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Cloning and Expression of DNA Coding for the Major House Dust Mite Allergen *Der p 1* in *Escherichia coli*

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Abstract. A cDNA clone coding for the major house dust mite allergen *Der p 1* was isolated from a lambda gt11 library. Its sequence correlates with known amino acid sequences of *Der p 1* and it produces a fusion protein which reacts with rabbit anti-*Der p 1* antiserum.

Progress in studying allergy to the house dust mite has been hindered by difficulties in obtaining standardised allergens [1]. About 6 antigens elicit most IgE anti-mite antibody responses [2, 3] and one of these, *Der p 1*, a 27-kilodalton glycoprotein found in mite faeces, reacts with about 60% of allergic sera usually as the predominant antigen. Also, because it is a constituent of faeces it is readily airborne and solubilised [4]. To facilitate studies on house dust mite allergy we have begun to clone and express allergens in *Escherichia coli*. We now report the cloning and expression of the major *Der p 1* allergen in *E. coli*.

Polyadenylated mRNA was isolated from the mite *Dermatophagoides pteronyssinus* cultured by Commonwealth Serum Laboratories (Parkville, Australia) and cDNA was synthesised by the RNase H method [5] using a kit (Amersham International, Bucks). After the addition of *EcoRI* linkers the cDNA was ligated into lambda gt11 and plated in *E. coli* Y1090 (r-) (Promega Biotec, Madison, Wisc.) to produce a library of 5×10^5 recombinants. Screening was performed by plaque radioimmunoassay [6] using a rabbit anti-*Der p 1* antiserum [7]. Reactivity was detected by developing with ¹²⁵I-staphylococcal protein A and autoradiography [8]. Phage-producing plaques reactive with the antiserum were checked for the ability of their DNA to hybridise with 17-mer oligonucleotides constructed on the basis of amino acid sequences obtained from three tryptic peptides obtained from faecal *Der p 1* [Simpson and Stewart, in prep.].

In brief, *Der p 1* was isolated by gel filtration, chromatofocusing and fast liquid protein chromatog-

raphy on a TSK 3000 SW column and then by reversed-phase high-performance liquid chromatography (HPLC). Reduction, carboxymethylation and tryptic digestion were performed by standard techniques [9] and peptides were isolated by HPLC using a Brownlee Bu-300 column (30 x 2.1 MMID) and a linear 60-min gradient of 0-60%B where solvent A was 10 mM KH₂PO₄, pH 6.5, and solvent B was acetonitrile. Amino acid sequences were determined on an applied biosystems (Model 470A) gas-phase sequencer [9]. Oligonucleotides were synthesised using an Applied Biosystem model 370A synthesiser and purified by reversed-phase HPLC. For hybridisation, oligonucleotides were labelled by polynucleotide kinase (Promega Biotec) using 20 pmol of DNA and 20 pmol γ -³²P-ATP at 1,400 Ci/mmol [10]. Labelled oligonucleotides were purified by 15% polyacrylamide gel electrophoresis (PAGE). Plaques were lifted onto nitrocellulose (Schleicher & Schull, Dassel, FRG) and denatured and baked [10]. Hybridisations were performed overnight in 6 x sodium chloride-sodium citrate (SSC), pH 7 [10], 0.1% sodium dodecyl sulphate (SDS) at 37°C at 10⁶ cpm/ml and washed for 1 h at 37°C in 6 x SSC containing 0.1% Triton X-100.

Several phage clones reacted with the rabbit anti-*Der p 1* serum and hybridised with all 3 oligonucleotide probes. One of these, lambda-gt11 p1(13T), was examined further. Its 0.87-kilobase DNA insert was subcloned in M13 mp18 and M13 mp19, and DNA sequencing was performed by the chain termination method [11]. The 306 partial nucleotide sequence in

AAAAACCGATTTTTG				1 Met ATG	Ser AGT	Ala GCA	Glu GAA 20	Ala GCT	Phe TTT	Glu GAA	His CAC	Leu CTC	10 Lys AAA	Thr ACT	Gln CAA
Phe TTC	Asp GAT 30	Leu TTG	Asn AAT	Ala GCT	Glu GAA	Thr ACT	Asn AAC	Ala GCC	Cys TGC	Ser AGT	Ile ATC 40	Asn AAT	Gly GGA	Asn AAT	Ala GCT
Pro CCA	Ala GCT	Glu GAA	Ile ATC	Asp GAT	Leu TTG 50	Arg CGA	Gln CAA	Met ATG	Arg CGA	Thr ACT	Val GTC	Thr ACT	Pro CCC	Ile ATT	Arg CGT 60
Met ATG	Gln CAA	Gly GGA	Gly GGC	Cys TGT	Gly GGT	Ser TCA	Cys TGT	Trp TGG	Ala GCT 70	Phe TTC	Ser TCT	Gly GGT	Val GTT	Ala GCC	Ala GCA
Thr ACT	Glu GAA	Ser TCA	Ala GCT 80	Tyr TAT	Leu TTG	Ala GCT	His CAC	Arg CGT	Asn AAT	Gln CAA	Ser TCA	Leu TTG	Asp GAT 90	Leu CTT	Ala GCT
Glu GAA	Gln CAA	Glu GAA	Leu TTA	Val GTC	Asp GAT	Cys TGT	Ala GCT	Ser TCC	Gln CAA	His CAC	Gly GGT	Cys TGT	His CAT	Gly GGT	Asp GAT
Thr ACC	Ile ATT	Pro CCA	Arg CGT												

Fig. 1. Nucleotide sequence and predicted amino acid sequence of N-terminal region of *Der p* 1 determined from the clone lambda gt11 P1(13T). Amino acid residues 19–45 correspond to the N-terminal amino acid sequence of faecal *Der p* 1 and residues 46–60 correspond to a known tryptic peptide sequence [Simpson and Stewart, in prep.].

	1									10										
<i>Der p</i> 1	Thr	Asn	Ala	Cys	Ser	Ile	Asn	-	Gly	Asn	Ala	Pro	Ala	Glu	Ile	Asp	Leu	Arg	Gln	Met
	*		*	*		*	*			*		*		*		*	*	*		
<i>Der f</i> 1	Thr	Ser	Ala	Cys	Arg	Ile	Asn	Ser	Val	Asn	Val	Pro	Ser	Glu	Leu	Asp	Leu	Arg	Ser	Leu

Fig. 2. Comparison of N-terminal sequence of *Der p* 1 and *Der f* 1. The amino acid sequence for *Der p* 1 is equivalent to amino acids 19–37 in figure 1 [the *Der f* 1 sequence is from reference 12]. A gap has been introduced into the *Der p* 1 sequence to maximise the homology. Asterisks indicate 11/20 positions of identity.

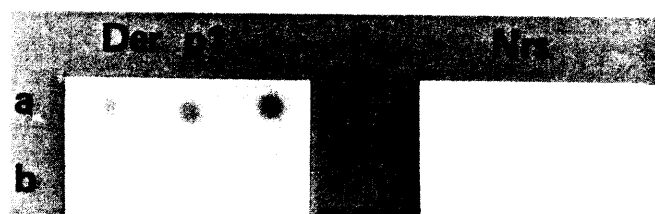


Fig. 3. Reactivity of lambda-gt11 p1(13T) with anti-*Der p* 1. Lysates from Y1089 lysogens induced for phage were reacted by dot-blot with rabbit anti-*Der p* 1 (*Der p* 1) or normal rabbit serum (*Nrs*). Dots (2 μ l) were made in triplicate from lysates of bacteria infected with lambda-gt11 p1(13T) (**a**) or lambda-gt11 (**b**). When developed with ^{125} I-protein A and autoradiography only the reaction between the lambda-gt11 p1 (13T) lysate and the anti-*Der p* 1 showed reactivity.

figure 1 shows the cDNA codes for *Der p* 1. After a short 5' non-coding sequence there is a putative leader sequence of 19 amino acids followed by the N-terminal threonine found in faecal *Der p* 1. Amino acid residues 19–45, as translated from the nucleotide sequence, correspond to the N-terminal sequence of *Der p* 1 [Simpson and Stewart, in prep.], and amino acids 45–59 to the sequence of a known tryptic peptide. The N-terminal can be compared with the N-terminal of the equivalent protein from *Dermatophagoides farinae* (*Der f* 1) [12]. There is identity in 11/20 positions of the sequences available for comparison (fig. 2).

To examine the protein produced by lambda-gt11

p1(13T) phage was lysogenised in Y1089 (r-) and the bacteria grown in broth culture at 30°C. Phage was induced by temperature switch and isopropyl thiogalactopyranoside [6] and the bacteria were suspended in phosphate-buffered saline to 1/20 of the culture volume, and sonicated for an antigen preparation. When examined by 7.5% SDS-PAGE it was found that lambda gt11 p1(13T) did not produce a 116-kilodalton β -galactosidase band but instead produced a 140-kilodalton band consistent with a fusion protein with the *Der p 1* contributing a 24-kilodalton moiety [6]. Rabbit anti-*Der p 1* was shown to react with the lysate from lambda-gt11 p1(13T) (fig. 3), but attempts to show reactivity with human serum IgE in this dot-blot system have been unsuccessful. The amount of soluble fusion protein in such lysates is however low [6] and further study must await purification and solubilisation. Such cloned material and the sequence data reported could be useful for the development of diagnostic and therapeutic materials.

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References

- 1 Ford, A.W.; Rawle, F.C.; Lind, P.; Spieksma, F.T.M.; Lowenstein, H.; Platts-Mills, T.A.E.: Standardisation of *Dermatophagoides pteronyssinus*. Assessment of potency and allergen content in the coded extracts. *Int. Archs Allergy appl. Immun.* 76: 58-67 (1985).
- 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.* 17: 263-273 (1983).

- 3 Krilis, S.; Baldo, B.A.; Basten, A.: Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. Part II. Identification of the major IgE binding antigens by crossed radioimmuno-electrophoresis. *J. Allergy clin. Immunol.* 74: 142-146 (1984).
- 4 Tovey, E.R.; Chapman, M.D.; Platts-Mills, T.A.E.: Mite faeces are a major source of house dust allergens. *Nature* 289: 592-593 (1981).
- 5 Gubler, U.; Hoffman, B.J.: A simple and very efficient method for generating cDNA libraries. *Gene* 25: 263-269 (1983).
- 6 Huynh, T.V.; Young, R.A.; Davis, R.W.: Constructing and screening cDNA libraries in gt10 and gt11; in Glover, DNA cloning, vol. 1, a practical approach, pp. 48-78 (IRL Press, Oxford, Washington 1985).
- 7 Stewart, G.A.; Thomas, W.R.: In vitro translation of messenger RNA from the house dust mite *Dermatophagoides pteronyssinus*. *Int. Archs Allergy appl. Immun.* 83: 384-389 (1987).
- 8 Thomas, W.R.; Rossi, A.A.: Molecular cloning of DNA coding for outer membrane proteins of *Haemophilus influenzae* type b. *Infect. Immunity* 52: 812-817 (1986).
- 9 Simpson, R.J.; Smith, J.A.; Moritz, R.L.; O'Hare, M.J.; Rudland, P.S.; Morrison, J.R.; Lloyd, C.J.; Grego, B.; Burgess, A.W.; Nice, E.C.: Rat epidermal growth factor: complete amino acid sequence. *Eur. J. Biochem.* 153: 629-637 (1985).
- 10 Maniatis, T.; Fritsch, E.F.; Sambrook, J.: Molecular cloning. A laboratory manual (Cold Spring Harbor Laboratory 1982).
- 11 Sanger, F.; Nicklen, S.; Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. *Proc. natn. Acad. Sci. USA* 74: 5463-5467 (1977).
- 12 Heyman, P.W.; Chapman, M.D.; Platts-Mills, T.A.E.: Antigen *Der f 1* from the house dust mite *Dermatophagoides farinae*: structural comparison with *Der p 1* from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J. Immun.* 137: 2841-2847 (1986).

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