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Production and characterization of mouse monoclonal antibodies to allergenic epitopes on *Lolp*I (Rye I)

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SUMMARY

This study describes the production and characterization of mouse monoclonal antibodies (Mab) to *Lolp*I (Rye I), the major allergen of rye-grass pollen. Hybridoma cell lines were screened for the secretion of Mab, the binding of which to labelled *Lolp*I could be inhibited by pooled human sera containing IgE and IgG anti-*Lolp*I antibodies. Twenty monoclonal hybridoma cell lines were expanded, and their product was purified and characterized. Each Mab bound to *Lolp*I but not to unrelated antigens. Cross-inhibition studies allowed the grouping of these 20 Mab into five families differing by their epitope specificity. One representative Mab of each family was tested for its ability to inhibit human IgE/IgG anti-*Lolp*-I from 15 allergic donors as well as for its inhibition by affinity-purified anti-*Lolp*I antibodies isolated from 14 donors. The results indicated that each family of Mab was specific for an epitope that was identical or close to that recognized by some human anti-*Lolp*I antibodies. It is further shown that human IgE/IgG anti-*Lolp*I are heterogeneous with some patients reacting preferentially with one or two epitopes defined by the Mab *Lolp*I, and others reacting with epitopes different from those identified by the Mab *Lolp*I.

INTRODUCTION

The majority of allergen extracts used in the diagnosis, treatment and research of allergic diseases are complex mixtures containing variable and generally low amounts of biologically relevant allergens (King, 1976). Recently, several monoclonal antibodies to common airborne allergens were produced with a view of allergen standardization (Baldo, Krilis & Basten, 1981; Kahn & Marsh, 1982; Krilis *et al.*, 1983; Smart *et al.*, 1983; Ekrammodoullah *et al.*, 1984) and purification of the major allergens from crude extracts (Esch & Klapper, 1984). Since the initial observation that mouse Mab and human antibodies to acetylcholine receptors may share a cross-reactive idiotype (Dwyer *et al.*, 1983), Mab to pollen allergen have been recently employed to detect auto-anti-idiotypes (aId) in the sera of allergic patients (Castracane, Hall & Rocklin, 1985). In our previous studies (Bose *et al.*, 1984; Bose, Marsh & Delespesse, 1986) we reported the presence of such auto-aId in rye-grass sensitive patients, and we further showed that some of these auto-aId are reacting with public idiotypic determinants expressed on IgE/IgG anti-*Lolp*I [the major allergen from rye grass (Marsh 1975)] from several allergic individuals. In the present study we report the production and the characterization of mouse Mab to *Lolp*I (Mab *Lolp*I) that were selected on the basis of their reactivity with allergenic epitopes, i.e. with epitope also recognized by human IgE/IgG anti-*Lolp*I antibodies. It is hoped that such Mab might be used as probes to identify human aId in the serum of rye-grass allergic patients, since sharing of idiotopes on the mouse and human antibodies has been demonstrated (Dwyer *et al.*, 1983; Castracane, Hall & Rocklin, 1985).

Abbreviations: BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetic acid; FCS, fetal calf serum; HDM, house dust mite extract; Ig, immunoglobulin; Mab, monoclonal antibodies; Mab *Lolp*I, anti-*Lolp*I monoclonal antibodies; PBS, phosphate-buffered saline; RAST, radioallergosorbent test; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Reagents

Purified *Lolp*I (Rye I) (Marsh, 1975; Friedhoff *et al.*, 1981), *Lolp*II (Rye II) and *Amb.* I (AgE) were kindly provided by Dr D. G. Marsh (Johns Hopkins University, Baltimore, MD). Purified P1 was a gift from Dr T. A. E. Platts-Mills (University of Virginia, Charlottesville, VI). House dust mite (HDM) was a crude extract of *Dermatophagoides pteronyssinus* received from P. Horan (Beecham Laboratories, Loughborough, Leicestershire, U.K. [batch 77/91(a)]). Tetanus toxoid (TT) was obtained from Connaught Laboratories, Willowdale, ON. The sheep antiserum to mouse Ig (immunoglobulins) was prepared by immunization with an ammonium sulphate precipitate of pooled sera from BALB/c and DBA/2 mice. The antiserum was

adsorbed on normal human Ig–Sepharose 4B. Antisera to mouse IgG1, IgG2a, IgG2b, IgG3, IgM and IgA (heavy chain specific) were obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands, whereas antiserum to κ and λ light chains were from Bionetics, Kensington, MD. Pristane (2,6,10,14-tetramethylpentadecane) was purchased from Aldrich Chemical Co., Milwaukee, WI. Freund's complete adjuvant was obtained from Difco Laboratories, Detroit, MI.

Radioiodination

*Lolp*I, *Lolp*II, *Amb.*I, P1 and TT were radioiodinated with carrier-free ^{125}I by the chloramine-T method (Klinman & Taylor, 1969). The radioactivity of labelled materials was more than 95% precipitable with trichloroacetic acid.

Serum samples

Serum samples from grass-allergic donors were kindly provided by Dr J. Duchateau (Clinical Immunology Laboratory, Brussels University Hospital, Belgium). The donors were selected on the basis of their RAST positivity (3–4+) to rye-grass pollen. The rye-grass non-allergic subjects were from Winnipeg, Canada; they had no history of allergy and no anti-*Lolp*I antibodies as tested by double-antibody RIA (Bose *et al.*, 1984). Sera from five non-allergic individuals who had been immunized with tetanus toxoid were used in some control experiments.

Production and selection of hybridoma cell lines

Female BALB/c mice (Jackson Laboratories, Bar Harbour, ME) were immunized subcutaneously with 5 μg of *Lolp*I emulsified in Freund's complete adjuvant and were boosted three times at 2-week intervals. Serum anti-*Lolp*I antibody was monitored by means of double antibody RIA. Three days prior to fusion, one mouse with the highest antibody titre was boosted intraperitoneally with 5 μg of *Lolp*I in PBS (phosphate-buffered saline, pH 7·2); its spleen was then isolated and 1×10^8 spleen lymphocytes were fused with 25×10^6 NS1 myeloma cells exactly as described earlier (Rector *et al.*, 1985). Supernatants from the primary cultures were screened for *Lolp*I-specific antibodies after 10, 17 and 24 days of fusion. On Day 24, the supernatants containing *Lolp*I-specific antibodies were tested in inhibition assay employing unlabelled antigen and rye-grass allergic human serum as inhibitors. The primary cultures showing strong inhibition of their binding to ^{125}I-*Lolp*I by allergic serum were tested as above after 10 and 14 days of culture, and the clones showing specific inhibition with both *Lolp*I and allergic serum were expanded and injected into the peritoneal cavity of pristane-primed BALB/c mice.

Purification of Mab Lolp*I*

Mab were purified by gel filtration (AcA-44 Ultrogel, LKB, Lyon, France) after ammonium sulphate precipitation of ascitic fluid. The fractions were pooled on the basis of their anti-*Lolp*I activity and purity as tested by double-antibody RIA and SDS–PAGE, respectively. This material was used for coating microtitre plates in solid-phase RIA. A portion of such Mab *Lolp*I preparation was then affinity-purified on *Lolp*I–Sepharose 4B (Bose *et al.*, 1984) and analysed in Ouchterlony analysis for its heavy and light chain classes.

Detection and characterization of Mab Lolp*I*

The presence of anti-*Lolp*I antibodies in mouse serum and hybridoma culture supernatant was tested by double-antibody RIA as described earlier (Bose *et al.*, 1984). Briefly, 100 μl of test sample were incubated overnight at room temperature with 25 μl of ^{125}I-*Lolp*I [8–10 ng containing 10^5 c.p.m., diluted in 8% normal mouse serum in assay buffer (PBS containing 0·5 M EDTA, 0·05% Tween-20 and 0·1% BSA)]. An excess of sheep anti-mouse Ig was then added and the incubation continued for 1 hr at 37° and overnight at 4°. The precipitate was washed three times with assay buffer and the radioactivity counted in a gamma counter. For the inhibition experiments with human serum the latter was preincubated with ^{125}I-*Lolp*I, whereas for antigen inhibition, labelled antigen was mixed with the test sample for 3 hr at room temperature.

Characterization of Mab *Lolp*I was performed using solid-phase RIA. Briefly, polyvinyl microtitre plates (Cooke Laboratories, Alexandria, VA) were successively coated with purified Mab *Lolp*I (diluted in 0·01 M carbonate–bicarbonate buffer, pH 9·2), blocked with 10% FCS in PBS, and incubated with ^{125}I-antigen (diluted in assay buffer supplemented with 10% FCS) for 16 hr at room temperature. The plates were then washed with PBS and the radioactivity in individual wells counted in a gamma counter. For inhibition experiments, the inhibitor was incubated overnight at room temperature with ^{125}I-*Lolp*I and then added to the Mab *Lolp*I-coated plates.

*RIA to study the allergenic cross-reactivity of human and mouse monoclonal anti-*Lolp*I antibodies*

Inhibition of ^{125}I-*Lolp*I binding to Mab *Lolp*I by human anti-*Lolp*I IgG was studied by the inhibition of solid-phase RIA as described above where immunoadsorbent-purified anti-*Lolp*I IgG and anti-TT IgG or purified human myeloma IgG antibodies (50 μg/ml final concentration) were used as inhibitors.

Inhibition of ^{125}I-*Lolp*I binding to human anti-*Lolp*I IgE by Mab *Lolp*I was performed by means of another solid-phase RIA as described earlier (Bose *et al.*, 1986). Briefly, mouse monoclonal anti-IgE (clone no. 89, 1 μg/ml in 0·01 M carbonate–bicarbonate buffer, 250 μl/well)-coated polyvinyl microtitre plates were incubated overnight at room temperature with undiluted rye-grass allergic serum (200 μl/well). The plates were then washed with PBS and supplemented with a mixture of Mab *Lolp*I or normal BALB/c IgG (both at 20 μg/ml final concentration) and ^{125}I-*Lolp*I (10^5 c.p.m./200 μl/well in 10% FCS in assay buffer, which were preincubated overnight at room temperature). After incubation for 8 hr at room temperature, the plates were washed with PBS and the radioactivity in each well was counted in a gamma counter.

RESULTS

Production and screening of hybridoma cell lines

All the BALB/c mice displayed a high antibody response to *Lolp*I, and the spleen of the best responding animal was employed for fusion. Primary culture wells were screened at Days 10, 17 and 24 after the fusion by double-antibody RIA. In 22 out of 288 culture supernatants showing high antibody activity, the binding of ^{125}I-*Lolp*I to the hybridoma antibodies was significantly inhibited by both *Lolp*I and pooled human allergic serum but not by pooled normal human serum. Four of these 22 primary cultures were employed for limiting dilutions. Table 1 shows that their culture supernatant contained anti-

Table 1. Identification of hybridoma producing anti-*Lolp*I antibodies

Primary culture supernatant screening						
		% inhibition†				
Primary culture no.	Titre of primary culture*	HDM	*Lolp*I	Normal serum	Allergic serum	Monoclonal antibody families‡
1	8749	0	71	0	27	Group I
2	12,134	0	9	0	16	Group II
3	5022	5	23	4	33	Group III
4	13,337	0	56	0·8	18	Groups IV and V

* Double-antibody RIA mean values from third screening of 24-day-old primary culture in the absence of inhibitor are given as c.p.m. of ^{125}I-*Lolp*I bound.

† Control for the inhibition was 10% FCS in PBS. Value given is the mean of two separate experiments.

‡ Monoclonal antibody clones produced by limiting dilution of the four primary cultures. See Table 2 for details.

*Lolp*I antibodies inhibitable by *Lolp*I (1 μg/ml) and by allergic serum but not by an unrelated antigen (HDM extract, 1 μg/ml final concentration) nor by normal human serum. Monoclonal hybridoma cell lines were tested 10 and 14 days after limiting dilution and 108/259 cultures displayed high anti-*Lolp*I activity (3–100 times above background). Ninety-four of the latter were inhibitable by the pooled allergic serum but not by normal serum. Twenty such cultures were expanded and injected into the peritoneal cavity of pristane-primed BALB/c mice.

Purification and characterization of Mab *Lolp*I

Monoclonal antibodies were isolated from ascitic fluid by ammonium sulphate precipitation and gel filtration on AcA-44 Ultrogel column. For the determination of the isotype, these preparations were further purified by affinity-chromatograph on *Lolp*I–Sepharose 4B. Heavy and light chain classes were determined by Ouchterlony and SDS–PAGE analyses. As shown in Table 2, five Mab *Lolp*I were IgA κ, whereas the remaining 15 were IgGl κ. Each antibody was tested in a solid-phase assay for its ability to bind ^{125}I- *Lolp*I. Figure 1 illustrates the dose–response curve determined by coating microtitre plate wells with solutions containing 0·1–100 μg/ml of a representative Mab *Lolp*I of each family (see later). Note that a weak response was obtained with Mab *Lolp*I V.8 from Family V. Because this antibody was active in the double-antibody assay, it is possible that its low activity in the solid-phase assay was due to its weak adherence to the polyvinyl well. The *Lolp*I specificity was tested by means of the same solid-phase assay by showing that Mab bound to radiolabelled *Lolp*I but not to labelled *Lolp*II, *Amb.*I, P1 or TT (data not shown). The reactivity of Mab *Lolp*I to native unlabelled *Lolp*I was shown in inhibition assays (Table 3) where 0·1–100 μg/ml of *Lolp*I (final concentration) inhibited the binding of ^{125}I-*Lolp*I to the Mab, whereas 100 μg/ml of TT or HDM extract have no effect.

Epitope specificity of Mab *Lolp*I

The fine specificity of each Mab *Lolp*I was compared to that of the others on the basis of its ability to inhibit the binding of ^{125}I-*Lolp*I to the 19 other Mab *Lolp*I (Fig. 2). The data in Table 4 indicate the quantity of each Mab *Lolp*I required to inhibit by 50% the binding of ^{125}I-*Lolp*I to a given Mab *Lolp*I attached to the solid phase. In these experiments inhibitors were employed at concentrations ranging from 0·01 μg/ml to 100 μg/ml. According to these data, five groups of Mab *Lolp*I may be defined (Table 2). Mab *Lolp*I belonging to one group inhibited each other very effectively, whereas they were not inhibited by, nor could they inhibit, the other groups of Mab *Lolp*I. The results suggest that each group of Mab *Lolp*I identifies a distinct epitope on *Lolp*I.

Comparison of the epitope specificity of anti-*Lolp*I human and mouse monoclonal antibodies

Because the Mab *Lolp*I were selected on the basis of their being inhibitable by a pool of human sera containing anti-*Lolp*I antibody, it is likely that they react with *Lolp*I epitopes that are identical or very close to those recognized by the human antibody. In the results shown in Table 5, one representative Mab *Lolp*I of each family was tested for its ability to be inhibited by anti-*Lolp*I IgG antibody from 14 individual allergic donors. Anti-TT IgG antibody from five TT immunized donors and one myeloma IgG were used to determine the specificity of the assay. Because the inhibition induced by anti-TT antibody and myeloma IgG ranged from 0 to 5%, an inhibition of 10% or more was considered to be significant. The results document the heterogeneity of human anti-*Lolp*I antibodies by showing that allergic sera differ by the number of Mab *Lolp*I that they are able to inhibit. It is of interest to note that serum no. 2 displayed only

Table 2. Characterization of Mab *Lolp*I

Group I		Group II		Group III		Group IV		Group V	
I.1*	(IgA, κ)	II.16*	(IgG1, κ)	III.10*	(IgG1, κ)	IV.6*	(IgG1, κ)	V.7	(IgG1, κ)
I.2	(IgA, κ)	II.17	(IgG1, κ)	III.11	(IgG1, κ)			V.8*	(IgG1, κ)
I.3	(IgA, κ)	II.18	(IgG1, κ)	III.12	(IgG1, κ)			V.9	(IgG1, κ)
I.4	(IgA, κ)	II.19	(IgG1, κ)	III.13	(IgG1, κ)				
I.5	(IgA, κ)	II.20	(IgG1, κ)	III.14	(IgG1, κ)				
				III.15	(IgG1, κ)				

* Representative antibodies of each group used for subsequent assays.

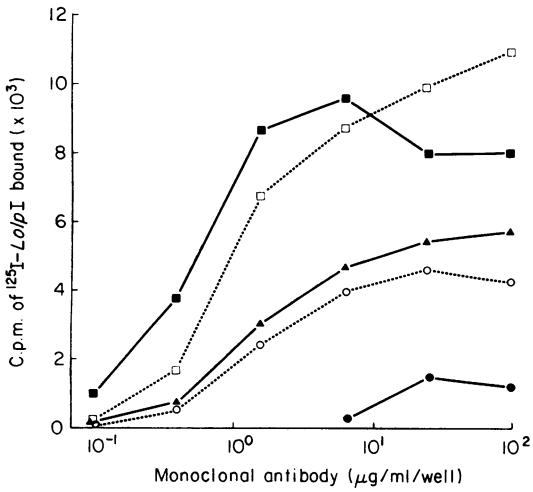

Figure 1. Binding curve for the reaction of ^{125}I-*Lolp*I with varying amounts of Mab *Lolp*I (from five different families) adsorbed to microtitre wells. Fifteen nanograms of ^{125}I-*Lolp*I were added to each well. Mab no. I.1 (▲——▲), II.16 (□ - - - □), III.10 (○ - - - ○), IV.6 (■——■) and V.8 (●——●).

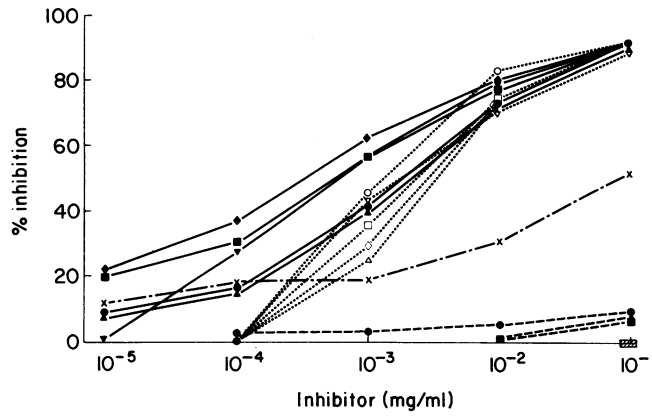

Figure 2. Inhibition of binding of ^{125}I-*Lolp*I to solid-phase Mab *Lolp*I no. I.3. Serial 10-fold dilutions of all 20 Mab *Lolp*I were used as inhibitors. The value obtained with 100 μg/ml of normal BALB/c IgG instead of an inhibitor was used as control. Mab no. I.1 (●——●), I.2 (▲——▲), I.3 (■——■), I.4 (▼——▼), I.5 (♦——♦), II.16 (○ - - - ○), II.17 (△ - - - △), II.18 (□ - - - □), II.19 (▽ - - - ▽), II.20 (◊ - - - ◊), III.10–15 (▨), IV.6 (× — - — ×), V.7 (● — —●), V.8 (▲ — — ▲) and V9 (■ — — ■).

Table 3. Inhibition of ^{125}I-*Lolp*I binding to Mab *Lolp*I by different antigens

		% inhibition‡							
		*Lolp*I				HDM		TT	
Mab*	Maximum binding†	0·1	1·0	10	100	10	100	10	100
I.1	3750	0	0	46	77	0	0	0	0
II.16	8468	3	15	60	89	6	0	3	0
III.10	2730	0	40	89	ND¶	0		0	
IV.6	7160	12	39	89	ND	0		0	
V.8§	10,376	0	0	27	77	0		0	

* Representative Mab *Lolp*I from five different families, see Table 2.

† C.p.m. of ^{125}I-*Lolp*I bound to Mab *Lolp*I in the absence of inhibitor.

‡ Percentage inhibition by antigens at 0·1–100 μg/ml final concentration.

§ Mab *Lolp*I V.8 was tested by double-antibody RIA.

¶ ND, not determined.

borderline inhibitory activity, suggesting that its anti-*Lolp*I antibodies reacted with epitopes different from those recognized by the four families of Mab *Lolp*I.

In a mirror experiment, a representative Mab *Lolp*I from each family was tested for its ability to inhibit the binding of human IgE anti-*Lolp*I to ^{125}I-*Lolp*I. Mab *Lolp*I were used at a final concentration of 20 μg/ml, and the value obtained by using normal BALB/c IgG instead of Mab *Lolp*I was employed to determine the maximum binding. As shown in Table 6, except for Mab *Lolp*I from Family V, Mab *Lolp*I from all the other four families inhibited a significant number of sera. Six out of 15 sera were weakly or not inhibited by any of the five Mab *Lolp*I (sera nos 22, 26, 28, 29, 31 and 34), whereas six other sera (nos 20, 23, 24, 27, 30 and 33) were significantly inhibited by two or more Mab *Lolp*I.

DISCUSSION

This study documents the production of monoclonal antibodies to five distinct antigenic determinants on *Lolp*I, the major allergen of rye-grass pollen. The data further suggest that these antigenic determinants or epitopes are also reacting with human IgE/IgG anti-*Lolp*I antibodies and may therefore be referred to as allergenic determinants. These epitopes are not expressed on some other common antigens or airborne allergens such as *Amb.*I (the major allergen of ragweed), P1 (the major allergen of house dust mite *Dermatophagoides pteronyssinus*), and *Lolp*I (a minor allergen of rye-grass pollen and tetanus toxoid). Indeed, Mab *Lolp*I bound to labelled *Lolp*I but not to labelled P1, *Amb.I*, *Lolp*I or TT, and conversely, the binding of labelled *Lolp*I to Mab is inhibited by *Lolp*I but not by HDM or TT. These observations do not rule out the possibility that some of the Mab *Lolp*I may cross-react with other grass-pollen antigens. Indeed, some Mab specific to a rye-grass pollen antigen were shown to cross-react with unrelated grass-pollen antigens such as reed canary, red top, orchard, fescue, velvet, sweet-vernal timothy, kentucky blue grass and others (Kahn & Marsh, 1983; Singh & Knox, 1985).

The twenty Mab *Lolp*I were grouped into five families differing by their epitope specificity. Hence, if two or more Mab *Lolp*I could inhibit each other in a reciprocal fashion, they were considered to be members of the same family; moreover, Mab *Lolp*I within one family did not compete with Mab *Lolp*I from other families for the binding to labelled *Lolp*I. It is likely that the epitopes identified by Group I Mab *Lolp*I (nos 1–5) are close to those reacting with Group II Mab *Lolp*I (nos 16–20); indeed, each Mab *Lolp*I from Group I or from Group II inhibits and is inhibited by the others. The only difference between Group I and Group II is that the latter (but not the former) inhibits the

Table 4. Determination of epitope specificity of Mab *Lolp*I*

Mab as inhibitors†	Monoclonal antibodies coupled to solid phase‡															
	I.1	I.2	I.3	I.4	I.5	III.10	III.11	III.12	III.13	III.14	III.15	II.16	II.17	II.18	II.19	II.20	IV.6
I.1	0·03	0·03	0·2	0·1	0·04	>2	>2	>2	>2	>2	>2	0·3	0·5	0·5	0·3	0·5	>2
I.2	0·03	0·04	0·2	0·1	0·04	>2	>2	>2	>2	>2	>2	0·3	0·6	0·6	0·3	0·5	>2
I.3	0·02	0·04	0·2	0·1	0·04	>2	>2	>2	>2	>2	>2	0·2	0·5	0·3	0·3	0·5	>2
I.4	0·01	0·03	0·1	0·1	0·03	>2	>2	>2	>2	>2	>2	0·2	0·4	0·2	0·3	0·2	>2
I.5	0·01	0·02	0·1	0·1	0·03	>2	>2	>2	>2	>2	>2	0·2	0·5	0·4	0·2	0·3	>2
III.10	>2	>2	>2	>2	>2	0·02	0·02	0·01	0·02	0·01	0·02	>2	>2	>2	>2	>2	>2
III.11	>2	>2	>2	>2	>2	0·04	0·02	0·01	0·02	0·02	0·02	>2	>2	>2	>2	>2	>2
III.12	>2	>2	>2	>2	>2	0·01	<0·01	0·01	<0·01	<0·01	0·02	>2	>2	>2	>2	>2	>2
III.13	>2	>2	>2	>2	>2	0·03	0·02	0·02	<0·01	<0·01	<0·01	>2	>2	>2	>2	>2	>2
III.14	>2	>2	>2	>2	>2	0·1	0·02	0·01	0·01	<0·01	<0·01	>2	>2	>2	>2	>2	>2
III.15	>2	>2	>2	>2	>2	<0·01	<0·01	<0·01	0·03	<0·01	<0·01	>2	>2	>2	>2	>2	>2
II.16	0·02	0·03	0·1	0·1	0·05	1·5	>2	0·2	>2	0·3	0·05	0·1	0·2	0·2	0·1	0·1	>2
II.17	0·02	0·03	0·1	0·1	0·05	>2	0·3	0·03	0·2	0·05	0·02	0·03	0·2	0·5	0·1	0·05	>2
II.18	0·02	0·03	0·1	0·1	0·05	>2	0·8	0·05	0·2	0·08	0·03	0·03	0·1	0·1	0·1	0·05	>2
II.19	0·02	0·05	0·1	0·1	0·05	>2	>2	0·2	0·8	1·2	0·2	0·1	0·2	0·1	0·2	0·1	>2
II.20	0·02	0·03	0·1	0·1	0·05	>2	1·2	0·2	0·8	0·01	0·03	0·05	0·2	0·05	0·1	0·1	>2
IV.6	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	0·01
V.7	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	1·5
V.8	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	1·5
V.9	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	1·5

* Each experiment was repeated twice in duplicate. Values represent the amount of inhibitor per well (in μg) required to obtain 50% inhibition.

† Each inhibitor was used at 0·01, 0·1, 1·0, 10 and 100 μg/ml (final concentration).

‡ Mab *Lolp*I nos V.7, 8 and 9 were only used as inhibitors due to their poor adherence to solid phase.

binding of ^{125}I-*Lolp*I to Mab *Lolp*I of Group III (nos 10–15); Group III differs from Group II because it does not block the binding of labelled *Lolp*I to Group II Mab *Lolp*I. These data suggest that the epilopes identified by Group III Mab *Lolp*I are located between those identified by Group I and Group III Mab *Lolp*I. An alternative explanation could be that Group II antibodies have higher affinity than Group I for the same or very close epitopes, as suggested by the antigen-binding studies (Fig. 1). Mab *Lolp*I from Groups IV and V do not interact at all with each other, nor with the previous groups, suggesting that they are specific to epitopes that are remote from the previous one. Using a similar approach, Chapman, Sutherland & Platts-Mills (1984) described two families of Mab to P1, specific to distinct epitopes on P1 molecules. The results observed in Table 4 showing Group I Mab of IgA class suggests the possibility that all five Mab of Group I might have originated from the same clone.

In order to test whether the five epitopes identified by the Mab *Lolp*I were also recognized by anti-*Lolp*I antibodies from allergic patients, human and Mab *Lolp*I were tested in two-way inhibition studies. In the first case, anti-*Lolp*I antibodies were purified by affinity-chromatography from the sera of 14 allergic patients, and each preparation of purified antibody (containing mainly IgG anti-*Lolp*I) was tested for its ability to inhibit the binding of ^{125}I-*Lolp*I to a representative Mab *Lolp*I from each family (except Family V, the antibodies of which do not adhere to the solid phase). Two Mab *Lolp*I (IV. 6 and III. 10) were inhibited by 12/14 preparations of purified human anti-*Lolp*I, suggesting that these epitopes are clinically relevant as they are recognized by the majority of the allergic patients. Note that the antibodies from patient no. 2 hardly inhibited any of the four Mab *Lolp*I, illustrating the heterogeneity of the antibody response by different patients. In these assays, 10% inhibition was considered to be significant, based on the observation that the same quantity (50 μg/ml, final concentration) of either myeloma IgG or affinity-purified IgG anti-TT led to inhibition ranging from 0 to 5·1%. It is of interest to note that affinity-purified antibodies and not whole sera were used in this assay, in order to eliminate the possible interference of auto-anti-idiotypes (aId) specific to anti-*Lolp*I. Indeed, such aId were described in allergic and non-allergic individuals, and some of them may inhibit the binding of ^{125}I-*Lolp*I to human IgE/IgG antibodies (Bose *et al.*, 1984). In the second series of inhibition experiments we tested the possibility to inhibit the binding of ^{125}I-*Lolp*I to IgG antibodies in sera from 15 different patients by means of Mab *Lolp*I. One Mab *Lolp*I representative of each

Table 5. Inhibition of ^{125}I-*Lolp*I binding to Mab *Lolp*I by human anti-*Lolp*I IgG antibodies

Inhibitor		% inhibition§			
Antibody*	Antibody titre†	I.1 (2400)‡	II.16 (6500)	III.10 (4210)	IV.6 (6680)
Anti-*Lolp*I IgG					
No. 1	43,296	35	34	46·1	21·7
No. 2	15,946	15·6	8	2·3	6·3
No. 3	14,965	0	0	34·3	32·2
No. 4	14,270	22·8	18·7	18·7	19·4
No. 5	11,620	0	8·1	50·9	3·4
No. 6	10,182	18·9	32·2	57·1	25·8
No. 7	8204	23·4	34·7	61·6	28·7
No. 8	6790	19·2	27	54·6	24·6
No. 9	6046	2·1	24·1	0·2	26·9
No. 10	5716	16·7	29	59·1	18·6
No. 11	5220	10·9	21	58·7	24
No. 12	4682	0·6	5	43·1	14·4
No. 13	4250	11·5	38	31·0	23·5
No. 14	3194	29·7	38·5	42	15·4
Anti-TT IgG					
No. 15	39,855	0	5·1	2	0
No. 16	39,485	1·5	0	0	0·8
No. 17	37,610	0	0	2·1	2
No. 18	35,369	0	0·5	0	1·4
No. 19	22,664	0	0	1·9	0
Myeloma IgG	—	0	0	0	0

* Inhibitors were used at 50 μg/ml (final concentration); anti-*Lolp*I IgG was affinity-purified from 14 allergic sera, anti-TT was affinity-purified from five hyperimmunized donors.

† C.p.m. of ^{125}I-*Lolp*I or ^{125}I-TT bound to 5 μg of immunoadsorbent purified antibodies, as determined by double-antibody RIA.

‡ Values given in parentheses are the maximum binding in the absence of inhibitor.

§ Percentage inhibition of ^{125}I-*Lolp*I binding to Mab *Lolp*I on solid phase. Values are the mean of two separate experiments done in duplicate.

Table 6. Inhibition of human anti-*Lolp*I IgE binding to ^{125}I-*Lolp*I by Mab *Lolp*I

Anti-*Lolp*I IgE		% inhibition‡				
Serum*	Maximum binding†	I.1	II.16	III.10	IV.6	V.8
No. 20	8996	8·3	27·1	24	31·2	10
No. 21	7944	9·0	13·1	21·7	0	0
No. 22	7465	12·2	3·4	8·5	0	1·3
No. 23	7012	1·3	20·0	25	18·6	10
No. 24	5268	28·3	33·4	21·4	33·1	7·8
No. 25	5179	4·2	0	25	5·1	9
No. 26	5173	0	0	16·3	0	1·8
No. 27	3973	4·4	27·3	23·2	19·5	6·8
No. 28	3956	5·0	6·6	10	1·3	4·4
No. 29	3863	0·6	7·0	0	0	0
No. 30	3677	24·8	24·2	10	25·0	12·4
No. 31	3106	2·4	0	12	0	0
No. 32	2623	2·9	35·1	8	17·9	5
No. 33	2172	0	23·4	20	13·3	0
No. 34	1603	3·3	8·1	8	0	7

* Sera from different hay fever patients.

† C.p.m. of ^{125}I-*Lolp*I bound to IgE anti-*Lolp*I in the presence of 20 μg/ml normal BALB/c IgG.

‡ Percentage inhibition by Mab *Lolp*I (20 μg/ml final concentration). Values are the mean of duplicate determinations. The experiment was repeated twice with concordant results.

family was tested at a final concentration of 20 μg/ml; the same amount of normal BALB/c IgG was used as a negative control and to determine the maximum binding. Inhibitions greater than 20% were obtained in 2/15, 7/15, 7/15 and 4/15 allergic sera by Mab *Lolp*I nos I.1, II.16, III.10 and IV.6, respectively, with the greatest inhibition being 35%. Note that Mab *Lolp*I no. 8 had no inhibiting activity. Moreover, 6/15 sera were significantly inhibited (> 20%) by two or more Mab *Lolp*I, whereas six other sera were not inhibited at all. Since human anti-*Lolp*I antibodies were shown to be directed against multiple allergenic determinants (Chapman *et al.*, 1984; Heyman *et al.*, 1985), we expected to see a low inhibition of ^{125}I-*Lolp*I binding to human IgE antibodies by *Lolp*I. It is therefore interesting to note that two *Lolp*I (nos. II.16 and III.10) inhibited 7/15 sera. Taken collectively, these inhibition studies indicate that it is now possible to analyse the heterogeneity of the human antibody response to *Lolp*I. Hence, by screening a large number of sera, it seems feasible to identify dominant allergenic epitopes. The same approach might also be employed to compare the fine specificity of antip*Lolp*I antibody from untreated patients to that from patients undergoing immunotherapy. The Mab *Lolp*I described in this study may also be used to test whether, in a given individual, the epitopes recognized by T cells are different from those identified by B cells, as has been suggested in earlier studies (Chou *et al.*, 1979; Fritz *et al.*, 1979; Ishizaka *et al.*, 1974). Finally, it should be mentioned that we are presently testing the possibility of using the Mab *Lolp*I described in this study as probes to identify human auto-aId. Indeed, as already mentioned, mouse and human antibodies may express the same idiotypes (Dwyer *et al.*, 1983; Castracane *et al.*,, 1985).

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