out most of the reactivity to *Der p* II with the *gst-Der p* II fusion although some residual activity remained with all sera. Interestingly, absorption also did not remove all reactivity when judged by the ability of serum to react with a 15-kD band in Western blotting, since it might have been expected that the denaturation-renaturation required for blotting would reduce the antigenic determinants expressed.

In conclusion, it has been possible to construct a fusion of *Der p* II to produce a recombinant (*gst*) protein which retains much of the IgE reactivity of normal *Der p* II. This can be isolated in large quantity and will help epitope analyses and other studies of this allergen.

References

- 1 Voohorst R, Spieksma FTM, Varekamp MJ, Leupen MJ, Lykema AW: The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identify with the house dust mite allergen. *J Allergy* 1967;39:325-328.
- 2 Lind P, Lowenstein H: Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983;17:263-273.
- 3 Krilis S, Baldo BA, Basten A: Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. II. Identification of the major IgE binding antigens by crossed radioimmuno-electrophoresis. *J Allergy Clin Immunol* 1984;74:142-146.
- 4 Tovey ER, Baldo BA: Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;79:93-102.
- 5 Van der Zee JS, van Swieten P, Jansen HM, Aalberse RC: Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988;81:884-896.
- 6 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE: Antigenic and structural analysis of group II allergens (*Der f II* and *Der p II*) from house dust mites (*Dermatophagoides spp*). *J Allergy Clin Immunol*, in press, 1989.
- 7 Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ: Sequence analysis of cDNA coding for a major house dust mite allergen *Der p I*. Homology with cysteine proteases. *J Exp Med* 1988;167:175-182.
- 8 Lind P: Purification and partial characterisation of two major allergens from the house dust mite: *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1984;76:224-230.
- 9 Thomas WR, Chua KY, Stewart GA, Doyle CR, Turner KJ, Simpson RJ, Dilworth RJ: Analysis and expression of cDNA clones coding for house dust mite allergens. *Adv Biosci* 1989;74:139-147.
- 10 Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR: Isolation of cDNA coding for the major mite allergen *Der p II* by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990;91:118-123.
- 11 Thomas WR, Rossi AA: Molecular cloning of DNA coding for outer membrane proteins of *Haemophilus influenzae* type b. *Infect Immun* 1986;52:812-817.
- 12 Chapman MD, Heymann PW, Platts-Mills TAE: Epitope mapping of two major inhalant antigens, *Der p I* and *Der f I* from mites of the genus *Dermatophagoides*. *J Immunol* 1987;139:1479-1484.
- 13 Smith DB, Johnson KS: Single step purification of polypeptide expressed in *Escherichia coli* as fusions with glutathione-S-transferase. *Gene* 1988;67:31-40.
- 14 Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory, 1982.
- 15 Laemmli UK: Cleavage of structural proteins during assembly of the head of T4 bacteriophage. *Nature* 1970;227:680-681.
- 16 Burnette WN: Western blotting: Electrophoretic transfer of proteins from sodium dodecylsulfate gels nitrocellulose to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 1981;112:195-203.
- 17 Stewart GA, Turner KJ, Baldo BA, Cripps AW, Ford A, Seagroatt V, Lowenstein H, Ekramoddoullah AKM: Standardisation of rye grass pollen (*Lolium perenne*) extract. An immunochemical and physicochemical assessment of six candidate international reference preparations. *Int Arch Allergy Appl Immunol* 1988;86:9-18.
- 18 Thompson SJ, Whitley HJ, Naysmith JD, Carswell F: IgE antibodies to *D. pteronyssinus* in atopic patients. *Immunology* 1988;64:311-314.
- 19 Thomas WR, Stewart GA, Simpson RJ, Chua KY, Plozza TM, Dilworth RJ, Nisbet A, Turner KJ: Cloning and expression of DNA coding for the major house dust mite allergen *Der p I* in *Escherichia coli*. *Int Arch Allergy Appl Immunol* 1988;85:127-129.
- 20 Goto T, Wang JC: Yeast DNA topoisomerase II is encoded by a single-copy essential gene. *Cell* 1983;36:1073-1080.
- 21 Coppel RL, Favalaro JM, Crewther PE, Burkot AE, Bianco AE, Stahl HD, Kemp DJ, Anders RF, Brown GV: A blood stage antigen of *Plasmodium falciparum* shares determinants with the sporozoite coat protein. *Proc Natl Acad Sci USA* 1985;82:5121-5125.
- 22 Kozak M: Comparison of protein synthesis in procaryotes, eucaryotes and organelles. *Microbiol Rev* 1983;47:1-45.

Received: July 4, 1989

Accepted: October 23, 1989

Correspondence to: Dr. W.R. Thomas
Clinical Immunology Research Unit
Princess Margaret Hospital
Thomas Street
Subiaco, 6008 W. Aust. (Australia)