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The IgE-Binding Self-Antigens Tubulin-α and HLA-DR- α are Overexpressed in Lesional Skin of Atopic Eczema Patients

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Abstract

Background: Atopic eczema is the most common chronic, relapsing, inflammatory skin disorder with an atopic background. Previous studies have shown that IgE-mediated reactivity to self-antigens plays a role in the pathogenesis of the disease. However, the expression of self-antigens associated with atopic eczema in the lesional skin is poorly investigated.

Aim of the study: This study was aimed to show that IgE-binding self antigens are over-expressed in atopic eczema lesions.

Methods: Tubulin-α and HLA-DR-α, two recently described self-antigens, were stained by immunohistochemistry in skin specimens from chronic and acute atopic eczema lesions, unaffected skin from the same patients or skin from healthy controls.

Results: The expression of tubulin-α and HLA-DR-α is up-regulated in atopic eczema lesions compared to non-lesional or healthy skin and correlates with the number of infiltrating immune cells and the degree of inflammation.

Conclusion: Upregulation of IgE-binding self-antigens in lesional skin of atopic eczema patients might further promote the existing inflammation and induce exacerbations of the disease in the absence of exposure to environmental allergens.

Keywords: Atopic eczema; Self-antigens; IgE; Autoreactivity

Background

Atopic eczema (AE) is the most common inflammatory skin disorder affecting up to 10-20 % of children and 1-3 % of adults in industrialized countries [1]. Multiple factors are involved in the pathogenesis of AE including genetic predisposition, impaired skin barrier function, microbial colonization, and sensitization against environmental allergens. In addition, IgE antibodies reacting with human self-antigens are supposed to be involved in the pathogenesis of the disease [2].

Several IgE-binding self-antigens associated with AE were identified, such as profilin, ribosomal protein P_2, manganese superoxide dismutase (MnSOD), cyclophilin, thioredoxin, and Hom s 1-5 (For a review see [2]). By screening a human cDNA library displayed on phage surface with immobilized serum IgE from AE patients we recently identified 140 additional IgE-binding self-antigens [3], demonstrating that a broad spectrum of IgE-binding self-antigens is associated with AE. Recombinant human self-antigens characterized in detail were shown to bind serum IgE of AE patients, to induce mediator release from basophils, and to stimulate the proliferation of PBMC [4-8]. Moreover, serum IgE of AE patients targets keratinocytes and normal human epidermis [9] and the well-characterized self-antigen MnSOD is sufficient to elicit eczematous reactions if applied to healthy skin areas of AE patients [10]. Interestingly, MnSOD expression is up-regulated in lesional, but not in healthy skin areas of AE patients sensitized to the self-antigen, providing strong evidence for the involvement of autoreactivity in the exacerbation of an existing inflammation [10].

During the present study we investigated the expression of two newly described self-antigens, tubulin-α and HLA-DR-α [3], by immunohistochemistry in skin biopsies taken from acute and chronic AE lesions and non-lesional skin of the same patients, or from healthy controls.

Methods

Patient selection

Biopsies were taken from 4 AE patients and 3 healthy controls of both sexes. Diagnosis of AE was made according to the recommendations of the EAACI and severity of the disease was determined by the SCORAD index as described [10]. Total and specific serum IgE against *Malassezia sympodialis* were determined by the ImmunoCap system (Phadia, Uppsala, Sweden). Healthy individuals had no history of allergy, asthma, or AE and normal total IgE levels. The characteristics of patients and controls are reported in Table 1. Permission to conduct this study was obtained from the Ethics Committee at Karolinska Hospital, Stockholm, and informed consent was given by all subjects.

Immunohistochemistry staining

Immunohistochemistry staining was performed on cryostat-embedded skin specimens taken from lesional and non-lesional skin of AE patients or of healthy controls. Frozen sections were fixed in 50 % and 100 % acetone and incubated in 0.3 % H_2O_2 in PBS to inhibit endogenous peroxidase activity. Non-specific binding was blocked with normal horse sera diluted 1:10 in 4 % BSA, followed by incubation with

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donor	sex	age (y)	SCORAD	Phadiatop	total IgE (kU/l)	IgE to *Malassezia* spp. (m70)
AE 1	m	49	48	pos	2700	24
AE 2	f	20	48	pos	2300	28
AE 3	m	43	61	pos	1700	7.5
AE 4	f	26	53	neg	81	<0.35
HC 1	m	41	-	neg	77	<0.35
HC 2	f	23	-	neg	11	<0.35
HC 3	f	23	-	neg	6.2	<0.35

AE, atopic eczema; HC, healthy control; SCORAD, severity scoring of atopic dermatitis

Table 1. Characteristics of patients and healthy controls.

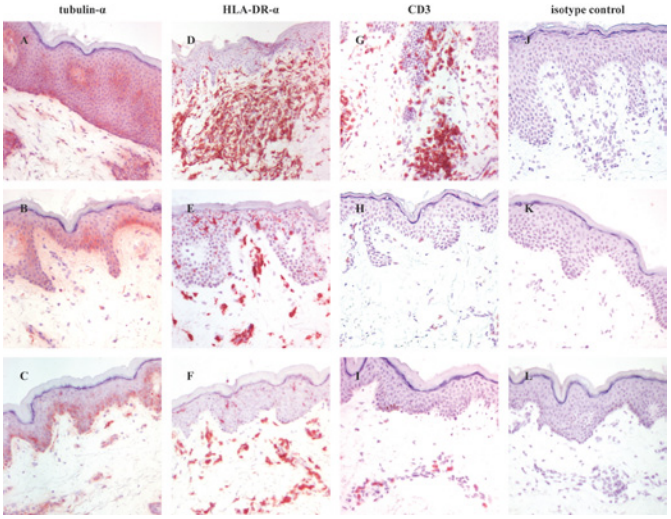

Figure 1: Upregulation of tubulin-α and HLA-DR-α expression in AE lesions. Tubulin-α and HLA-DR-α were stained by immunohistochemistry in the lesional (A, D), non-lesional skin (B, E) of the same AE patients and in healthy skin (C, F). Infiltrating $CD3^+$ cells (G, H, J) reflect the degree of inflammation. Results obtained for one representative AE patient and one healthy control are shown. Isotype control staining for lesional skin (J), non lesional skin of the same patient (K), and skin of a healthy individual (L) is shown.

avidin and biotin blocking solutions (Vector Laboratories, Burlingame, US). Human tubulin-α, HLA-DR-α, and CD3 were detected with specific primary antibodies: mouse anti-human tubulin-α, clone TU-01 (AbD Serotec, Kidlington, UK) diluted 1:250; mouse anti-human HLA-DR-α, clone TAL.1B5 (Dako, Glostrup, Denmark) diluted 1:12.5; mouse anti-human CD3, clone SK7 (BD Biosciences, Franklin Lakes, US) diluted 1:16. Mouse IgG1 (Dako) was used as isotype control. After incubation with biotinylated horse anti-mouse IgG secondary antibody (Vector Laboratories) diluted 1:10, biopsies were developed with ABC-elite solution (Vector Laboratories) and 3-amino-9-ethylcarbazole (Sigma, St. Louis, US) as substrate. All slides were counterstained with Mayer's hematoxylin and examined by standard bright field optics.

Results

Human tubulin-α, and HLA-DR-α were stained by immunohistochemistry in skin biopsies taken from chronic lesions, positive APT reactions and non-affected skin of four AE patients or from healthy skin of three non-atopic individuals.

A strong upregulation of tubulin-α expression was found in epidermal cells of chronic (Figure 1A) and acute AE lesions of all four patients tested. In contrast, tubulin-α expression was strongly reduced in keratinocytes from non-affected skin of the same patients (Figure 1B) and confined to stratum granulosum in the skin of the healthy individuals (Figure 1C).

HLA-DR-α was highly expressed on infiltrating cells such as B cells, dermal dendritic cells, and Langerhans cells in chronic AE lesions (Figure 1D) and positive APT reactions (data not shown). In biopsies taken from non-affected skin of the same AE patient (Figure 1E) or from healthy donors (Figure 1F) HLA-DR-α expression was strongly reduced compared to inflamed skin. Moreover, the degree of HLA-DR-α staining correlated quite well with the degree of inflammation, determined by the numbers of infiltrating $CD3^+$ T cells (Figure 1G) whereas only few $CD3^+$ T cells were detectable in non lesional skin of AE patients or in skin of healthy individuals (Figure 1H, I). Staining of the skin with mouse IgG1 used as isotype control were negative in all biopsies tested and independent from inflammatory processes as expected (Figure 1J, L).

Discussion

IgE-mediated autoreactivity is assumed to play a role in the multi-factorial pathogenesis of AE [16] and a broad spectrum of IgE-binding self-antigens has been described [3]. They include proteins with a high degree of homology to environmental allergens [4-7,12] as well as self-antigens without any sequence homology to known allergens [8]. Autoreactivity to self-antigens sharing sequence homology to environmental allergens can be explained by molecular mimicry as clearly shown for MnSOD [5,10], cyclophilin [12], and thioredoxin [7]. These proteins are inducible by oxidative stress [13], a condition characteristic for inflamed skin of AE patients [14], and in fact it has been shown that MnSOD is moderately expressed in the skin of healthy individuals or in healthy skin areas of AE patients, but strongly up-regulated in lesional skin areas [10]. Moreover, application of human MnSOD to unaffected skin areas of AE patients in atopy patch tests is sufficient to elicit eczematous reactions in AE patients sensitized to MnSOD, highlighting the role of IgE-mediated autoreactivity in the exacerbation and/or perpetuation of AE [10]. However, overexpression of other IgE-binding self-antigens in the inflammatory skin areas of AE patients has not been reported. During the present study we analyzed the expression of two IgE-binding self-antigens, tubulin-α and HLA-DR-α, in lesional and non-affected skin of AE patients and in the skin of healthy individuals by immunohistochemistry. Both, tubulin-α and HLA-DR-α are detectable in epidermal keratinocytes and infiltrating immune cells, respectively. The expression of the self-antigens is up-regulated in skin specimens taken from chronic AE lesions compared to unaffected skin of the same patients or skin from healthy individuals (Figure 1). These findings corroborate previous work, demonstrating that the IgE-binding self-antigen MnSOD is up-regulated in inflamed skin of AE patients. The overexpression of self-antigens in AE lesions provides targets for autoreactive serum IgE antibodies at the site of inflammation allowing the formation of IgE immune complexes that could target effector cells like mast cells and basophils. Clear evidences supporting this assumption are the ability of IgE-binding self-antigens to induce mediator release from basophils [3] and immediate type I skin reactions [5-8,12]. Activation of effector cells results in the release of preformed mediators, production of cytokines, and initiation of an allergic tissue reaction resulting in induction and maintenance of inflammatory skin responses [15].

Conclusions

In summary, over-expression of IgE-binding self-antigens in lesional skin of AE patients seems to be a common phenomenon, which

can explain the significant correlation observed between autoreactivity and severity of the disease [11]. Because a relevant subset of about 30 % AE patients shows IgE reactivity to a variety of human self-antigens, it is likely that these reactions contribute to the exacerbation of AE in a subset of patients.

Competing Interests

The authors declare that they have no competing interest.

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