

Original article

IgE-binding components of cultured human keratinocytes in atopic eczema/dermatitis syndrome and their crossreactivity with *Malassezia furfur*

Background: Atopic eczema/dermatitis syndrome (AEDS) patients display immunoglobulin E (IgE) reactivity to several antigens, e.g. saprophytic yeasts as *Malassezia furfur*. AEDS patients also show IgE autoreactivity towards cells of their own tissue including epidermis.

Purpose of the study: The aim of this study was to investigate the IgE autoreactivity of AEDS patients to cultured keratinocytes and to reveal potential crossreacting epitopes in cultured keratinocytes and *M. furfur*.

Material and methods: Serum samples of 27 AEDS patients were analyzed, of these 13 were *M. furfur* radioallergosorbent test (RAST) positive and 14 negative. Four urticaria, three psoriasis, and seven nonatopic patients were included as controls. The studies were performed by using IgE immunoblotting and immunoblotting inhibition methods.

Results: Ten IgE-binding protein bands were detected in cultured human keratinocytes by IgE immunoblotting using sera from adult AEDS patients. Anti-keratinocyte IgE antibodies were more associated with elevated S-IgE level than *M. furfur* RAST. Clear crossreactivity with *M. furfur* could not be shown.

Conclusions: The possible pathomechanism of anti-keratinocyte IgE antibodies is not due to IgE epitope mimicry of saprophytic yeast and local tissue in AEDS skin.

**O. Kortekangas-Savolainen¹,
S. Peltonen^{1,2}, K. Pummi², K. Kalimo¹,
J. Savolainen³**

Departments of ¹Dermatology, ²Medical Biochemistry and ³Clinical Allergy, University of Turku, Turku, Finland

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Outi Kortekangas-Savolainen
Department of Dermatology
University of Turku
FIN-20520 Turku
Finland

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Severe generalized atopic eczema/dermatitis syndrome (AEDS) in adult age is generally associated with high total immunoglobulin E (IgE) levels with specific IgE antibodies directed against external allergens, including commensal yeasts, such as *Candida albicans*, *Saccharomyces cerevisiae* and especially *Malassezia furfur* (1–6). IgE autoantibodies to several proteins of human origin have been demonstrated in AEDS patients by IgE-immunoblotting (7). In immunohistochemistry monoclonal antibodies to *Hom s 1*, the first human autoallergen, bind to several human tissues, including keratinocytes (8). Saprophytic *M. furfur* typically grows infiltrating keratinocyte layers. It also induces maturation of immature monocyte cells and Th2 type response (9–11).

The role of autoantibodies has remained disputed. One possible explanation could be IgE epitope mimicry of saprophytic *M. furfur* and keratinocytes. The purpose of the present study was to reveal keratinocyte autoreactivity in patients with *M. furfur*-specific IgE. In addition, we analyzed the presence of crossreacting IgE epitopes in cultured keratinocytes and *M. furfur* by immunoblotting inhibition.

Material and methods

Patient sera

Altogether 27 IgE-mediated AEDS patients were included in the study (12). Of these patients 13 were *M. furfur*-RAST (ImmunoCap, Pharmacia, Uppsala, Sweden) positive (>0.7 kU/l) and 14 *M. furfur*-RAST negative (<0.35 kU/l). In addition, four urticaria and three psoriasis patients were included. Sera from six healthy subjects were included as control samples for immunoblotting. These study subjects are presented in Table 1.

Keratinocytes

The skin samples were obtained from plastic surgical operation of a healthy female aged 35 years. The operation was carried out for cosmetic reasons at the University Hospital of Turku, Finland. Primary cultures of keratinocytes were established by a modification of the method of Boyce and Ham (13). Keratinocytes (third passage) were grown in serum-free low Ca^{2+} (<0.1 mM) keratinocyte growth medium (Clonetics Corp., San Diego, CA) until about 80% confluency. The cells were washed in phosphate-buffered saline and lysed in 500 μl of lysis buffer containing 62.5 mM Tris-HCl, pH 6.8, 2.3% sodium dodecyl sulfate, and 8% glycerol. Protein concentrations of

Table 1. Study subjects

	<i>n</i>	M/F	Age*	S-IgE*	<i>Malassezia furfur</i> RAST*
AEDS	27	10/17	33 (11)	3308 (4452)	26.1 (27.9)
Urticaria	4	1/3	57 (16)	127 (87)	n.d.
Psoriasis	3	1/2	54 (12)	n.d.	n.d.
Healthy	6	3/3	37 (10)	5 (5)	0.3 (0.0)

* Values are mean $\pm$ SD.

n.d., not done.

the samples were detected with DC Protein Assay (Bio-Rad Laboratories, Hercules, CA).

Malassezia furfur antigen extract

Malassezia furfur strain ATCC 42132 (American Type Culture Collection) was cultured on agar plates according to Leeming and Notman modified as described earlier (6, 14). Yeast cells were disrupted by X-press (Järfälla, Sweden) and extracted as previously described (6).

IgE-immunoblotting

Keratinocyte suspension (500 μ l) was mixed with 25 μ l of mercaptoethanol and boiled for 10 min. The keratinocyte proteins and *M. furfur* extract were run in a homogenous 10% gel overnight together with the molecular weight markers (15, 16). The proteins were transferred onto nitrocellulose according to Towbin (17). Nitrocellulose filters were incubated with patient sera, and the bound IgE was visualized using ^{125} I-labeled anti-IgE antibody (ImmunoCAP) (16, 18). IgE-immunoblotting inhibition was performed by preincubation of the studied sera with increasing concentrations of inhibiting antigen (19).

Statistics

Statistical significance in contingency table data was analyzed by Fisher's exact test.

Results

Coomassie blue staining revealed over 50 bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels of the keratinocyte extract (Fig. 1). Immunoblotting analysis showed 10 IgE-binding bands (Fig. 2). Ten of the 27 AEDS patients were positive in immunoblotting. Most frequently detected IgE-binding antigen was a 46 KDa band that was detected in six of 10 sera. Other IgE-binding components in cultured keratinocytes were 32 (1 serum), 36 (2), 40 (1), 42 (4), 44 (1), 50 (1), 60 (1), 67 (2) and 79 KDa (2) bands (Fig. 2). No binding was seen in sera from patients with urticaria, psoriasis, and healthy controls (data not shown).

The IgE binding to cultured keratinocytes was more associated with elevated total serum IgE (S-IgE > 1000 U/ml) than positive *M. furfur* RAST (Fig. 2 and Table 2). Anti-keratinocyte IgE was seen in eight of 10 subjects with elevated S-IgE, but only in two of 17 subjects with

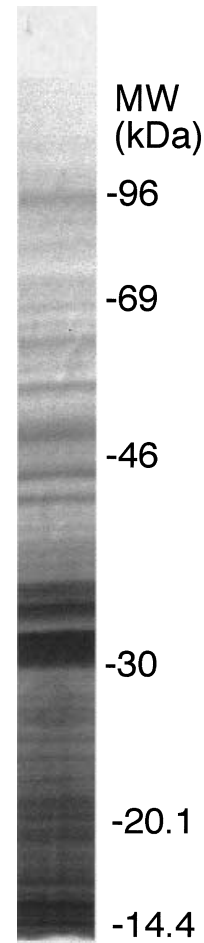


Figure 1. Lysate of cultured keratinocytes run in 10% SDS-PAGE gel stained with Coomassie brilliant blue.

S-IgE < 1000 U/ml ($P < 0.001$). Keratinocyte-specific IgE was seen in seven of 13 *M. furfur* RAST-positive and in three of 14 RAST-negative subjects ($P = 0.12$).

To find possible crossreacting components in keratinocytes and *M. furfur* extract an IgE-immunoblotting-inhibition experiment was performed using a subset of immunoblotting positive sera displaying most IgE binding to keratinocytes (Fig. 3). No clear inhibition of keratinocyte IgE-immunoblots was observed with increasing concentrations of *M. furfur* extract. Almost complete autoinhibition of *M. furfur* IgE-immunoblots was seen by increasing concentrations of *M. furfur* extract.

Discussion

This study demonstrates that AEDS patients with high levels of IgE also present with IgE autoantibodies to epidermal keratinocytes. Although epidermal reactivity is characteristic of AEDS, the relationship between IgE antibodies and cultured keratinocytes has not previously been investigated. In 1991 Taylor et al. demonstrated that

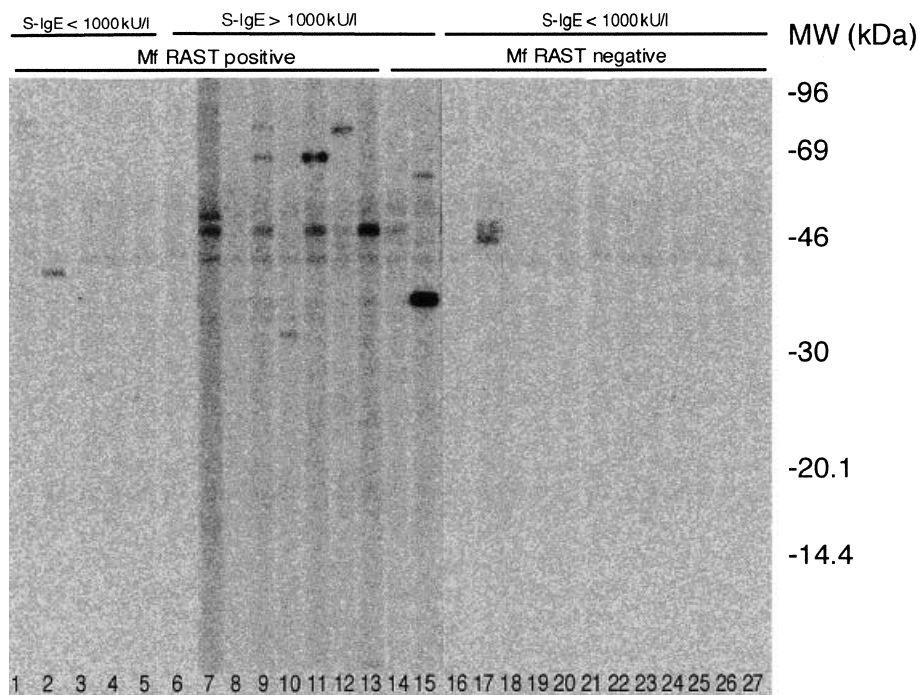


Figure 2. Lysate of cultured keratinocytes run in 10% SDS-PAGE gel blotted onto nitrocellulose and incubated with sera (dilution 1 : 4) from AEDS patients. The IgE-binding to antigens on the nitrocellulose membranes probed with ^{125} I-anti-IgE.

peripheral blood mononuclear cells from AEDS patients proliferated in response to autologous epidermal cells from AEDS lesional skin (20). IgE autoreactivity to human proteins was shown by Valenta et al. who demonstrated increased levels of IgE antibodies against human proteins in AEDS patients but not in chronic urticaria, allergic rhinoconjunctivitis, systemic lupus erythematosus or graft vs host patients (7). The IgE antibodies recognized proteins in endothelial cells, platelets, fibroblast and epithelial cells, as shown by immunoblotting technique. The only epithelial cell line studied was established from epidermal mammary carcinoma and displayed nearly 20 IgE binding bands in the molecular weight range of 10–100 KDa.

In the present study we have detected IgE autoantibodies against cultured normal human epidermal keratinocytes. To our knowledge, this is the first report to utilize keratinocytes in an IgE autoreactivity study. Serum from AEDS patients revealed several IgE binding bands, the most frequent with a molecular weight of 46 KDa. Some autoreactive proteins have previously been characterized: IgE autoantibodies against a transcription factor p75 were originally found on immunostaining against nuclear fine speckles (21). In addition, a purified recombinant *Hom s 1* 55 KDa allergen binds IgE from sera of AEDS patients (8). Immunohistochemistry revealed a prominent *Hom s 1* staining in suprabasal keratinocytes of the skin in both healthy and AEDS subjects. None of our bands directly corresponds to the described 55 KDa band. However, the molecular weight

determination in SDS-PAGE is not accurate. Further characterization of the protein components was outside of the scope of this study.

IgE antibodies to *M. furfur* have been described in AEDS in several studies with several others reporting efficacy of antifungal treatments (1–3, 6, 22–25). The IgE antibodies against *M. furfur* are likely to be of immunopathogenic importance as *M. furfur* preferentially induces Th2 type cytokine responses (9, 11, 26). *M. furfur* also activates immature dendritic cells and induces their maturation, which enhances lymphocyte proliferation (10, 27). *Malassezia furfur* is thus likely to contribute to the early phase of the skin inflammatory process in AEDS. Recent findings also suggest a non-IgE-dependent mechanism for *M. furfur* in AEDS inflammation as *M. furfur* *in vitro* induces production of proinflammatory cytokines in keratinocytes (28). The good therapeutic effect of antifungal treatment in AEDS may thus reflect the involvement of *M. furfur* in the AEDS inflammation by several mechanisms, both IgE-mediated and non-IgE-mediated.

While anti-*M. furfur* IgE appears of importance, the role of IgE autoantibodies has remained pathophysiologically disputed. A role for IgE containing immunocomplexes has been proposed (29). As the specificity of autoantibodies is not clear, this study was designed to evaluate the potential crossreactivity to a common epidermal saprophytic yeast. However, in the IgE immunoblotting inhibition no cross-reacting epitopes were found in spite of parallelly achieved successful autoinhibition. The IgE autoantibodies are likely

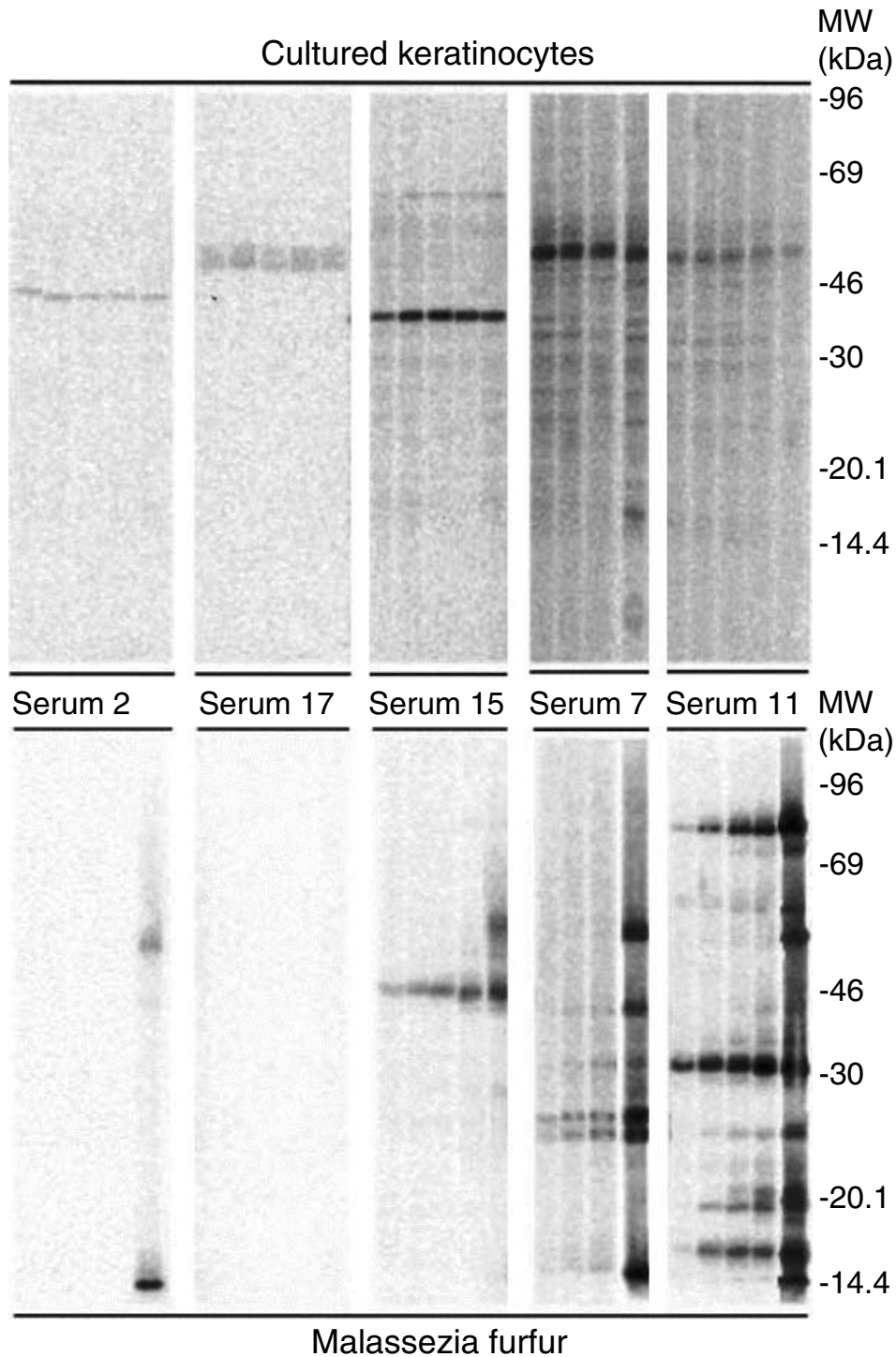


Figure 3. IgE-immunoblotting inhibition with lysate of cultured keratinocytes and *Malassezia furfur* extract run in 10% SDS-PAGE gel blotted onto nitrocellulose. Preincubation of the selected AEDS sera (dilution 1 : 4) with increasing (five concentrations from right to left: 0, 0.08, 0.4, 2 and 10 mg/ml) concentrations of *M. furfur* extract as inhibiting antigen. The IgE-binding to antigens on the nitrocellulose membranes probed with ^{125}I -anti-IgE.

to represent a secondary phenomenon induced by Th2 type dominant phase of the inflammation in the skin in which *M. furfur* is also involved. It is noteworthy that the

highest frequency of anti-keratinocyte IgE was seen in patients with the highest serum total IgE values. These patients display IgE responses against a wide variety of

Table 2. IgE-binding to keratinocyte antigens by immunoblotting in AEDS patients according to S-IgE and *Malassezia furfur* RAST

	IgE-binding to keratinocytes	
	Yes	No
S-IgE > 1000		
<i>M. furfur</i> RAST positive	6	2
<i>M. furfur</i> RAST negative	2	0
S-IgE < 1000		
<i>M. furfur</i> RAST positive	1	4
<i>M. furfur</i> RAST negative	1	11

Fisher's exact test for:

S-IgE vs IgE-binding to keratinocytes $P < 0.001$.*Malassezia furfur* RAST vs IgE-binding to keratinocytes $P = 0.12$.

environmental antigens, but the clinical and immunopathogenic importance of many of these antibodies is not known. Although we failed to demonstrate any IgE epitope mimicry with *M. furfur*, the role of keratinocyte-specific IgE in AEDS remains to be elucidated.

In conclusion, several IgE-binding components were detected in cultured human keratinocytes by IgE immunoblotting using sera from adult AEDS patients. Antikeratinocyte IgE antibodies were more associated with elevated S-IgE than anti-*M. furfur* IgE, and did not crossreact with *M. furfur*. This shows that AEDS patients display a variety of IgE reactivity, both autoantibodies and antibodies to external antigens. So far the anti-keratinocyte IgE, unlike anti-*M. furfur* IgE, has not been clearly associated with immunopathologic phenomena in AEDS.

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