

IgE-Mediated Reactions to Autoantigens in Allergic Diseases

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IgE-binding autoantigens · Autoimmunity ·
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Abstract

Background: There is increasing evidence that IgE-mediated reactivity against autoantigens could play a role in the pathology of severe chronic atopic diseases.

Methods: Human mitochondrial manganese superoxide dismutase (MnSOD), acidic ribosomal P₂ protein and cyclophilins were cloned by PCR, expressed as inclusion body proteins in *Escherichia coli*, purified by Ni²⁺-chelate affinity chromatography, refolded in vitro and used for further experiments. **Results:** Human MnSOD, P₂ ribosomal protein and cyclophilins respectively, show sequence identity and similarity of >50% and 70% to the corresponding allergens of *Aspergillus fumigatus*. The human proteins were able to bind IgE from sera of individuals sensitized to the mould proteins and to elicit strong skin reactions of the immediate type in these individuals. **Conclusions:** These observations provide evidence for autoreactivity to human proteins in allergic persons chronically sensitized to environmental allergens from *A. fumigatus* which share a high degree of sequence homology to the corresponding human proteins.

Introduction

Atopy as an IgE-mediated overreaction of the immune system against normally harmless environmental substances is well known to affect a large part of the population in industrialized countries [1]. In some chronic and severe forms of atopic diseases such as asthma, atopic dermatitis or allergic bronchopulmonary aspergillosis, clinical exacerbation of the diseases can occur also in absence of exposure to the environmental allergens. These observations have led to consider sensitization against autoantigens as a possible pathophysiological cause of severe atopic diseases [2]. However, only the recent application of molecular biology provided strong evidence for a relevant role of IgE-mediated autoimmunity in the pathogenesis of allergic diseases. Cloning and sequencing of cDNAs encoding allergenic molecules revealed structural and functional similarities between environmental allergens and human proteins [3–5]. In the case of mitochondrial manganese superoxide dismutase (MnSOD) [3] and profilin [4] reactivity of IgE antibodies from patients sensitized to the environmental allergens to the corresponding human proteins has been demonstrated. Moreover, human MnSOD is able to elicit specific immediate type I skin reactions in individuals sensitized to the *Aspergillus fumigatus* enzyme [3]. These observations together with the ability of serum IgE from atopics to recognize nitrocellulose-blotted human proteins [6] strongly support the idea that autoantigens are of pathogenic relevance for the chronicity of certain severe forms of allergic diseases.

Table 1. PCR primers used to amplify human proteins

Protein	Primer	Oligonucleotide sequence	Restriction enzyme
MnSOD	5'-hMnSOD	5'-GGA AGATCT AAGCACAGCCTCCCCGACCTGCC-3'	<i>Bgl</i> II
	3'-hMnSOD	5'-CCCA AGCTTTT ACTTTTGAAGCCATGTATCTTTC-3'	<i>Hind</i> III
P ₂ ribosomal protein	5'-hP ₂	5'-GGGG ATCC ATGCGCTACGTCGCCTCCTACC-3'	<i>Bam</i> HI
	3'-hP ₂	5'-GCT CTAGATTA ATCAAAAAGGCCAAATCCC-3'	<i>Xba</i> I
Cyclophilin B	5'-hCycb	5'-GGGG ACTT ATGAAGGTGCTCCTTGCCGCCGCC-3'	<i>Bam</i> HI
	3'-hCycb	5'-CCCA AGCTT CTACTCCTTGCGCATGGC-3'	<i>Hind</i> III
Profilin	5'-hProf	5'-CCGG ATCCA ATGGCCGGGTGGAACGGCTACATC-3'	<i>Bam</i> HI
	3'-hProf	5'-GGGA AGCTTT CAGTACTGGGAACGCCGAAGG-3'	<i>Hind</i> III

Materials and Methods

Cloning, Production and Characterization of Recombinant Human Proteins

Human cDNA was isolated from a commercial λ -Zap II cDNA library (Stratagene, La Jolla, Calif., USA) by in vivo excision of the pBluescript phagemid [7]. Genes encoding the proteins of interest were amplified by PCR using the primers reported in table 1 and the following conditions: 95 °C, 60 s; 56 °C, 60 s; 72 °C, 60 s for 30 cycles and a terminal extension cycle (10 min 72 °C). Purified PCR products were cloned into the high level expression vector pDS56 and transformed in *Escherichia coli* to produce [His]₆-tagged fusion proteins [3]. Recombinant proteins were purified over Ni²⁺-NTA columns [8] and refolded by stepwise dialysis against buffer with decreasing urea concentration followed by a final dialysis step against H₂O. Purity and molecular weight were assessed by SDS-PAGE (4–20%) and pure protein samples were lyophilized for long-term storage.

IgE-Binding Properties and Skin Tests

Specific binding of serum IgE from individuals sensitised to *A. fumigatus* to recombinant human proteins was analyzed by an allergen-specific ELISA [9]. Intradermal skin tests used to show the allergenicity of the protein preparations in vivo were performed as described [9]. The study was carried out according to a clinical protocol approved by the ethical committee of Davos.

Results and Discussion

The aim of this study was to clone, produce and assess the ability of human proteins to elicit positive type 1 skin reactions in individuals sensitized to environmental allergens. MnSOD [3, 12], ribosomal P₂ protein and cyclophilin [11] share a high degree of sequence identity to *A. fumigatus* allergens, and profilin [4] is homologous to Bet v 2 from birch pollen.

Cloning, Production and Characterization of Recombinant Human Proteins

The human genes encoding MnSOD, ribosomal P₂ protein, cyclophilin B and profilin were amplified from a human cDNA library using the primers reported in table 1. The primers contain recognition sites for restriction enzymes that allow a direct subcloning of the fragments into high-expression vectors to produce (His)₆-tagged fusion proteins. Inclusion body proteins, refolded by stepwise dialysis against decreasing urea concentration were soluble in deionized water. Human MnSOD and cyclophilin B showed the expected enzymatic activity in specific assays [12, 13]. Human profilin was shown to be able to bind to poly-*L*-proline-Sepharose [4].

IgE and Skin Test Reactivity of the Recombinant Human Proteins

The IgE-binding capacity of the purified human recombinant proteins was compared by an antigen-specific ELISA to the IgE binding capacity of the corresponding environmental allergens. As demonstrated for the MnSOD [12], a close correlation between IgE-binding to both, environmental allergens and human proteins in patients sensitized to the aeroallergens was found (data not shown). However, IgE binding of a (recombinant) protein in vitro, although a strong evidence, is not enough to prove its capacity to act as an allergen in vivo. We have tested the ability of the four human and environmental proteins to elicit type 1 reactions in intradermal skin tests [9]. Figure 1 shows an example of a skin reaction elicited by the P₂ ribosomal protein in a patient sensitised to the P₂ ribosomal protein of *A. fumigatus*. Challenges with human MnSOD [2], P₂ ribosomal protein and cyclophilin B at very low concentrations (0.01 µg range) were all able to elicit strong skin reactions in patients sensitized to the homologous mould proteins

Table 2. Characteristics of different recombinant human proteins and skin test reactivity in patients sensitized to the corresponding allergens from *A. fumigatus* or birch profilin (Bet v 2)

	Protein			
	MnSOD	P ₂ ribosomal	cyclophilin B	profilin
EMBL accession No.	2780818	13061	118090	130979
Identity, %	51.5 ^a	62.2 ^a	55.3 ^a	34 ^b
MW, kD ^c	21.6	11.2	21.5	14.0
Amino acids, n	198	115	208	140
Yield, mg/l	180	66	121	48
Solubility, mg/ml	>2	>2	>2	>2
IgE-binding	++	++	++	++
Skin test reactivity ^d	8/8	10/10	6/6	0/4

^a To the corresponding allergens of *A. fumigatus* [11].
^b To birch profilin [4].
^c Estimated from SDS-PAGE gels.
^d Patients with positive intradermal skin test/number of patients tested.

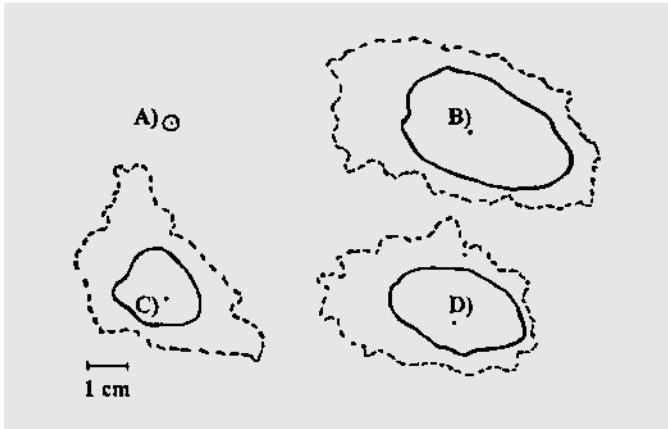


Fig. 1. Record of a typical intradermal skin reaction outlined with a fine-tipped felt pen and transferred to paper with transparent tape. Wheals depicted with a solid line, erythemas with a dotted line. The scale (cm) is indicated. **A** Physiological saline (negative control). **B** Histamine (positive control). **C** *A. fumigatus* P₂ ribosomal protein (0.01 µg); **D** Human P₂ ribosomal protein (0.01 µg).

(table 2). In contrast, skin reactivity to human profilin was not detectable in 4 individuals sensitized to Bet v 2. Further experiments will be required to clarify whether the low sequence identity between birch and human profilin (34%, table 2) or whether structural features of the recombinant profilin account for the lack of reactivity. However, the skin test reactivity of the other three human proteins clearly demonstrates that they can act as autoallergens in vivo. These observations raise questions about possible mechanisms responsible for these autoimmune reactions. All the

proteins investigated here belong to the class of phylogenetically highly conserved intracellular proteins. Therefore certain cross-reactivity at humoral and cellular level might be expected [2]. The fact that sera of MnSOD-sensitized individuals recognise a whole pattern of MnSODs, including the *Drosophila* enzyme [12] which is unlikely to be a source of exposure in humans indicates molecular mimicry as a mechanism for the observed cross-reactivity. However, cytoplasmic proteins are unlikely to be accessible for antigen-antibody interactions under normal circumstances and therefore the clinical relevance of these autoimmune reactions remains to be elucidated. Nevertheless, intracellular proteins may be continuously released by necrotic cells in chronic inflammation or punctually during viral infections accounting for exacerbation of the allergic reactions.

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