

Automated specific IgE assay with recombinant allergens: evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia CAP System

R. CRAMERI, J. LIDHOLM*, H. GRÖNLUND*, D. STÜBER†, K. BLASER and G. MENZ‡

Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland, *Pharmacia Diagnostics AB, Uppsala, Sweden, †Hoffmann-La Roche Ltd, Basel and ‡The Hochgebirgsklinik, Davos-Wolfgang, Switzerland

Summary

Background We report the results of a study comparing the recombinant *Aspergillus fumigatus* allergen I (rAsp f I) to commercial *A. fumigatus* extracts in serological assays, Pharmacia CAP System and skin tests.

Objective The study was designed to test the feasibility of using recombinant allergens in an automated serology system for determination of allergen-specific IgE.

Methods Patients with allergic bronchopulmonary aspergillosis (ABPA), asthmatics with *A. fumigatus* allergy and control subjects, who included allergic asthmatics without allergy to *A. fumigatus* and healthy subjects, were investigated. All subjects were characterized with respect to their total IgE level, radio allergosorbent test to *A. fumigatus* and skin test reactivity to both commercial *A. fumigatus* extracts and recombinant rAsp f I protein.

Results All patients with ABPA ($n = 30$) showed positive skin test reactions with commercial *A. fumigatus* extracts, and 24 were sensitized to rAsp f I by the same criterion. The 10 patients with asthma and *A. fumigatus* allergy showed positive skin reactions to at least one commercial extract, and five reacted to rAsp f I. All control subjects ($n = 19$) scored negatively in skin tests to *A. fumigatus* extracts and rAsp f I, and showed no detectable rAsp f I-specific IgE. ImmunoCAP carrying immobilized rAsp f I were evaluated using sera from all individuals described and the results compared with those obtained with the rAsp f I-specific ELISA for IgE. The data obtained with the two rAsp f I-specific detection systems correlated closely ($r = 0.997$) and were in perfect agreement with the skin test results.

Conclusion The data show that rAsp f I can be used as immobilized allergen in the Pharmacia CAP System indicating the feasibility of using recombinant allergens for an automated serological diagnosis of allergic diseases. However, every recombinant allergen needs to be evaluated individually for its performance if applied to a new diagnostic technology.

Keywords: *Aspergillus fumigatus*, recombinant allergens, CAP System, rAsp f I, IgE detection, skin tests, automated serology

Clinical and Experimental Allergy, Vol. 26, pp. 1411–1419. Submitted 16 November 1995; revised 20 May 1996; accepted 17 June 1996.

Introduction

The discovery that IgE antibodies are involved in allergic reactions in humans [1–4] has provided the rationale for the development of IgE based laboratory tests for the diagnosis of allergic diseases [5–7]. Specific serum IgE determination is now used routinely in the diagnosis of IgE-mediated allergic diseases. With the introduction of the radio allergosorbent test (RAST) [5], the accuracy of the methods was further improved [8]. In many of the systems available for specific IgE determination, the IgE quantitation is not on an absolute scale [8], which makes comparisons between different *in vitro* IgE test systems difficult. The Pharmacia (Uppsala, Sweden) CAP System, however, is calibrated against the World Health Organization (WHO) standard for IgE, and allows quantitative expression of total or allergen-specific IgE [9]. The quality of solid-phase immunoassays of specific IgE largely depends on the quality of the allergen preparation used [10,11]. Therefore, efforts have been made to standardize allergen preparations [12–14] and guidelines regarding the standardization of extracts proposed [15].

Using well-characterized patients and standardized allergen preparations, an excellent overall correlation between skin test results and RAST can be obtained [16,17]. Although skin testing is a reliable method [15], the standardization of extracts may be difficult due to variability among source materials and extraction processes used, batch-to-batch variations and storage [18–20]. The application of recombinant DNA techniques to produce IgE-binding proteins offers the possibility of producing highly pure, perfectly standardized allergens with defined biochemical properties in virtually unlimited amounts [21,22]. We have chosen *Aspergillus fumigatus* as an example of a complex allergenic system involving more than 40 IgE binding components [23], to investigate the feasibility of relying on recombinant molecules for the diagnosis of allergic conditions.

Aspergillus fumigatus is associated with an extensive spectrum of diseases in humans, ranging from invasive systemic aspergillosis, aspergilloma and saprophytic colonization of the lung, to allergic diseases such as IgE-mediated asthma, allergic rhinitis and sinusitis, hypersensitivity pneumonitis and allergic bronchopulmonary aspergillosis [24]. Unfortunately, efficient diagnosis of pulmonary diseases related to the fungus are hampered by the lack of standardized *A. fumigatus* antigen/allergen preparations [25]. Although the development of new technologies to display cDNA libraries on phage surface [26,27] has speeded up cloning and identification of allergens from *A. fumigatus* [28,29], the structure and biological function of these proteins are largely unknown. One exception is represented by the *A. fumigatus* allergen I (Asp f I), reported

to be a member of the mitogillin family of cytotoxins [30,31] which has been cloned [32], produced at high level in *Escherichia coli* and demonstrated to be of relevance for about 70% of the subjects sensitized to *A. fumigatus* [33–35]. In our effort to establish recombinant allergens as standardized reagents for the diagnosis of allergies, we have evaluated the performance of rAsp f I in the Pharmacia CAP System.

Materials and methods

Subjects

A group of patients with asthma with ($n = 10$) or without ($n = 9$) *A. fumigatus* sensitization, 30 patients with allergic bronchopulmonary aspersellosis (ABPA) and 10 healthy control subjects were enrolled in the study. The patients with asthma met the guidelines for the diagnosis and management of asthma [36]. The patients with ABPA had asthma and fulfilled at least six of the seven diagnostic criteria proposed by Rosenberg *et al.* [37] and Patterson *et al.* [38]. The healthy controls had no history of allergy or asthma and showed normal serum IgE levels. Subject characteristics including age, sex, total IgE and RAST are given in Table I. None of the subjects had taken medications that might interfere with the outcome of the tests before and during the investigations.

Diagnosis of allergy

The diagnosis of allergy and characterization of the patients were based on case history and skin prick tests (SPT). Only well characterized patients with a history of sensitization to *A. fumigatus* or allergic asthmatics with a history of allergy to cat, dog, grass or birch allergens but with RAST class 0 and negative skin test to *A. fumigatus* entered this study (Table I).

Skin-prick tests. Skin prick tests were performed with commercial preparations purchased from Allergopharma (Hamburg, Germany) and Bencard (SmithKline Beecham, Neuss, Germany) and used as recommended by the manufacturers. Histamine dihydrochloride (Streuli & Co., Uznach, Switzerland), at a concentration of 0.1%, and physiological saline solution (SmithKline Beecham) were used as positive and negative controls, respectively. For SPT with rAsp f I, an aliquot of lyophilized rAsp f I [32,33], was dissolved in physiological saline solution and tested at a single concentration of 100 µg/ml as described [33]. Skin tests were considered to be positive when the wheal size induced by the allergen preparation had a surface of at least 9 mm² after SPT [33].

Table 1. Patient characteristics

	Patients with asthma		
	Allergics	<i>Aspergillus fumigatus</i> -allergic	ABPA
No.	9	10	30
Age (years)	42 ± 12	36 ± 15	51 ± 12
Sex ratio (F : M)	5 : 4	6 : 4	17 : 13
Total IgE (kU/l)	228 ± 173	446 ± 130	1734 ± 423
RAST*	—	2.4 ± 0.7	3.5 ± 0.7
SPT (no. positive)†	—	10	30

**Aspergillus fumigatus*-specific IgE determined with the Pharmacia CAP System.

†History of a positive skin-prick test (SPT) against *A. fumigatus*.

ABPA, Allergic bronchopulmonary aspergillosis;

RAST, radio allergosorbent test.

Approval. Ethical approval for skin testing human subjects with recombinant allergens was obtained from the ethical committee. A full explanation of the procedure was given to all participants before testing, and written consent was obtained.

Total IgE and RAST. Total serum IgE levels were determined by radioimmunoassay (RIA) with Pharmacia CAP System IgE RIA according to the manufacturer's instructions. The titration of *A. fumigatus*-specific IgE was performed using Phadebas RAST (Pharmacia) according to the package insert, and results converted into RAST classes as described by the manufacturers.

Production of rAsp f I protein and rAsp f I ImmunoCAP

rAsp f I was produced as N-terminal [His]₆-tagged fusion protein in *E. coli* cells harbouring the rAsp f I construct [32], yielding 15 g of crude rAsp f I in a 100 l fermentation [32,39]. The gene product was purified by Ni²⁺-chelate affinity chromatography [39]. The fully denatured protein was refolded *in vitro* by stepwise dialysis against phosphate-buffered saline, pH 7.4, and distilled water. Determination of protein concentration was performed using the BioRad (Richmond, CA, USA) protein assay with bovine serum albumin as standard. Purity was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and Western blot analysis using monoclonal antibody 4A6 raised against native Asp f I [30] and sera from individuals allergic to *A. fumigatus* [32], as well as by determination of the enzymatic activity [32,40]. Pure biologically active samples of rAsp f I were lyophilized for long-term storage and used for ELISA, skin tests and for the production of rAsp f I ImmunoCAP.

Titration of specific serum IgE

Pharmacia CAP System. IgE antibodies to *A. fumigatus* were measured with Pharmacia CAP System using ImmunoCAP m3 (*A. fumigatus* extract) according to the manufacturer's instructions. Results were obtained as kilo units of allergen-specific IgE per litre (kU_A/l). rAsp f I ImmunoCAP assays were run with the same fluoro-immunoassay version and results expressed quantitatively in kU_A/l of specific anti-rAsp f I IgE.

ELISA. The binding of anti-rAsp f I IgE was analysed by a standard direct solid-phase ELISA using mouse monoclonal antibody TN-142 anti-human IgE [32] and results were expressed as arbitrary ELISA units per millilitre (EU/ml), calibrated with an in-house reference standard [33].

Statistical analysis

Statistical analysis was performed with the two-tailed Mann-Whitney *U*-test. Correlation coefficients and statistical significance were determined with Pearson's linear regression analysis.

Results

Characterization of the patient and control groups

Skin test reactivity to commercial *A. fumigatus* extracts and to rAsp f I, total serum IgE and RAST were used as criteria to classify the status of the individuals tested with respect to the *A. fumigatus* sensitization (Table 1). All subjects showed a positive histamine response and were non-reactive to physiological saline solution in SPT.

All patients with ABPA (*n* = 30) as well as all subjects

Table 2. Total IgE levels, radio allergosorbent test (RAST), *rAsp f* I-specific IgE results and skin test reactivity

Serum	Total IgE (kU/l)	RAST class <i>Aspergillus</i>	m3 CAP (kU _A /l)	<i>rAsp f</i> I IgE (EU/ml)	<i>rAsp f</i> I CAP (kU _A /l)	Skin-prick test*	
						A	B
Healthy controls (<i>n</i> = 10)	62 (5–210)†	0	< 0.35	< 5	< 0.35	–	–
Allergic asthmatics (<i>n</i> = 9)	228 (20–624)†	0	< 0.35	< 5	< 0.35	–	–
<i>A. fumigatus</i> allergic asthmatics							
1	371.0	2	1.22	<5	<0.35	+	+
2	61.4	2	0.70	<5	<0.35	–	–
3	79.1	2	0.78	<5	<0.35	+	+
4	282.0	2	0.75	<5	<0.35	+	+
5	523.0	2	1.79	<5	<0.35	–	–
6	734.9	4	18.40	140.6	6.59	+	+
7	346.0	3	6.66	233.0	11.90	+	+
8	157.0	2	1.89	21.6	0.89	+	+
9	1461.0	2	2.50	84.6	4.32	+	+
10	441.0	3	3.94	23.8	1.15	+	+
Mean values†	445.6 (61–1461)		3.86 (0.7–18.4)	100.7 (21.6–233.0)‡	4.97 (0.98–11.9)‡		
ABPA patients							
1	2643.0	4	43.70	184.0	8.99	+	+
2	1508.0	3	16.20	47.1	2.45	+	+
3	420.0	4	40.50	153.9	7.57	+	+
4	153.0	3	14.60	46.0	2.24	+	+
5	808.9	4	35.60	203.3	9.81	+	+
6	490.0	3	4.48	457.3	22.30	+	+
7	9150.0	4	>100	224.5	9.69	+	+
8	130.0	4	17.90	90.1	4.28	+	+
9	194.0	2	0.99	11.0	0.49	+	+
10	883.0	3	4.71	40.7	1.97	+	+
11	194.0	2	1.54	17.0	0.54	+	+
12	1293.0	3	10.90	873.0	44.70	+	+
13	283.0	2	1.02	12.3	0.48	+	+
14	4030.0	4	25.70	334.0	12.60	+	+
15	228.0	3	10.70	56.0	2.46	+	+
16	1456.0	4	52.30	357.8	17.20	+	+
17	2100.0	4	41.70	168.0	7.77	+	+
18	1508.0	4	19.20	67.0	3.05	+	+
19	581.0	4	69.00	562.3	28.30	+	+
20	609.0	3	15.90	29.5	1.26	+	+
21	6050.0	4	>100	470.5	23.20	+	+

22	1283.0	3	4.02	558.0	26.00	+	+	+
23	4720.0	3	8.04	29.5	1.37	+	+	+
24	581.0	4	64.08	151.7	8.57	+	+	+
25	628.0	4	38.70	<5	<0.35	+	+	+
26	191.0	4	36.10	<5	<0.35	+	+	+
27	1375.0	4	25.60	<5	<0.35	+	+	+
28	7720.0	4	68.50	<5	<0.35	+	+	+
29	191.0	3	8.60	<5	<0.35	+	+	+
30	628.0	4	27.40	<5	<0.35	+	+	+
Mean values†	1734.3 (130–9150)		30.3 (1.02–>100)	214.4 (11–873)‡	10.3 (0.48–44.7)‡	NA	NA	NA
Reference serum	1756.0	4	24.30	100.0	4.85	NA	NA	NA

*To commercial *A. fumigatus* extracts from Allergopharma (A), Bencard (B) or rAsp f I.

†Values expressed as mean, minimum and maximum level indicated in parentheses.

‡Values below the detection level (<5 EU/ml and <0.35 kU_A/l) were not considered for the calculation of the mean values.

NA, not applicable; ABPA, allergic bronchopulmonary aspergillosis.

with a history of sensitization to *A. fumigatus* ($n = 10$) reacted positively to at least one of the commercial *A. fumigatus* extracts tested in SPT (Table 2). Skin-prick test reactivity to rAsp f I was clearly positive in 24 of the patients with ABPA and in five *A. fumigatus*-allergic asthmatics giving an overall sensitization to Asp f I of about 70%. The incidence of Asp f I sensitization among the *A. fumigatus* allergic individuals was in concordance with earlier reports [30,33]. These two groups of patients had a RAST class to *A. fumigatus* of at least 2 at the time of the study (Table 2).

All healthy control subjects ($n = 10$) and all patients with asthma without a history of *A. fumigatus* sensitization showed no reaction to commercial extracts, nor to rAsp f I on SPT. Specific IgE antibodies to *A. fumigatus* were not detectable in these two groups (RAST class 0).

Specific IgE antibodies to rAsp f I: correlation between ELISA, Pharmacia CAP System and skin test results

Sera obtained from the subjects listed in Table 2 were analysed for rAsp f I-specific IgE by ELISA and using the newly developed rAsp f I ImmunoCAP. For specific IgE determinations to *A. fumigatus* extract the commercially available m3 ImmunoCAP was used.

From the total of 40 patients sensitized to *A. fumigatus*, 29 had specific serum IgE against pure recombinant Asp f I protein. All *A. fumigatus* allergic asthmatics or patients with ABPA who had detectable IgE against rAsp f I also responded in SPT to rAsp f I (Table 2). Thus, rAsp f I elicited an immediate type I reaction in skin test in all patients who had a detectable level of rAsp f I-specific IgE, whereas patients with IgE values below the cut-off level were consistently non-reactive to rAsp f I in skin test. The comparison of IgE values obtained by ELISA with those of the rAsp f I ImmunoCAP determinations showed that all sera with <5 EU/ml rAsp f I-specific IgE scored below the cut-off value of the Pharmacia CAP System of <0.35 kU_A/l, in agreement with the skin test results. The rAsp f I-specific ImmunoCAP determination of IgE in sera from individuals with values >5 EU/ml varied between 0.5 and 45 kU_A/l. These variations reflect the levels of rAsp f I-specific IgE which are highly variable from patient to patient and correlate fairly well with the magnitude of the wheal surface obtained in skin tests [33].

All healthy control subjects ($n = 10$) and allergic asthmatics without a history of sensitization to *A. fumigatus* had <5 EU/ml rAsp f I-specific IgE as determined by ELISA and were below the detection limit in rAsp f I-ImmunoCAP assays (< 0.35 kU_A/l) confirming the skin test results (Table 2). In fact, no skin reactivity to commercial *A. fumigatus* extracts or to rAsp f I was observed in the control subjects. The excellent correlation reaching

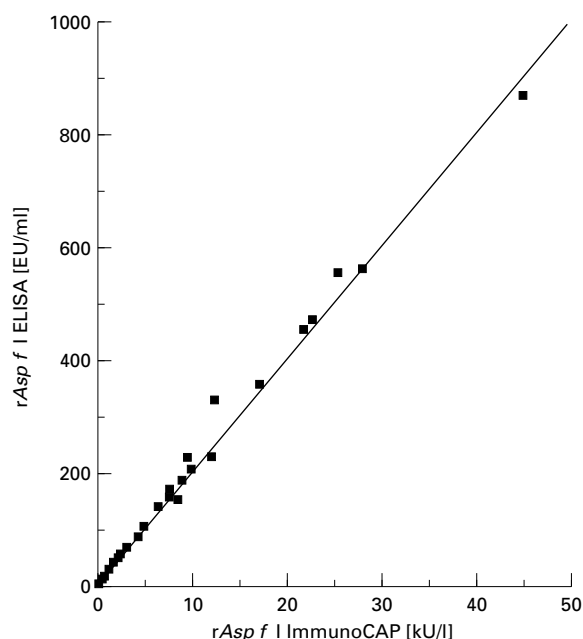


Fig. 1. Correlation between IgE results obtained with the rAsp f I-specific ELISA and rAsp f I ImmunoCAP determinations. $r = 0.997$; $P < 0.0001$.

$r = 0.997$ between the two rAsp f I-specific IgE detection methods (Fig. 1) suggests that the rAsp f I allergen can be incorporated in the Pharmacia CAP System with retained antigenic activity.

As expected, the overall correlation between the values obtained with rAsp f I ImmunoCAP and with the m3 ImmunoCAP prepared with *A. fumigatus* extracts is not conclusive (Fig. 2). This result is related to the incidence of Asp f I sensitization among the *A. fumigatus*-allergic individuals which is in the range of 60–85% and to the variable polyvalent sensitization of these subjects towards different allergenic components present in *A. fumigatus* extracts. ImmunoCAPs are prepared using a constant amount of protein. Therefore, the Asp f I ImmunoCAPs are expected to contain about 200 times more Asp f I protein than the ImmunoCAPs prepared with extract which contains low amounts of Asp f I [33]. This could explain the fact that two *A. fumigatus*-allergic asthmatics (nos 7 and 9, Table 2) and three patients with ABPA (nos 6, 12 and 22, Table 2), who were prevalently sensitized to Asp f I (data not shown), gave substantially higher values ($\text{kU}_\text{A}/\text{l}$) against the recombinant protein than the whole extract in CAP.

DISCUSSION

Laboratory tests for the determination of allergen-specific IgE antibodies are of well-established value in the clinical

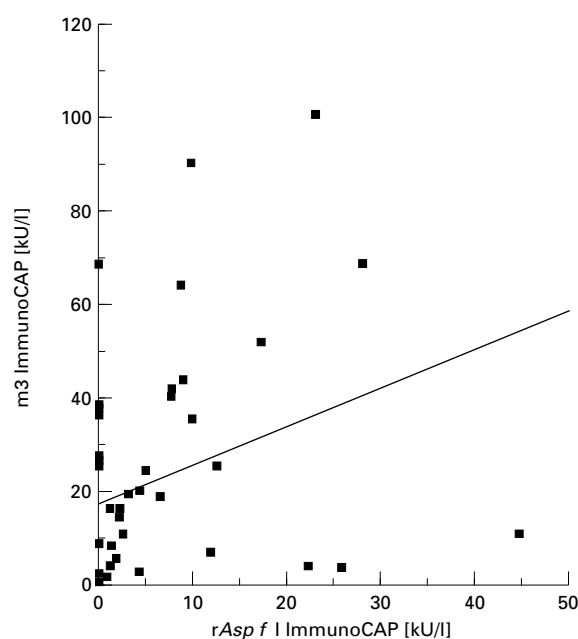


Fig. 2. Correlation between IgE results obtained with commercially available m3 ImmunoCAP prepared with *Aspergillus fumigatus* extract and rAsp f I ImmunoCAP determinations. $r = 0.314$; $P = 0.0425$.

evaluation of allergic diseases [3,5]. However, the accuracy and efficacy of the method require high and consistent quality of the allergen preparation used [11,12] which often necessitates extensive standardization due to several reasons including batch-to-batch variations of the source materials [19,20]. Modern genetic engineering techniques allow the cloning and production of recombinant proteins of consistent quality and purity in virtually unlimited amounts. During the past few years more than 100 complete or partial sequences of allergenic proteins have been reported [41], and some allergens have been produced at high level in different systems [21,32,42] and evaluated for their diagnostic value [33–35,43]. The results so far are encouraging and demonstrate the potential advantages of recombinant technologies over classical methods for the preparation of standardized allergens. Use of recombinant allergens may help to solve many of the problems related to extraction procedures, stability and standardization of allergenic extracts [44]. Accordingly, recombinant allergens may contribute to a significant improvement of the tools for diagnosis of allergic conditions, provided that they become available in a pure, fully antigenic form suitable for routine assessments.

In this study we report the evaluation of a single recombinant allergen immobilized to the Pharmacia CAP System, a fully automated system for the measurement of circulating IgE antibodies. One advantage of this system is

the calibration against the international reference standard for IgE recognized by the WHO. This permits expression of IgE levels in quantitative terms [9].

The investigations entailed SPT and serological analyses of 59 individuals: 30 patients with ABPA, 10 with allergic asthma and *A. fumigatus* sensitization, nine allergic asthmatics without *A. fumigatus* sensitization and 10 healthy controls. All subjects were tested for skin responses by challenge with commercial *A. fumigatus* extracts and with rAsp f I protein. Of the total of 40 individuals sensitized to *A. fumigatus* (30 ABPA and 10 *A. fumigatus*-allergic asthmatics), all subjects reacted to commercial extracts on SPT, whereas 29 individuals (72.5%) responded positively to skin challenges with rAsp f I (Table 2).

The results are in agreement with earlier reports showing that the Asp f I allergen elicits IgE antibody responses in 60–85% of the subjects sensitized to *A. fumigatus* [30,33]. None of the 19 individuals from the negative control groups reacted to commercial *A. fumigatus* extracts or to rAsp f I on SPT (Table 2). rAsp f I-specific IgE was detected by an allergen-specific ELISA only in subjects responding with immediate type I reactions in skin tests with rAsp f I, whereas the 11 patients with negative skin test results as well as the 19 subjects from the negative control groups had rAsp f I-specific IgE values below the detection limit of the test (<5 EU/ml).

The results obtained with the rAsp f I ImmunoCAP were in perfect accordance with the results from SPT and with the ELISA determinations. All sera of subjects unable to respond to rAsp f I challenge on SPT had no detectable rAsp f I-specific IgE on ELISA and scored below the cut-off value of the ImmunoCAP (≤ 0.35 kU_A/l). The rAsp f I ImmunoCAP determination in sera of patients with positive SPT to rAsp f I and ELISA values > 5 EU/ml varied between 0.5 and 45 kU_A/l (Table 2). The close correlation between the rAsp f I-specific values obtained with ELISA and ImmunoCAP determinations (Fig. 1) shows that the rAsp f I protein exhibits similar immunological properties after immobilization to different solid phases.

The results of this study corroborate earlier reports and indicate that recombinant allergens should be suitable for the development of reagents allowing a fully automated diagnosis of allergic conditions. However, every recombinant allergen has to be individually evaluated for its performance if applied to a new diagnostic technology to demonstrate that the IgE-binding activity is not influenced by the chosen immobilization procedure. As expected, a single allergen is not sufficient to set up a reliable diagnosis able to cover all subjects sensitized to *A. fumigatus* (Table 2). The fungus can produce more than 40 allergenic components and elicits a highly variable pattern of IgE reactivity depending on the status of sensitization of the individuals tested [45].

A panel of recombinant *A. fumigatus* allergens cloned

from a phage surface display library [27,28] will allow the investigations to be extended in order to test the feasibility of using a defined mixture of recombinant allergens to diagnose allergy to *A. fumigatus*. Moreover, single recombinant allergens will open up the possibility of investigating patient-specific reactivity patterns and perhaps correlating specific reactivity patterns with different pulmonary complications related to the fungus.

Acknowledgements

The authors are grateful to Dr C. H. Heusser (Ciba-Geigy, Basel, Switzerland) for providing TN-142 anti-human IgE monoclonal antibody, to B. Real for the excellent technical assistance and to Dr A. Faith and S. Hemmann for careful reading of the manuscript. Supported in part by a Swiss National Science Foundation Grant (no. 31–39429.93 to R. Crameri).

References

- 1 Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. Correlation of reaginic activity with γ E-globulin antibody. *J Immunol* 1966; 97:840–53.
- 2 Johansson SGO. Raised levels of a new immunoglobulin class (IgND) in asthma. *Lancet* 1967; 2:951–3.
- 3 Spitz E, Gelfand EW, Sheffer AL, Austen KF. Serum IgE in clinical immunology and allergy. *J Allergy Clin Immunol* 1972; 49:337–47.
- 4 Stanworth DR. The discovery of IgE. *Allergy* 1993; 48:67–71.
- 5 Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an *in vitro* test for antibodies. *Lancet* 1967; 2:1105–7.
- 6 Merret J, Merret TG. Phadiatop — a novel IgE antibody screening test. *Clin Allergy* 1987; 17:409–16.
- 7 Kleine-Tebbe J, Eickholt M, Gätjen M, Brunnée T, O'Connor A, Kunkel G. Comparison between MAGIC LITE^R- and CAP^R-system: two automated specific IgE antibody assays. *Clin Exp Allergy* 1992; 22:475–84.
- 8 Bernstein IL. Proceedings on the task force on guidelines for standardizing old and new technologies used for the diagnosis and treatment of allergic diseases. *J Allergy Clin Immunol* 1988; 82:499–504.
- 9 Axen R, Drevin H, Kober A, Yaman L. A new laboratory diagnostic system applied to allergy testing. In: Johansson SGO, ed. Proceedings of a clinical workshop on IgE antibodies and the Pharmacia CAP system in allergy diagnosis. Uppsala, Sweden:Pharmacia, 1988:3–5.
- 10 Berg T, Johansson SGO. *In vitro* diagnosis of atopic allergy. V. The value of repeated testing using several batches of allergen extract. *Int Arch Allergy* 1976; 51:471–8.
- 11 Schröder H. RAST-based techniques for allergen assay. In: Brede HD, Goings H, eds. Proceedings of the first Paul Ehrlich seminar on regulatory control and standardization of allergenic extracts. Stuttgart: G. Fischer Verlag, 1980:139–44.
- 12 Aas K, Bakman A, Belin L, Weeke B. Standardization of

- allergen extracts with appropriate methods: the combined use of skin prick testing and radioallergosorbent test. *Allergy* 1978; 29:130–7.
- 13 Dreborg S, Einarson R. The major allergen content of allergenic preparations reflects their biologic activity. *Allergy* 1992; 47:418–23.
 - 14 Kauffman HF, van der Heide S, van der Laan S, Hovenga H, Beaumont F, de Vries K. Standardization of allergenic extracts of *Aspergillus fumigatus*: liberation of IgE-binding components during cultivation. *Int Arch Allergy Appl Immunol* 1985; 76:168–73.
 - 15 Dreborg S, Frew A (eds). Position paper: Allergen standardization and skin tests. *Allergy* 1993; 48:48–82.
 - 16 Eriksson NE, Ahsted A, Belin L. Comparison between RAST, skin tests, and provocation tests. *Int Arch Allergy Appl Immunol* 1976; 52:335–46.
 - 17 Niemeijer NR, Fluks AF, de Monchy JGR. Optimization of skin testing. II. Evaluation of concentration and cutoff values, as compared with RAST and clinical history, in a multicenter study. *Allergy* 1993; 48:498–508.
 - 18 Bousquet J, Djoukhard F, Hewitt B, Guerin B, Michel FB. Comparison of the stability of a mite extract and a pollen extract stored in normal conditions of use. *Clin Allergy* 1985; 15:29–35.
 - 19 Logbottom JL, Austwick PKC. Antigens and allergens of *Aspergillus fumigatus*. I. Characterization by quantitative immunoelectrophoretic techniques. *J Allergy Clin Immunol* 1986; 78:9–17.
 - 20 Schumacher M, Jeffery S. Variability of *Alternaria alternata*: biochemical and immunochemical characteristics of culture filtrates from seven isolates. *J Allergy Clin Immunol* 1976; 58:263–77.
 - 21 Dudler T, Chen W-Q, Wang S *et al*. High-level expression in *Escherichia coli* and rapid purification of enzymatically active honey bee venom phospholipase A₂. *Biochim Biophys Acta* 1992; 1165:201–10.
 - 22 Baldo BA. Structural features of allergens large and small with emphasis on recombinant allergens. *Curr Opin Immunol* 1991; 3:841–50.
 - 23 Wellenbeck I, Aukrust L, Einarsson R. Antigenic variability of different strains of *Aspergillus fumigatus*. *Int Arch Allergy Appl Immunol* 1984; 73:166–72.
 - 24 Bardana EJ. The clinical spectrum of aspergillosis. Part 2: classification and description of saprophytic, allergic and invasive variants of human disease. *CRC Crit Rev Clin Lab Sci* 1980; 13:85–159.
 - 25 Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 1991; 4:439–56.
 - 26 Crameri R, Suter M. Display of biologically active proteins on the surface of filamentous phages: a cDNA cloning system for selection of functional gene products linked to the genetic information responsible for their production. *Gene* 1993; 137:69–75.
 - 27 Crameri R, Hemmann S, Blaser K. pJuFo: a phagemide for display of cDNA libraries on phage surface suitable for selective isolation of clones expressing allergens. In: Schon A, Kraft D, eds. *Molecular biology of allergens and the atopic immune response*. New York: Plenum, 1996 (in press).
 - 28 Crameri R, Jaussi R, Menz G, Blaser K. Display of expression products of cDNA on phage surfaces. A versatile screening system for selective isolation of genes by specific gene-product/ligand interaction. *Eur J Biochem* 1994; 226:53–8.
 - 29 Crameri R, Blaser K. Cloning allergens from *Aspergillus fumigatus*: the filamentous phage approach. *Int Arch Allergy Immunol* 1995; 107:460–1.
 - 30 Arruda KL, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990; 172:1529–32.
 - 31 Lamy B, Moutaouakil M, Latge J-P, Davies J. Secretion of a potential virulence factor, a fungal ribonucleotoxin, during human aspergillosis infections. *Mol Microbiol* 1991; 5:1811–5.
 - 32 Moser M, Crameri R, Menz G *et al*. Cloning and expression of recombinant *Aspergillus fumigatus* allergen I/a (rAsp f I/a) with IgE binding and type I skin test activity. *J Immunol* 1992; 149:54–60.
 - 33 Moser M, Crameri R, Brust E, Suter M, Menz G. Diagnostic value of recombinant *Aspergillus fumigatus* allergen I/a for skin testing and serology. *J Allergy Clin Immunol* 1994; 93:1–11.
 - 34 Disch R, Menz G, Blaser K, Crameri R. Different reactivity to recombinant *Aspergillus fumigatus* allergen I/a in patients with atopic dermatitis or allergic asthma sensitised to *Aspergillus fumigatus*. *Int Arch Allergy Immunol* 1995; 108:89–94.
 - 35 Nikolaizic WH, Moser M, Crameri R *et al*. Identification of ABPA in CF patients by recombinant *Aspergillus fumigatus* allergen I/a serology. *Am J Respir Crit Care Med* 1995; 152:634–9.
 - 36 Sheffer AL (ed). Guidelines for the diagnosis and management of asthma. *J Allergy Clin Immunol* 1991; 82:427–38.
 - 37 Rosenberg M, Patterson R, Mintzer R, Cooper J, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann Int Med* 1977; 86:405–14.
 - 38 Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. *Ann Int Med* 1982; 96:286–91.
 - 39 Stüber D, Matile H, Garotta G. System for high level production in *Escherichia coli* and rapid purification of recombinant proteins: application to epitope mapping, preparation of antibodies and structure-function analysis. In: Lefkovits I, Pernis B, eds. *Immunologic methods*. New York: Academic Press, 1990:121–52.
 - 40 Endo Y, Huber PW, Wool IG. The ribonuclease activity of the cytotoxin α -sarcin. *J Biol Chem* 1983; 258:2662–7.
 - 41 Bousquet J, Lehrer S, Lowenstein H, Norman PS, DeWeck AL. Special report from the IUIS/WHO Allergen Standardization Committee. *Allergy Clin Immunol News* 1994; 6:38–44.
 - 42 Chua KY, Parminder KK, Thomas WR, Vaughan PR, Macreade IG. High-frequency binding of IgE to *Der p* I

- allergen expressed in yeast. *J Allergy Clin Immunol* 1992; 89:95–102.
- 43 Müller UR, Dudler T, Schneider T *et al.* Type I skin reactivity to native and recombinant phospholipase A₂ from honey bee venom is similar. *J Allergy Clin Immunol* 1995; 96:395–402.
- 44 Creticos PS, Norman PS. Why standardized extracts are necessary. *Clin Rev Allergy* 1986; 4:355–61.
- 45 Borga A. Allergens of *Aspergillus fumigatus*. PhD thesis, Karolinska Institute, Repro Print AB, Stockholm, 1990.