

IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis

Peter Schmid-Grendelmeier, MD,^{a,b} Sabine Flückiger, PhD,^c Rainer Disch, MD,^d Axel Trautmann, MD,^a Brunello Wüthrich, MD,^b Kurt Blaser, PhD,^a Annika Scheynius, MD,^e and Reto Crameri, PhD^a Davos and Zürich, Switzerland, and Stockholm, Sweden

Background: Autoreactivity of patients with atopic dermatitis (AD) to human proteins has been postulated as a decisive pathogenetic factor for AD.

Objective: In this study, it was investigated whether the stress-inducible enzyme manganese superoxide dismutase (MnSOD) of human and fungal origin might act as an autoallergen in atopic dermatitis.

Methods: Patients with AD (n = 69; mean SCORAD [SCORing Atopic Dermatitis], 27) and other inflammatory skin diseases as well as with inhalant allergies were investigated. The presence of specific IgE against recombinant MnSOD of fungal and human origin and the fungal extracts of *Aspergillus fumigatus* and *Malassezia sympodialis* was measured by CAP, ELISA, skin prick test, and in subset of patients also by atopy patch tests (APTs) and PBMC proliferation assays. Cross-reactivity between allergens was determined by CAP inhibition. The presence of MnSOD in human skin in various inflammatory skin conditions was investigated by immunohistochemistry.

Results: Specific IgE antibodies against human MnSOD correlating with the disease activity were found in 29 out of 67 patients with AD. The human protein was able to induce *in vitro* T-cell reactivity and eczematous reactions in APT in MnSOD-sensitized patients with AD. MnSOD was upregulated in various inflammatory skin reactions and APT skin specimens. Cosensitization to structurally related and cross-reacting fungal MnSOD and the skin-colonizing yeast *M. sympodialis* was observed in all patients sensitized against human MnSOD.

Conclusion: Human MnSOD may play a role as an autoallergen in a subset of patients with AD, including nonatopic eczema. By molecular mimicry leading to cross-reactivity such sensitization might be induced primarily by exposure to environmental fungal MnSOD of *M. sympodialis*. (J Allergy Clin Immunol 2005;115:1068-75.)

Key words: Autoallergy, *Aspergillus fumigatus*, atopic dermatitis, atopy, fungi, *Malassezia sympodialis*, intrinsic type, nonatopic eczema, recombinant allergens

Atopic dermatitis (AD) is a common chronic relapsing skin disorder with multifactorial causes.^{1,2} Recently, IgE-mediated autoreactivity against human proteins has been postulated as a possible mechanism involved in the exacerbation of AD.³⁻⁵ Human manganese superoxide dismutase (MnSOD) has been shown to elicit positive skin reactions and specific T-cell proliferation in patients with allergic bronchopulmonary aspergillosis (ABPA) sensitized to the *Aspergillus fumigatus* MnSOD^{6,7} and to cross-reactive MnSODs of different origin.⁸ The physiologic role of MnSOD in eukaryotic organisms is to protect mitochondrial DNA against oxidative damage,⁹ and therefore, the enzyme is upregulated during periods of oxidative stress, mechanical trauma, or UV radiation.^{10,11} Circumstantial evidence exists that the same factors may play a role in the pathogenesis of AD,¹² concomitant with the increased tendency of patients with AD to react to common environmental antigens.^{1,2}

Manganese superoxide dismutase has been reported as an allergen of the pathogenic mold *A. fumigatus*, the etiologic agent identified in 80% of all *Aspergillus*-related diseases.¹³ Although the pathophysiologic mechanisms leading to *Aspergillus*-related complications are largely unknown, sensitization to MnSOD has been shown to represent a specific marker for ABPA,¹⁴ an intense inflammatory and allergic response in the lung.¹⁵ On the other hand, recent studies point out the importance of bacterial antigens in the pathogenesis of AD.¹⁶ Specifically, proteins from staphylococci and the skin-colonizing yeast *Malassezia* seem to be relevant pathogens in AD because of their IgE-inducing and binding abilities.

In this study, we investigated whether human MnSOD might also induce IgE-mediated and/or T-cell-mediated autoreactivity in patients with AD. In addition, we sought a possible relationship between sensitization against MnSOD of fungal and human origin because of a strong structural relationship between those molecules.

METHODS

Patient selection

Diagnosis of AD was made according to the criteria of Hanifin and Rajka.¹⁷ Disease activity was estimated by using SCORAD, a scoring

From ^athe Swiss Institute of Allergy and Asthma Research, Davos; ^bthe Allergy Unit, Department of Dermatology, University Hospital of Zürich; ^cBioVisioN Schweiz AG, Davos; ^dAlexanderhausklinik, Davos; and ^ethe Department of Medicine, Unit of Clinical Allergy Research, Karolinska Institute and Hospital, Stockholm.

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Reprint requests: Peter Schmid-Grendelmeier, MD, Allergy Unit, Department of Dermatology, University Hospital, Gloriastr 31, CH-8091 Zürich, Switzerland. E-mail: peter.schmid@usz.ch.

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Abbreviations used

ABPA: Allergic bronchopulmonary aspergillosis
AD: Atopic dermatitis
APT: Atopy patch test
EU: ELISA unit
hMnSOD: Human manganese superoxide dismutase
MnSOD: Manganese superoxide dismutase
SPT: Skin prick test

system involving extension, severity, itching, and sleeping disturbance.¹⁸ From all 69 patients with AD, other allergic diseases such as rhinitis or asthma were reported and skin prick test (SPT) and total and specific serum IgE against common inhalant and fungal allergens (sx1, mx1) determined.

Patients with *A fumigatus*-related allergic asthma or ABPA met the American Thoracic Society criteria for the diagnosis of asthma.¹⁹ They were included if they had a metacholine-induced $\geq 20\%$ decrease in FEV₁ after inhalation and a $\geq 15\%$ increase in FEV₁₀ after 2 inhalations of albuterol. The *A fumigatus*-sensitized patients with asthma had a history of sensitization to the fungus, and ABPA was excluded according to the clinical history. The patients with ABPA fulfilled at least 6 of the 7 diagnostic criteria proposed by Patterson et al.²⁰ Patients with psoriasis and healthy control subjects had no history of allergy, asthma, or AD and normal IgE levels (Table I).

An ethical approval for taking blood samples and taking skin biopsies of various skin diseases and testing human subjects with recombinant proteins was obtained from the ethics committee of the University of Zürich. A full explanation of the procedure was given to all participants, and their written consent was obtained before testing.

Allergens and allergen extracts

cDNAs coding for the *A fumigatus* MnSOD (Asp f 6) and human MnSOD (hMnSOD) proteins were amplified by PCR, subcloned into the high-level expression plasmid pQE30 (Qiagen, Hilden, Germany), verified by sequencing, and used to produce highly pure hexahistidine-tagged recombinant MnSOD as described.⁵ Limulus amoebocyte lysate tests for purity were performed, and endotoxin doses (LPS) in all recombinant allergen constructs were well under the cutoff value. *A fumigatus* extract was purchased from ALK-Abelló (Hørsholm, Denmark) as a commercially available SPT solution; *M sympodialis* extract (strain ATCC 42132) was provided by Prof A. Scheynius, Karolinska Institute, Stockholm.

Determination of total and allergen-specific IgE

Total serum IgE levels and allergen-specific serum IgE to *A fumigatus* (m3) and *M sympodialis* (m70) extracts were determined by the ImmunoCAP system as recommended by the manufacturer (Pharmacia, Uppsala, Sweden). In addition, specific IgE against common inhalant allergens (sx1, Pharmacia) and food allergens (fx5, Pharmacia) was determined. Allergen-specific IgE raised against rAsp f 1, rAsp f 6, and rhMnSOD was determined by an allergen-specific ELISA as described elsewhere.²¹ Absorbency was measured at 405 nm with a Molecular Devices reader (Menlo Park, Calif) and optical densities converted into arbitrary ELISA units (EU)/mL calibrated against an in-house serum pool arbitrarily defined as 100 EU/mL.¹⁴ Values below 1 EU/mL were set as 1 EU/mL for graphic display and nonparametric statistical analysis (Mann-Whitney U test and Spearman rank correlation test). *P* values $< .05$ were considered as statistically significant.

CAP inhibition assays

For ImmunoCAP inhibitions, serum of a patient highly sensitized to *M sympodialis* was diluted to a value of 1 kU_A/L specific IgE with PBS. The diluted serum was incubated with serially increasing doses of the allergens rAsp f 6, rhMnSOD, or BSA overnight at 4°C. Binding of residual allergen-specific serum IgE to *M sympodialis* ImmunoCAPs (m70) was quantitatively determined according to the package inserts (Pharmacia).

Skin tests

Skin prick tests were performed with commercial *A fumigatus* extract (ALK-Abelló), an in-house *M sympodialis* extract, and the recombinant proteins rAsp f 6 and rhMnSOD. Recombinant allergens were diluted in 0.9% sterile sodium chloride solutions at a concentration of 100 µg/mL. Solutions of 0.9% saline and 0.01% histamine hydrochloride (ALK-Abelló) served as positive and negative control, respectively. All SPT were performed in double on the volar forearm applied in opposite directions and evaluated after 20 minutes, as described.²² Reactions were considered positive when a mean diameter of >4 mm was achieved. In patients with AD and asthma or ABPA, SPT was also performed with a battery of common inhalant and food allergens (including house dust mite, birch, hazel, alder, ash, grass and mugwort pollen, *Alternaria alternata*, *Penicillium penicillium*, *Candida albicans*, *Cladosporium herbarum*, cow's milk, and hen's egg).

Atopy patch tests (APTs) were performed on nonlesional skin of the back. All allergens were tested on untreated nonlesional skin and, in parallel, on nonlesional skin tape-stripped 10 times before allergen application.²² Each recombinant allergen (20 µL of each recombinant fungal MnSOD Asp f 6 and rhMnSOD) at 100 µg/mL and 1 mg/mL concentration was applied on paper discs in 12-mm Finn chambers, in parallel with 20 µL *A fumigatus* and *M sympodialis* extract at a concentration of 5 mg/mL. Saline and petrolatum served as negative controls. Patch tests were removed after 48 hours and read 15 minutes and 24 hours after removal.

Proliferative response of PBMCs

PBMCs were isolated from heparinized peripheral venous blood by Ficoll density gradient centrifugation, washed 3 times, and resuspended in RPMI 1640 supplemented with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 50 µmol/L 2-mercaptoethanol, 1% minimum essential medium nonessential amino acids and vitamins, 100 µg/mL streptomycin, 100 U/mL penicillin (all from Life Technologies, Basel, Switzerland), and 10% heat-inactivated FCS (Sera-Lab, Harlan, United Kingdom). Samples of 5×10^5 cells/100 µL were stimulated with different concentrations ranging from 0.01 µg/mL to 10 µg/mL recombinant Asp f 6, MnSOD, *A fumigatus*, or *M. sympodialis* extract, respectively, in triplicate for 6 days. Proliferation was measured as incorporation of tritiated thymidine (DuPont-NEN, Regensdorf, Switzerland) during the final 8 hours of culture. A stimulation index >3 was considered positive.

Immunohistochemistry staining

Immunohistochemistry staining was performed on cryostat-embedded skin specimens. Sections of lesional skin of AD in acute and chronic stage with various sensitization patterns (3 patients MnSOD-positive, 3 patients with AD MnSOD-negative/house dust mite-positive, and 2 patients without detectable IgE-mediated sensitization from the intrinsic type), noninvolved skin specimens, and skin of APT reactions after 48 hours of allergen application were investigated. Skin samples before and after 7 days of treatment with topical corticosteroids in 3 patients with AD were also compared. Skin specimens of patients with ABPA (2 patients), lesional skin of psoriasis (5 patients), and skin of cutaneous T-cell lymphoma (2 patients)

TABLE I. Characteristics and sensitization patterns of the patient groups investigated [mean (SD)]

	AD	Psoriasis	Allergy to <i>A fumigatus</i>	ABPA	Healthy controls
Number	69	13	13	11	5
Mean age, y (±SD)	29.3 (±5.6)	31.3 (±8.6)	23.6 (±11.2)	21.4 (±7.9)	22.3 (±4.9)
Female:male	41:28	8:5	7:6	6:5	3:2
IgE Asp f 1, EU/mL	2.8 (±1.7)	<0.35	43 (±12.3)	138 (±29.4)	<0.35
Positive SPT to Asp f 1	2	0	13	11	0
IgE Asp f 6, EU/mL	117 (±29.8)	<0.35	4 (±2.1)	123 (±26.4)	5 (±2.6)
Positive SPT to Asp f 6	29	0	0	11	0
IgE hMnSOD, EU/mL	93 (±26.6)	2.14 (±1.10)	1.9 (±1.2)	102 (±36.2)	2.13 (±1.3)
Positive SPT to hMnSOD	29	0	0	11	0
IgE <i>A fumigatus</i> , kU/L	4.25 (±1.8)	<0.35	19.2 (±7.1)	24.6 (±8.4)	<0.35
Positive SPT to <i>A fumigatus</i>	2	0	13	11	0
IgE <i>M sympodialis</i> , kU/L	16.5 (±9.5)	<0.35	<0.35	1.04 (±0)	<0.35
Positive SPT to <i>M sympodialis</i>	34	0	0	1	0
Total IgE, kU/L	3904 (±1444)	97.4 (±28.8)	417.2 (±79.9)	4821 (±1882)	76.8 (±55.3)

TABLE II. Characteristics of patients with AD

	IgE hMnSOD positive*	IgE hMnSOD negative†
Number	29	40
Mean age, y	31.2	27.9
Sex, female:male	15:14	23:17
Mean SCORAD, points	30.7	22.3
IgE hMnSOD, EU/mL	156 (±86.6)	
IgE <i>A fumigatus</i> (m3) positive	27	2
IgE <i>A fumigatus</i> (m3), kU/L	4.34 (±2.1)	3.1
IgE <i>M sympodialis</i> (m70) positive	29	5
IgE <i>M sympodialis</i> , kU/L	18.6 (±9.9)	4.38 (±2.2)
Exclusively IgE <i>M sympodialis</i> (m70) positive‡	2	2
Total IgE, kU/L	4261 (±1716)	3645 (±1903)
Total IgE range, kU/L	72-9215	64-7338
Extrinsic type of AD§	27	34
Intrinsic type of AD	2	6

*hMnSOD: ELISA and SPT positive.

†hMnSOD: ELISA, SPT negative.

‡Specific IgE against Sx1 and Fx5 negative.

§Total IgE serum > or < 200 kU/L, Sx1 and/or Fx 5 positive (≥0.35 kU/L), 1 or several SPTs to common inhalant allergens positive.

||Total IgE serum < 200 kU/L, Sx1 and Fx5 and SPT to common inhalant allergens negative.²⁶

were stained. Endogenous peroxidase activity was blocked with a 0.3% H₂O₂ solution in methanol. Nonspecific binding was blocked with 10% normal rabbit serum (Chemicon, Temecula, Calif) followed by incubation for 2 hours with antihuman MnSOD antibody (Calbiochem, catalog #574596; Merck Biosciences Ltd., Nottingham, United Kingdom) or goat IgG as isotype control. Sections were further incubated with biotin-labeled rabbit anti-goat IgG and stained with the alkaline phosphatase-antialkaline phosphatase method by using a commercial kit (Dako, Zug, Switzerland). All sections were examined and photographed by using standard bright-field optics.

RESULTS

IgE responses to MnSOD and fungal allergens

Twenty-nine out of 69 patients with AD investigated mounted IgE-specific immune responses to hMnSOD,

resulting in an incidence of sensitization in the range of 36% (Table I). Patients with AD were not sensitized to Asp f 1, a major allergen of *A fumigatus* recognized at high frequency by patients with IgE-mediated aspergillosis (Table I). ImmunoCAP determinations showed that patients with *A fumigatus* allergy or ABPA display a high polyclonal IgE response to the fungal extract, in contrast with the results for patients with psoriasis and healthy controls, which were negative throughout. Patients with AD showed a moderate response to *A fumigatus* but a strong IgE response to *M sympodialis*, which was absent in patients with aspergillosis (Table I). Specific IgE against rAsp f 6, the *A fumigatus* MnSOD,⁵ and hMnSOD was high in patients with ABPA, as expected, but below the cutoff value of 7 EU/mL¹³ in *A fumigatus*-sensitized individuals without ABPA, individuals with psoriasis, and healthy controls, but surprisingly very high in a subset of the patients with AD investigated. All of the 29 AD patients with detectable serum levels of IgE raised against hMnSOD also showed IgE reactivity to Asp f 6 and reacted positively to skin challenges with both recombinant proteins (Table I).

Of all patients with AD, 8 were not sensitized against other inhalant or food allergens (Table II) and had normal total IgE levels. From these patients fulfilling the criteria of the intrinsic type of AD newly named *nonatopic eczema*,^{23,24} 4 were sensitized against *M sympodialis*.²⁵ Two of these patients also reacted to hMnSOD. These 2 female patients did show mild but clear sensitization proven by positive IgE levels, lymphocytic transformation test, and APT but not SPT with rhMnSOD and also *M sympodialis*. They both had rather severe AD (SCORAD 21 and 35 points) but normal total IgE serum levels.

Interestingly, AD activity assessed with SCORAD correlated moderately with the levels of hMnSOD-specific and *M sympodialis*-specific serum IgE levels, but not with total IgE levels ($R = 0.213$; $P = .246$) in analyses involving the whole patient population investigated. Specific IgE raised against *A fumigatus* extract did not correlate with the SCORAD (data not shown). However,

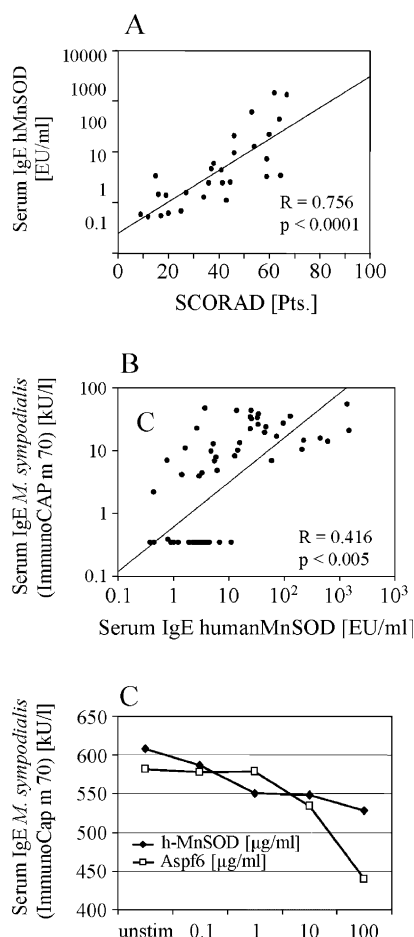


FIG 1. A, The relationship between disease activity expressed by SCORAD and specific IgE against MnSOD in the subgroup sensitized against hMnSOD. B, Values of specific IgE against *M. sympodialis* analyzed by ImmunoCAP are compared with specific IgE against hMnSOD measured by ELISA. C, The inhibition of detectable IgE levels against *M. sympodialis* by fungal MnSOD (Asp f 6) and human MnSOD is shown, pointing out the cross-reactivity between these proteins and *M. sympodialis* extract.

the same analysis performed for the subset of patients with AD sensitized to *M. sympodialis* showed a statistically relevant correlation between SCORAD and hMnSOD ($R = 0.756$; $P < .0001$; Fig 1, B), respectively serum IgE-levels against *M. sympodialis*-specific serum IgE levels and hMnSOD ($R = 0.416$; $P < .005$).

Investigation of cross-reactivity between hMnSOD and environmental fungal allergens

We found high serum levels of specific IgE against *M. sympodialis* extract (ImmunoCAP m70) in all 29 patients with AD showing detectable serum IgE levels directed against hMnSOD. In contrast, only 2 of the patients with AD reacted positively in SPT challenges with *A. fumigatus* extract (Table I). A statistical analysis of the serologic data showed a significant correlation between specific IgE raised against hMnSOD and Asp f 6 ($r = 0.635$; $P < .001$) and between *M. sympodialis* serum IgE and MnSOD ($r = 0.416$; $P < .005$, Fig 1, B), but

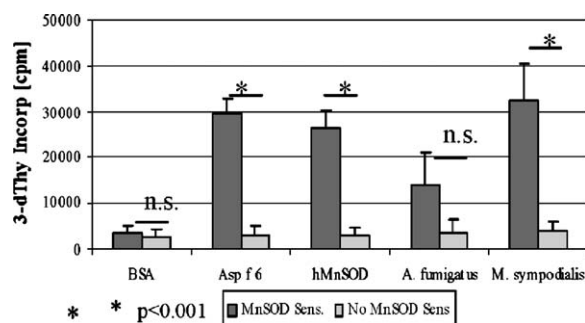


FIG 2. PBMC proliferation after 6 days of exposure with optimal concentrations of the recombinant Asp f 6, hMnSO, and fungal extracts of *A. fumigatus* and *M. sympodialis* in 3 patients with AD, showing reactivity against MnSOD and *M. sympodialis* in SPT, specific IgE, and APT in comparison with 3 patients with AD not sensitized against these allergens.

not between *A. fumigatus* and hMnSOD-specific IgE ($r = 0.231$; $P = .183$). Inhibition assays showed that hMnSOD and rAsp f 6 were able to reduce significantly the IgE binding capacity of serum from *M. sympodialis*-sensitized patients with AD to *M. sympodialis*-specific ImmunoCAPs in a concentration-dependent manner, indicating that MnSOD is a major allergenic component of this extract (Fig 1, C). In contrast, inhibition experiments with MnSODs using *A. fumigatus* ImmunoCAPs did not influence significantly the IgE-binding capacity of treated sera to *A. fumigatus* extract as a consequence of the extremely polyclonal response to a complex pattern of IgE-binding proteins of this mold.

Proliferative responses of T cells from individuals sensitized to hMnSOD

The proliferative responses of PBMCs from 3 hMnSOD-sensitized individuals with AD to rhMnSOD, rAsp f 6, and the fungal extracts of *A. fumigatus* and *M. sympodialis* were investigated (Fig 2). The mean proliferation at optimal concentrations showed highly significant differences between patients with AD with IgE antibodies against hMnSOD compared with individuals lacking such antibodies, indicating that the recombinant antigens did not induce nonspecific effects.

Allergenicity of the recombinant MnSOD proteins in vivo

All 69 patients with AD, 13 patients sensitized to *A. fumigatus* without clinical signs of ABPA, 11 patients with ABPA, 13 patients with psoriasis, and 5 healthy control individuals were investigated for their ability to respond in SPT and/or APT to rhMnSOD, rAsp f 6, and crude extracts of *A. fumigatus* and *M. sympodialis*. All ImmunoCAP-positive individuals reacted to challenges with the corresponding fungal extracts, whereas ImmunoCAP-negative individuals had negative results to skin challenges throughout, as expected.²⁶ None of the individuals lacking detectable serum IgE antibodies to hMnSOD protein reacted against the recombinant



FIG 3. Atopy patch test reactivity after 48-hour application of the various fungal allergens and hMnSOD. The results clearly demonstrate an eczematous reaction to *M sympodialis* and hMnSOD in a patient with AD accordingly sensitized. The applied substances are (1) petrolatum, (2) rAsp f 6, (3) *A fumigatus* extract, (4) *M sympodialis* extract, (5) recombinant human MnSOD, and (6) saline.

MnSODs, and all individuals who had positive MnSOD-specific IgE serum levels in ELISA reacted positively to skin challenges with the recombinant enzymes (Table I).

Although in all patients with AD SPTs were performed, it unfortunately was not possible to perform APTs in all patients with ADs showing high levels of MnSOD-specific IgE in serum, because of the lack of unaffected skin areas available for testing. However, in 3 of 5 patients with AD sensitized against human MnSOD, it was possible to induce strong eczematous reactions by APT, applying *M sympodialis* extract or rhMnSOD at a concentration of 5 mg/mL and 1 mg/mL, respectively, in nonlesional skin areas. The same patients showed moderate APT reactions to rAsp f 6 and *A fumigatus* extracts as shown in Fig 3. APT with hMnSOD and Asp f 6 in 4 patients with AD sensitized to *M sympodialis* lacking IgE responses to MnSOD, 5 patients with AD without serum IgE to the fungal extracts or hMnSOD, 4 patients with *A fumigatus*-related allergic asthma without signs of AD and MnSOD-specific IgE, and 5 control individuals without allergy were negative throughout, confirming the specificity of the reactions. Interestingly, in 3 patients with ABPA showing specific IgE against hMnSOD, APT with extracts and MnSOD of human and fungal origin were both negative.

MnSOD is upregulated in inflammatory skin areas

Manganese superoxide dismutase was detectable in healthy skin and in affected skin of patients with in-

flammatory skin diseases such as AD (Fig 4, B-F), but also contact dermatitis, psoriasis, and cutaneous lymphoma (Fig 4, I-L). However, an increased higher upregulation of MnSOD expression was found in lesional skin of severe AD (Fig 4, D). In addition, upregulation of MnSOD could be induced by APT applying either *M sympodialis* extract or hMnSOD epicutaneously for 48 hours (Fig 4, G) in parallel with the induction of eczematous reactions (Fig 3). The expression of MnSOD was also decreased in patients with AD after application of topical steroids for 1 week according to the clinical degree of inflammation, but independent of sensitization against human MnSOD. Skin of patients with ABPA did not show an increased expression of MnSOD (Fig 4, H).

DISCUSSION

The current study demonstrates that patients with severe AD have a high rate of specific sensitization to human MnSOD. The allergenicity of hMnSOD was shown by detection of specific IgE, induction of antigen-mediated T-cell proliferation, and positive immediate-type reactions to skin challenges.

Moreover, in patients with less severe AD, it was possible to induce eczematous reactions through APT, applying human MnSOD for 48 hours to unaffected skin areas (Fig 3). This is a direct demonstration that a self antigen is sufficient to elicit eczematous reactions in AD patients sensitized to the antigen. IgE-mediated self-reactivity has been postulated as a possible pathogenic factor responsible for the exacerbation and/or perpetuation of AD in several studies.³⁻⁵ This report now provides *in vivo* evidence to support this claim.

Manganese superoxide dismutase is an essential enzyme²⁷ upregulated under many stress conditions like UV radiation, mechanical trauma, and any form of acute or chronic inflammation, including AD.¹⁰⁻¹² In this study, the upregulation of MnSOD expression in AD could be clearly demonstrated by immunohistochemistry. T-cell-mediated tissue damage, an important pathomechanism in AD,²⁸ can promote further release of the cytoplasmic located MnSOD contributing to maintenance and potentiation of local inflammatory processes. The downregulation of MnSOD after topical application of corticosteroids also reflects the role of inflammation in its cellular expression.

Interestingly, the amount of specific IgE directed against human MnSOD was strongly correlated with disease activity as reflected by SCORAD (Fig 1, A). Such a phenomenon has been reported by other authors²⁹ and might reflect primary tissue destruction leading to the release and secondary immune response to such autoallergenic proteins.

Manganese superoxide dismutase was earlier identified as an allergen in patients with ABPA, but not in *A fumigatus*-sensitized individuals without clinical signs of ABPA or in healthy controls.^{13,14} These observations confirm the cross-reactivity between MnSODs reported for patients with ABPA, but exclude *A fumigatus* as

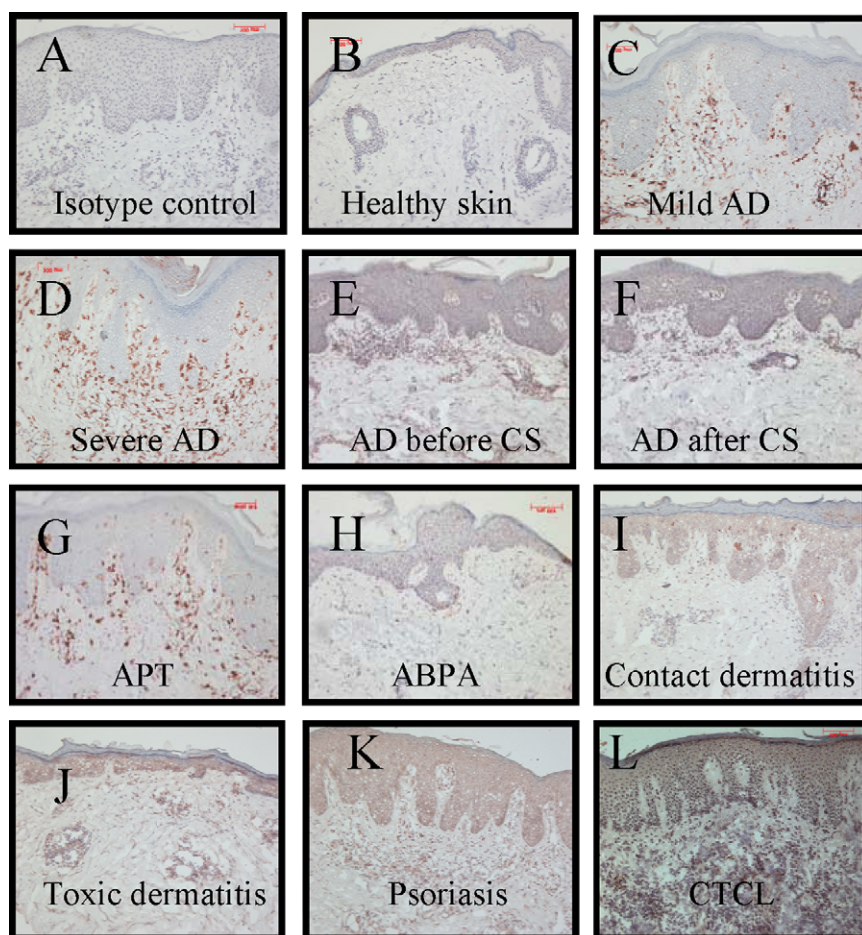


FIG 4. Manganese superoxide dismutase staining in skin. By immunohistochemistry, the stage and disease-dependent expression of MnSOD in various human skin tissue samples are demonstrated. There is a mild expression of MnSOD in healthy skin (**B**) that rapidly increases in mild (**C**) and even more in severe AD (**D**). Application of topical corticosteroids (**CS**) in AD leads to decreased expression of MnSOD after 7 days (**E**, before; **F**, after). Interestingly, upregulation can also be induced by application of rhMnSOD in APT detectable after only 48 hours (**G**). The expression of hMnSOD is relatively low in ABPA (**H**) and other inflammatory skin diseases (**I-L**) except cutaneous T-cell lymphoma (**CTCL**) (**L**), pointing out the dependance of MnSOD production on inflammatory lymphatic cells.

primary sensitizing agent for the high incidence of autoreactivity to hMnSOD observed in patients with AD.

Therefore, a crucial question is how sensitization to hMnSOD, expected to be recognized as self-antigen and thus ignored by the immune system, might occur in AD. Interestingly, all patients with AD showing serum IgE against hMnSOD were concomitantly sensitized to the skin-colonizing yeast *M sympodialis* (Table I), and IgE raised against hMnSOD and fungal extract correlated fairly well (Fig 1, B). Moreover, hMnSOD was able to compete for IgE binding to *M sympodialis* extracts, as shown in inhibition experiments (Fig 1, C). In another study, it was also recently shown that because of sequence similarities, a high IgE-mediated cross-reactivity between MnSOD from *M sympodialis* and hMnSOD exists.³⁰ Therefore, it is very likely that primary sensitization occurs through sensitization to the environmental MnSOD

of *M sympodialis*. Certain abnormalities in AD including perturbed epidermal barrier and increased reactivity of infiltrating T cells³¹ may favor sensitization against allergens of a skin-colonizing organism such as *M sympodialis*.²⁵ Also, colonization of the skin by *M sympodialis* is increased in AD, as shown by Nabkabayashi et al.³² A substantial subset of patients with AD (36%) in our collective was sensitized to *M sympodialis* as well. All of the 29 patients with AD sensitized against the fungal MnSOD rAsp f 6 reacted also to hMnSOD in SPT (Table I). Taken together, these investigations strongly suggest a specific role for the skin-colonizing yeast *M sympodialis* in patients with AD sensitized to MnSOD and support the increasing evidence for an involvement of *M sympodialis*-specific IgE responses in the pathogenesis of the disease in a relevant subset of patients with AD.²⁵

Manganese superoxide dismutase is an essential enzyme, and therefore, it was detectable in healthy skin and in affected skin of patients with inflammatory skin diseases such as contact dermatitis, psoriasis, and cutaneous lymphoma (Fig 4). MnSOD expression in skin seems increased only in local inflammation, because no upregulation was seen in clinically normal skin of patients with ABPA. Interestingly, we also found 2 patients sensitized against hMnSOD who otherwise would have fulfilled the criteria of the so-called intrinsic type or nonallergic form of AD, recently named *nonatopic eczema*^{23,24} (Table II). This subtype of AD by definition does not show IgE-mediated sensitization against common inhalant or food allergens and has normal total IgE serum levels. The result of patients with AD reacting against *M sympodialis* in APT and T-cell proliferation assays but not to other allergens was found in another large series of patients tested with allergens of *M sympodialis*.³³ Therefore, some of the patients subsummarized as intrinsic type might be sensitized against other, rarer or formerly unidentified microbial allergens such as *M sympodialis*, *Staphylococcus aureus*, or autoallergens not included in the standard batteries of SPT and specific IgE.^{34,35}

In summary, the data presented provide strong evidence for humoral and cell-mediated autoimmune reactivity to human MnSOD in a relevant number of patients with AD. Reactivity against self-allergens is correlated with disease severity. Also, MnSOD expression can be downregulated by topical steroid treatment. Thus, consequent and early anti-inflammatory therapy with corticosteroids or calcineurin inhibitors might prevent potentially harmful auto-reactivity against self-allergens such as MnSOD. Because environmental fungal allergens might induce sensitizations by molecular mimicry, further studies as performed with bacteria such as staphylococci might be necessary to determine their role in AD.^{16,36} Various recent studies have reported beneficial effects of antifungals in AD.³⁶⁻³⁸ The potential importance of fungal elements on the skin in inducing potentially harmful autoreactivity might justify in addition the use of antifungals in some subgroups of patients with AD.

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