

Cyclophilins, a new family of cross-reactive allergens

Sabine Flückiger¹, Helmi Fijten¹, Paul Whitley², Kurt Blaser¹ and Reto Crameri¹

¹ Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

² Department of Biology and Biochemistry, University of Bath, Bath, GB

Type I allergic reactions occur by immediate release of anaphylactogenic mediators due to cross-linking of IgE bound to the high-affinity Fc ϵ RI on the surface of effector cells of sensitized individuals after allergen exposure. IgE-mediated hypersensitivity against normally innocuous environmental antigens is of clinical importance because of an increasing incidence of asthma and severe atopic diseases causing raising health care burdens to the society. A vast variety of different molecular structures has been shown to be able to induce hypersensitivity reactions. However, the high structural homology between phylogenetically conserved allergenic proteins present in different, apparently unrelated sources of exposure seems to play an important role in IgE-mediated poly-sensitization. These allergen families, formally termed pan-allergens, represent proteins sharing a high degree of sequence homology. Here we report cloning, production and serological investigations of a new pan-allergen family, the cyclophilins, found to be cross-reactive across species including humans. IgE-mediated cross-reactivity against autoantigens may contribute to perpetuation of severe atopic disorders even in the absence of exogenous allergen exposure. The molecular definition of pan-allergen families may substantially contribute to reduce the number of structures needed for diagnosis and therapy of allergic diseases based on highly pure, standardized recombinant allergens.

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1 Introduction

Cyclophilin (CyP) was first isolated in 1984 from bovine thymus as a specific high-affinity binding protein for the immunosuppressant agent cyclosporin A (CsA) [1]. In 1989 CyP was reported to correspond to the enzyme peptidyl-prolyl *cis-trans* isomerase. This enzyme family plays a crucial role in protein folding [2, 3] and includes many homologous proteins with multiple isoforms abundantly found in prokaryotic and eukaryotic organisms [4]. The remarkable phylogenetic conservation and the presence of CyP in all organisms investigated suggest that enzymatic catalysis of peptidyl-prolyl isomerization is a critical cellular function [5]. Because the affinities of CsA derivatives for CyP correlate with their immunosuppressive activities, CyP has been implicated in the helper T cell signal transduction pathway [6, 7]. More specifically, CsA has been shown to inhibit interleukin-2 gene tran-

scription stimulated by the nuclear factor of activated T cells [8], blocking translocation of a preexisting cytoplasmic subunit of the nuclear factor to the nucleus [9]. Recent studies indicate that CsA also blocks the activation of JNK and p38 signaling pathways triggered by antigen recognition, making CsA a highly specific inhibitor of T cell activation [10]. However, CyP has also been isolated as an IgE-binding protein from the basidiomycete *Psilocybe cubensis* [11], from the mould *Aspergillus fumigatus* [12] and from the yeast *Malassezia furfur* [13]. Recently, cyclophilin has been demonstrated to be a minor allergen involved in birch allergy [14]. These cyclophilins show a high degree of sequence homology with the corresponding human enzymes CyP A, B and C [15]. A high degree of sequence identity between allergens and the corresponding human proteins has been reported in several cases [15–18], whereas strong humoral and cell-mediated autoreactivity has been clearly shown in subjects suffering from mould-associated chronic asthma or severe atopic dermatitis [16, 17]. However, the clinical relevance of these reactions has never been demonstrated by provocation tests with autoantigens, although intradermal skin challenges showed sensitization to allergen-homologous human proteins *in vivo* [16, 17]. IgE-mediated cross-reactivity

[122107]

Abbreviations: **ABPA:** Allergic bronchopulmonary aspergillosis **CyP:** Cyclophilin **CsA:** Cyclosporin A **EU/ml:** ELISA units/ml **GuCl:** Guanidinium chloride

between homologous allergens from phylogenetically distant species is likely to be responsible for clinical syndromes like mugwort-birch-celery-spice or pollen-associated food allergies [19, 20]. In this report we describe IgE-binding capacity and cross-reactivity of CyP from different sources, including man, showing that CyP can be considered as a novel pan-allergen family.

2 Results

2.1 Cloning, production and characterization of recombinant CyP

To isolate IgE-binding proteins of *M. furfur* a cDNA library displayed on the surface of filamentous phage M13 was constructed and selectively enriched for allergen-displaying phage using serum IgE from *M. furfur* sensitized individuals as ligand [13]. Among the large population of phage encoding different allergenic proteins, one clone containing an open reading frame spanning 486 bp revealed significant homology with CyP. The allergen, formally termed Mal f 6, shows 69% sequence identity with *A. fumigatus* CyP (Asp f 11) [12] encoded by an open reading frame of 513 bp isolated using the same technology. To investigate the cross-reactivity between CyP from different species *C. albicans* and *S. cerevisiae* CyP were cloned by PCR using primers designed from the known sequences (Table 1). In an analogous procedure human CyP B [15], CyP A and CyP C were cloned from human cDNA to test their ability to bind IgE from

individuals sensitized to CyP allergens (Table 1). Alignment of the amino acid sequences deduced from the cDNA encoding the Mal f 6 and Asp f 11 allergens with the other CyP sequences used in this work shows a high degree of sequence identity (Fig. 1).

Mal f 6 recombinant protein was produced in *E. coli* BL21 DE3 pLysS as described [13]. All other CyP-encoding inserts were subcloned into the high level expression vector pQE30 and transformed by electroporation into *E. coli* strains M15 to produce hexahistidine-tagged fusion proteins [21].

The Ni²⁺-chelate affinity-purified recombinant CyP were found to be >95% pure as shown by denaturing SDS-PAGE and Coomassie blue staining. The estimated molecular masses of 17 kDa for Mal f 6, 21 kDa for Asp f 11, 18 kDa for *C. albicans* and *S. cerevisiae* CyP, and 18, 21 and 23 kDa for the human CyP A, B and C, respectively, were in good agreement with the calculated values for the His-tagged fusions (data not shown).

Enzymatic activity of the recombinant CyP was demonstrated by their ability to catalyze the *cis-trans* isomerization of *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide. The reaction was monitored by the increase in absorbency at 390 nm due to free 4-nitroaniline generated by α -chymotrypsin cleavage of the *trans*-isomer [22] and recorded over a period of 2 min (Fig. 2). To monitor the thermal isomerization of the substrate a control experiment without CyP was run in parallel (Fig. 2). Asp f 11, C.

Table 1. PCR primers

Primer	Oligonucleotide sequence (restriction sites underlined)	Restriction enzyme
5'-cCyP	CGGGATCCATGTCTACCGTTTACTTTGATG	<i>Bam</i> HI
3'-cCyP	CCCAAGCTTTTACAATTGACCAGATTCTTCAATG	<i>Hind</i> III
5'-sCyP	CGGGATCCATGTCCCAAGTCTATTTTGATG	<i>Bam</i> HI
3'-sCyP	CCCAAGCTTTTATAATTCACCGGACTTGGC	<i>Hind</i> III
5'-hCyP A	CGGGATCCATGGTCAAGCCCACCGTGTTTC	<i>Bam</i> HI
3'-hCyP A	CCCAAGCTTTTATTCGAGTTGTCCACAGTCAGC	<i>Hind</i> III
5'-hCyP C	CGGGATCCATGGGCCCGGGTCCTCCG	<i>Bam</i> HI
3'-hCyP C	CCCAAGCTTTTCACCAATCAGCGATCACAACC	<i>Hind</i> III

c = *C. albicans*; s = *S. cerevisiae*; h = *H. sapiens*

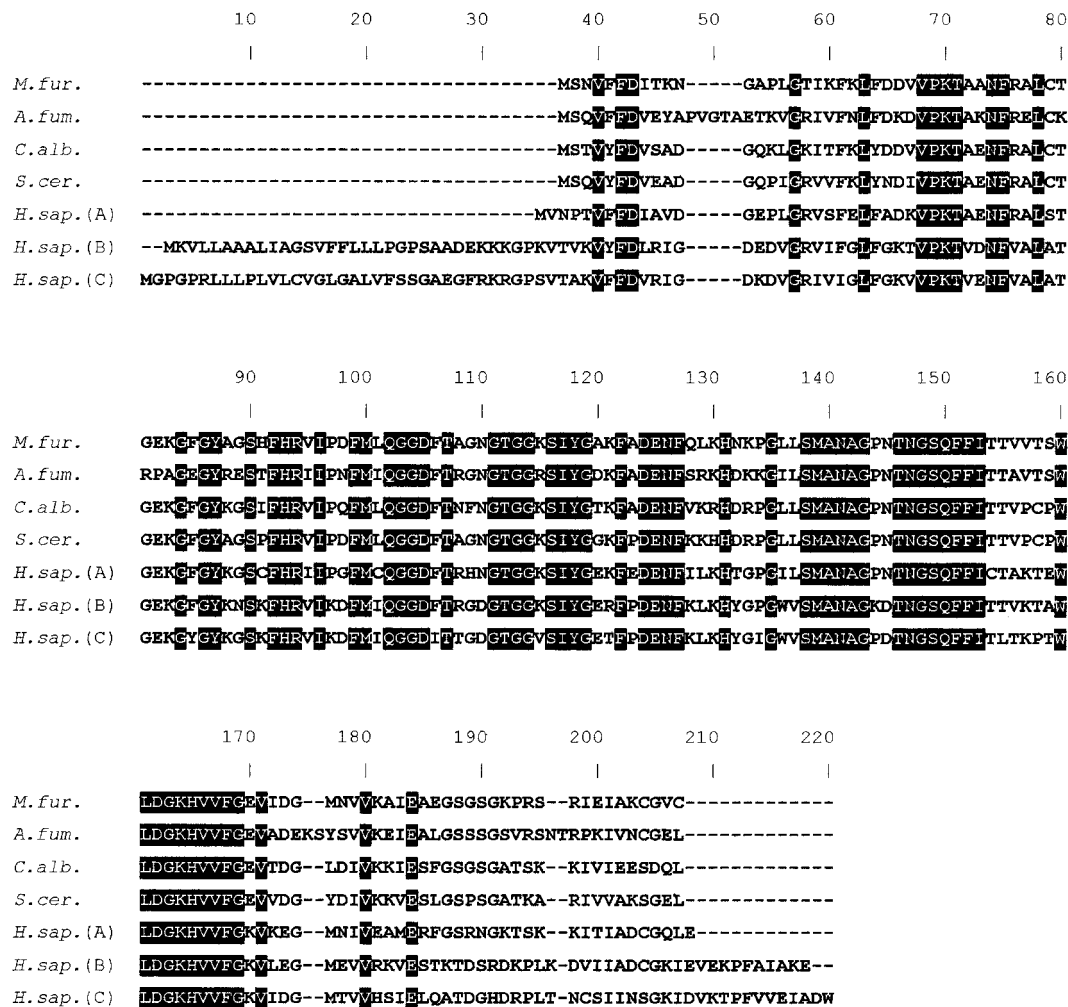


Fig. 1. Alignment of the deduced amino acid sequences of *M. furfur* (*M. fur.*), *A. fumigatus* (*A. fum.*), *C. albicans* (*C. alb.*), *S. cerevisiae* (*S. cer.*) and the human CyP A, B and C (*H. sap.*). Amino acid residues matching in all sequences are marked by shading, gaps are indicated by dashes.

albicans CyP and the human CyP A, B and C were found to be enzymatically active indicating a native-like folding of the enzymes.

2.2 IgE immunoblot analysis

The IgE-binding capacity of the recombinant CyP was investigated by Western blotting using a serum pool of individuals either sensitized to *M. furfur* or suffering from allergic bronchopulmonary aspergillosis (ABPA). As shown in Fig. 3, CyP were found to bind IgE from sera of *M. furfur*- or *A. fumigatus*-sensitized individuals, with Asp f 11 and Mal f 6 showing the strongest binding capacity for IgE, whereas human CyP C showed negligible IgE binding.

2.3 Inhibition ELISA

In a further step we investigated whether CyP share common IgE-binding epitopes by inhibition ELISA. As an example depicted in Fig. 4, the binding of serum IgE from an *A. fumigatus*-sensitized individual to Asp f 11 coated on solid phase was completely inhibited by increasing amounts of Asp f 11 added to the fluid phase, whereas BSA as control protein did not affect IgE binding. Analysis of the inhibition properties of Mal f 6 revealed that the *M. furfur* CyP is able to inhibit binding of IgE to solid-phase-coated Asp f 11 in a dose-dependent manner (Fig. 4). In similar experiments, Mal f 6 was also capable to inhibit IgE-binding to the other recombinant CyP, suggesting shared IgE-binding epitopes among this enzyme family (data not shown).

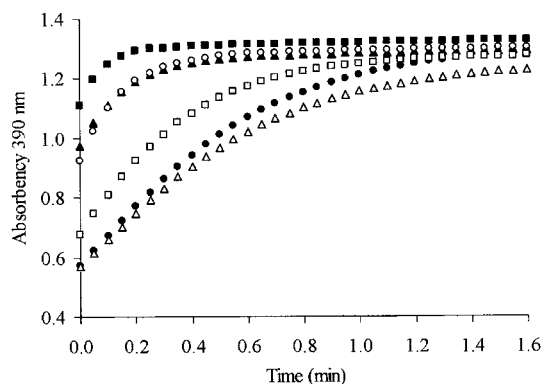


Fig. 2. Time course of the peptidyl-prolyl *cis-trans* isomerization by recombinant CyP (25 nM final concentration) and in the absence of CyP (Δ). The substrate *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide was isomerized by cyclophilin, the *trans*-isomer of the substrate was cleaved by α -chymotrypsin and the increase in absorbency at 390 nm recorded. $\blacktriangle$: Asp f 11; $\bullet$: *C. albicans* CyP; $\circ$: human CyP A; $\blacksquare$: human CyP B; $\square$: human CyP C.

2.4 Extended serological investigations and skin tests

To analyze the incidence of sensitization to Asp f 11 and Mal f 6, we selected 30 and 48 individuals sensitized to *A. fumigatus* and *M. furfur*, respectively, and 10 control individuals without a history of atopy. From the 30 *A. fumigatus*-sensitized individuals, 27 (90%) showed Asp f 11-specific IgE values >16 ELISA units (EU)/ml compared to the marginal IgE reactivity of the control group

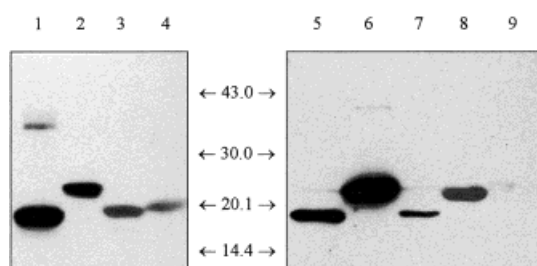


Fig. 3. Immunoblot analysis for specific IgE against recombinant CyP. Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes and incubated with a serum pool of *M. furfur*-sensitized individuals (left panel) or ABPA patients (right panel). The molecular mass standard (kDa) is indicated. Lane 1: Mal f 6 (2 μ g); lane 2: Asp f 11 (2 μ g); lane 3: *C. albicans* CyP (6 μ g); lane 4: *S. cerevisiae* CyP (6 μ g); lane 5: Mal f 6 (2 μ g); lane 6: Asp f 11 (1 μ g); lane 7: human CyP A (6 μ g); lane 8: human CyP B (6 μ g); lane 9: human CyP C (6 μ g).

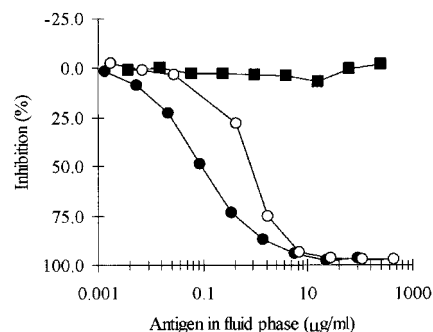


Fig. 4. Inhibition of IgE-binding to Asp f 11 coated on a solid phase. Serum of an *A. fumigatus*-sensitized patient with ABPA was preincubated with increasing amounts of Asp f 11 ($\bullet$), Mal f 6 ($\circ$) and BSA as negative control ($\blacksquare$). Preincubated serum samples were transferred to wells coated with Asp f 11 and IgE binding was analyzed by ELISA.

to Asp f 11 (mean 5.4 ± 7.3 EU/ml). These individuals were regarded as sensitized to Asp f 11 considering three times the mean EU/ml value of the control group as background value for the ELISA determination of allergen-specific IgE. The ability of recombinant Asp f 11 to induce type I reactions *in vivo* was assessed by intradermal skin tests. Five individuals with serum IgE levels >30 EU/ml against Asp f 11, four individuals allergic to the fungus with Asp f 11-specific serum IgE levels <15 EU/ml and three nonallergic control individuals were investigated for their ability to respond to increasing intradermal challenge with recombinant Asp f 11. As expected, none of the four nonallergic controls reacted against the recombinant protein confirming the specificity of the test. A positive skin reaction was detected only in *A. fumigatus*-sensitized individuals who had Asp f 11-specific IgE levels >30 EU/ml, whereas the amounts of recombinant protein needed to elicit a classical type I reaction ranged from 1 to 10 ng, depending on the subject. These results indicate that Asp f 11 is able to induce release of pro-inflammatory mediators by cross-linking of allergen-specific IgE bound to effector cells. From the 48 *M. furfur*-sensitized individuals, 44 (92%) showed Mal f 6-specific IgE values >26 EU/ml, compared to the marginal IgE reactivity of the control group to Mal f 6 (mean 8.8 ± 5.6 EU/ml). *M. furfur*-sensitized individuals showing Mal f 6-specific IgE values >26 EU/ml were regarded as CyP-sensitized. As shown in Fig. 5A and B, Asp f 11- and Mal f 6-specific IgE values in *A. fumigatus*- and *M. furfur*-sensitized individuals correlated fairly well ($r=0.820$, $p<0.001$ and $r=0.589$, $p<0.001$, respectively) providing further evidence for cross-reactivity between the allergens. Furthermore, statistically relevant correlations were demonstrated between Mal f 6-specific IgE and IgE raised against *C. albicans* CyP (Fig. 5C, $r=0.733$, $p<0.001$) and *S. cerevisiae* CyP (Fig. 5D, $r=0.518$,

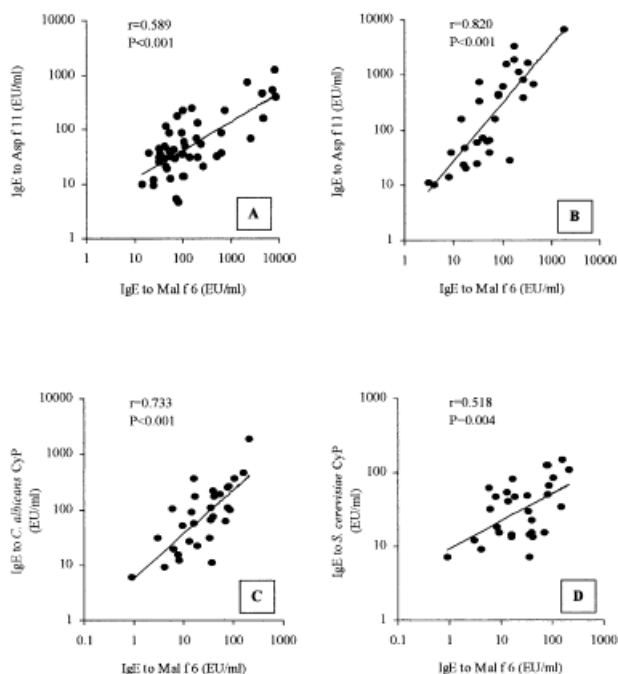


Fig. 5. Correlation between specific IgE against (A) Mal f 6 and Asp f 11 in 48 sera of individuals sensitized to *M. furfur*, (B) Mal f 6 and Asp f 11 in 30 sera of individuals sensitized to *A. fumigatus*, (C) Mal f 6 and *C. albicans* CyP in 30 sera of individuals sensitized to *A. fumigatus*, and (D) Mal f 6 and *S. cerevisiae* CyP in 30 sera of individuals sensitized to *A. fumigatus*. Values for each serum are expressed as EU/ml and plotted in a linear diagram. Spearman rank order correlation coefficients and statistical significance are given.

$p=0.004$) in the group of *A. fumigatus*-sensitized individuals, strongly indicating IgE-mediated cross-reactivity between these enzymes. Similar correlations were obtained comparing the specific IgE values of Asp f 11 with the specific IgE values obtained with sera of *A. fumigatus*-sensitized individuals using *C. albicans* and *S. cerevisiae* CyP (data not shown).

3 Discussion

Recently we demonstrated that sera from *A. fumigatus*-sensitized individuals suffering from ABPA or severe chronic atopic dermatitis contain IgE antibodies against a vast variety of *A. fumigatus* IgE-binding proteins [12]. Manganese-dependent superoxide dismutase [16] and acidic ribosomal P₂ protein [17], representing evolutionary highly conserved proteins, also showed cross-reactivity with the corresponding human homologues [15] and with a variety of homologous proteins derived from different, phylogenetically distant species [23, 24]. Closely related molecular structures have been postu-

Table 2. Characteristics of recombinant CyP

Organism	Residues ^{a)}	MW (kD) ^{a)}	Identity with Mal f 6 (%)
<i>M. furfur</i>	162	17.2	100
<i>A. fumigatus</i>	171	18.8	69
<i>C. albicans</i>	162	17.6	75
<i>S. cerevisiae</i>	162	17.4	74
<i>H. sapiens</i> (A)	165	18.0	71
<i>H. sapiens</i> (B)	208	22.7	63
<i>H. sapiens</i> (C)	212	22.8	60

a) Amino acid residues without [His]₆-tag.

lated to be responsible for many clinical syndromes including food-pollen allergy [25], latex-mould syndrome [26] and exacerbation or perpetuation of severe atopic diseases through cross-reactive interactions with allergen-homologous human autoantigens [16–18]. Therefore, knowledge about cross-reactive structures is essential to understand the pathophysiology of allergic diseases, to predict clinically relevant sensitization to allergen sources, which contain immunologically related allergens, and for the development of new diagnostic and therapeutic concepts. Identification of cross-reactive molecular structures is likely to allow a substantial reduction of the number of allergens required for a component resolved diagnosis and therapy of allergic conditions [27]. Here we report the identification of cyclophilin as a new pan-allergen family. This enzyme was first isolated as allergen from the basidiomycete *Psilocybe cubensis*, and later also as IgE-binding protein from different cDNA phage surface display libraries, including human cDNA [15, 28]. The corresponding proteins, recombinantly produced in *E. coli*, were shown to be enzymatically active, indicating native-like folding, a prerequisite for IgE-binding [29]. The ability of CyP to act as allergen *in vivo* was shown by positive skin reactions of *A. fumigatus*-sensitized individuals, clearly demonstrating sensitization to this molecular structure. Cross-reactivity between CyP derived from *A. fumigatus*, *M. furfur*, *C. albicans*, *S. cerevisiae* and human CyP A, CyP B and, marginally, CyP C was shown by Western blot analysis and competitive inhibition experiments. Moreover, determination of CyP-specific serum IgE in *A. fumigatus*- or *M. furfur*-sensitized individuals with CyP of different origin as antigen showed statistically relevant correlations (Fig. 5), corroborating the display of shared B cell epitopes between the CyP. Based on these results, CyP can be considered as a novel functional pan-allergen and a major allergen from both, *M. furfur* and *A.*

fumigatus according to the high incidence of sensitization exceeding 50% of the investigated allergic individuals [30]. Cross-reactivity of IgE antibodies can be expected for proteins sharing more than 50% sequence identity as a consequence of molecular mimicry resulting from shared features on the level of both, primary and tertiary structure of the homologous proteins [29] and can explain large parts of the results obtained during this work. However, the IgE-mediated cross-reactivity to the human CyP is more difficult to explain. The presence of a human protein homologous to an allergen available to the immune system would be expected to induce immune tolerance at least to the shared epitopes. CyP is an intracellular enzyme and as such not expected to be available to the immune system under normal circumstances. However, in some pathological situations like chronic inflammation the proteins can become available to the immune system as a consequence of tissue damage due to the inflammatory process [17, 18]. Therefore, exposure to self antigens containing cross-reactive determinants can occur *in vivo* and contribute to the perpetuation of allergic symptoms [16–18]. In fact, cross-reactive IgE antibodies were so far only detected in severe chronic allergic conditions like allergic bronchopulmonary aspergillosis [15, 16] or severe forms of atopic dermatitis [17, 18]. The availability of the sequences encoding the different CyP allergens together with the solved three-dimensional structures of the human CyP will allow molecular modeling of the structures to predict solvent-exposed residues shared between the different proteins as candidates responsible for the observed cross-reactivity to be submitted to experimental verification by site directed mutagenesis.

4 Materials and methods

4.1 Cloning, production and characterization of recombinant CyP

Phage surface display technology [31] was used to clone CyP from *A. fumigatus* [12], formally termed Asp f 11, and from *M. furfur* [13], formally Mal f 6, according to the recommendations of the international allergen nomenclature committee [30]. The genes encoding *C. albicans* and *S. cerevisiae* CyP were amplified by PCR from a genomic DNA prepared as described [32] using the primers reported in Table 1. PCR cycling conditions were 30 cycles of 95°C for 60 s, 60°C for 60 s, and 72°C for 60 s followed by a terminal extension at 72°C for 10 min. Human CyP B was cloned by PCR as described [15]. Human CyP A and CyP C were cloned from human cDNA isolated from a commercial λ -ZapII cDNA library (Stratagene, La Jolla, CA) by *in vivo* excision of the p-Bluescript phagemid [33] using the primers reported in Table 1 and the same cycling conditions as described above. All PCR amplification products were puri-

fied on 1.2% agarose gel, the bands of interest excised and the DNA isolated using GenElute Agarose Spin Columns (Supelco, Bellefonte, PA). The purified PCR products were digested with *Bam*HI and *Hind*III, cloned into the high level expression vector pQE30 and transformed into competent *E. coli* M15 pREP4 cells [21, 34]. After sequence verification, correct clones were used to produce recombinant CyP as N-terminal hexahistidine-tagged fusion proteins [21]. Cell pellets were lysed with 6 M GuCl and recombinant proteins purified in one step over Ni²⁺-chelate affinity columns (Qia-gen, Hilden, Germany). Molecular size and purity of the recombinant proteins were analyzed by SDS-PAGE (4–20% gels, Novex, San Diego, CA) under denaturing, reducing conditions.

Peptidyl-prolyl *cis-trans* isomerase activity of the recombinant CyP was measured using the assay described by Kofron et al. [22] with minor modifications. Briefly, 25 μ l of 0.5 μ M CyP solution (final concentration 25 nM) were diluted with 437.5 μ l 50 mM Hepes, 100 mM NaCl, pH 8.0 and cooled on ice. Then 25 μ l of 10 mg/ml α -chymotrypsin (Sigma, St. Louis, MO) in 1 mM HCl were added and the reaction was started by adding this mixture to 12.5 μ l of 4 mM *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (Sigma) in trifluoroethanol/470 mM lithium chloride in a photometer cuvette. After a delay of 10 s mixing time the increase in absorbency at 390 nm was recorded every 2 s for 2 min with an UvikonXL spectrophotometer (Bio-Tek instruments). The background due to thermal *cis-trans* isomerization of the substrate was measured in blank samples without CyP addition.

4.2 Subjects, routine assessments and skin tests

Sera from 48 individuals sensitized to *M. furfur* [13] and from 30 individuals sensitized to *A. fumigatus* [12] selected according to clinical history and immediate skin reactivity to commercial extracts were analyzed together with sera from 10 healthy controls. The titration of allergen-specific IgE was performed using Phadebas RAST (Pharmacia, Uppsala, Sweden) according to the package insert and converted into RAST classes as described by the manufacturer. Skin-prick tests were performed with commercial preparations from Allergopharma (Hamburg, Germany) and Bencard (Smith-Kline Beecham, Neuss, Germany) and considered positive if a wheal surface >9 mm² was induced [34]. Skin tests with recombinant proteins were performed as described [35]. The study was carried out according to a clinical protocol approved by the appropriate ethical committee. A full explanation of the procedure was given to all participants before testing and subsequently a written consent was obtained.

4.3 IgE immunoblots

Recombinant CyP were separated on polyacrylamide gels (4–20%, Novex) under denaturing, reducing conditions,

transferred to Hybond™ECL™ nitrocellulose membranes (Amersham, Little Chalfont, GB) and processed as described [16, 32]. After incubation with patients' sera diluted 1:10 as 'first antibody', binding of specific IgE was detected with mouse anti-human IgE mAb TN-142 in blocking buffer (0.5 µg/ml, 2 h, room temperature) and developed with peroxidase-conjugated goat anti-mouse IgG (Dako, Copenhagen, Denmark) in blocking buffer (0.14 µg/ml, 1 h, room temperature). Bands were visualized with SuperSignal® West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) by exposing the membranes to Hyperfilm™ECL™ (Amersham).

4.4 Titration of allergen-specific serum IgE and inhibition ELISA

IgE-binding to recombinant CyP was determined by a standard direct solid phase ELISA in polystyrene microtiter plates (Maxisorp™, Nunc, Roskilde, Denmark) coated overnight at 4°C with 10 µg/ml protein in PBS pH 8.0. Allergen-specific serum IgE was detected with mouse anti-human IgE mAb TN-142 and visualized with alkaline phosphatase-conjugated goat anti-mouse IgG H + L chains (Pierce) as described [34, 35]. Results were expressed as EU/ml calibrated against the absorbency (OD_{405nm}) of an in-house reference standard arbitrarily defined as 100 EU/ml for each allergen tested [35].

For inhibition ELISA coating and blocking was performed as for standard ELISA. In a separate untreated 96-well plate (Costar®, Corning, NY) CyP and bovine serum albumin (BSA) as a control were serially diluted 1:4 in blocking buffer and incubated with 1:10 diluted patients' sera for 2 h at 37°C. The preincubated sera were transferred to the coated plates and incubated for 2 h at 37°C. Residual IgE-binding capacity was determined as described above. Percentage of inhibition was calculated from the absorbency of the serial dilutions containing BSA in fluid phase set as 0% inhibition.

4.5 Statistics

Comparison of IgE levels against different allergens was done by nonparametric Mann-Whitney U-test; *p* values of <0.05 were considered to be significant. Correlation coefficients were calculated by Spearman rank order.

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References

- 1 Handschumacher, R. E., Harding, M. W., Rice, J., Drugge, R. J. and Speicher, D. W., Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* 1984. **226**: 544–547.
- 2 Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. and Schmid, F. X., Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 1989. **337**: 476–478.
- 3 Takahashi, N., Hayano, T. and Suzuki, M., Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 1989. **337**: 473–475.
- 4 Trandinh, C. C., Pao, G. M. and Saier, M. H., Structural and evolutionary relationships among the immunophilins: two ubiquitous families of peptidyl-prolyl cis-trans isomerases. *FASEB J.* 1992. **6**: 3410–3420.
- 5 Fruman, D. A., Burakoff, S. J. and Bierer, B. E., Immunophilins in protein folding and immunosuppression. *FASEB J.* 1994. **8**: 391–400.
- 6 Elliott, J. F., Lin Y., Mizel, S. B., Bleackley, R. C., Harnish, D. G. and Paetkau, V., Induction of interleukin 2 messenger RNA inhibited by cyclosporin A. *Science* 1984. **226**: 1439–1441.
- 7 Kronke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, R. A. and Green, W. C., Cyclosporin A inhibits T cell growth factor gene expression at the level of mRNA transcription. *Proc. Natl. Acad. Sci. USA* 1984. **81**: 5214–5218.
- 8 Banerji, S. S., Parsons, J. N. and Tocci, M. J., The immunosuppressant FK-506 specifically inhibits mitogen-induced activation of the interleukin-2 promoter and the isolated enhancer elements NFIL-2A and NF-AT1. *Mol. Cell. Biol.* 1991. **11**: 4074–4087.
- 9 Rovira, P., Mascarelli, L. and Truffa-Bachi, P., The impact of immunosuppressive drugs on the analysis of T cell activation. *Curr. Med. Chem.* 2000. **7**: 673–692.
- 10 Matsuda, S. and Koyasu, S., Mechanisms of action of cyclosporin. *Immunopharmacology* 2000. **47**: 119–125.
- 11 Horner, W. E., Reese, G. and Lehrer, S. B., Identification of the allergen Psi c 2 from the basidiomycete *Psilocybe cubensis* as a fungal cyclophilin. *Int. Arch. Allergy Immunol.* 1995. **107**: 298–300.
- 12 Cramer, R., Epidemiology and molecular basis of the involvement of *Aspergillus fumigatus* in allergic diseases. *Contrib. Microbiol.* 1999. **2**: 44–56.
- 13 Lindborg, M., Magnusson, C. G., Zargari, A., Schmidt, M., Scheynius, A., Cramer, R. and Whitley, P., Selective cloning of allergens from the skin colonizing yeast *Malassezia furfur* by phage surface display technology. *J. Invest. Dermatol.* 1999. **113**: 156–161.
- 14 Cadot, P., Diaz, J. F., Proost, P., Van Damme, J., Engelborghs, Y., Stevens, E. A. M. and Ceuppens, J. L., Purification and characterization of an 18-kd allergen of birch (*Betula verrucosa*) pollen: identification as cyclophilin. *J. Allergy Clin. Immunol.* 2000. **105**: 286–291.
- 15 Appenzeller, U., Mayer, C., Menz, G., Blaser, K. and Cramer, R., IgE-mediated reactions to autoantigens in allergic diseases. *Int. Arch. Allergy Immunol.* 1999. **118**: 193–196.
- 16 Cramer, R., Faith, A., Hemmann, S., Jaussi, R., Ismail, C., Menz, G. and Blaser, K., Humoral and cell-mediated autoimmunity in allergy to *Aspergillus fumigatus*. *J. Exp. Med.* 1996. **184**: 265–270.

- 17 Mayer, C., Appenzeller, U., Seelbach, H., Achatz, G., Oberkofler, H., Breitenbach, M., Blaser, K. and 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.* 2000. **189**: 1507–1512.
- 18 Valenta, R., Seiberler, S., Natter, S., Mahler, V., Mossabeh, R., Ring, J. and Stingl, G., Autoallergy: a pathogenetic factor in atopic dermatitis? *J. Allergy Clin. Immunol.* 2000. **105**: 432–437.
- 19 Leitner, A., Jensen-Jarolim, E., Grimm, R., Wüthrich, B., Ebner, H., Scheiner, O., Kraft, D. and Ebner, C., Allergens in pepper and paprika. Immunologic investigation of the celery-mugwort-spice syndrome. *Allergy* 1998. **53**: 36–41.
- 20 Kelso, J. M., Pollen-food syndrome. *Clin. Exp. Allergy* 2000. **30**: 905–907.
- 21 Hochuli, E., Bannwarth, W., Döbli, H., Gentz, R., and Stüber, D., Genetic approach to facilitate purification of recombinant proteins with a novel metal chelate adsorbent. *Bio/Technol.* 1988. **6**: 1321–1325.
- 22 Kofron, J. L., Kuzmic, P., Kishore, V., Colon-Bonilla, E. and Rich, D. H., Determination of kinetic constants for peptidyl prolyl cis-trans isomerases by an improved spectrophotometric assay. *Biochemistry* 1991. **30**: 6127–6134.
- 23 Mayer, C., Hemmann, S., Faith, A., Blaser, K. and 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.
- 24 Achatz, G., Oberkofler, H., Lechnauer, E., Simon, B., Unger, A., Kandler, D., Ebner, C., Prillinger, H., Kraft, D. and Breitenbach, M., Molecular cloning of major and minor allergens of *Alternaria alternata* and *Cladosporium herbarum*. *Mol. Immunol.* 1995. **32**: 213–227.
- 25 Valenta, R. and Kraft, D., Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J. Allergy Clin. Immunol.* 1996. **97**: 893–895.
- 26 Wagner, S., Sowka, S., Mayer, C., Cramer, R., Focke, M., Kurup, V., Scheiner, O. and 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**: 120–127.
- 27 Valenta, R., Lidholm, J., Niederberger, V., Hayek, B., Kraft, D. and Grönlund, H., The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy* 1999. **29**: 896–904.
- 28 Cramer, R. and Walter, G., Selective enrichment and high-throughput screening of phage surface-displayed cDNA libraries from complex allergenic systems. *Combin. Chem. High Throughput Screen.* 1999. **2**: 63–72.
- 29 Aalberse, R. C., Structural biology of allergens. *J. Allergy Clin. Immunol.* 2000. **106**: 228–238.
- 30 King, T. P., Hoffman, D., Lowenstein, H., Marsh, D. G., Platts-Mills, T. A. E. and Thomas, W., Allergen nomenclature. *J. Allergy Clin. Immunol.* 1995. **95**: 5–14.
- 31 Cramer, R., Jaussi, R., Menz, G. and Blaser, K., Display of expression products of cDNA libraries on phage surface. A versatile system for selective isolation of genes by gene-product/ligand interaction. *Eur. J. Biochem.* 1994. **226**: 53–58.
- 32 Hemmann, S., Blaser, K. and Cramer, R., Allergens of *Aspergillus fumigatus* and *Candida boidinii* share IgE-binding epitopes. *Am. J. Respir. Crit. Care Med.* 1997. **156**: 1956–1962.
- 33 Short, J. M., Fernandez, J. M., Sorge, J. A. and Huse, W. D., λZap: an expression vector with in vivo excision properties. *Nucleic Acids Res.* 1988. **15**: 7583–7600.
- 34 Moser, M., Cramer, R., Menz, G., Schneider, T., Dudler, T., Virchow, C., Gmachl, M., Blaser, K. and Suter, M., Cloning and expression of recombinant *Aspergillus fumigatus* allergen I/a (rAsp f 1/a) with IgE binding and type 1 skin test activity. *J. Immunol.* 1992. **149**: 454–460.
- 35 Hemmann, S., Menz, G., Ismail, C., Blaser, K. and Cramer, R., Skin test reactivity to 2 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.

Correspondence: Reto Cramer, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, CH-7270 Davos, Switzerland
 Fax: +41-81-410-0840
 e-mail: crameri@siaf.unizh.ch