

Mutational analysis of amino acid residues involved in IgE-mediated cross-reactivity between the *Malassezia sympodialis* allergen Mala s 11 and its human homologue manganese superoxide dismutase

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INTRODUCTION/BACKGROUND

Atopic eczema (AE) is a chronic inflammatory skin disease characterised by pruritic inflammatory skin lesions [1]. Although the pathogenic mechanisms underlying AE remain largely unknown several factors such as genetic predisposition, exposure to environmental allergens and skin colonization with microorganisms appear to be of importance [2]. The opportunistic yeast *Malassezia sympodialis* is a member of the normal cutaneous flora [3]. Approximately 50% of adult AE patients have serum IgE specific for *M. sympodialis* allergens or show immediate-type skin reactions against crude extracts of this yeast [4] while such reactivity is very rare in other allergic diseases [5], suggesting that sensitization to *M. sympodialis* is associated with AE. Ten allergens from *M. sympodialis* have been cloned to date [4]. One allergen of particular interest is Mala s 11, which has a high degree of sequence identity to manganese superoxide dismutase (MnSOD) from various species including *Homo sapiens* (50%) and *Aspergillus fumigatus* (56%) [6]. MnSOD protects mitochondrial DNA against oxygen related radicals by dismutation of superoxide to oxygen and hydrogen peroxide [7]. MnSOD from *A. fumigatus*, denoted Asp f 6, is an allergen associated with allergic bronchopulmonary aspergillosis (ABPA) [8]. Humoral and cell-mediated cross-reactivity between MnSOD from *H. sapiens*, *A. fumigatus*, *D. melanogaster* and *S. cerevisiae* has been demonstrated [9]. A comparative study of the crystal structures of the *H. sapiens*-derived MnSOD (hMnSOD) and its homologous *A. fumigatus*-derived MnSOD revealed patches of identical amino acids displayed on the surface of both enzymes that potentially constitute cross-reactive IgE-binding epitopes [8]. Based on these studies, on the high degree of sequence homology and on the recent finding that hMnSOD is sufficient to induce eczematous reactions in healthy skin areas of AE patients sensitised to *M. sympodialis* [10], it can be assumed that cross-reactivity also occurs between Mala s 11 and hMnSOD. The aims of the current study were to i) show the existence of such cross-reactivity and ii) identify amino acid residues that are involved in IgE-mediated cross-reactivity between hMnSOD and the highly homologous *M. sympodialis* allergen.

MATERIAL & METHODS

Sequence alignment and molecular modelling were used to map identical amino acids exposed to the surface on both Mala s 11 and human MnSOD. The amino acids identified as potentially involved in cross-reacting B-cell epitopes were mutated using site direct mutagenesis and mutated versions of Mala s 11 was produced in *E. coli*. Native like folding was verified by enzymatic activity tests and circular dichroism. The ability of hMnSOD and of mutated Mala s 11 variants to inhibit IgE-binding to wild-type (WT) Mala s 11 was tested in inhibition ELISA using plasma from AE patients sensitised to Mala s 11 and hMnSOD.

RESULTS

Plasma samples from five AE patients sensitised to *M. sympodialis* with specific IgE-binding to both recombinant Mala s 11 (rMala s 11) and recombinant hMnSOD (rhMnSOD) were used to study the ability of rMala s 11 and rhMnSOD to inhibit IgE-binding to each other in inhibition ELISAs. In contrast to BSA used as a negative control, rMala s 11 and rhMnSOD in the fluid phase exhibited comparable inhibition of IgE-binding to solid phase-coated rMala s 11 for the five plasma samples (Figure 1a). IgE binding to solid-phase coated rhMnSOD was also inhibited by rMala s 11 added in fluid phase for all five plasma samples tested (Fig 1), demonstrating that both proteins share IgE-binding epitopes.

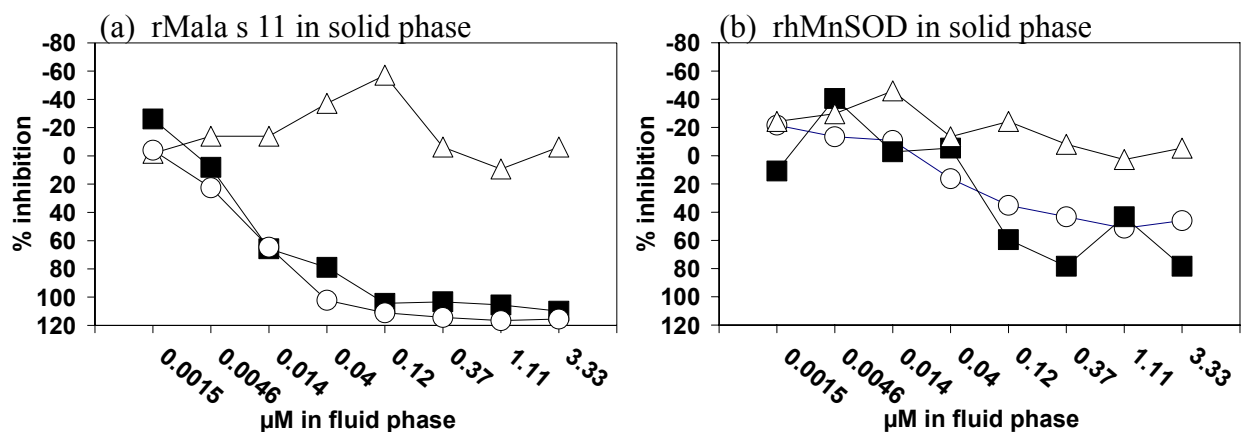


Fig. 1: Inhibition of IgE-binding to rMnSODs in the solid phase. Plasma from an AE patient sensitised to rMala s 11 and rhMnSOD was pre-incubated with increasing amounts of rMala s 11 (○), rhMnSOD (■) or BSA (△). Pre-incubated plasma was transferred to plates pre-coated with rMala s 11 (a) or rhMnSOD (b) and IgE binding was analysed by ELISA. Representative results from one donor out of five tested are shown.

A molecular model of Mala s 11 was created using the previously solved crystal structure of hMnSOD [11] as a template (Figure 2). To determine which of the residues shared between Mala s 11 and hMnSOD could be of importance for IgE-binding we identified conserved residues that are exposed to the solvent on the surface of the molecular model of Mala s 11. Four independent regions (numbered 1-4) including 17 key residues were identified as potential cross-reacting areas (Figure 2).

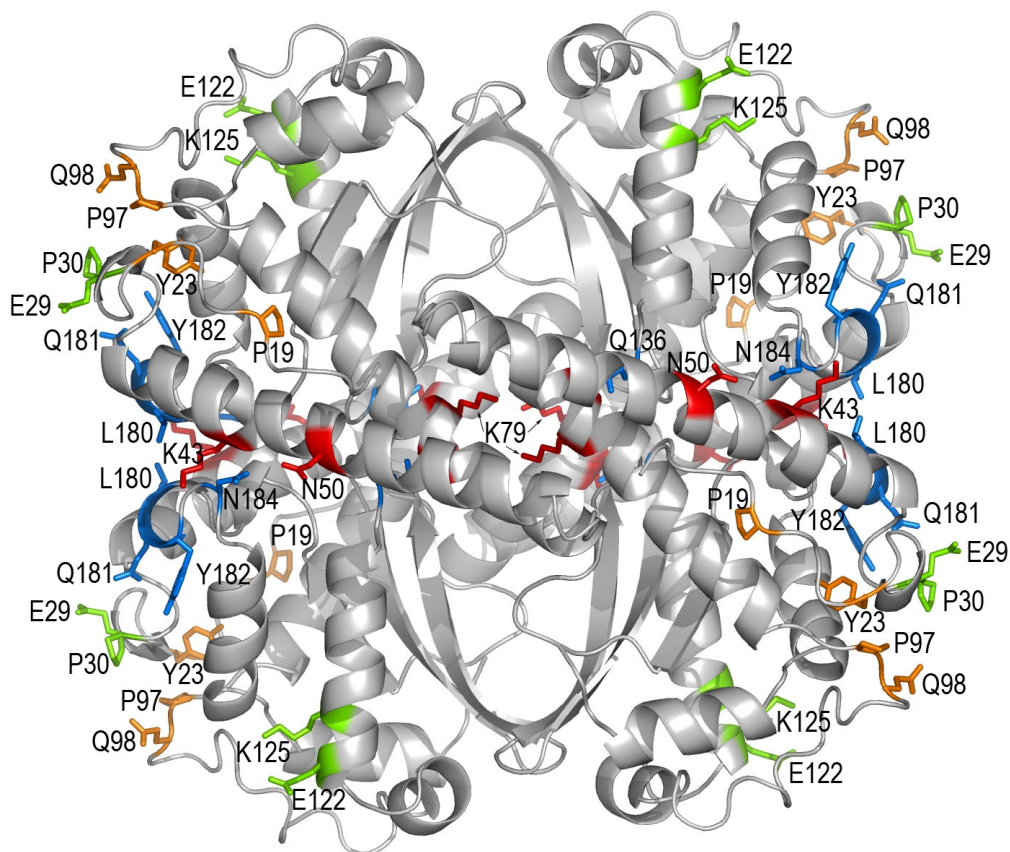


Fig. 2: Molecular model of the Mala s 11 tetramer presented as a ribbon diagram with the residues potentially involved in cross-reactivity between Mala s 11 and hMnSOD marked in red (region 1), green (region 2), blue (region 3) and orange (region 4).

The IgE binding capacity of mutated rMala s 11 versions was compared to that of WT rMala s 11 in inhibition ELISA assays. Five ELISA assays, each using a different patient sample, were performed using plasma from AE patients sensitised to both rMala s 11 and rhMnSOD. rMala s 11 inhibited IgE-binding to itself in a dose-dependent manner in all assays, whereas the negative control protein BSA did not inhibit IgE-binding to rMala s 11. For the assay performed with plasma from AE patient No. 1, lower IgE-binding was observed for all mutated rMala s 11 versions compared to the binding capacity of WT rMala s 11 (Figure 3). In the remaining four patients, only rMala s 11, in which mutations were introduced in region 2, resulted in lower IgE-binding when compared to WT rMala s 11 (Figure 3). Thus our results indicate that region 2, comprising residues E29, P30, E122 and K125, is of importance for IgE-binding to Mala s 11.

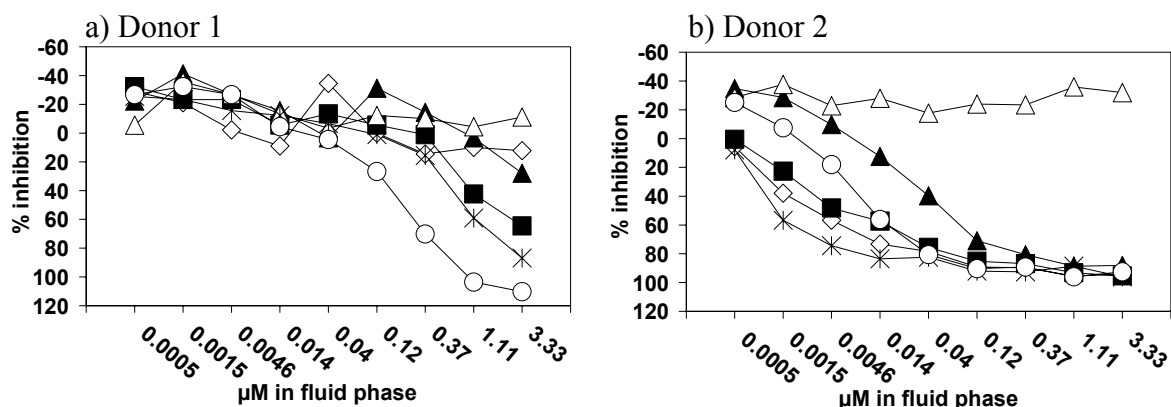


Fig. 3: Inhibition of IgE binding to WT rMala s 11 in solid phase. In each assay plasma from an AE patient sensitised to rMala s 11 and rhMnSOD was pre-incubated with increasing amounts of WT rMala s 11 (○) or versions of rMala s 11 mutated in region 1 (*), region 2 (▲), region 3 (■), region 4 (◇) or BSA (Δ) as control. Pre-incubated plasma was transferred to plates pre-coated with rMala s 11 and IgE-binding was analysed with ELISA. a) Plasma sample in which all mutated versions showed lower IgE binding in comparison to WT rMala s 11 (○). b) One representative out of four assays performed where only mutations in regions 2 (▲) led to lower IgE binding in comparison to WT rMala s 11 (○).

CONCLUSIONS & OUTLOOK

Using inhibition ELISA assays we demonstrate that rMala s 11 is able to inhibit IgE-binding to rhMnSOD and *vice-versa*, indicating that these two homologous structures share common IgE epitopes. These results suggest that the mechanisms underlying autoreactive responses to hMnSOD in AE are due to molecular mimicry. In addition, we identified a set of residues that are involved in IgE binding to Mala s 11. This provides a more detailed understanding of cross-reactivity at the molecular level. With the results presented here could facilitate the design of environmental allergens devoided of cross-reactive epitopes for a possible use in immunotherapeutic treatments aimed to suppress unwanted reactivity to self antigens remains to be elucidated.

REFERENCES

- 1 Gupta, A. K. et al. (2004). "Skin diseases associated with *Malassezia* species." *J Am Acad Dermatol* **51**(5): 785-98.
- 2 Akdis, C. A. et al. (2006). "Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report." *J Allergy Clin Immunol* **118**(1): 152-69.
- 3 Scheynius, A. et al. (2002). "Atopic eczema/dermatitis syndrome and *Malassezia*." *Int Arch Allergy Immunol* **127**(3): 161-9.
- 4 Schmid-Grendelmeier, P. et al. (2006). "The role of sensitization to *Malassezia sympodialis* in atopic eczema." *Chem Immunol Allergy* **91**: 98-109.
- 5 Casagrande, B. F. et al. (2006). "Sensitization to the yeast *Malassezia sympodialis* is specific for extrinsic and intrinsic atopic eczema." *J Invest Dermatol* **126**(11): 2414-21.
- 6 Andersson, A. et al. (2004). "Cloning, expression and characterization of two new IgE-binding proteins from the yeast *Malassezia sympodialis* with sequence similarities to heat shock proteins and manganese superoxide dismutase." *Eur J Biochem* **271**(10): 1885-94.
- 7 Hwang, C. S., Y. U. Baek, et al. (2003). "Protective roles of mitochondrial manganese-containing superoxide dismutase against various stresses in *Candida albicans*." *Yeast* **20**(11): 929-41.
- 8 Flückiger, S. et al. (2002). "Comparison of the crystal structures of the human manganese superoxide dismutase and the homologous *Aspergillus fumigatus* allergen at 2-Å resolution." *J Immunol* **168**(3): 1267-72.
- 9 Flückiger, S., L. Scapozza, et al. (2002). "Immunological and structural analysis of IgE-mediated cross-reactivity between manganese superoxide dismutases." *Int Arch Allergy Immunol* **128**(4): 292-303.
- 10 Schmid-Grendelmeier, P. et al. (2005). "IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis." *J Allergy Clin Immunol* **115**(5): 1068-75.
- 11 Borgstahl, G. E. et al. (1992). "The structure of human mitochondrial manganese superoxide dismutase reveals a novel tetrameric interface of two 4-helix bundles." *Cell* **71**(1): 107-18.