

Recombinant Allergens for Skin Testing

Peter Schmid-Grendelmeier Reto Crameri

Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

Key Words

Recombinant allergen · Skin prick test · Intradermal test · Allergy · Diagnosis

Abstract

Skin testing is a basic diagnostic procedure widely used to explore immediate-type reactions to allergen preparations in vivo. Despite their reliability, if standardized extracts are used, skin tests suffer from limited reproducibility due to difficulties in preparing consistently standardized extracts from natural raw material. Starting from allergen-encoding cDNAs, large amounts of highly pure allergens with a high batch-to-batch consistency satisfying the quality requirements of medicinal products manufactured by recombinant DNA technology can be produced. These reagents are expected to be qualitatively superior to the commercially available allergen preparations used for the in vitro and in vivo diagnosis of allergic conditions. In this article the current literature available on skin testing with such recombinant allergens (rAllergens) is reviewed and critically analyzed. To date many different rAllergens of various pollens, moulds, mites, bee venom, latex and celery have been used in skin testing in more than 1,600 allergic and control individuals. Skin prick tests as well as intradermal skin tests with rAllergens prove to be highly specific and safe. The diagnostic sensitivity of single rAllergens is generally lower than those obtained with allergen extracts, but can be considerably increased by using rAl-

lergen panels covering the most important allergenic structures present in a given complex allergenic extract. Moreover, quantitative skin testing with single rAllergens allows interesting insights into correlations between the in vivo and in vitro sensitization to a given allergen. In conclusion, skin testing with rAllergens offers a highly specific and safe additional diagnostic tool to elucidate patient- and disease-specific sensitization patterns which will be needed for the development of patient-tailored immunotherapeutic treatments.

Copyright © 2001 S. Karger AG, Basel

Introduction

Immediate-type allergies, also called type I allergies, are a daily problem in each allergologist's practice. The diagnosis of IgE-mediated allergy is based on clinical history and skin test reactivity to appropriate allergen preparations corroborated by in vitro allergen-specific IgE determinations. Rather seldom, the diagnosis is verified by additional provocation tests.

Skin prick tests (SPT) and intracutaneous or intradermal skin tests (IDT) performed with commercially available extracts are the most commonly used techniques. According to the technique used, a lower (SPT) or higher (IDT) sensitivity can be achieved. The scratch skin test method should be used only in limited cases due to a low specificity [1]. Skin tests are easy to perform and allow a visualization of sensitization within a few minutes. A

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1252–0096\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Peter Schmid-Grendelmeier
Swiss Institute for Allergy and Asthma Research (SIAF)
Obere Strasse 22
CH–7270 Davos (Switzerland)
Tel. +41 81 410 08 52, Fax +41 81 410 08 40, E-Mail peter.schmid@hin.ch

wide choice of skin test solutions is commercially available, including numerous inhalant, food and other allergens. Also crude material such as raw food (prick-to-prick technique) or samples of house dust or animal dander can be used as test solution [2]. Skin tests have also been shown to be generally safe, sensitive and reliable if standardized allergen extracts are used. Both the American Academy of Allergy, Asthma and Immunology [3] and the European Academy of Allergology and Clinical Immunology [1] strongly recommend the use of standardized extracts for the diagnosis of allergic conditions. However, it is widely acknowledged that there are several pitfalls associated with current standardization methods (see below). In fact only a few internationally recognized reference allergen extracts are available [3]. The goal of standardization is to produce allergen extracts consistent from manufacturer to manufacturer and with a high batch-to-batch reproducibility. This will improve sensitivity and specificity of the diagnosis which is a prerequisite for improvement in safety and efficacy of extracts used for immunotherapy [4]. The application of new technologies to allergen standardization, particularly the use of recombinant allergens (rAllergens) as standardized single components, bears a high potential to facilitate objective comparisons of test results obtained in different studies. Due to simplicity, safety and rapid results obtained, skin test procedures are among the most helpful basic tools available for management of allergic patients. Here we summarize the state of the art resulting from the use of rAllergens in clinical practice.

Use of Crude Allergens or Allergen Extracts for Skin Testing – and Its Limitations

Allergen extracts prepared from natural sources are complex mixtures containing, besides the relevant allergenic proteins in unknown concentrations, many non-allergenic proteins, carbohydrates, and low-molecular-weight substances like histamine [5] or immunosuppressive compounds [6]. The quality of the extracts is influenced by many factors including quality of the starting raw material [7], extraction procedure [8], protease content [9] and storage conditions [10]. These factors strongly influence the final composition, potency and stability of allergen preparations and, as a consequence thereof, the outcome of diagnostic tests *in vitro* [11] and *in vivo* [12]. Most extracts derived from natural sources vary in allergen composition and content and generate discrepant results between simultaneously performed *in vitro* IgE

determination and skin tests [13]. Standardization of allergen extracts is therefore essential to avoid variation in sensitivity and specificity of allergy diagnoses [1]. The quality of a given extract does not depend only on the extraction procedure [8, 14], but also from the starting material used for preparation. It makes a huge difference if whole-body extracts of honey bees or just the poison is used as allergen source [15], if dander or pelt extracts or saliva of cats are used [16] or if a mould extract is prepared from a young or from an old culture [14, 17, 18]. Moreover, natural allergen extracts can be contaminated with allergens from other sources [19], and their composition is subject to variation resulting from proteases co-extracted during preparation [20]. Proteolytic enzymes may or may not be allergenic components of the extract [21, 22], but cause in any case degradation and loss of potency of the extracts either used for diagnostic or immunotherapeutic purposes [10]. Batch-to-batch variations in allergen extracts represent one of the main problems for the reproducibility of the diagnosis of allergy [12] and hamper direct comparison of results obtained in different studies [23, 24].

Standardization of allergen extracts has improved considerably during the last decades concomitantly with the progress achieved in the fields of biochemistry and protein chemistry. For most inhalant allergens, biological standardization procedures based on intradermal skin testing of the most sensitive patients have been introduced [1, 3]. To date, standardized extracts approved by the FDA are available for cat hair, cat pelt, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, short ragweed and for most of the *Hymenoptera* venoms [3]. Many reasonably standardized pollen extracts are commercially available [3]. Most allergen preparations derived from complex allergenic sources like fungi, moulds, yeasts, food allergens and some pollens are still not yet available as internationally recognized, standardized products [2, 3]. In contrast to most pollen and *Hymenoptera* venoms, these allergenic sources contain complex patterns of allergenic molecules ranging from 20 to over 80 different molecular structures [25, 26]. Many of these single allergens are unstable and therefore strongly influenced by the storage procedure [27]. On the other hand, the time-dependent production of different allergens during different phases of cultivation of moulds and fungi makes a standardization of these extracts an almost impossible task [14, 17, 18]. It is well recognized that due to the reasons mentioned, significant differences in both sensitivity and specificity between extracts purchased from different manufacturers do exist [28]. Extracts used to prepare immunoCAPs, the most widely used method

for the determination of allergen-specific IgE in vitro which is standardized against the WHO standard for IgE [29], are not available as skin test solutions. Therefore, in most cases a comparison between skin reactivity and allergen-specific serum IgE is of limited value. Besides unassessed toxic effects, unspecific histamine release and potential infectiosity of crude material used in skin testing, the unknown concentration of each single allergenic molecule in commercial extracts should be regarded as a major problem in comparative studies. Skin reactivity depends basically on two variables: the allergen concentration of the test preparation used that can be directly influenced by standardization and the sensitivity of the subject which cannot be directly influenced at the time point of the first diagnosis. Thus, the importance of standardization of allergen extracts which can be directly influenced by manufacturing procedures can hardly be overestimated.

Recombinant Allergens

The general aspects of rAllergens for diagnostic and therapeutic use has recently been extensively reviewed elsewhere [30–34]. The rapid progress achieved in the fields of protein analysis and allergen cloning offers new opportunities for improved standardization of allergen preparations. Many of the relevant allergens from pollen [35], dust mites [36], animal danders [37], moulds and fungi [38, 39], insect venoms [40] and foods [41] have now been defined at molecular level through gene cloning. Starting from the encoding cDNAs the corresponding recombinant antigens/allergens can be biotechnologically expressed in heterologous hosts at laboratory scale up to industrial production and easily purified to homogeneity [42].

Recombinant allergens produced in several expression systems like bacteria [42], yeast [43] or insect viruses [44] often show allergenic properties comparable to those of the biochemically purified natural allergens. However, in contrast to the natural allergens present in extracts, single rAllergens can be carefully quantified in terms of protein concentration and IgE reactivity. Although cloning and characterization of single allergens may be time-consuming, the advantages are manifold. The biotechnological processes allow the production of large quantities of highly pure proteins with reproducible batch-to-batch consistency satisfying the requirements needed for pharmacological use. Therefore, problems related to contamination of the preparation with IgE-binding natural components

[19], altered allergenicity due to extraction procedures and variability on allergen content can be circumvented. Moreover, the same allergen preparation can be used for both in vitro and in vivo tests allowing a direct comparison of the diagnostic results obtained with different methods [45–47]. Beside diagnostic applications, rAllergens are powerful reagents to study the mechanisms involved in allergic reactions [48] and to study structure-function relationships [49]. The elucidation of the primary structure of allergens has already strongly contributed to our understanding of cross-reactivity among apparently unrelated allergenic sources. Shared T- and B-cell epitopes between structurally related allergens present in different allergenic sources [50] offer a rational explanation for the old known problem of polysensitization [51]. It has become evident that recognition of a protein by IgE antibodies strongly depends on the three-dimensional structure of the allergen and that receptors on T cells recognizes allergens as processed linear peptides in association with MHC class II molecules on antigen-presenting cells [52, 53]. Although the allergenicity of proteins does not depend directly on their primary structure, determination of the three-dimensional structures of allergens, either by NMR techniques, x-ray crystallography, or computer-based modelling provided further evidence for the importance of structural shapes in IgE binding [52, 54–59]. Molecular biology provides us with powerful tools suitable to study allergy in details which need to be fully exploited to improve diagnosis and treatment of allergic diseases.

The Potential of rAllergens for Diagnostic Application

The crucial question for the use of rAllergens in diagnosis – and also therapy – of allergic conditions is if the recombinant proteins show a comparable allergenicity to their natural counterparts. Due to incorrect folding, lack of post-translational modification, or cloning of isoforms, differences in the relevant IgE-binding capacity compared to the natural counterpart may arise [43]. However, it has been largely demonstrated that rAllergens with comparable or even superior IgE-binding capacity to their native counterpart can be produced [60, 61]. Of course, the performance of each single allergen needs to be assessed individually if applied to a new diagnostic technology [29]. Nevertheless, rAllergens have demonstrated to be far superior to conventional extracts in serologic detection of allergic bronchopulmonary aspergillosis [62], a complex

clinical syndrome related to exposure to *Aspergillus fumigatus* difficult to be discriminated from *A. fumigatus* allergy [63]. The fact that rAllergens can be used as immobilized allergens in the Pharmacia CAP System allows an automated serologic diagnosis of allergic conditions [29, 64]. This makes a direct correlation of the quantitatively determined allergen-specific serum IgE with the results obtained in skin tests possible [46, 65].

Repertoire cloning using advanced technologies based on phage surface display and high throughput screening of enriched libraries [66, 67] allowed the elucidation of virtually all IgE-binding molecules produced by *A. fumigatus*, probably one of the most complex allergenic sources. The availability of the allergen repertoire in the form of single standardized recombinant components will allow to determine individual reactivity patterns for each single subject tested. This component-resolved diagnosis (CRD) opens new perspectives for the preparation of patient-tailored mixtures to be used in specific immunotherapy [60, 68].

A good example showing the practical potential of basic allergy research for clinical applications is bee venom allergy. Mapping of T-cell epitopes allowed exact definition of the fragments of PLA₂ involved in T-cell help [48]. The chemically synthesized peptides were successfully used to immunize bee-venom-allergic subjects without side effects [69]. This provides the proof of concept for clinical exploitation of knowledge derived from studies devoted to elucidate the mechanisms of immune responses in vitro studying the responses of cell cultures to allergen exposure [48]. A great potential for future applications in immunotherapy derives from the possibility to manipulate the coding sequences of allergens to produce hypoallergenic variants, e.g. molecules retaining the important structural features for the induction of T-cell-mediated immune responses but lacking the capability to bind IgE [70]. For various allergenic sources including birch and grass pollen allergens, as well as peanut and mite allergens, hypoallergenic variants have been genetically engineered, produced and partly evaluated in clinical studies [71–80].

However, the final proof that a recombinant allergen acts as such in vivo has to be delivered by clinical tests. The by far simplest and least dangerous experimental assessment of the allergenicity of a substance is its use in skin tests [1, 3, 81]. Mostly due to ethical and legal restrictions regulating the use of recombinantly produced proteins in humans, skin tests with rAllergens have been rarely performed. The first skin tests with rAllergens were described in 1992 [82], many years after cloning of the first allergen from house dust mite [83]. Recently, more and more work has been done in this direction and rAllergens from pollen,

mites, various fungi, bee venom, celery and latex (table 1) have been evaluated in skin tests. The rapidly increasing data acquired in this field are summarized below.

rAllergens Evaluated in Skin Tests

A wide range of allergens covering different molecular structures have been tested for their performance in skin tests and will be summarized according to the corresponding allergenic source. Detailed data regarding the skin test method and allergen concentration used, number of individuals tested and pertinent references are reported in table 1.

Pollens

From the over 700 publications reported in the literature describing rAllergens, more than 250 deal with cloning and characterization of pollen allergens, highlighting the predominant role of pollen as a cause of allergic diseases. With the exception of one report describing the reactivity of rOle e 1 from olive pollen [84], and several studies evaluating rPhl p 1, 2, 3 and 6 from timothy grass [60, 85, 86], all other reports concern the major birch pollen allergen Bet v 1 and the minor allergen Bet v 2 of *Betula verrucosa* (see details reported in table 1). The Bet v 1 family consists of a considerable number of highly homologous pathogenesis-related proteins identified in species from the entire plant kingdom [35]. Bet v 1-homologous proteins from apple, celery, carrot and cherry consist of 153–159 amino acid residues and share sequence similarities of approximately 60% [35]. The three-dimensional structure of Bet v 1 has been solved [54, 55] and recently co-crystallization of Bet v 1 with a Bet v 1-specific Fab fragment has been reported [52]. Thus, Bet v 1 represents the best investigated allergen at all. Many isoforms of Bet v 1 have been reported, some of them lacking the ability to bind IgE but retaining the full capability of stimulating T-cell proliferation [70]. These naturally occurring isoforms as well as genetically engineered variants lacking IgE-binding capability are considered as potential candidates for improvement of allergen-specific immunotherapy [71–76]. Bet v 1 fragments as well as dimerized and trimerized molecules have been examined for their ability to elicit type I skin reactions in vivo delineating new possibilities for the generation of potentially safe vaccines by genetic engineering [61, 72, 76]. Profilin, a minor allergen of the birch *B. verrucosa* (Bet v 2), is a phylogenetically highly conserved protein regulating actin polymerization [87] which has been demonstrated to be an allergen present in many sources [88]. This ubiquitous 14-kD actin-binding protein family has been proposed to be one

Table 1. Recombinant allergens used for skin testing

Allergen	Production host	cDNA source	Type of skin test	Patients ^a	Concentration used	Side effects	Ref.
<i>Mite</i>							
rDer p 5	<i>E. coli</i>	<i>D. pteronyssinus</i>	IDT	45 mite allergics	1 mg/l	No	97
rDer p 2	<i>E. coli</i>	<i>D. pteronyssinus</i>	SPT	11 healthy controls	1, 10, 100 mg/l	No	98
rDer p 2				60 mite allergics			
rDer p 5				12 healthy controls			
rDer p 7	<i>E. coli</i>	<i>D. pteronyssinus</i>	SPT	170 mite allergics	1, 10, 100 mg/1	No	99
rDer p 2				7 allergics	5 mg/l	No	100
rBlo t 5				<i>D. pteronyssinus</i>	7 healthy controls	10 ⁻⁵ –10 mg/l	No
rDer p 5	<i>E. coli</i>	<i>D. pteronyssinus</i>	SPT	75 mite allergics	20 mg/l		
rDer p 5			IDT	31 mite allergics	10 ⁻⁵ –10 ⁰ mg/l		
rBlo t 5	<i>E. coli</i>	<i>B. tropicalis</i>	SPT	75 mite allergics	5–50 mg/l	No	101
rDer f 11			IDT	31 mite allergics			
			<i>E. coli</i>	<i>D. farinae</i>			
rCra-A	<i>E. coli</i>	<i>P. americana</i>	SPT	22 cockroach allergics	100 mg/l	No	155
				15 healthy controls			
	<i>E. coli</i>	<i>B. tropicalis</i>	SPT	51 mite allergics	5–50 mg/l	No	96
rBlo t 5	<i>E. coli</i> / Baculovirus	<i>L. destructor</i>	SPT	44 allergics	1, 10, 100 mg/l	No	95
rDer p 2				19 atopics			
rLep d 2				19 healthy controls			
rTyr p 2	<i>E. coli</i>	<i>T. putrescentiae</i>					
<i>Pollen</i>							
rBet v 1	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	35 birch allergics	3–50 mg/l	No	93
				16 atopics			
rBet v2	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	7 healthy controls	r Bet v 1 5/20 mg/l r Bet v2 5/20 mg/l	No	92
rBet v 1				20 birch allergics			
rBet v 2				2 healthy controls			
rBet v 1	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	51 birch allergics	100 mg/l	No	156
				8 healthy controls	10 ⁻² –10 ⁻⁶ mg/l	No	60
				28 birch allergics	1–10 mg/l		
rBet v 1	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	27 atopics	1, 10, 100 mg/l	No	72
rBet v 2				10 healthy controls			
				23 birch allergics			
Fragments	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	12 healthy controls	10 mg/l	No	61
Trimer ^b				48 birch allergics			
rBet v 1d				11 atopics			
		10 healthy controls					
rBet v 1	<i>E. coli</i>	<i>B. verrucosa</i>	SPT	29 birch allergics	nBet v1 r Bet v1: 10/50 mg/l	No	76
Fragments				10 healthy controls			
Dimer, trimer ^b							
			IDT		0.1 mg/l		
rOle e 1	<i>P. pastoris</i>	<i>O. europaea</i>	SPT	33 olive allergics	0.1–100 mg/l	No	84
				10 atopics			
				10 healthy controls			
rPhl p 1	<i>E. coli</i>	<i>P. pratense</i>	SPT	52 grass allergics	1–10 mg/l	No	60
rPhl p 2				1 atopic			
rPhl p 3				10 healthy controls			
<i>Fungal</i>							
rAsp f I/a	<i>E. coli</i>	<i>A. fumigatus</i>	SPT	9 ABPA	100 mg/l	No	82
				5 asp. allergics			
				6 atopics			
	<i>E. coli</i>		SPT	7 healthy controls	10 ⁻² –10 ⁻⁵ mg/l	No	45
rAsp f I/a				15 ABPA			
				10 asp. allergics			
	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	6 atopics	0.1–10 mg/l	No	46
				7 healthy controls			
rAsp f 3				11 ABPA			
				8 asp. allergics			
				4 healthy controls			

Table 1 (continued)

Allergen	Production host	cDNA source	Type of skin test	Patients ^a	Concentration used	Side effects	Ref.
rAsp f I/a	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	15 atopic dermatitis 10 asp. allergics 10 healthy controls	10 ⁻⁵ –10 mg/l	No	107
rAsp f I	<i>E. coli</i>	<i>A. fumigatus</i>	SPT	30 ABPA 10 Asp f-allergic	100 mg/l	No	64
rAsp f 6	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	4 asp. f 6 all 2 atopics 2 healthy controls	10 ⁻⁵ –1 mg/l	No	103
rAsp f I/a	<i>E. coli</i>	<i>A. fumigatus</i>	SPT	6 CF-ABPA 25 CF-asp. allergics 13 CF controls	100 mg/l	No	13
rAsp f 1	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	60 ABPA	0.1–1 mg/l	No	62
rAsp f 3	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	40 asp. allergics 20 healthy controls			
rAsp f 6	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	11 ABPA 8 asp. allergics 8 healthy controls	0.1–1 mg/l	No	46
rAsp f 4	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	12 ABPA 12 asp. allergics 5 healthy controls	10 ⁻² –10 mg/l	No	65
rAsp f 6	<i>E. coli</i>	<i>A. fumigatus</i>	IDT	8 asp. allergics 2 healthy controls	10 ⁻⁵ –1 mg/l	No	104
rCop c 1	<i>E. coli</i>	<i>C. comatus</i>	SPT	5 <i>C. comatus</i> allergics 5 atopic controls	11 pg/μl–479 ng/μl	No	111
<i>Bee venom</i>							
rPLA 2	<i>E. coli</i>	<i>A. mellifera</i>	IDT	6 BV allergics 6 healthy controls	10 ⁻⁸ –10 ⁻² mg/l	No	116
refolded/nonrefolded	<i>E. coli</i>	<i>A. mellifera</i>	IDT	85 BV allergics 21 healthy controls	10 ⁻⁸ –10 ⁻² mg/l	No	117
<i>Latex</i>							
rHev b	<i>E. coli</i>	<i>H. brasiliensis</i>	SPT	29 latex allergics 5 atopics 5 healthy controls	Hev b 2, 5, 7 1,000 mg/l Hev b 6, 8, 500 mg/l Hev b 3, 200 mg/l Plus serial 10-fold dilutions	No	134
<i>Celery</i>							
rApi g 1	<i>E. coli</i>	<i>A. graveolens</i>	SPT IDT	24 celery & birch allergics (CH) 12 celery allergics (F) 5 healthy controls	10, 100, 1,000 mg/l 10 ⁻² –10 mg/l	No	121

^a Atopic: individuals without sensitization to the investigated allergen suffering from allergy to other sources; asp. allergics = individuals sensitized to *A. fumigatus*; ABPA = patients suffering from allergic bronchopulmonary aspergillosis; CF = patients suffering from cystic fibrosis.

^b Genetically engineered fragments, dimers and trimers of Bet v 1.

of the factors accounting for the observed cross-reactivity occurring between pollinosis and vegetable-food allergy [89]. Profilin was the first human molecular structure shown to be able to bind IgE from birch-sensitized individuals in vitro [90]. However, skin reactivity of the human recombinant protein could not yet be demonstrated [91]. In contrast, Bet v 2 shows skin reactivity in some birch-sensitized individuals [29, 60, 92, 93], and it would be of interest to test different profilins with regard to their cross-reactivity in vivo.

Mites

Mite allergens can be classified into different structural groups according to their sequence homology [36, 83]. Although cysteine proteases (group 1) represent major allergens of the mites cloned from different species [36, 83], they have not yet been used for skin tests. On the other hand, group 2 allergens including Der p 2 of *D. pteronyssinus*, Lep d 2 from *Lepidoglyphus destructor*, Der f 2 from *D. farinae* [94] and Tyr p 2 from *Tyrophagus putrescentiae*, have been evaluated in skin tests. Group 2

allergens, although not yet defined in terms of biological function, are recognized by the majority of the mite-sensitized allergies [95–99]. Unfortunately, group 5 and group 7 allergens could also not be assigned to a functional class of proteins with the exception of their ability to bind IgE from sensitized individuals [36]. rDer p 5 and rDer p 7 from *D. pteronyssinus* and Blo t 5 from *Blomia tropicalis* have been confirmed to be able to elicit type 1 reactions in clinical studies [95–98, 100, 101]. However, in view of the shared sequence homology, members of a distinct group of structurally related allergens can be expected to be cross-reactive as demonstrated in vitro for some of the mite allergens [102]. Cross-reactive allergens bear the potential to conspicuously contribute to a reduction of the number of structures needed for a patient-tailored immunotherapy [60, 68].

Moulds and Fungi

Moulds are among the most complex allergenic sources and are able to produce large amounts of IgE-binding molecules covering many different structures. Repertoire cloning with advanced technologies revealed that *A. fumigatus*, *Cladosporium herbarum*, *Coprinus comatus* and *Malassezia furfur* are able to synthesize an impressive number of species-specific and cross-reactive allergens [66, 67]. Moreover, *A. fumigatus* MnSOD [103], P₂ acidic ribosomal protein [104] and thioredoxin [unpubl. results] show IgE-mediated cross-reactivity to their human counterpart. In view of the difficulties encountered in standardizing fungal extracts [66, 67] and the huge number of allergenic molecular structures covered by moulds, recombinant allergens are especially important in this field. rAsp f 1, a fungal ribotoxin with solved three-dimensional structure [105], has been widely tested in clinical studies involving asthmatic patients with or without allergic bronchopulmonary aspergillosis (ABPA) [62], suffering from cystic fibrosis (CF) [47, 106] and atopic dermatitis [107]. Skin tests in over 300 patients and controls have been performed with this allergen. No adverse reactions have been reported so far. Additional *A. fumigatus* allergens tested clinically are a peroxisomal protein cross-reactive with the homologous yeast structures [46, 50] and rAsp f 4 [47, 65], a protein with unknown biological function serving as specific marker for ABPA and manganese superoxide dismutase (rAsp f 6) [62, 65]. Moreover, 4 patients sensitized to the *A. fumigatus* P₂ ribosomal protein (rAsp f 8) showed positive skin reactions and T-cell proliferation to both the fungal and human P₂ ribosomal protein, demonstrating the involvement of autoreactivity in chronic fungus-related allergic diseases [103, 104]. The

two allergens rAsp f 4 and rAsp f 6 have been used as disease-specific allergens to facilitate the diagnosis of ABPA, both in serology and skin tests [47, 62, 63, 65]. In terms of diagnostic specificity, the recombinant *A. fumigatus* allergens have proven to be superior to allergen extracts, allowing a direct correlation between allergen-specific IgE in serum and wheal and flare reaction in skin test [46, 65, 108]. Alt a 1, the major allergen of *Alternaria alternata* [109, 110], and Alt a 5 corresponding to enolase [53] showed the ability to elicit type I reactions in a limited number of patients [53, 109]. All the 7 allergic patients tested could be diagnosed using the two allergens, whereas 10 control individuals scored negative in skin test with mould extract and rAllergens. Although the results need to be confirmed in larger studies, these results indicate that the number of structures required to diagnose allergy to *A. alternata* could be rather limited. The only fungal allergen so far tested in the clinics, Cop c 1 from the basidiomycete *C. comatus* [111], showed skin reactivity in the picomole range (11 pg/μl–450 ng/μl) in sensitized individuals fulfilling the criteria to be classified as a clinically relevant allergen.

Bee Venom

Honey bee venom contains basically four allergens, phospholipase A₂ (PLA₂) [112], hyaluronidase [44], acidic phosphatase [36, 113] and mellitin [114], and represents, therefore, a relatively simple allergenic source. PLA₂ was the first allergen crystallized at all [115] and the cloned PLA₂ molecule has served as a model system to study and to elucidate the basic mechanisms of allergic reactions [48]. It was demonstrated that PLA₂ as major single allergen of bee venom was about 10 times more potent in skin test than the venom itself [116]. rPLA₂ was slightly less potent than nPLA₂, probably due to small differences in folding, however, the lack of glycosylation of the recombinant protein and of the enzymatic activity did not significantly influence the allergenicity of the protein [117]. The clinical tests with rPLA₂ clearly showed the requirement of a correct three-dimensional structure for the allergenicity of the protein, since non-refolded recombinant protein was unable to elicit type I reactions [116, 117]. Moreover, it was demonstrated that, although in vivo and in vitro diagnostic tests with bee venom are more sensitive, diagnostic tests with rPLA are clearly more specific [117]. Thus it is possible to produce rAllergens showing in vivo activity similar to that of purified natural allergens. Site-directed mutagenesis of the PLA₂ molecule was used to generate fully allergenic, enzymatically inactive protein [118] offering an easy method for the conversion of toxic

allergens into nontoxic products without influencing their allergenicity. Other insect venom allergens have so far not been evaluated in skin tests.

Celery

Allergy to celery is almost always associated with sensitization to birch and/or mugwort pollen [119]. Structurally, IgE cross-reactivity between celery and other plant allergens is related to at least three classes of proteins [120]. Api g 1, a major celery allergen [121], belongs to the type 10 class of pathogenesis-related proteins [122], profilin (Api g 4), a member of ubiquitously present actin-binding proteins [123] and high-molecular-weight allergens [120]. Little is known about the higher-molecular-weight allergens, except that they cross-react with other plant and food allergens [124]. Api g 1 [125] and Api g 4 [126] have been cloned and produced as rAllergen. The only celery structure evaluated so far in skin tests is Api g 1, a homologous to Bet v 1 [121]. This interesting study documents that rApi g 1 allows accurate in vivo diagnosis of celery allergy but only in areas where birch trees are common. In areas where birch is rare, sensitization to rApi g 1 could not be detected. These results show that allergy to celery is not necessarily dependent on sensitization to birch and more work will be required to elucidate the additional structures involved in celery allergy.

Latex

Many IgE-binding proteins have been identified in the latex of *Hevea brasiliensis* and at least ten different proteins have been identified at molecular level [127]. Latex as such is not a natural source of exposure and, therefore, latex allergy is limited to restricted groups of risk including occupational exposure in healthcare workers [128] and spina bifida patients [129–131]. Structurally, many latex allergens are related to other allergens detected in fruits [132] and moulds [133]. They include b-1,3-glucanase (Hev b 2), chitinase (Hev b 6.02) and profilin (Hev b 8) also present in fruits [132] as well as enolase (Hev b 9) and MnSOD (Hev b 10) recently described to be cross-reactive with the corresponding mould allergens [133]. The allergens Hev b 2, 3, 5, 6, 7 and 8 have been evaluated in SPT in a group of 29 latex-allergic individuals [134]. This is, so far, the only report describing the evaluation of latex rAllergens in a clinical study. Although limited in terms of tests performed, the investigation showed that all rAllergens tested were able to elicit type 1 reactions and that a combination of Hev b 5, 6, and 7 was sufficient to diagnose latex allergy with a sensitivity of 93 and 100% specificity. Also in this case, the sensitivity and specificity

of SPT with rAllergens was superior to those reported for latex-specific serum IgE measurements in a large population which reached 73 and 97%, respectively [131].

Specificity and Sensitivity of rAllergens in Skin Testing

The specificity of an allergen preparation is the most important criterion because false-positive reactions need to be excluded for a safe diagnosis. A high specificity allows to confirm sensitization but can miss weak sensitizations that should be detected in routine screenings. In the studies analyzed, rAllergens were compared to allergen extracts and in most cases also to the clinical symptoms. The use of rAllergens for skin tests gives an excellent specificity which often reaches 100% for both parameters (table 2). However 'sensitivity' of skin tests with rAllergens is generally lower than those obtained with commercial extracts. Skin tests with single rAllergens are, particularly in IDT, extremely sensitive as shown by the elicitation of type 1 reactions in the pico- to femtomole range [135]. The lower 'sensitivity' results from the lower incidence of sensitization to the single allergen in the population tested. As shown in different studies, parallel tests with increasing numbers of rAllergens in the group of patients tested drastically increase the sensitivity of the diagnosis and will reach 100% if all allergens present in a given extract can be produced as recombinant proteins and tested simultaneously. Combined with their excellent specificity, rAllergens will allow a high standard CRD of sensitization.

Type of Skin Test and Optimal Allergen Dosage

Standard SPT and IDT can both be used also with rAllergens. Most of the studies reported so far used SPT alone or in combination with IDT, while in about one third of the investigations only IDT were performed. SPT are simpler to perform, faster, more comfortable for the patient and, most importantly, considered to be at lower risk than IDT for provocation of anaphylactic reactions [81, 136]. Therefore, SPT are recommended for routine assessments, epidemiological studies and in surveys involving large populations [1, 3]. However, IDT is 10^2 – 10^3 times more sensitive than SPT and should therefore be used to re-assess sensitization in individuals scoring negative in SPT [136]. The optimal allergen concentration to be used in SPT has not been determined so far. Concen-

Table 2. Specificity and sensitivity of skin tests with rAllergens

rAllergens	Specificity ^a , %	Sensitivity, % ^b	Type of skin test	Max. ^c used $\mu\text{g/ml}$	Ref.
<i>Mite</i>					
rDer p 2	0/43 (100)	256/346 (64)	SPT, IDT	100	96–98, 100, 101
rDer p 5	0/12 (100)	36/60 (60)	SPT		96, 98, 100
rDer p 7	n.g.	31/60 (52)	SPT	100	98
rDer f 11	n.g.	15/21 (62)	SPT	1	94, 157
rBlo t 5	n.g.	25/53 (47)	SPT, IDT	10	96, 100, 101
rLep d 2	0/38 (100)	24/41 (59)	SPT	100	95
rTyr p 2	0/38 (100)	14/25 (56)	SPT	100	95
rPer a 1	1/15 (94)	12/22 (54)	SPT	100	155
<i>Pollen</i>					
rBet v 1	0/80 (100)	301/343 (87)	SPT, IDT	100	33, 60, 61, 72, 92, 93, 145, 158
rBet v 2	0/12 (100)	20/49 (41)	SPT	20	60, 92
rPhle p 1	0/10 (100)		SPT	20	60
rPhle p 2	0/10 (100)		SPT	20	60
rPhle p 3	0/10 (100)		SPT	20	60
rOle e 1	0/20 (100)	33/33 (100)	SPT	100	84
<i>Fungal</i>					
rAsp f 1	1/103 (99)	220/244 (90)	SPT, IDT	100	29, 62, 82, 106–108
rAsp f 3	0/20 (100)	ABPA: 67/69 (97) Af allergics: 35/48 (73)	IDT	10	46, 62
rAsp f 4	0/5 (100)	ABPA: 8/12 (75) Af allergics: 0/12 (0)	IDT	10	65
rAsp f 6	0/5 (100)	ABPA: 5/12 (42) Af allergics: 0/12 (0)	IDT	10	65
rAsp f 8	0/6 (100)	4/4	IDT	100	104
rCop c 1	n.g.	5/5	SPT	0.475	111
<i>Bee venom</i>					
rPL A 2	0/21 (100)	81/81 (95)	IDT	1	117
<i>Latex</i>					
rHev b 2, 5, 7	0/10 (100)	27/29 (93)	SPT	1000	134
rHev b 6, 8				500	
rHev b 3				200	
<i>Celery</i>					
rApi g 1	0/5 (100)	24/24 (100)	SPT, IDT	100	35

^a Specificity is defined as number of positive skin tests with rAllergen/number of controls with negative skin test with the corresponding commercial extract (n.g. = not given).

^b Sensitivity is defined as the number of positive skin tests with rAllergens/number of positive skin tests with conventional allergen extract.

^c Maximal allergen concentration used in skin tests, either SPT or IDT.

trations used vary between 5 and 100 $\mu\text{g/ml}$, although concentrations up to 1,000 $\mu\text{g/ml}$ have been occasionally used (see tables 1 and 2). Taking all available data together, it seems to be reasonable to use 100 $\mu\text{g/ml}$ to achieve a good sensitivity and keep an excellent specificity in SPT. IDT have usually been performed applying serial 10-fold

dilutions starting with absolute allergen amounts corresponding to 1 ng. Taking into account the anaphylactic risk derived from IDT and the large number of tests performed, 1-ng challenges seem to be safe to start the provocation tests, although some patients may give positive skin reactions also at lower concentrations [61, 65, 135].

Sources of rAllergens Used in Skin Tests

For the expression of recombinant allergens, prokaryotic and eukaryotic hosts such as *Escherichia coli*, yeasts or baculovirus are used [34]. Proteins may not fold correctly if expressed in prokaryotic systems, while they might fold easier into a native-like structure in eukaryotic expression systems [44]. However, the great majority of rAllergens used so far in skin tests in humans have been produced in *E. coli*. This enterobacterium is the most widely used organism to produce recombinant proteins of pharmaceutical grade and is considered as safe for such applications. Nowadays, powerful techniques have been developed to purify recombinant proteins and in spite of some limitations related to the lack of post-translational modifications [43] and protein folding, *E. coli* still represents the first choice for biotechnological applications.

Beside *E. coli*, the yeasts *Saccharomyces cerevisiae* [43, 90, 137] and *Pichia pasteuris* [84, 130, 138–140] have been used to efficiently express allergens not easily produced by *E. coli*. In two cases, rLep d 2 [95] and hyaluronidase from bee venom [44], the allergenic proteins have been produced using insect cells infected with baculovirus. The mite allergen rLep d 2 was also compared to the recombinant counterpart produced in *E. coli* and no difference in skin test reactivity was found [95].

Although the quality of the rAllergens expressed in baculovirus is reported to be superior to the corresponding proteins expressed in *E. coli* [44], their use in vivo cannot be recommended. It is known that baculovirus is able to quantitatively infect human liver hepatocytes [141] and it cannot be excluded that traces of DNA contaminations in the allergen preparations could be of risk for the individuals tested. Unfortunately, none of the rAllergens are available as standard solution for skin testing and only a few of them are available as ImmunoCAPs for determination of allergen-specific IgE.

Quantitative Skin Testing (End-Point Titration) with rAllergens

The end-point titration method in skin testing has been shown to estimate allergenic activities with good reproducibility. Skin test results obtained with this method also correlate with bioassays in vivo and RAST inhibition measurements performed in parallel [142]. rAllergens can be applied in exactly quantifiable amounts as virtually pure proteins. Therefore, the use of rAllergens for end-point titration studies in skin testing allows interesting

insights into the correlation between allergen load, skin test reactivity and in vitro findings. The best documented studies in this field have been performed using recombinant bee venom PLA₂ [116].

Correlation between Allergen-Specific Serum IgE and Skin Reactivity

rAllergens as highly pure proteins can be used for the development of commercial reagents for fully automated detection of allergen-specific serum IgE. So far, rBet v 1, 2 and 4 from *B. verrucosa*, rPhl p 1, 2, 4, 5, 6, 7, 11 and 12 from *Phleum pratense* and rAsp f 1, 2, 3, 4 and 6 from *A. fumigatus* are commercially available for routine assessments in the Pharmacia CAP System. The results obtained with rAsp f 1 [13], rAsp f 3 [46], rAsp f 4 and rAsp f 6 [65] ImmunoCAPs were in perfect accordance with the results from SPT or IDT and with the manual ELISA determinations. Interestingly, skin test studies with all these four rAllergens showed that all patients with a positive wheal-and-flare reaction to a given rAllergen also showed detectable levels of allergen-specific serum IgE antibodies. Furthermore, the levels of allergen-specific IgE significantly correlated with the magnitude of the wheal surface [46, 65]. Thus, a direct correlation between in vitro and in vivo tests has been shown with rAllergens, in contrast to fungal extracts where discrepancies between results of skin tests and allergen-specific IgE determinations have been reported [143]. The quantitative determination of allergen-specific IgE compared to the skin test outcomes allowed determining a threshold value of allergen-specific serum IgE required for a positive skin reaction close to 2 µg/l [65], whereas the lowest amount of allergen able to elicit a positive skin reaction is <1 ng. This demonstrates the high biologic activity of the rAllergens used.

Provocation Tests with Recombinant Allergens

Besides skin testing, provocation tests can be used to determine the allergenicity of a protein preparation in vivo. Provocation tests are even more sensitive than skin tests allowing a biologically relevant assessment of the allergenicity of a given protein [144]. So far, provocation tests with rAllergens have been reported only for Bet v 1, the major birch pollen allergen, and for the isoforms Bet v 1 d, a naturally occurring variant of Bet v 1 lacking the ability to bind IgE from sera of Bet v 1 sensitized individ-

uals [61]. Thirty-three of 48 birch-pollen-allergic patients reacted positively in conjunctival provocation with Bet v 1, but scored negative with the Bet v d isoforms [61]. Bet v 1d elicited positive skin test reactions only at a 100- to 1,000-fold increased concentration compared to Bet v 1. This indicates a relevantly reduced capability for IgE binding of the Bet v 1d isoform. However, the ability of Bet v 1d to induce T-cell proliferation was comparable to the proliferation induced by rBet v 1 also at low concentrations. In another study, nasal and bronchial challenges with nBet 1 and rBet v 1 were performed in 13 patients allergic to birch pollen, in addition to SPT with both allergen forms [145]. Although slightly higher concentrations of the recombinant form was necessary to elicit positive challenge reactions, no significant differences between the native and recombinant allergen were found. These findings demonstrate that engineered isoforms of a defined allergen can show significantly reduced IgE-binding capacity in vitro and in vivo as shown by skin and provocation tests retaining, however, a high immunogenicity for T cells. Therefore, modified forms of genetically-engineered recombinant allergens depleted from IgE binding might be promising candidates for use in specific immunotherapy.

Risk and Side Effects of Skin Testing with Recombinant Allergens

Large local reactions, or rarely also systemic allergic reactions, are well-known risks of skin tests [146]. Especially skin tests with crude material such as food allergens (Crustacea, peanuts) or skin tests performed in highly sensitized patients (e.g. latex) have been reported to induce such deleterious reactions [129, 147]. One of the major problems in skin testing with undefined allergen preparations results from the unknown amounts of allergens applied which do not allow an estimation of the risk associated with the test. In contrast, rAllergens can be applied in exactly defined amounts contributing to an exact definition of the amount of allergen required for a positive skin test reaction [65]. So far, no side effects related to the use of rAllergens in skin tests have been reported in spite of the fact that more than 1,600 allergic individuals have been tested with 21 different rAllergens cloned from 14 different allergenic sources. Obviously, such side effects have to be expected if higher doses of rAllergens would be used in skin testing to achieve a higher sensitivity, especially in intradermal tests. Performing SPT or titration series starting with very low concentrations in IDT sub-

stantially reduce this risk for both conventional and rAllergens. In summary, it can be concluded that skin tests with rAllergens does not bear a higher risk compared to skin testing with conventional, commercially available extracts. Moreover, false-positive skin tests with rAllergens are extremely rare as reported (see table 2), contributing to safe diagnoses of allergic conditions.

Ethical and Legal Aspects

Besides all similar risks encountered also with conventional extracts, recombinant products theoretically bear the following additional risks [148]: (1) Products from naturally occurring genes expressed in foreign hosts may deviate structurally, biologically or immunologically from their natural counterparts. (2) The choice of the expression system and purification procedure may result in products contaminated with DNA or endotoxins. (3) Uncontrolled culture conditions may favour the expression of modified gene products.

Apparently, the use of rAllergens does not bear an increased risk of side effects compared with commercial extracts. However, ethical aspects concerning the use of recombinant material produced in heterologous hosts such as bacteria or yeasts may be raised. Standardized methods of protein expression and purification widely used for the production of pharmacological grade proteins for human use warrant that exclusively the wanted rAllergen is contained in the final preparation used for skin tests. Of course, a theoretical risk deriving from DNA contaminations of the producing host should be considered in ethical assessments. This risk is not to be considered higher than the risk derived from the use of other medicinal products like antibiotics which are also subject to DNA contaminations derived from the producing organism [149]. rAllergens expressed in baculovirus bear a certain, not yet assessed, risk derived from DNA contaminations. As clearly shown, this virus is able to quantitatively infect human liver hepatocytes [141].

Mostly due to restrictions by ethical and legal restrictions, skin tests have been performed only in a limited number of countries including Austria [60], Brazil [100], Canada [134], France [71, 76, 93], Singapore [101], Spain [84], Sweden [28, 72, 150, 151], Switzerland [46, 47, 61–63, 82, 103, 106, 107, 111, 116, 152–154], Taiwan [94, 155] and Venezuela [98, 99]. Authors from other nations, including the USA, have collected extensive data with recombinant allergens; however, the skin tests with the rAllergens had to be performed elsewhere.

The following precautions should, however, be included to minimize potential risks associated with the use of rAllergens in skin tests: (1) Producers should exactly describe the host cell and the expression vector used. (2) Full details of the nucleotide sequence of the gene insert and flanking regions should be given. (3) The procedure used for fermentation and purification of the expressed protein including criteria used for purity controls should be described and performed under good manufactory practice-like conditions. (4) Toxicity and mitogenicity should be assessed in human cell cultures [46]. (5) For clinical studies the patients have to be well selected and symptoms occurring spontaneously or after challenge have to be recorded, controlled and validated.

Conclusions

rAllergens for skin testing represent a new powerful diagnostic tool. They can be biotechnologically produced in large amounts as highly pure reagents with a high batch-to-batch consistency satisfying the quality requirements of medicinal products manufactured by recombinant DNA technology. These products are qualitatively superior to many commercially available allergen extracts currently used for the in vitro and in vivo diagnosis of allergic diseases.

To date, many different rAllergens of various pollens, moulds, mites, bee venom, latex and celery have been used in skin testing in more than 1,600 allergic and control individuals. SPT as well as IDT with rAllergens proved to be highly specific and safe. The diagnostic sensitivity of single rAllergens is generally lower than those obtained with allergen extracts, but can be considerably increased using rAllergen panels covering the most important allergenic structures present in a given complex allergenic extract. Moreover, quantitative skin testing with single rAllergens allows interesting insights into correla-

tions between the sensitization determined in vivo and in vitro. However, there are also limitations for the widespread use of rAllergens. Identification and cloning of all relevant allergens from a complex source can be very time- and resource-consuming, despite modern techniques. The highly specific answer that rAllergens elicit in skin test reactions together with low sensitivity requires panels of rAllergens from each allergenic source to identify allergic patients with a high sensitivity. Of course, recombinant allergens also bear the same risk to elicit anaphylactic reactions as conventional extracts if used in vivo. Depending on the host where rAllergens are produced, a certain risk of contamination with DNA or endotoxins cannot be excluded. However, allergens produced in *E. coli* can be considered safe. Presently a wider use of rAllergens in vivo is limited by ethical and legal restrictions, as in many countries their application in patients is not yet allowed. However, in the long series of skin tests with rAllergens reviewed in this article, neither anaphylactic, toxic nor infectious side effects were reported.

In conclusion, skin testing with rAllergens offers a highly specific, fast and safe additional diagnostic tool to elucidate patient- and disease-specific sensitization patterns. Allowing precise, reproducible skin test reactions, rAllergens are expected to contribute to a relevant improvement of allergy diagnosis in the near future.

Acknowledgement

We thank Dr. Hans Grönlund, Stockholm, Sweden for critical review of the manuscript and for fruitful discussions. We are grateful to Prof. Kurt Blaser, Prof. Brunello Wüthrich and Prof. Günter Burg for their continuous support and to all clinicians involved in the in vivo evaluation of rAllergens.

Supported by the Swiss National Science Foundation grants No. 31-50515.97 and 31-63381.00. P. Schmid-Grendelmeier is a recipient of a grant 'For the Promotion of Academic Young People' of the University of Zürich.

References

- 1 The European Academy of Allergology and Clinical Immunology: Position paper. Allergen standardization and skin tests. *Allergy* 1993; 48:48–82.
- 2 Cuesta-Herranz J, Lazaro M, Figueredo E, Igea JM, Umpierrez A, De-Las-Heras M: Allergy to plant-derived fresh foods in a birch- and ragweed-free area. *Clin Exp Allergy* 2000;30: 1411–1416.
- 3 American Academy of Allergy, Asthma and Immunology (AAAAI): The use of standardized allergen extracts. *J Allergy Clin Immunol* 1997;99:583–586.
- 4 Platts-Mills TA, Mueller GA, Wheatley LM: Future directions for allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:335–343.
- 5 Williams PB, Nolte H, Dolen WK, Koepke JW, Selner JC: The histamine content of allergen extracts. *J Allergy Clin Immunol* 1992;89:738–745.
- 6 Mullbacher A, Eichner RD: Immunosuppression in vitro by a metabolite of a human pathogenic fungus. *Proc Natl Acad Sci USA* 1984;81: 3835–3837.
- 7 Esch RE: Allergen source materials and quality control of allergenic extracts. *Methods* 1997; 13:2–13.

- 8 Portnoy J, Pacheco F, Ballam Y, Barnes C: The effect of time and extraction buffers on residual protein and allergen content of extracts derived from four strains of *Alternaria*. *J Allergy Clin Immunol* 1993;91:930–938.
- 9 Soldatova LN, Paupore EJ, Burk SH, Pastor RW, Slater JE: The stability of house dust mite allergens in glycerinated extracts. *J Allergy Clin Immunol* 2000;105:482–488.
- 10 Nelson HS, Ikle D, Buchmeier A: Studies of allergen extract stability: The effects of dilution and mixing. *J Allergy Clin Immunol* 1996;98:382–388.
- 11 Vieths S, Hoffmann A, Holzhauser T, Muller U, Reindl J, Haustein D: Factors influencing the quality of food extracts for in vitro and in vivo diagnosis. *Allergy* 1998;53:65–71.
- 12 Dreborg S: The batch-to-batch variation of the potency of dog- and cat-allergen-coated lancets. Evaluation by skin prick testing. *Allergy* 1993;48:373–376.
- 13 Nikolaizik WH, Cramer R, Blaser K, Schöni MH: Skin test reactivity to recombinant *Aspergillus fumigatus* allergen I/a in patients with cystic fibrosis. *Int Arch Allergy Immunol* 1996;111:403–408.
- 14 Kauffman HF, van der Heyden PJ, van der Laan S, van der Heide S, Beaumont F, de Vries K: Antibody determination against *Aspergillus fumigatus* by means of the enzyme-linked immunosorbent assay. II. Physico- and immunochemical properties of the polystyrene-binding components. *Int Arch Allergy Appl Immunol* 1985;78:174–181.
- 15 Schwartz HJ, Lockey RF, Sheffer AL, Parrino J, Busse WW, Yunginger JW: A multicenter study on skin-test reactivity of human volunteers to venom as compared with whole body Hymenoptera antigens. *J Allergy Clin Immunol* 1981;67:81–85.
- 16 Anderson MC, Baer H, Ohman JL Jr: A comparative study of the allergens of cat urine, serum, saliva and pelt. *J Allergy Clin Immunol* 1985;76:563–569.
- 17 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;76:168–173.
- 18 Bisht V, Singh BP, Arora N, Sridhara S, Gaur SN: Allergens of *Epicoecum nigrum* grown in different media for quality source material. *Allergy* 2000;55:274–280.
- 19 Van der Veen MJ, Mulder M, Witterman AM, van Ree R, Aalberse RC, Jansen HM, van der Zee JS: False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J Allergy Clin Immunol* 1996;98:1028–1034.
- 20 Rosenbaum MR, Esch RE, Schwartzman RM: Effects of mold proteases on the biological activity of allergenic pollen extracts. *Am J Vet Res* 1996;57:1447–1452.
- 21 Akiyama K, Shida T, Yasueda H, Mita H, Yanagihara Y, Hasegawa M, Maeda Y, Yamamoto T, Takesako K, Yamaguchi H: Allergenicity of acid protease secreted by *Candida albicans*. *Allergy* 1996;51:887–892.
- 22 Moser M, Menz G, Blaser K, Cramer R: Recombinant expression and antigenic properties of a 32-kilodalton extracellular alkaline protease, representing a possible virulence factor from *Aspergillus fumigatus*. *Infect Immun* 1994;62:936–942.
- 23 Wood RA, Phipatanakul W, Hamilton RG, Eggleston PA: A comparison of skin prick tests, intradermal skin tests, and RASTs in the diagnosis of cat allergy. *J Allergy Clin Immunol* 1999;103:773–779.
- 24 Adler BR, Assadullahi T, Warner JA, Warner JO: Evaluation of a multiple food specific IgE antibody test compared to parental perception, allergy skin tests and RAST. *Clin Exp Allergy* 1991;21:683–688.
- 25 Cramer R: Epidemiology and molecular basis of the involvement of *Aspergillus fumigatus* in allergic diseases. *Contrib Microbiol* 1999;2:44–56.
- 26 Steringer I, Aukrust L, Einarsson R: Variability of antigenicity/allergenicity in different strains of *Alternaria alternata*. *Int Arch Allergy Appl Immunol* 1987;84:190–197.
- 27 Varjonen E, Björkstén F, Savolainen J: Stability of cereal allergens. *Clin Exp Allergy* 1996;26:436–443.
- 28 Nielsen NH, Dirksen A, Mosbech H, Laubjerg J, Biering I, Soborg M: Skin prick testing with standardized extracts from three different manufacturers. A comparative randomized study. *Allergol Immunopathol* 1992;20:246–248.
- 29 Cramer R, Lidholm J, Grönlund H, Stüber D, Blaser K, Menz G: Automated specific IgE assay with recombinant allergens: Evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia Cap System. *Clin Exp Allergy* 1996;26:1411–1419.
- 30 Valenta R, Vrtala S, Laffer S, Spitzauer S, Kraft D: Recombinant allergens. *Allergy* 1998;53:552–561.
- 31 Kraft D, Ferreira F, Vrtala S, Breiteneder H, Ebner C, Valenta R, Susani M, Breitenbach M, Scheiner O: The importance of recombinant allergens for diagnosis and therapy of IgE-mediated allergies. *Int Arch Allergy Immunol* 1999;118:171–176.
- 32 Spertini F: Diagnostic and therapeutic value of synthetic or recombinant allergens. *Rev Med Suisse Romande* 1999;119:235–239.
- 33 Pauli G, Deviller P: Recombinant allergens: Diagnostic use and therapeutic perspectives. *Rev Mal Respir* 2000;17:293–303.
- 34 Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A: Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* 2000;106:409–418.
- 35 Hoffmann-Sommergruber K: Plant allergens and pathogenesis-related proteins. What do they have in common? *Int Arch Allergy Immunol* 2000;122:155–166.
- 36 Thomas WR, Smith W: Towards defining the full spectrum of important house dust mite allergens. *Clin Exp Allergy* 1999;29:1583–1587.
- 37 Spitzauer S: Allergy to mammalian proteins: At the borderline between foreign and self? *Int Arch Allergy Immunol* 1999;120:259–269.
- 38 Weichel M, Flückiger S, Cramer R: Molecular characterisation of mould allergens involved in respiratory complications; in Pandali SG (ed): Recent Research Developments in Respiratory and Critical Care Medicine. Trivandrum/India, Research Signpost, 2001, in press.
- 39 Horner WE, Helbling A, Salvaggio JE, Lehrer SB: Fungal allergens. *Clin Microbiol Rev* 1995;8:161–179.
- 40 King TP, Spangfort MD: Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol* 2000;123:99–106.
- 41 Ebner C: Genetically modified food: A danger or a benefit for atopics? *Curr Probl Dermatol* 1999;28:81–87.
- 42 Suter M, Moser M, Menz G, Blaser K, Cramer R: Cloning, production, purification and biological activity of an 18 kDa *Aspergillus fumigatus* allergen; in Kraft D, Sehon A (eds): Molecular Biology and Immunology of Allergens. Boca Raton, CRC Press, 1993, pp 267–269.
- 43 Chua KY, Kehal PK, Thomas WR, Vaughan PR, Macreadie IG: High-frequency binding of IgE to the Der p allergen expressed in yeast. *J Allergy Clin Immunol* 1992;89:95–102.
- 44 Soldatova LN, Cramer R, Gmachl M, Kemeény DM, Schmidt M, Weber M, Mueller UR: Superior biologic activity of the recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with *Escherichia coli*. *J Allergy Clin Immunol* 1998;101:691–698.
- 45 Moser M, Cramer 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.
- 46 Hemmann S, Ismail C, Blaser K, Menz G, Cramer R: Skin-test reactivity and isotype-specific immune responses to recombinant Asp f 3, a major allergen of *Aspergillus fumigatus*. *Clin Exp Allergy* 1998;28:860–867.
- 47 Hemmann S, Nikolaizik WH, Schöni MH, Blaser K, Cramer R: Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. *Eur J Immunol* 1998;28:1155–1160.
- 48 Blaser K, Carballido J, Faith A, Cramer R, Akdis C: Determinants and mechanisms of human immune responses to bee venom phospholipase A₂. *Int Arch Allergy Immunol* 1998;117:1–10.
- 49 Bufer A: The biological function of allergens: Relevant for the induction of allergic diseases? *Int Arch Allergy Immunol* 1998;117:215–219.
- 50 Hemmann S, Blaser K, Cramer R: Allergens of *Aspergillus fumigatus* and *Candida boidinii* share IgE-binding epitopes. *Am J Respir Crit Care Med* 1997;156:1956–1962.

- 51 Silvestri M, Rossi GA, Cozzani S, Pulvirenti G, Fasce L: Age-dependent tendency to become sensitized to other classes of aeroallergens in atopic asthmatic children. *Ann Allergy Asthma Immunol* 1999;83:335–340.
- 52 Mirza O, Henriksen A, Ipsen H, Larsen JN, Wissenbach M, Spangfort MD, Gajhede M: Dominant epitopes and allergic cross-reactivity: Complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v 1. *J Immunol* 2000;165:331–338.
- 53 Simon-Nobbe B, Probst G, Kajava AV, Oberkofler H, Susani M, Cramer R, Ferreira F, Ebner C, Breitenbach M: IgE-binding epitopes of enolases, a class of highly conserved fungal allergens. *J Allergy Clin Immunol* 2000;106:887–895.
- 54 Gajhede M, Osmark P, Poulsen FM, Ipsen H, Larsen JN, Joost van Neerven RJ, Schou C, Lowenstein H, Spangfort MD: X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996;3:1040–1045.
- 55 Spangfort MD, Larsen JN, Gajhede M: Crystallization and preliminary X-ray investigation at 2.0 Å resolution of Bet v 1, a birch pollen protein causing IgE-mediated allergy. *Proteins* 1996;26:358–360.
- 56 Rouvinen J, Rautiainen J, Virtanen T, Zeiler T, Kauppinen J, Taivainen A, Mantylarvi R: Probing the molecular basis of allergy. Three-dimensional structure of the bovine lipocalin allergen Bos d 2. *J Biol Chem* 1999;274:2337–2343.
- 57 Lascombe MB, Gregoire C, Poncet P, Tavares GA, Rosinski-Chupin I, Rabillon J, Goubran-Botros H, Mazie JC, David B, Alzari PM: Crystal structure of the allergen Equ c 1. A dimeric lipocalin with restricted IgE-reactive epitopes. *J Biol Chem* 2000;275:21572–21577.
- 58 Fedorov AA, Ball T, Valenta R, Almo SC: X-ray crystal structures of birch pollen profilin and Phl p 2. *Int Arch Allergy Immunol* 1997;113:109–113.
- 59 Perez-Canadillas JM, Santoro J, Campos-Olivas R, Lacadena J, Martinez del Pozo A, Gaviñanes JG, Rico M, Bruix M: The highly refined solution structure of the cytotoxic ribonuclease alpha-sarcin reveals the structural requirements for substrate recognition and ribonucleolytic activity. *J Mol Biol* 2000;299:1061–1073.
- 60 Heiss S, Mahler V, Steiner R, Spitzauer S, Schweiger C, Kraft D, Valenta R: Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;113:830–837.
- 61 Arquint O, Helbling A, Cramer R, Ferreira F, Breitenbach M, Pichler WJ: Reduced in vivo allergenicity of Bet v 1d isoform, a natural component of birch pollen. *J Allergy Clin Immunol* 1999;104:1239–1243.
- 62 Cramer R, Hemmann S, Ismail C, Menz G, Blaser K: Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. *Int Immunol* 1998;10:1211–1216.
- 63 Cramer R: Recombinant *Aspergillus fumigatus* allergens: From the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998;115:99–114.
- 64 Cramer R, Lidholm J, Menz G, Grönlund H, Blaser K: Automated serology with recombinant allergens. A feasibility study. *Adv Exp Med Biol* 1996;409:111–116.
- 65 Hemmann S, Menz G, Ismail C, Blaser K, Cramer R: Skin test reactivity to two recombinant *Aspergillus fumigatus* allergens in *A. fumigatus*-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitization. *J Allergy Clin Immunol* 1999;104:601–607.
- 66 Cramer R, Walter G: Selective enrichment and high throughput screening of phage surface-displayed cDNA libraries from complex allergenic systems. *Comb Chem High Throughput Screen* 1999;2:63–72.
- 67 Cramer R, Kodzius R, Konthur Z, Lehrach H, Blaser K, Walter G: Tapping allergen repertoires by advanced cloning technologies. *Int Arch Allergy Immunol* 2001;124:43–47.
- 68 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
- 69 Müller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, Blaser K: Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A₂ induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;101:747–754.
- 70 Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, Hoffmann-Sommergruber K, Scheiner O, Kraft D, Breitenbach M, Rheinberger HJ, Ebner C: Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: Potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996;183:599–609.
- 71 Pauli G, Purohit A, Oster JP, de Blay F, Vrtala S, Niederberger V, Kraft D, Valenta R: Clinical evaluation of genetically engineered hypoallergenic rBet v 1 derivatives. *Int Arch Allergy Immunol* 1999;118:216–217.
- 72 van Hage-Hamsten M, Kronqvist M, Zetterstrom O, Johansson E, Niederberger V, Vrtala S, Grönlund H, Gronneberg R, Valenta R: Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: Results obtained with a mix of two recombinant Bet v 1 fragments and recombinant Bet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol* 1999;104:969–977.
- 73 Vrtala S, Hirtenlehner K, Susani M, Hufnagl P, Binder BR, Vangelista L, Pastore A, Sperr WR, Valent P, Ebner C, Kraft D, Valenta R: Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: Candidates for specific immunotherapy. *Int Arch Allergy Immunol* 1999;118:218–219.
- 74 Valenta R, Vrtala S, Focke-Tejkl M, Bugajska S, Ball T, Twardosz A, Spitzauer S, Grönlund H, Kraft D: Genetically engineered and synthetic allergen derivatives: Candidates for vaccination against type I allergy. *Biol Chem* 1999;380:815–824.
- 75 Nopp A, Hallden G, Lundahl J, Johansson E, Vrtala S, Valenta R, Gronneberg R, van Hage-Hamsten M: Comparison of inflammatory responses to genetically engineered hypoallergenic derivatives of the major birch pollen allergen Bet v 1 and to recombinant Bet v 1 wild type in skin chamber fluids collected from birch pollen-allergic patients. *J Allergy Clin Immunol* 2000;106:101–109.
- 76 Pauli G, Purohit A, Oster JP, De Blay F, Vrtala S, Niederberger V, Kraft D, Valenta R: Comparison of genetically engineered hypoallergenic rBet v 1 derivatives with rBet v 1 wild-type by skin prick and intradermal testing: Results obtained in a French population. *Clin Exp Allergy* 2000;30:1076–1084.
- 77 Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, Bannon GA: Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999;103:535–542.
- 78 Kleber-Janke T, Cramer R, Appenzeller U, Schlaak M, Becker WM: Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999;119:265–274.
- 79 Chapman MD, Smith AM, Vailes LD, Arruda LK: Recombinant mite allergens. New technologies for the management of patients with asthma. *Allergy* 1997;52:374–379.
- 80 Takai T, Mori A, Yuuki T, Okudaira H, Okumura Y: Non-anaphylactic combination of partially deleted fragments of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. *Mol Immunol* 1999;36:1055–1065.
- 81 Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology: Position paper. Skin tests used in type I allergy testing. *Allergy* 1989;44:1–59.
- 82 Moser M, Cramer R, Menz G, Schneider T, Dudler T, Virchow C, Gmachi M, Blaser K, Suter M: 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;149:454–460.
- 83 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 1. Homology with cysteine proteases. *J Exp Med* 1988;167:175–182.
- 84 Quiralte J, Gonzalez E, Arias De Saavedra JM, Villalba M, Florido JF, Saenz De San Pedro B, Rodriguez R: Immunological activity of recombinant Ole e 1 in patients with *Olea europaea* pollinosis. *Int Arch Allergy Immunol* 2000;122:101–107.

- 85 Vrtala S, Fischer S, Grote M, Vangelista L, Pastore A, Sperr WR, Valent P, Reichelt R, Kraft D, Valenta R: Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from timothy grass (*Phleum pratense*) pollen. *J Immunol* 1999;163:5489–5496.
- 86 Rossi RE, Monasterolo G, Operti D, Operti R, Berlen R: Evaluation of IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, and Phl p 5) in a random sample of patients with specific IgE to *Phleum pratense*. *Allergy* 2000;55:181–184.
- 87 Weber A: Actin binding proteins that change extent and rate of actin monomer-polymer distribution by different mechanisms. *Mol Cell Biochem* 1999;190:67–74.
- 88 Aalberse RC: Structural biology of allergens. *J Allergy Clin Immunol* 2000;106:228–238.
- 89 Caballero T, Martin-Esteban M: Association between pollen hypersensitivity and edible vegetable allergy: A review. *J Investig Allergol Clin Immunol* 1998;8:6–16.
- 90 Valenta R, Duchene M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 1991;253:557–560.
- 91 Appenzeller U, Meyer C, Menz G, Blaser K, Cramer R: IgE-mediated reactions to autoantigens in allergic diseases. *Int Arch Allergy Immunol* 1999;118:193–196.
- 92 Niederberger V, Pauli G, Grönlund H, Froschl R, Rumpold H, Kraft D, Valenta R, Spitzauer S: Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen: A quantitative IgE inhibition study with sera from different populations. *J Allergy Clin Immunol* 1998;102:579–591.
- 93 Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, Ferreira F, Kraft D, Valenta R: Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: Diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100–1109.
- 94 Tsai L, Sun Y, Chao P, Ng H, Hung M, Hsieh K, Liaw S, Chua K: Sequence analysis and expression of a cDNA clone encoding a 98-kDa allergen in *Dermatophagoides farinae*. *Clin Exp Allergy* 1999;29:1606–1613.
- 95 Kronqvist M, Johansson E, Magnusson CG, Olsson S, Eriksson TL, Gafvelin G, van Hage-Hamsten M: Skin prick test and serological analysis with recombinant group 2 allergens of the dust mites *L. destructor* and *T. putrescentiae*. *Clin Exp Allergy* 2000;30:670–676.
- 96 Jorge PPO, Tobias, KRC, Ferrirani VPL, Smith AM, Chapman MD, Arruda LK: Recombinant allergens for diagnosis of mite allergy in children with asthma and/or rhinitis: Comparison with commercial extracts. *J Allergy Clin Immunol* 2000;105:S169.
- 97 Lin KL, Hsieh KH, Thomas WR, Chiang BL, Chua KY: Characterization of Der p 5 allergen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. *J Allergy Clin Immunol* 1994;94:989–996.
- 98 Lynch NR, Thomas WR, Garcia NM, Di Prisco MC, Puccio FA, L'Opez R I, Hazell LA, Shen HD, Lin KL, Chua KY: Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1997;114:59–67.
- 99 Lynch NR, Thomas WR, Chua Y, Garcia N, Di Prisco MC, Lopez R: In vivo biological activity of recombinant Der p II allergen of house-dust mite. *Int Arch Allergy Immunol* 1994;105:70–74.
- 100 Arruda LK, Vailes LD, Platts-Mills TA, Fernandez-Caldas E, Montealegre F, Lin KL, Chua KY, Rizzo MC, Naspitz CK, Chapman MD: Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med* 1997;155:343–350.
- 101 Chew FT, Yi FC, Chua KY, Fernandez-Caldas E, Arruda LK, Chapman MD, Lee BW: Allergic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1999;29:982–988.
- 102 Aalberse RC: Allergens from mites: Implications of cross-reactivity between invertebrate antigens. *Allergy* 1998;53:47–48.
- 103 Cramer R, Faith A, Hemmann S, Jaussi R, Ismail C, Menz G, Blaser K: Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. *J Exp Med* 1996;184:265–270.
- 104 Mayer C, Appenzeller U, Seelbach H, Achatz G, Oberkofler H, Breitenbach M, Blaser K, Cramer R: Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P₂ protein in individuals sensitized to *Aspergillus fumigatus* P₂ protein. *J Exp Med* 1999;189:1507–1512.
- 105 Martinez S, Smith JL: Crystallization and preliminary characterization of mitogillin, a ribosomal ribonuclease from *Aspergillus restrictus*. *J Mol Biol* 1991;218:489–492.
- 106 Nikolaizik WH, Moser M, Cramer R, Little S, Warner JO, Blaser K, Schöni MH: Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology. *Am J Respir Crit Care Med* 1995;152:634–639.
- 107 Disch R, Menz G, Blaser K, Cramer R: Different reactivity to recombinant *Aspergillus fumigatus* allergen I/a in patients with atopic dermatitis or allergic asthma sensitized to *Aspergillus fumigatus*. *Int Arch Allergy Immunol* 1995;108:89–94.
- 108 Moser M, Cramer R, Menz G, Suter M: Recombinant *Aspergillus fumigatus* allergen I/a (rAsp f I/a) in the diagnosis of *Aspergillus*-related diseases. *Agents Actions Suppl* 1993;43:131–137.
- 109 Unger A, Stoger P, Simon-Nobbe B, Susani M, Cramer R, Ebner C, Hintner H, Breitenbach M: Clinical testing of recombinant allergens of the mold *Alternaria alternata*. *Int Arch Allergy Immunol* 1999;118:220–221.
- 110 Aden E, Weber B, Bossert J, Teppke M, Frank E, Wahl R, Fiebig H, Cromwell O: Standardization of *Alternaria alternata*: Extraction and quantification of Alt a 1 by using an mAb-based 2-site binding assay. *J Allergy Clin Immunol* 1999;104:128–135.
- 111 Brander KA, Borbely P, Cramer R, Pichler WJ, Helbling A: IgE-binding proliferative responses and skin test reactivity to Cop c 1, the first recombinant allergen from the basidiomycete *Coprinus comatus*. *J Allergy Clin Immunol* 1999;104:630–636.
- 112 Dudler T, Chen WQ, Wang S, Schneider T, Annand RR, Dempcy RO, Cramer R, Gmachl M, Suter M, Gelb MH: High-level expression in *Escherichia coli* and rapid purification of enzymatically active honey bee venom phospholipase A₂. *Biochim Biophys Acta* 1992;1165:201–210.
- 113 Hoffman DR: Allergens in bee venom. III. Identification of allergen B of bee venom as an acid phosphatase. *J Allergy Clin Immunol* 1977;59:364–366.
- 114 King TP, Lu G, Agosto H: Antibody responses to bee melittin (Api m 4) and hornet antigen 5 (Dol m 5) in mice treated with the dominant T-cell epitope peptides. *J Allergy Clin Immunol* 1998;101:397–403.
- 115 Scott DL, White SP, Otwinowski Z, Yuan W, Gelb MH, Sigler PB: Interfacial catalysis: The mechanism of phospholipase A₂. *Science* 1990;250:1541–1546.
- 116 Müller UR, Dudler T, Schneider T, Cramer R, Fischer H, Skrbic D, Maibach R, Blaser K, Suter M: Type I skin reactivity to native and recombinant phospholipase A₂ from honeybee venom is similar. *J Allergy Clin Immunol* 1995;96:395–402.
- 117 Muller U, Fricker M, Wymann D, Blaser K, Cramer R: Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A₂. *Clin Exp Allergy* 1997;27:915–920.
- 118 Wymann D, Akdis CA, Blesken T, Akdis M, Cramer R, Blaser K: Enzymatic activity of soluble phospholipase A₂ does not affect the specific IgE, IgG4 and cytokine responses in bee sting allergy. *Clin Exp Allergy* 1998;28:839–849.
- 119 Wuthrich B, Stager J, Johansson SG: Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990;45:566–571.
- 120 Bauer L, Ebner C, Hirschwehr R, Wuthrich B, Pichler C, Fritsch R, Scheiner O, Kraft D: IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: Immunoblot investigation of the birch-mugwort-celery syndrome. *Clin Exp Allergy* 1996;26:1161–1170.
- 121 Hoffmann-Sommergruber K, Demoly P, Cramer R, Breiteneder H, Ebner C, Laimer Da Camara Machado M, Blaser K, Ismail C, Scheiner O, Bousquet J, Menz G: IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1. *J Allergy Clin Immunol* 1999;104:478–484.

- 122 Breiteneder H, Ebner C: Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000;106:27–36.
- 123 Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelmann H, Kraft D, et al: Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
- 124 Ganglberger E, Radauer C, Grimm R, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O, Jensen-Jarolim E: N-terminal sequences of high molecular weight allergens from celery tuber. *Clin Exp Allergy* 2000;30:566–570.
- 125 Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, Susani M, Ahorn H, Ebner C, Kraft D, Scheiner O: Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484–489.
- 126 Scheurer S, Wangorsch A, Haustein D, Vieths S: Cloning of the minor allergen Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. *Clin Exp Allergy* 2000;30:962–971.
- 127 Breiteneder H: Latex allergens. *Int Arch Appl Allergol* 2001;121:98–107.
- 128 Toraason M, Sussman G, Biagini R, Meade J, Beezhold D, Germole D: Latex allergy in the workplace. *Toxicol Sci* 2000;58:5–14.
- 129 Niggemann B, Breiteneder H: Latex allergy in children. *Int Arch Allergy Immunol* 2000;121:98–107.
- 130 Breiteneder H, Sowka S, Wagner S, Krebitz M, Hafner C, Kinaciyan T, Yeang HY, Scheiner O: Cloning of the patatin-like latex allergen Hev b 7, its expression in the yeast *Pichia pastoris* and its immunological characterization. *Int Arch Allergy Immunol* 1999;118:309–310.
- 131 Hamilton RG, Biagini RE, Krieg EF: Diagnostic performance of Food and Drug Administration-cleared serologic assays for natural rubber latex-specific IgE antibody. The Multi-Center Latex Skin Testing Study Task Force. *J Allergy Clin Immunol* 1999;103:925–930.
- 132 Brehler R, Theissen U, Mohr C, Luger T: Latex-fruit syndrome: Frequency of cross-reacting IgE antibodies. *Allergy* 1997;52:404–410.
- 133 Wagner S, Swoka S, Mayer C, Cramer R, Focke M, Kurup V, Scheiner O, Breiteneder H: Identification of a *Hevea brasiliensis* latex manganese superoxide dismutase (Hev b 10) as a cross-reactive allergen. *Int Arch Allergy Immunol* 2001;125:120–124.
- 134 Yip L, Hickey V, Wagner B, Liss G, Slater J, Breiteneder H, Sussman G, Beezhold D: Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000;121:292–299.
- 135 Suter M, Dudlet T, Schneider T, Gelb M, Moser M, Cramer R, Menz G, Müller U: Rekombinantes Bienengift-Allergen Phospholipase A₂. Ein Modell für die Standardisierung von rekombinanten Allergenen. *Allergologie* 1994;17:414–418.
- 136 Dreborg S: The skin prick test in the diagnosis of atopic allergy. *J Am Acad Dermatol* 1989;21:820–821.
- 137 Hakkaart GA, Harmsen MM, Chua KY, Thomas WR, Aalberse RC, Van Ree R: Expression of the house dust mite allergen Der p 2 in the baker's yeast *Saccharomyces cerevisiae*. *Clin Exp Allergy* 1998;28:45–52.
- 138 Van Ree R, van Leeuwen WA, Bulder I, Bond J, Aalberse RC: Purified natural and recombinant Fel d 1 and cat albumin in vitro diagnostics for cat allergy. *J Allergy Clin Immunol* 1999;104:1223–1230.
- 139 Vailes LD, Kinter MT, Arruda LK, Chapman MD: High-level expression of cockroach allergen, Bla g 4, in *Pichia pastoris*. *J Allergy Clin Immunol* 1998;101:274–280.
- 140 McDermott MJ, Weber E, Hunter S, Stedman KE, Best E, Frank GR, Wang R, Escudero J, Kuner J, McCall C: Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1). *Mol Immunol* 2000;37:361–375.
- 141 Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag M, Strauss M: Efficient gene transfer into human hepatocytes by baculovirus vectors. *Proc Natl Acad Sci USA* 1995;92:10099–10103.
- 142 Grammer LC, Shaughnessy MA, Shaughnessy JJ, Patterson R: Brief report: Evaluation of polymerized ragweed extracts by intradermal end point titration, RAST inhibition, and parallel line bioassay. *J Allergy Clin Immunol* 1985;76:123–127.
- 143 Van de Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC: Discrepancies between the skin test and IgE antibody assays: Study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgE. *J Allergy Clin Immunol* 1988;82:270–281.
- 144 Nelson HS, Oppenheimer J, Buchmeier S, Kordash TR, Freshwater LL: An assessment of the role of intradermal skin testing in the diagnosis of clinically relevant allergy to timothy grass. *J Allergy Clin Immunol* 1996;97:1193–1201.
- 145 Godnic-Cvar J, Susani M, Breiteneder H, Berger A, Havelec L, Waldhor T, Hirschwehr R, Valenta R, Scheiner O, Rudiger H, Kraft D, Ebner C: Recombinant Bet v 1, the major birch pollen allergen, induces hypersensitivity reactions equal to those induced by natural Bet v 1 in the airways of patients allergic to tree pollen. *J Allergy Clin Immunol* 1997;99:354–359.
- 146 Dreborg S: The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy* 1993;48:473–475.
- 147 Devenney I, Falth-Magnusson K: Skin prick tests may give generalized allergic reactions in infants. *Ann Allergy Asthma Immunol* 2000;85:457–460.
- 148 Kenimer J, Arntzen FC, Lowenstein H, Hausstein D, Geffer M: Regulations governing the licensure of recombinant allergens and peptides. *ACI News* 1994;6:60–63.
- 149 Webb V, Davies, J: Antibiotic preparations contain DNA: A source of drug resistance genes. *Antimicrob Agents Chemother* 1993;37:2379–2384.
- 150 Elfimer L, Svensson M, Lidholm J, Pauli G, Valenta R: Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol* 1997;113:249–251.
- 151 Laffer S, Spitzauer S, Susani M, Pairleitner H, Schweiger C, Grönlund H, Menz G, Pauli G, Ishii T, Nolte H, Ebner C, Schon AH, Kraft D, Eichler HG, Valenta R: Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J Allergy Clin Immunol* 1996;98:652–658.
- 152 Menz G, Willer G, Cramer R: Allergic bronchopulmonary aspergillosis. *Pneumologie* 2000;54:375–384.
- 153 Cramer R, Hemmann S, Ismail C, Menz G, Blaser K: Recombinant allergens for diagnosis of allergic bronchopulmonary aspergillosis. *Pneumologie* 1998;52:183–185.
- 154 Mayer C, Hemmann S, Faith A, Blaser K, Cramer R: Cloning, production, characterization and IgE cross-reactivity of different manganese superoxide dismutases in individuals sensitized to *Aspergillus fumigatus*. *Int Arch Allergy Immunol* 1997;113:213–215.
- 155 Wang NM, Lee MF, Wu CH: Immunologic characterization of a recombinant American cockroach (*Periplaneta americana*) Per a 1 (Cr-PII) allergen. *Allergy* 1999;54:119–127.
- 156 Menz G, Dolecek C, Schönheit-Kenn U, Ferreira F, Moser M, Schneider T, Suter M, Boltz-Nitulescu G, Ebner C, Kraft D, Valenta R: Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v1, the major birch pollen allergen. *Clin Exp Allergy* 1996;26:50–60.
- 157 Tsai LC, Chao PL, Hung MW, Sun YC, Kuo IC, Chua KY, Liaw SH, Chua KY, Kuo IC: Protein sequence analysis and mapping of IgE and IgG epitopes of an allergenic 98-kDa *Dermatophagoides farinae* paramyosin, Der f 11. *Allergy* 2000;55:141–147.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.