

Monoclonal antibodies against *Fel d* I and other clinically relevant cat allergens

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1. Summary

Several mouse monoclonal antibodies (MAbs) were obtained which specifically recognized allergen molecules from cat dander extract. Two of them (C5.8 and C5.24) were specific for the main cat allergen, *Fel d* I. One (C5.25) recognized an important allergen with approximate molecular weight of 30000 Da and a pI between 3.9 and 4.3. A third group of MAbs comprised several hybridomas which were specific either for cat albumin or cat immunoglobulin. The immunochemical characterization and the clinical significance of the cat dander components recognized by these MAbs is presented and discussed.

2. Introduction

Allergens derived from the domestic cat give rise to severe allergic reactions in humans [1]. A single allergen, identified in cat pelts, cat saliva and sebaceous glands of cat skin, and designated cat allergen 1 (*Fel d* I according to the WHO/IUIS Allergen Nomenclature Subcommittee [2]) has been reported as the most important cat allergen [3, 4]. This allergen has been purified by biochemical [3] and immuno-affinity techniques using polyclonal antibodies [5]. On the other hand, studies on cat pelt ex-

tracts have indicated that cat albumin was an allergen of minor importance and that there were additional intermediate allergens [4, 6].

Lately, the use of monoclonal antibodies (MAbs) has become a powerful tool for purification and characterization of allergenic structures as described for allergens of *Ambrosia* pollen [7], *Dermatophagoides* mites [8–10] and *Parietaria judaica* pollen [11]. Likewise, recent studies have demonstrated the usefulness of *Fel d* I MAbs for purification of this allergen from house dust extracts [12].

In the present paper, we describe the production and characterization of MAbs against *Fel d* I and other IgE-binding components from cat dander. Some biochemical properties of these components as well as their clinical significance are discussed.

3. Materials and Methods

3.1. Preparation of antigens

Cat dander extract (CDE) and partially purified *Fel d* I were prepared as indicated [13].

Cat albumin and cat IgG were purified from cat serum by classical biochemical techniques.

3.2. Polyclonal antibodies

Rabbit antibodies against CDE were purchased from ALK Laboratories (Copenhagen, Denmark). Crossed immunoelectrophoresis (CIE) of CDE was carried out essentially as indicated [13].

Goat antibodies against mouse Ig (GAM) were obtained by affinity chromatography of a serum pool from goats previously immunized with mouse IgG.

Key words: Monoclonal antibodies; Cat allergens; Cat allergy

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3.3. ^{125}I -labelling

Goat antibodies against mouse IgG were radioiodinated to a specific activity of about $10\ \mu\text{Ci}/\mu\text{g}$ according to Fraker and Speck [14]. Similarly, $20\ \mu\text{g}$ of CDE protein were labelled with $1\ \text{mCi}$ of ^{125}I using the same method.

3.4. Production of monoclonal antibodies

Female BALB/c mice were immunized with an intraperitoneal injection of either $100\ \mu\text{g}$ of CDE or $50\ \mu\text{g}$ of partially purified *Fel d* I, previously polymerized with glutaraldehyde [15], in $100\ \mu\text{l}$ of PBS emulsified with $200\ \mu\text{l}$ of Freund's complete adjuvant. Fifteen days later, mice were boosted with an identical amount of antigen in Freund's incomplete adjuvant, and the same process was again repeated 60 days later. A month after the last injection the mice with a suitable antibody titre were injected intravenously with the same amount of CDE or *Fel d* I in PBS. Fusions were carried out 3 days later as indicated [16]. About 2 weeks after the fusion, hybridomas supernatants were screened, and the positive hybrids were cloned and subcloned in soft agar.

The isotype of the MAbs was determined by immunodiffusion using rabbit anti-mouse subclass antibodies (Nordic, Tilburg, The Netherlands).

3.5. Immunoassays

Hybridomas were screened for specific antibodies with ability to recognize CDE. $50\ \mu\text{l}$ of hybridoma culture supernatant was added to CDE or *Parietaria judaica* extract covalently linked to BrCN activated paper discs, and incubated for 1 h at room temperature. After washing the discs, they were incubated for 1 h with $50\ \mu\text{l}$ of ^{125}I -GAM. Then they were washed again and the radioactivity bound to them was measured. The assays for specific antibodies against cat albumin or cat IgG were carried out in a similar way, but the solid phase consisted of polyvinyl chloride (PVC) wells coated with $50\ \mu\text{l}$ of purified cat albumin or cat IgG ($10\ \mu\text{g}/\text{ml}$) and saturated with 1% BSA.

To determine whether or not the structures recognized by the MAbs had, at the same time, capacity to recognize specific human IgE from individual patients we proceeded as follows: PVC wells were coated with $70\ \mu\text{l}$ of GAM ($10\ \mu\text{g}/\text{ml}$) and after saturation with 1% BSA, they were incubated for 2 h with $70\ \mu\text{l}$ of hybridoma supernatant and then incubated

with $70\ \mu\text{l}$ of CDE ($0.5\ \text{mg}/\text{ml}$) for 2 h at room temperature. After washing the wells, they were incubated overnight with $70\ \mu\text{l}$ of individual serum diluted 1:3 in PBS from cat-allergic patients (RAST class 3–4 to CDE paper discs). The wells were again washed and then incubated with about 35000 cpm of ^{125}I -labelled anti-human-IgE (Pharmacia, Sweden). After washing, the radioactivity bound to the wells was measured.

3.6. Immunoprecipitation

PVC wells were coated with $50\ \mu\text{l}$ of GAM ($10\ \mu\text{g}/\text{ml}$) and after saturation with 1% BSA, the wells were incubated for 1 h with $50\ \mu\text{l}$ of hybridoma supernatant and then with 2.5×10^5 cpm of ^{125}I -CDE. After washing, the radioactivity bound to the solid phase was detached by incubating for 5 min at 90°C with $30\ \mu\text{l}$ of either SDS-PAGE or IEF sample buffers, and then subjected to SDS-PAGE under reducing conditions [17] or analytical IEF (pH range 3.5–9.5) in the presence of 8 M urea and 2-mercaptoethanol [18]. Finally, the gels were dried and autoradiographed.

Table 1
Specificity of monoclonal antibodies to CDE.

MAb	CDE ^{a,b}	<i>Parietaria judaica</i> ^{a,b} extract	Cat albumin ^{a,c}	Cat IgG ^{a,c}
C3.3	11920	1193	1290	14160
C3.7	9920	1255	1214	13905
C3.10	17335	1063	6676	1600
C3.14	18705	1320	1684	12640
C3.18	8780	1275	1736	13260
C3.22	11820	1190	5008	1720
P3X63	1470	1040	1744	1715
C5.8	9100	1265	680	1235
C5.19	14850	1120	4810	1495
C5.24	10140	1075	755	1040
C5.25	7455	1160	640	945
C5.32	14075	1330	1330	12535
P3X63	1260	1115	607	852

Specificity of the MAbs obtained in the two fusions. The radioimmunoassays were achieved as indicated in Section 3.

^a cpm of ^{125}I -GAM bound to MAbs. ^b CDE or *Parietaria judaica* was covalently bound to activated paper discs. ^c PVC wells were coated with both purified cat albumin or cat IgG. Non-specific binding was determined using P3X63 mouse myeloma culture supernatant.

4. Results

4.1. Specificity of the MAbs anti-CDE

Table 1 shows the specificity of the more important MAbs from two different fusion experiments: one using spleen cells from mouse immunized with the whole CDE (the corresponding MAbs are indicated as C3), and another fusion using mouse immunized with partially purified *Fel d* 1 (the MAbs from this fusion were designated C5). It can be observed that the MAbs recognized the CDE in a specific way, as they were unable to recognize *Parietaria judaica* coupled discs. On the other hand, when a similar experiment was carried out, but using in the solid phase purified cat albumin or cat IgG, it was observed (Table 1) that all the C3 MAbs were specific for these proteins. However, as to the C5 MAbs indicated in the table, one (C5.19) was specific

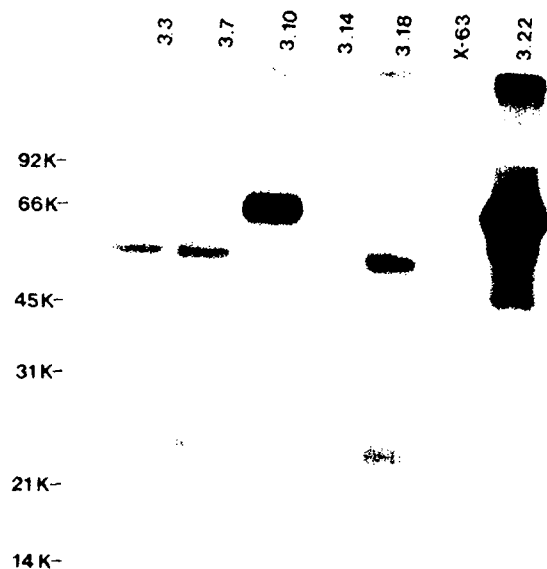


Fig. 1. Autoradiography, after SDS-PAGE, of the immunoprecipitates obtained with ^{125}I -labelled CDE and the C3 MAbs. The immunoprecipitates were detached from the PVC wells with Laemmli's sample buffer and subjected to SDS-PAGE in a 12% acrylamide concentration. As shown, all the MAbs obtained in the C3 fusion were specific either for cat albumin or cat immunoglobulin. X-63: Negative control with P3X63 mouse myeloma culture supernatant.

for cat albumin, another (C5.32) was specific for cat IgG, and the other three were supposed to recognize different components in the cat dander extract.

4.2. Biochemical characterization of the molecules recognized by the anti-CDE MAbs

In order to characterize the molecules recognized by the anti-CDE MAbs, immunoprecipitation experiments were performed using ^{125}I -labelled CDE. Fig. 1 shows the SDS-PAGE pattern of the immunoprecipitates obtained with the C3 MAbs. C3.3, C3.7, C3.14 and C3.18, specific for cat IgG as indicated in Table 1, immunoprecipitated two polypeptides of molecular weights around 50000 and

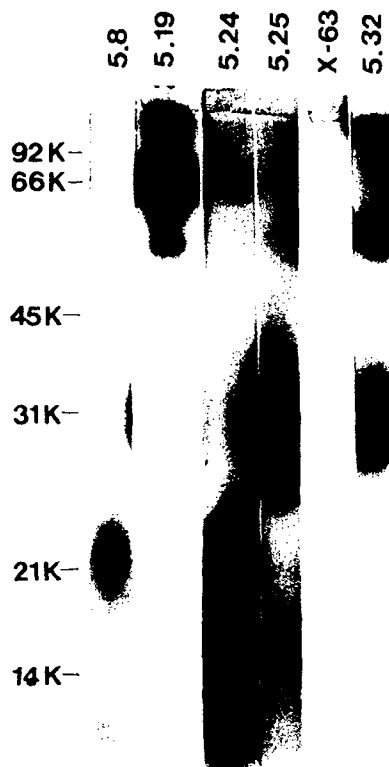


Fig. 2. Autoradiography, after SDS-PAGE, of the immunoprecipitates obtained with ^{125}I -labelled CDE and the C5 MAbs. Both C5.8 and C5.24 were specific for *Fel d* 1. C5.25 recognized *Fel d* Bd 4.1/30K. C5.19 was specific for cat albumin (65K) while C5.32 was specific for cat immunoglobulin as demonstrated by the presence of cat Ig heavy and light chains. Non-specific binding was determined by using P3X63 mouse myeloma culture supernatant (X-63).

25000 Da, which must correspond to the heavy and the light chains of cat IgG. The other two MAbs (C3.10 and C3.22) mainly recognized a protein of 65000 Da, which corresponds to cat albumin.

Figure 2 shows the corresponding SDS-PAGE pattern of the immunoprecipitates obtained with the C5 MAbs. It can be observed that C5.19 and C5.32 recognized cat albumin and cat immunoglobulin, respectively. MAbs C5.8 and C5.24 precipitated a set of polypeptides with molecular weights around 20000 and 10000 Da, which could belong to *Fel d* I. MAb C5.25 identified other structure with a molecular weight of about 30000 Da.

The isoelectric focusing of the immunoprecipitated components by the C5 MAbs is shown in Fig. 3.

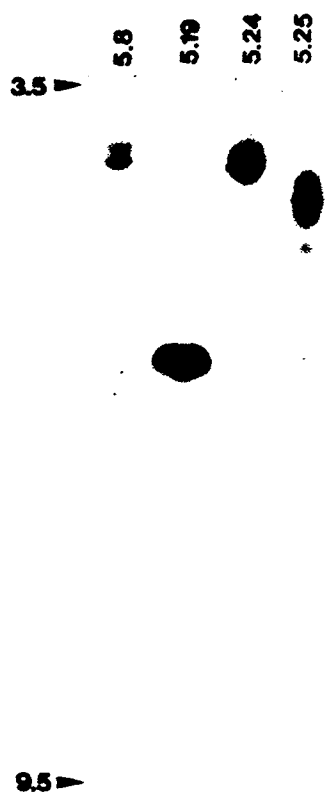


Fig. 3. Isoelectric focusing, in the presence of 8 M urea, of the components immunoprecipitated by C5 MAbs. The immunoprecipitates were detached from the PVC wells with IEF sample buffer (10% saccharose, 2.5% ampholyte, 1% NP-40, 8 M urea, 5% 2-mercaptoethanol) and subjected to analytical IEF in a pH range from 3.5 to 9.5. The gel was then dried and autoradiographed.

MAb C5.19 recognized a protein of $pI = 4.6$, in agreement with the cat albumin pI , whereas MAbs C5.8 and C5.24 precipitated a set of proteins focused at pH around 3.8, a pI which is claimed for *Fel d* I [5]. Finally, several proteins focussed between pH 3.9 and 4.3 were identified by MAb C5.25.

In order to confirm the specificity of MAbs C5.8 and C5.24, a CRIE-like experiment was carried out. CIE plates of the CDE were overlaid with the hybridoma culture supernatant, and then the plates were incubated with ^{125}I -GAM. The results are indicated in Fig. 4 and proved that both MAbs recognized the CIE-arc corresponding to cat allergen 1 or *Fel d* I. A faint radiostaining of other arcs can also be observed; however, when the P3X63 mouse myeloma culture supernatant was used as negative control, the same faint radiostaining was observed, indicating that it was caused by a non-specific binding.

4.3. Allergenic significance of the structure recognized by the anti-CDE MAbs

A set of individual sera from cat-allergic patients was tested, as indicated in Section 3, for specific IgE against the proteins recognized by the C5 MAbs. Table 2 shows that most of the sera had specific IgE against the allergen recognized by MAbs C5.8 and C5.24. On the other hand, approximately 50% of the patients studied had developed IgE antibodies to an allergen identified by MAb C5.25. Finally, only a few patients have specific IgE against cat albumin or cat IgG.

5. Discussion

In this report, the production and characterization of several MAbs against *Fel d* I and other allergens from CDE is described. The success in the production of *Fel d* I MAbs was based on the use of spleen cells from mice immunized with a partially purified *Fel d* I preparation, that had been polymerized with glutaraldehyde. When the mice were immunized with the whole CDE, we only obtained MAbs against cat albumin and cat IgG. Taken into account that the content of *Fel d* I in CDE was higher than the content of cat IgG, it seems that the immunogenicity in mice of the main cat allergen is low, at least in native conditions.

MAbs C5.8 and C5.24 seem to be specific of the

Table 2
Presence in individual sera of specific IgE to the components recognized by anti-CDE
MAbs.

Serum No.	P3X-63 ^{a,b}	Specificity				
		<i>Fel d 1</i>		Cat albumin	<i>Fel d</i> Bd 4.1/30K	Cat Ig
		C5.8 ^a	C5.24 ^a	C5.19 ^a	C5.25 ^a	C5.32 ^a
1	490	2485	5150	450	565	540
2	435	6320	13005	490	2230	590
3	730	4690	8515	704	3760	750
4	445	2690	5360	455	505	420
5	1320	9085	13210	1450	2595	1770
6	465	5670	11035	550	995	565
7	455	5930	10125	485	565	655
8	575	4875	7165	560	515	605
9	410	1645	3365	370	440	405
10	440	415	670	450	510	445
11	400	1895	7905	490	3325	445
12	445	900	1655	360	410	430
13	500	2985	9065	445	525	625
14	465	1260	2565	440	1685	425
15	400	1700	3340	415	700	490
16	625	3855	7170	610	1885	500
17	475	3490	6110	465	1605	530
18	465	1690	2795	495	795	440
19	400	1010	4640	500	1215	440
20	440	5455	10335	730	6675	525
21	525	2670	9370	505	6140	590
22	455	900	3630	380	440	555
23	430	835	4355	430	430	510
24	380	2225	4290	385	1010	455
25	435	2550	5305	350	1045	455
26	350	ND	ND	9924	ND	3335
27	400	ND	ND	5655	ND	385
28	525	ND	ND	9875	ND	430
ND	430	350	465	405	455	425

^a cpm of ¹²⁵I-anti-IgE bound to the solid phase. ^b Non-specific binding was determined using P3X63 mouse myeloma culture supernatant. We have considered as positive binding those results which showed a cpm twice that of the negative control. ND = Not determined. NC = Negative control with a serum pool from non-allergic individuals.

main cat allergen, *Fel d 1* or cat allergen 1, as demonstrated by the following experiments. (a) They were able to recognize, in a specific way, paper discs coupled with CDE. On the other hand, they did not recognize cat albumin or cat IgG in a solid-phase RIA. (b) These MAbs immunoprecipitated a set of proteins with molecular weight around 20000 and 10000 Da and a pI of about 3.8, in agreement with the molecular weight and pI of the CDE proteins with the highest IgE-binding ability [13], and the

results of other authors [5]. (c) They recognized the CIE-arc corresponding to *Fel d 1*. (d) Finally, a study in a sample of cat-allergic patients proved that most of them had specific IgE against the CDE components recognized by the MAbs C5.8 and C5.24, thus coinciding with the fact that most cat-allergic patients have specific IgE against *Fel d 1* [4]. Furthermore we obtained one MAb, C5.25, that identified a set of proteins of molecular weight about 30000 Da and pI of about 3.9 to 4.3. These

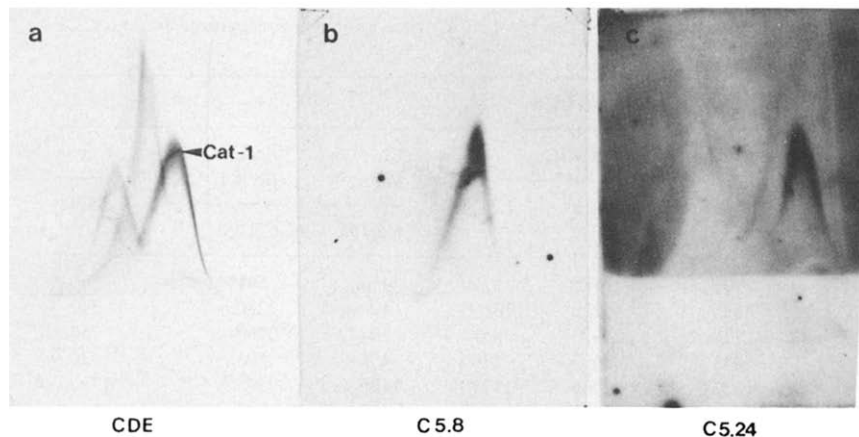


Fig. 4. Crossed immunoelectrophoresis of CDE. In the first dimension of CIE, 15 μ l of CDE (2.5 mg/ml) was electrophoresed at 450 V for 25 min. The second dimension was carried out against anti-CDE rabbit antibody during 18 h at 150 V. (a) CIE pattern of CDE after staining with Coomassie brilliant blue. (b) Autoradiography of the CIE plate after incubating with C5.8MAb and then with 5.0×10^5 cpm of ^{125} I-GAM. (c) The same as in (b), except that the monoclonal antibody used was C5.24. Non-specific binding was tested by using P3X63 mouse myeloma culture supernatant as negative control.

molecules must have allergenic activity because about 50% of the cat-allergic patients studied had specific IgE against them. This allergen could be the second in importance after *Fel d I*, and following the nomenclature system recommended by the IUIS [2] can be designated as *Fel d Bd 4.1/30K*. This structure might be one of the cat dander antigens identified by means of CIE/CRIE as allergens of intermediate importance [6].

Finally, we have obtained several MAbs against cat albumin and cat IgG, and, using these as specific probes, it was demonstrated that only a few patients of the sample studied had specific IgE against them, indicating that cat albumin and cat IgG are minor allergens, in agreement with the results of other authors [6, 19].

The availability of these antibodies will allow their use in the identification, purification and quantification of the corresponding antigens, which can be very useful in the standardization of cat allergen extracts as well as in the biochemical and immunochemical study of cat allergens.

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