

Structural aspects of cross-reactive IgE-binding fungal and self antigens

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INTRODUCTION/BACKGROUND

Allergic diseases represent one of the most common causes of chronic illness in industrialized countries. In the last few decades the incidence of atopic diseases has dramatically increased. IgE is the key immunoglobulin responsible for allergic symptoms through cross-linking of its high affinity receptor upon interaction with allergens. Therefore, identification of B cell epitopes is important for a better understanding of allergenicity and cross-reactivity. The environmental allergen repertoire is complex and covers a vast variety of different structural entities. Among these families of phylogenetically highly conserved structures like MnSOD, P₂ acidic ribosomal protein, profilin, cyclophilin, and thioredoxin, can act as potent allergens. Fungal allergens have been recognised to be involved in IgE-mediated allergic diseases and to share cross-reactive structures. Allergic bronchopulmonary aspergillosis (ABPA) and atopic eczema (AE) are the most severe atopic diseases related to hypersensitivity to proteins from *Aspergillus fumigatus* and *Malassezia sympodialis*. Not surprising, some pan-allergen structures are present also in the human proteome. Comparisons showed that fungal cyclophilin are fully cross-reactive. Binding of serum IgE as well as type I skin reactions to the human homologous of those allergens can be detected *in vitro* and *in vivo*, indicating a possible implication of humoral and cell-mediated autoreactivity in severe atopic diseases. We have solved the crystal structures of the fungal cyclophilins [1, 2] which, together with the solved structures of the homologous human protein, allow exact comparison of conserved residues between pairs of self/non-self antigens. Conserved and solvent exposed residues which are clustered in surface regions are probably responsible for cross-reactivity. Conformation, size, and chemical properties of patches formed by solvent exposed, identical amino acids are compatible with the structure of B cell epitopes and explain at molecular level the cross-reactivity observed between environmental allergens and homologous self antigens.

MATERIAL & METHODS

We cloned and produced cyclophilins from *Aspergillus fumigatus* (Asp f 11, Asp f 27), *Malassezia sympodialis* (Mala s 6), and human CyPA and CyPB [1, 2]. The recombinant proteins were examined in immunoassays for IgE binding. Sera from patients suffering from AE sensitized to *M. sympodialis* and from patients sensitized to *A. fumigatus*, selected according to clinical history and immediate skin prick test (SPT) reactivity to the respective fungal extract, were analyzed together with sera from 15 healthy controls in standard ELISA, inhibition ELISA and Western blot analysis. Intradermal skin tests (IDT) with human recombinant CyP A, CyP B and Asp f 11 were performed. Mala s 6 and Asp f 11 were crystallized, and the 3D structures solved. The solvent-accessible areas of cyclophilins were calculated and compared.

RESULTS

We show that phylogenetically conserved cyclophilins are cross-reactive. Competitive inhibition ELISA and Western blotting experiments show that IgE antibodies from patients

with ABPA or AE can not only bind cyclophilins from different fungi but also human cyclophilins (Fig. 1).

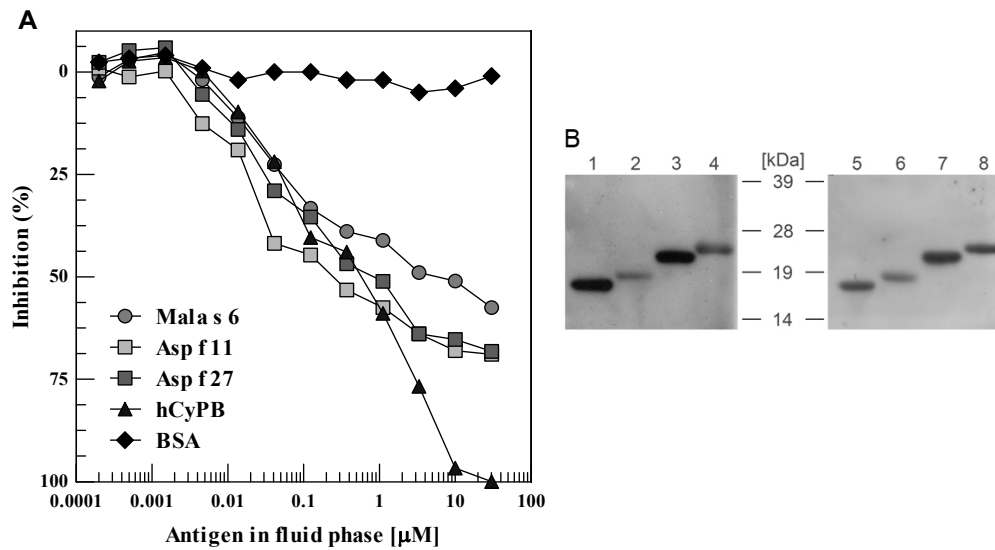


Fig. 1: (A) Competitive inhibition of IgE-binding to recombinant human CyP B coated on a solid phase. Serum from *A. fumigatus*-sensitized patient was pre-incubated with increasing amounts of recombinant antigens. (B) Specific IgE binding of recombinant CyPs analysed by Western blotting using serum pools of individuals sensitized to *M. sympodialis* (left) and *A. fumigatus* (right). Lane 1 and 5: Mala s 6, Lane 2 and 6: Asp f 27, Lane 3 and 7: Asp f 11, Lane 4 and 8: human CyP B.

Skin tests with recombinant fungal allergens and recombinant human Cyp A and B demonstrate their ability to induce immediate skin reactions. From the individuals tested four ABPA patients showed IgE antibodies against all CyPs, three healthy controls, and three ABPA patients had CyP-specific IgE antibodies below background. The results show that positive IDTs were obtained only in patients with detectable serum IgE levels against cyclophilin. In contrast, non-allergic controls and ABPA patients without detectable serum IgE antibodies to CyP were negative, demonstrating highly specific hypersensitivity responses in CyP-sensitized individuals (Table 1).

Table 1: Induction of immediate skin reactions with recombinant CyP

Values are in mm^2 calculated according to the formula $[(D1 + D2)/2]^2$ [22]. hCyP, human CyP.

Individual	Weal surface area (mm^2)					IgE (EU/ml)*		
	Histamine	NaCl	Asp f 11	hCyp A	hCyp B	Asp f 11	Mala s 6	hCyp B
ABPA patients sensitized to CyP								
Patient 1	361	0	625	1215	1089	319	146	137
Patient 2	324	0	400	169	225	216	76	129
Patient 3	484	0	572	246	225	262	95	111
Patient 4	245	0	256	151	121	156	111	98
ABPA patients not sensitized to CyP								
Patient 5	484	0	0	0	0	19	23	28
Patient 6	289	0	0	0	0	16	6	8
Patient 7	529	0	0	0	0	18	26	9
Healthy controls								
Patient 8	436	0	0	0	0	10	15	21
Patient 9	387	0	0	0	0	14	12	16
Patient 10	414	0	0	0	0	11	13	15

* Relative level of allergen-specific IgE; cut-off value 45 EU/ml (see Figure 3).

Mala s 6 shares 60% identity with human CyP B. The structure of Mala s 6 was solved by X-ray crystallography. At structural level, Mala s 6 and human CyP B superpose well with an root mean square deviation of 1.05 Å for all C α atoms (Figure 2A). Because Mala s 6 and

human CyP B show cross-reactivity, they must share common IgE-binding epitopes. Only those residues that are at least partly exposed to solvent can contribute to antigen–antibody interactions in native proteins. Thus solvent-accessible residues conserved in both proteins are potentially involved in the IgE-mediated cross-reactivity. The residues which are identical and at least 30% solvent-exposed in Mala s 6 and human CyP B were mapped on the solvent-accessible surfaces (Figure 2B) and shown to form two patches that fulfill the criteria for an IgE-binding epitope.

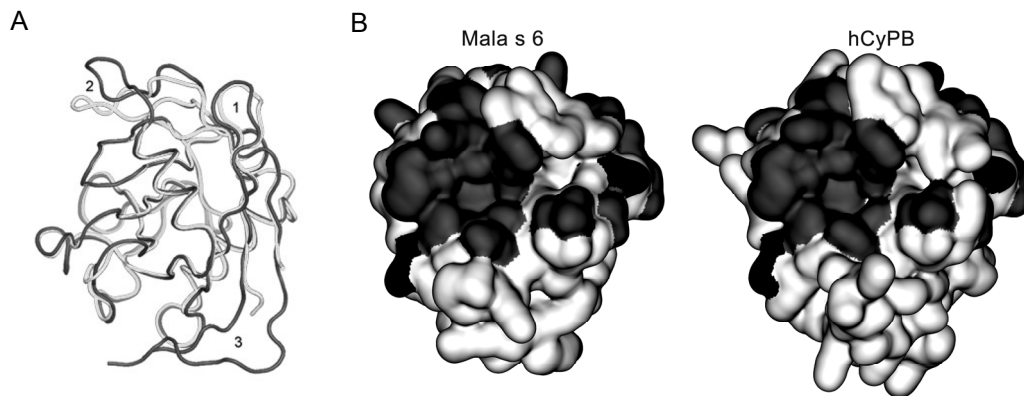


Fig. 2: (A) Superposition of Mala s 6 (light) on human CyP B (dark) reveals large structural similarities with minor deviations: the loop after the first β -strand (1) and the loop after the second α -helix (2) adopt different conformations. The N- and C-terminal extensions of human CyP B form an additional β -sheet (3) (B) Solvent exposed surfaces of Mala s 6 and the human CyP B. Amino acids which are conserved in the structures and exposed to the solvent define two putative IgE-binding epitopes (black)

CONCLUSIONS & OUTLOOK

Our work demonstrate that IgE antibodies raised against environmental cyclophilins also cross-react with the corresponding homologous human self antigens. Cross-reactivity was demonstrated *in vitro* by ELISA, Western blot analyses and competitive inhibition ELISA, and *in vivo* by intradermal skin tests. Structural data provide information allowing a rational explanation of cross-reactivity. Evidence is increasing that self antigens are a contributing factor to the exacerbation of long-standing, inflammatory atopic diseases [3].

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