

IgE and IgG₄ autoantibodies against DFS70/LEDGF in atopic dermatitis

KANAKO WATANABE, YOSHINAO MURO, KAZUMITSU SUGIURA, & YASUSHI TOMITA

Division of Connective Tissue and Autoimmunity, Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

(Submitted 23 September 2010; revised 6 December 2010; accepted 15 December 2010)

Abstract

Background. DFS70/LEDGF is a known survival factor, growth factor, and HIV-1 transporter. It is also an autoantigen recognized by IgG autoantibodies (aAbs) in patients with atopic dermatitis (AD). In the past few decades, various IgE aAbs against self-proteins have been identified in AD. Although DFS70 is considered to be one of these proteins, to date, there has not been a detailed analysis of the significance of this putative autoantigen.

Objective and methods. Using recombinant protein that was generated using a baculovirus and insect cell system, we established an ELISA that can measure IgE-anti-DFS70 aAbs and IgG₄-anti-DFS70 aAbs. Sera obtained from 61 AD patients and 20 healthy donors were analyzed using the ELISA.

Results. Nine of 61 AD patients were determined to be IgE-anti-DFS70 aAb-positive. IgG₄-anti-DFS70 aAbs were detected in nine of 61 patients. The serum thymus and activation-regulated chemokine concentrations were significantly higher in the group that was positive for both aAbs compared to the aAb-negative group, although the total serum IgE levels were not significantly different between these groups.

Conclusion. We conclude that serum IgE-anti-DFS70 aAbs and IgG₄-anti-DFS70 aAbs may be related to the severity of AD.

Keywords: atopic dermatitis, autoantibody, DFS70, IgE, IgG₄, LEDGF

Introduction

Atopic dermatitis (AD) is a very common, chronic, relapsing allergic dermatitis that is characterized by severe dry skin, redness, and pruritus. Several predisposing factors have been identified, such as the inability of the skin to protect against environmental antigens or loss-of-function mutations in filaggrin [1].

In the past few decades, various IgE autoantibodies (aAbs) against self-proteins have been identified in atopic diseases including AD [2–7]. Dense fine speckles 70 kDa protein (DFS70)/Lens epithelium-derived growth factor (LEDGF) was identified as one of these self-proteins [8,9] as well as Hom S1–5 [10], thioredoxin (TRX) [11,12], and manganese superoxide dismutase (MnSOD) [13]. It has been also reported that allergen-specific IgG₄ Abs act as blocking antibodies and induce tolerance in IgE-mediated allergic diseases [14,15], and allergen-specific IgE and IgG₄ are often discussed together with regard to AD [16,17]. In addition, previous reports have shown that the clinical activity of non-atopic autoimmune skin

diseases, such as bullous pemphigoid and pemphigus vulgaris, relates not only to the IgG class but also to IgE and IgG₄ autoantibodies against BP180 and desmoglein 3, respectively [18,19].

DFS70 is a nuclear antigen with a characteristic pattern in immunofluorescence assay showing a nuclear distribution of dense, fine, speckles (DFS) [20]. DFS70 was identified as a growth factor that stimulates cell growth and activates the expression of heat shock and stress-related genes [21], and was shown to interact with HIV-1 integrase and help target this protein to chromatin [22,23]. In addition, DFS70 is also an autoantigen that is recognized by IgG aAbs in healthy individuals [24] as well as patients with alopecia [25], chronic fatigue syndrome [8], and AD [8,9]. Although 29.6–71.4% of AD patients have been determined to be IgG-anti-DFS70 aAb-positive [8,9], the prevalence of IgE-anti-DFS70 aAbs in AD patients has not been determined.

In this study, we newly collected 61 sera samples from AD patients, established an ELISA to measure serum IgE-anti-DFS70 aAbs and IgG₄-anti-DFS70

Correspondence: Y. Muro, Division of Connective Tissue and Autoimmunity, Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel: +81-52-744-2314. Fax: +81-52-744-2318.
E-mail: ymuro@med.nagoya-u.ac.jp

aAbs, and investigated how these aAbs relate to the clinical disease severity. Thymus and activation-regulated chemokine (TARC) was previously identified as a biomarker for AD, and serum TARC concentrations have been shown to significantly correlate with severity scoring of atopic dermatitis (SCORAD) and the total serum IgE levels [26,27].

Materials and methods

Serum samples

The profiles of subjects are shown in Table I. Sera from 61 AD patients were obtained from the Department of Dermatology, Nagoya University Hospital. All of the subjects were Japanese and included 30 men and 31 women with ages ranging from 13 to 59 years (mean age 26.2 ± 9.8). They fulfilled the criteria of Hanifin and Rajka [28]. Sera from 20 healthy individuals without antinuclear Abs were used as controls. We could not obtain detailed information regarding medication, disease duration, and/or disease severity from all of the patients at the time of blood collection. Written informed consent was obtained from each subject, and the Ethics Committee of Nagoya University, Graduate School of Medicine approved the use of human materials.

Recombinant protein

We chemically synthesized an artificial gene that consisted of the chitinase signal sequence, DFS70 gene (323–530aa, including the autoepitope region of DFS70 autoantigen [29]), and a $6 \times$ His-Tag sequence. This synthesized gene was inserted into the pAcSG2 baculovirus transfer vector (BD Biosciences, Tokyo, Japan) and co-transfected into the insect cell line Sf9 with BaculoGold linearized baculovirus DNA (BD Biosciences). Recombinant DFS70 baculovirus was produced and amplified to obtain a high-titer viral stock. High-Five insect cells were infected with the high-titer recombinant virus and incubated to induce protein expression.

The culture supernatant was collected, concentrated, and dialyzed with a dialysis membrane (Cellu Sep T2, Membrane Filtration Product, Inc., Seguin, TX, USA) in phosphate-buffered saline (PBS). The obtained recombinant protein was purified using a TALON Metal Affinity Resin column (Clontech Laboratories, Inc., Mountain View, CA, USA).

The purity was $>90\%$ based on SDS-PAGE (data not shown). The purified protein obtained by these procedures is also used in the commercially available DFS ELISA kits (MBL Co., Ltd., Nagoya, Japan). *Escherichia coli* expressing recombinant human TRX was purchased from Oriental Yeast Co., Ltd., Tokyo, Japan.

Protein G sepharose and acid treatment of sera

When measuring the specific IgE levels in the sera using a direct ELISA, competition with coexisting IgG often impedes an exact IgE determination. For this reason, serum samples that were examined using an ELISA for IgE Abs specific to DFS70 and TRX were pre-treated with protein G (PG) sepharose to remove IgG Abs as previously described [30–32], with slight modifications. Sera ($10 \mu\text{l}$), PBS ($75 \mu\text{l}$) and a 50% slurry of PG sepharose (GE Healthcare UK Ltd., Buckinghamshire, England) in PBS ($30 \mu\text{l}$) were mixed and incubated with shaking for 1.5 h at room temperature. After removing the PG sepharose by centrifugation (1000 rpm for 1 min), $95 \mu\text{l}$ of supernatant was collected. Acid treatment was also performed for some serum samples to liberate IgE from the IgG–IgE complexes as previously described [33].

ELISA for IgE aAbs to DFS70 and TRX

Specific binding of serum IgE aAbs to recombinant DFS70 and TRX was analyzed using a direct solid-phase ELISA. Forty-eight wells of 96-well polystyrene plates (Medisorp, Nunc, Roskilde, Denmark) were coated with the DFS70 or TRX recombinant proteins in PBS (1 and $0.5 \mu\text{g/ml}$, respectively) and incubated overnight at 4°C . After washing three times with PBS containing 0.05% Tween 20 (T-PBS), all wells on the plate were incubated with 3% bovine serum albumin (BSA) (Wako, Osaka, Japan) in T-PBS ($100 \mu\text{l/well}$) for 2 h at room temperature. Uncoated wells were used to measure the background levels for each sample. After three washes, diluted serum samples that had been treated with PG sepharose as described above were applied to two wells ($45 \mu\text{l}$ each) with and without the recombinant protein and then incubated overnight at 4°C .

After five washes, a horseradish peroxidase (HRP)-conjugated goat anti-human IgE Ab (Kierkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) was used as secondary Ab ($100 \mu\text{l/well}$) at a 1:50,000 dilution. After incubating for 2 h at room temperature, the plates were washed 10 times and then incubated with Ultra TMB (Pierce, Rockford, IL, USA) as the substrate according to the manufacturer's protocol. Then, optical density (OD) at 450 nm was determined using Multiskan JX (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Table I. Atopic dermatitis patient and control profiles.

	Atopic dermatitis patient	Healthy control
Number	61	20
Male/female	30/31	4/16
Age (year)	13–59	22–57
Mean age $\pm$ SD	26.2 ± 9.8	33.1 ± 9.5
Median age (year)	25	29.5

For each sample, the OD of the uncoated well was subtracted from the OD of the coated well and then subjected to data analysis. OD values greater than the mean +3SD values of the normal controls were considered positive in this study. All ELISA results were transformed into ELISA units using the following formula: ELISA units = $100 \times (\text{corrected OD of serum from each sample}) / \text{the cut-off OD value}$.

ELISA for IgG- and IgG₄-anti-DFS70 aAbs

Specific IgG aAbs against DFS70 were measured using a commercial ELISA kit (MBL) and the results were evaluated as an index value that was calculated according to the manufacturer's instructions. The cut-off level was determined as the mean +3SD values of the normal controls in the kit (15 index values). Each well of the commercial plates was coated with 0.1 µg of recombinant DFS70. The serum levels of IgG₄-anti-DFS70 aAbs were measured similarly using the same kit according to the manufacturer's instructions with slight modifications.

Briefly, the serum samples were diluted 1:50 in assay diluent (100 µl), added to the wells, and incubated for 1 h at room temperature. After five washes, the plates were incubated with an HRP-conjugated mouse anti-human IgG₄ Ab (SouthernBiotech, Birmingham, AL, USA) diluted 1:10,000 in assay diluent for 2 h at room temperature. After five washes, Ultra TMB (Pierce) was used as a substrate according to the manufacturer's protocol, and then the OD at 450 nm was determined. Each sample was examined in duplicate. OD values greater than the mean +5SD values of the normal controls were considered positive. All data were transformed into ELISA units using the formula described here.

Measuring serum TARC concentrations and total IgE levels

Commercial kits were used to measure the TARC concentrations (R&D Systems, Inc. Minneapolis, NE, USA) following the manufacturer's protocol. Total serum IgE levels were determined using routine laboratory examinations. The total serum IgE levels and TARC concentrations were expressed as logarithmically transformed values as described previously [35], because they followed a normal distribution.

Immunohistochemistry of normal and AD human skin sections

Normal human and AD skin samples were obtained from the left anterior arm of a 15-year-old male (normal), 36-year-old female (AD), and 41-year-old female (AD) with written informed consent. These human skin samples were examined by immunohistochemistry as previously described [35]. Briefly, the VECTASTAIN Elite ABC-PO (rabbit IgG) kit

(Vector Laboratories, Inc., Burlingame, CA, USA) was used for staining. Thin sections (6 µm) were cut from paraffin blocks of human skin.

The sections were immersed in a 0.4% pepsin solution for 30 min, and soaked for 20 min at room temperature in 0.3% H₂O₂/methanol to block endogenous peroxidase activity. After washing in PBS with 0.01% Triton X-100, the sections were incubated for 30 min in PBS buffer with 4% BSA and then probed with polyclonal anti-DFS70 antiserum named No. 6369 [25] (1:1000 dilution) in PBS buffer containing 1% BSA overnight at 4°C. After washing in PBS buffer, the thin sections were exposed to avidin-conjugated goat anti-rabbit Ig Abs for 1 h and then washed with PBS buffer. After washing, the tissue sections were immersed in VECTASTAIN Elite ABC Reagent for 30 min and then washed in PBS buffer. The Ab complexes were visualized by the addition of 1% H₂O₂.

Statistical analyses

Statistical analyses were performed using SPSS (SPSS, Inc., Chicago, IL, USA). Differences were analyzed with an unpaired *t*-test and Mann-Whitney *U*-test, and correlations between two parameters were analyzed by Spearman's correlation coefficients. A *p*-value less than 0.05 was considered statistically significant.

Results

Effect of PG sepharose and acid treatment on the ability to detect DFS70-specific IgE aAbs by ELISA

Aside from the 61 samples examined in this study, additional serum samples from AD patients were used to determine the effects of protein G sepharose and acid pretreatment on IgE-anti-DFS70 aAb ELISAs. The changes in the OD values before and after treating the serum with PG sepharose are shown in Figure 1a. The OD values of some of the examined serum samples were elevated after PG sepharose treatment. However, acid treatment sometimes lowered the OD values (Figure 1b), which is probably because human IgE antibodies are more sensitive and more easily denatured by acid. For this reason, we pretreated the serum samples only with PG sepharose.

Detection of IgG-anti-DFS70 aAbs by ELISA

IgG-anti-DFS70 aAbs were detected using commercial ELISA kits and the index values were calculated using the manufacturer's protocol. Twenty one percent (13 of 61) of the AD patients were positive for IgG-anti-DFS70 aAbs (Figure 2a), including 4 males and 9 females. All IgG-anti-DFS70 aAb-positive AD sera were subsequently confirmed to be positive by Western blotting (WB) as described previously [24] (data not shown). Twenty control sera were determined to be IgG-anti-DFS70 aAb-negative by WB in a previous study [24], although one

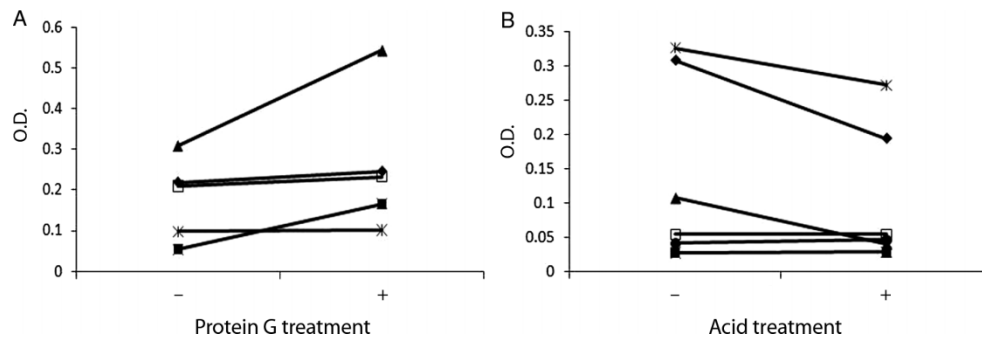


Figure 1. Effect of protein G sepharose and acid treatment on DFS70-specific IgE detection by ELISA. Five serum samples from AD patients were pre-treated with (+) or without (-) PG sepharose, and then the levels of DFS70-specific IgE were determined by ELISA (A). Serum samples from 6 AD patients were pre-treated with (+) or without (-) acidification (B). The markers representing each serum sample are independent in (A) and (B).

of these serum samples was positive by this ELISA system.

Detection of IgE-anti-DFS70 and IgE-anti-TRX aAbs by ELISA

The presence of IgE-anti-DFS70 and anti-TRX aAbs in the serum samples of AD patients and normal controls was assessed by ELISA. OD values greater

than the mean +3SD values of the controls were considered positive. IgE-anti-DFS70 aAbs were detected in 15% (9 of 61) of AD patients (Figure 2b), which included seven males and two females.

The ELISA for IgE aAbs against TRX, which is a well-known IgE-binding autoantigen [7,10,11,12], was performed using the same protocol to confirm that our method could be used to evaluate other IgE aAbs. IgE-anti-TRX aAbs were detected in 5% (3 of 61) of AD patients (Figure 2c) using these methods, and this rate of positivity was lower than earlier studies [7,12]. Only one patient was positive for both IgE-anti-DFS70 and IgE-anti-TRX aAbs.

Measurement of IgG₄-subclass anti-DFS70 aAbs by ELISA

IgG₄-anti-DFS70 aAbs were detected in 15% (9 of 61) of AD patients (Figure 2d), including five males and four females. OD values greater than the mean +3SD values of the controls were considered positive. There was no correlation between the IgG₄-anti-DFS70 titer and patient age.

Association between DFS70-specific IgE, whole IgG, and IgG₄ aAbs with serum TARC concentrations and total IgE Levels

As several studies have reported [26,27], there was a significant correlation between the serum TARC concentrations and total IgE levels ($r = 0.567$, $p < 0.001$, data not shown). The serum TARC concentrations were significantly higher in IgE and IgG₄-anti-DFS70 aAb-positive patients than in aAb-negative patients (Figure 3a,e), whereas there was no significant difference in the total IgE level between these groups (Figure 3b,f). The IgG-anti-DFS70 aAb-positive and -negative patients did not differ with respect to either the serum TARC concentrations or the total IgE levels (Figure 3c,d).

There was neither a positive nor negative correlation between the levels of IgE- and IgG₄-anti-DFS70 aAbs (data not shown). There was no correlation between

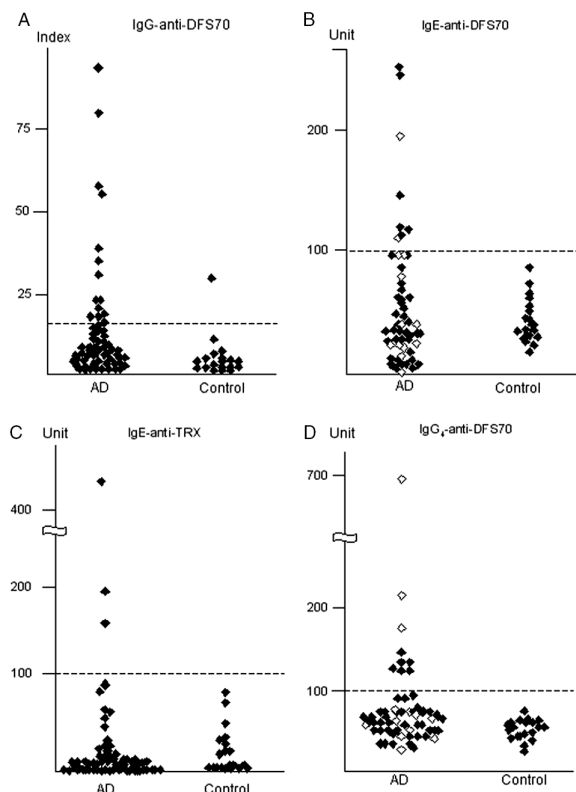


Figure 2. IgG-anti-DFS70 (A), IgE-anti-DFS70 (B), IgE-anti-TRX (C), and IgG₄-anti-DFS70 (D) aAb levels in sera from AD patients and healthy controls. The IgE-anti-DFS70, IgE-anti-TRX, and IgG₄-anti-DFS70 aAb levels are shown as OD values, while the IgG-anti-DFS70 aAb levels are shown as index values. The dotted line shows the cut-off value in each assay (15 index values for IgG-anti-DFS70 aAbs (A) and mean +3SD values for the healthy controls (B, C, and D)). The open diamonds in (B) and (D) indicate the individuals who were positive for IgG-anti-DFS70 aAbs.

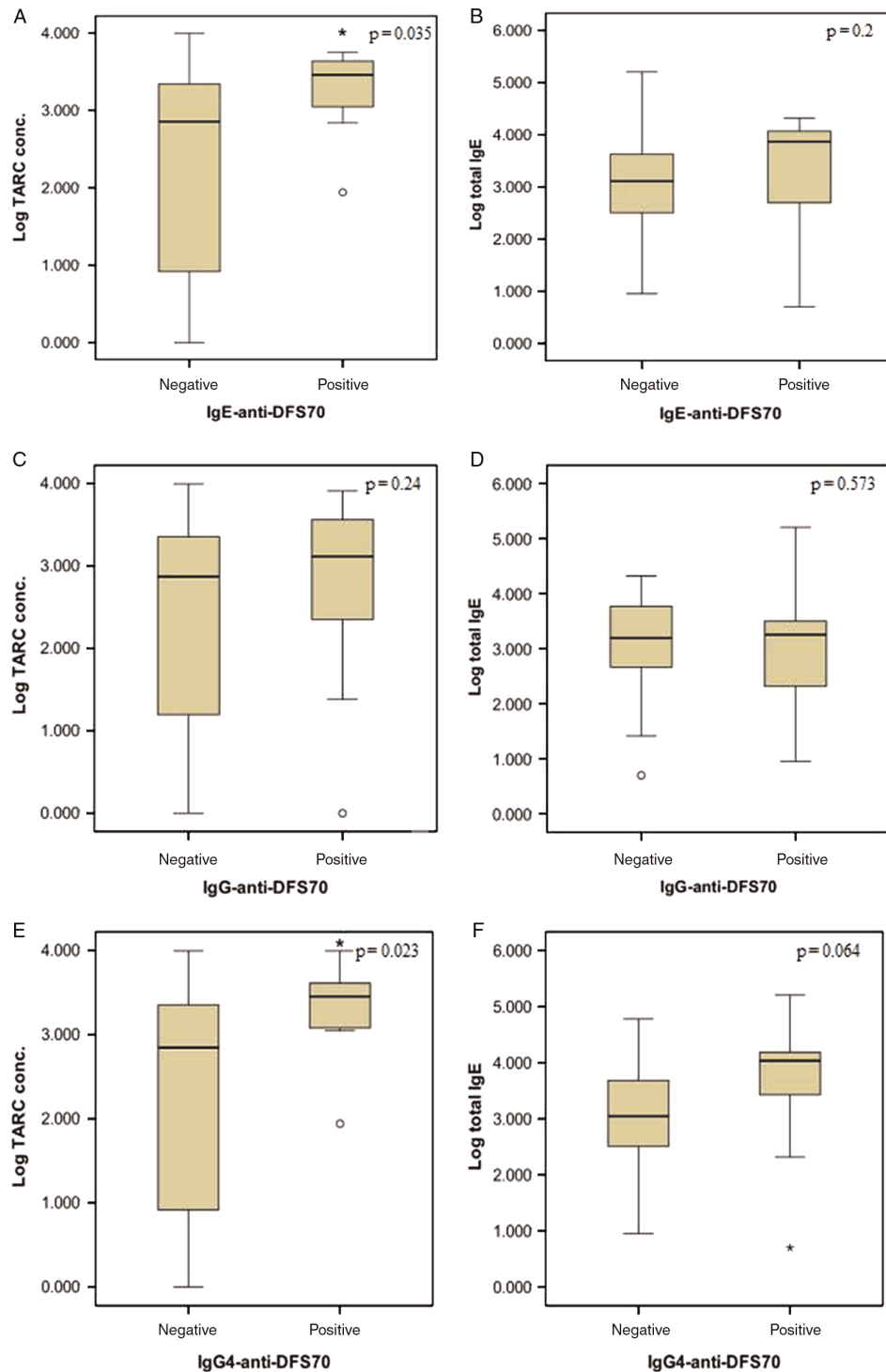


Figure 3. Serum TARC concentrations and total IgE levels in IgE-anti-DFS70 aAb-positive patients and -negative patients (A, B), IgG-anti-DFS70 aAb-positive patients and -negative patients (C, D), and IgG₄-anti-DFS70 aAb-positive and -negative patients (E, F). Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. The total serum IgE levels (IU/ml) and TARC concentrations (pg/ml) were expressed as logarithmically transformed values. Differences were analyzed by a Mann–Whitney *U*-test and statistically significant differences are indicated (* $p < 0.05$).

the serum levels of IgE-anti-DFS70 aAbs and serum TARC concentrations in sera from 61 AD patients ($r = 0.17$, $p > 0.05$). However, serum levels of IgG₄-anti-DFS70 aAbs correlated with serum TARC concentrations ($r = 0.36$, $p < 0.01$, data not shown).

Immunohistochemical analyses

The localization of DFS70 was visualized by immunohistochemical analyses of normal and AD human skin sections. The cytoplasm of epidermal cells in normal skin was more strongly labeled than the nuclei

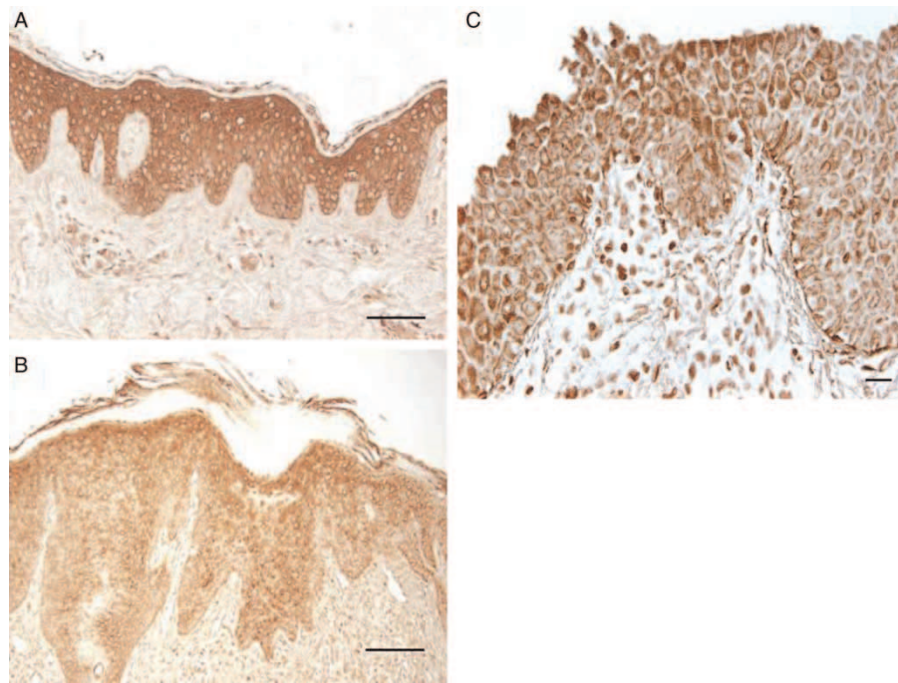


Figure 4. Immunohistochemical analyses of DFS70 in human skin sections. Thin skin tissues from a healthy individual (A), an AD patient without anti-DFS70 aAbs (B), and an AD patient with IgG-anti-DFS70 aAbs (C) were immunohistochemically stained with a polyclonal antiserum to DFS70. Bar = 200 μ m.

(Figure 4a). This staining pattern was also detected in skin lesions from AD patients, and there was no indication that DFS70 leaked into the extracellular space (Figure 4b,c). Based on the ELISAs, the serum of the 41-year-old woman with AD who provided a skin biopsy specimen was determined to be IgG-anti-DFS70 aAb-positive, while the other two were negative for IgG, IgG₄, and IgE-anti-DFS70 aAbs. The staining patterns did not remarkably differ between the skin sections from the IgG-anti-DFS70 aAb-positive and -negative AD patients. Mononuclear cells infiltrating into the upper layer of the dermis were strongly stained in skin sections from both AD patients.

Discussion

The pathogenesis of AD is likely to result from a combination of various factors, and recent studies suggest that one possible trigger is IgE-mediated reactions to self-antigens [2–5]. The autoreactivity of AD patients to self-antigens has been postulated to be a crucial pathogenetic factor in AD. Some known IgE-binding self-antigens such as MnSOD, ribosomal P2 protein, cyclophilin, or TRX have high homology to exogenous allergens and induce skin reactions in patients who are hypersensitive to the corresponding environmental allergens [7].

Furthermore, MnSOD alone can induce an eczematous reaction in a skin patch test in AD patients [13]. Another group of IgE-reactive autoantigens, which has been termed the Hom s antigens, was identified in a disease-oriented approach. However,

the prevalence of aAbs to each Hom s autoantigen in AD patients has not been determined or has only been examined with a small sample size (Hom s 2; 27.3% [36], Hom s 4; 16.7% [37]).

Nevertheless, it was recently argued that IgE autoreactivity to antigens in this group is an important sign or marker of disease severity, particularly in AD patients [38]. Zeller et al. recently probed the repertoire of IgE-binding self-antigens by screening a human phage surface-displayed cDNA library with serum IgE from AD patients. The IgE-binding capacity of the seven selected self-antigens, including actin, tubulin, eIF6, HLA-DR, RP1, cyclophilin B, and TRX, was confirmed by ELISAs using recombinant proteins and sera from 71 AD patients. The prevalence ranged from 8.7 to 29.0%, and 71.8% of 71 AD patients showed variable patterns of IgE autoreactivity to one or more of the tested self-antigens.

Ochs et al. identified IgE-class aAbs against DFD70 by performing Western blot analyses with sera from patients with AD [8]. To determine the prevalence of IgE-anti-DFS70 aAbs and investigate its possible role as a marker of AD, we established novel ELISA systems that measure serum IgE- and IgG₄-anti-DFS70 aAbs.

Twenty-three of 61 AD patients were positive for one or two anti-DFS70 aAbs (Figure 5). Several sera were anti-DFS70—negative based on the kit but were positive for the IgG₄-subclass, indicating that some IgG₄-anti-DFS70 aAbs were potentially missed because the anti-IgG reagent in the kit does not contain a sufficient amount of antibodies against the IgG₄-subclass.

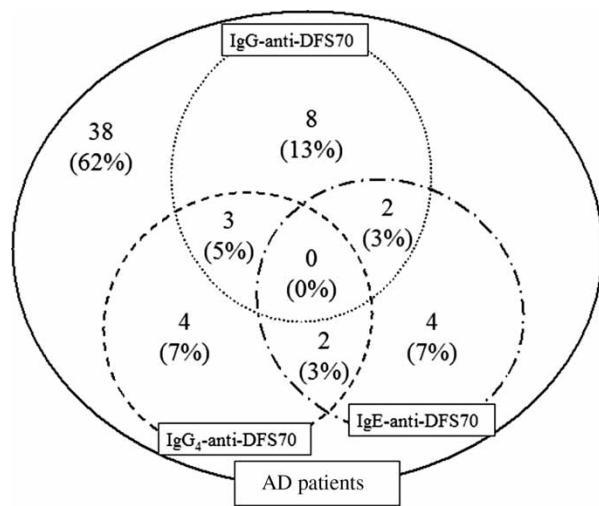


Figure 5. Distribution of 61 AD patients according to their positivity for IgG-, IgE-, and IgG4- anti-DFS70 autoantibodies. The number and frequency in each category is shown.

The prevalence of IgE-anti-DFS70 aAbs was 15%. The prevalence of IgE-anti-TRX positivity based on our ELISA conditions was lower than those in other studies [7,10,12], although this difference might be due to our patient population or other factors.

The DFS70 antigen used in our ELISA system was not the full-length protein, but contained a conformational autoepitope that is recognized by IgG-class aAbs and is not contained in the alternatively spliced form p52 [29].

Therefore, we cannot exclude the possibility that IgE-class aAbs against other domains such as the p52-specific domain could exist and that the positivity of IgE-anti-DFS70 aAbs might be underestimated. Nonetheless, our analysis demonstrated that serum TARC concentrations, which are a well-known biomarker of AD [26,27], were significantly higher in the IgE-anti-DFS70 aAb-positive group than in the aAb-negative group. This result suggests that IgE-anti-DFS70 aAbs might be an indicator of the severity of AD and implies that the IgG-class major autoepitope domain of DFS70 might also be important for the production of IgE-class aAbs.

The prevalence of IgG4-anti-DFS70 aAbs was also 15%. The coexistence of IgE aAbs was observed in 3 of 9 patients with these aAbs. Interestingly, the serum TARC concentrations in the IgG4-anti-DFS70 aAb-positive group were also significantly higher compared to the aAb-negative group. Furthermore, serum levels of IgG4-anti-DFS70 aAbs correlated with serum TARC concentrations in sera from 61 AD patients. In the field of IgE-mediated allergies against environmental antigens and food allergens, the presence of high levels of specific IgG4 Abs signifies a long disease duration and/or acquisition of tolerance [14,16,17].

However, our results show that there is a possible positive relationship between disease severity and

IgG4-anti-DFS70 aAb levels in AD patients. There was no association between IgG4-anti-DFS70 aAb levels and patient age, although the precise disease durations in our patients were unknown. In light of the association between T helper (Th) 2 cells and AD [1], the occurrence of Th2-regulated antigen-specific antibody subtypes, i.e. IgE and IgG4, is understandable. In fact, recent investigations indicate that the levels of eIF-specific IgG4 in AD patients directly correlated with the levels of specific IgE [7]. The serum total IgE levels, which are also a noted clinical marker for AD, did not differ significantly between the IgE-anti-DFS70 aAb-positive and -negative groups. The same result was observed regarding IgG4-anti-DFS70 aAbs.

Although the pathological role of IgE and IgG4 aAbs against DFS70 in AD patients is still largely unknown, immunohistochemical analyses showed that DFS70 is present in epidermal cells and infiltrating monocytes in skin sections from AD patients. This finding indicates that the release of DFS70 from damaged tissue or invading cells may trigger responses mediated by IgE or T cells. The skin sections used in this study were not from AD patients with IgE or IgG4-anti-DFS70 aAbs, and additional studies are needed to clarify the distribution of DFS70 in the skin of AD patients with IgE and IgG4-anti-DFS70 aAbs.

In the present study, the positivity of IgE-anti-DFS70 aAbs in AD patients was equivalent to or higher than those of the well-known self-antigens mentioned above. Therefore, we propose that DFS70 is an important IgE-binding self-protein in AD that potentially affects disease severity. Unfortunately, because AD is a chronic disease, most of the AD patients examined in this study were treated by home doctors and they only occasionally visited our hospital. For this reason, we could not obtain sequential serum samples or detailed information on the disease duration or severity for all the patients. Hereafter, we will investigate the time-dependent changes in aAb titers in patients with more detailed clinical information.

In conclusion, the current study indicates that both IgE- and IgG4-anti-DFS70 aAbs, rather than whole IgG-anti-DFS70 aAbs, are related to the severity of AD and these aAbs might be novel serological markers of a certain subset of AD.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- [1] Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. *J Dermatol Sci* 2010;58:1–7.
- [2] Valenta R, Maurer D, Steiner R, Seiberler S, Sperr WR, Valent P, Spitzauer S, Kapiotis S, Smolen J, Stingl G. Immunoglobulin E response to human proteins in atopic patients. *J Invest Dermatol* 1996;107:203–208.

- [3] Natter S, Seiberler S, Hufnagl P, Binder BR, Hirschl AM, Ring J, Abeck D, Schmidt T, Valent P, Valenta R. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. *FASEB J* 1998;12:1559–1569.
- [4] Seiberler S, Bugajska-Schretter A, Hufnagl P, Binder BR, Stöckl J, Spitzauer S, Valent P, Valenta R. Characterization of IgE-reactive autoantigens in atopic dermatitis. 1. Subcellular distribution and tissue-specific expression. *Int Arch Allergy Immunol* 1999;120:108–116.
- [5] Seiberler S, Natter S, Hufnagl P, Binder BR, Valenta R. Characterization of IgE-reactive autoantigens in atopic dermatitis. 2. A pilot study on IgE versus IgG subclass response and seasonal variation of IgE autoreactivity. *Int Arch Allergy Immunol* 1999;120:117–125.
- [6] Altrichter S, Kriehuber E, Moser J, Valenta R, Kopp T, Stingl G. Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis. *J Invest Dermatol* 2008;128:2232–2239.
- [7] Zeller S, Rhyner C, Meyer N, Schmid-Grendelmeier P, Akdis CA, Cramer R. Exploring the repertoire of IgE-binding self-antigens associated with atopic eczema. *J Allergy Clin Immunol* 2009;124:278–285.
- [8] Ochs RL, Muro Y, Si Y, Ge H, Chan EK, Tan EM. Autoantibodies to DFS 70 kd/transcription coactivator p75 in atopic dermatitis and other conditions. *J Allergy Clin Immunol* 2000;105:211–220.
- [9] Ayaki M, Ohoguro N, Azuma N, Majima Y, Yata K, Ibaraki N, Singh DP, Ko V, Shinohara T. Detection of cytotoxic anti-LEDGF autoantibodies in atopic dermatitis. *Autoimmunity* 2002;35:319–327.
- [10] Zeller S, Glaser AG, Vilhelmsson M, Rhyner C, Cramer R. Immunoglobulin-E-mediated reactivity to self antigens: A controversial issue. *Int Arch Allergy Immunol* 2008;145:87–93.
- [11] Schmid-Grendelmeier P, Scheynius A, Cramer R. The role of sensitization to *Malassezia sympodialis* in atopic eczema. *Chem Immunol Allergy* 2006;91:98–109.
- [12] Limacher A, Glaser AG, Meier C, Schmid-Grendelmeier P, Zeller S, Scapozza L, Cramer R. Cross-reactivity and 1.4-Å crystal structure of *Malassezia sympodialis* thioredoxin (Mala s 13), a member of a new pan-allergen family. *J Immunol* 2007;178:389–396.
- [13] Schmid-Grendelmeier P, Flückiger S, Disch R, Trautmann A, Wüthrich B, Blaser K, Scheynius A, Cramer R. IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. *J Allergy Clin Immunol* 2005;115:1068–1075.
- [14] Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G₄: An odd antibody. *Clin Exp Allergy* 2009;39:469–477.
- [15] Gondo A, Saeki N, Tokuda Y. IgG₄ antibodies in patient with atopic dermatitis. *Br J Dermatol* 1987;117:301–310.
- [16] Noh G, Ahn HS, Cho NY, Lee S, Oh JW. The clinical significance of food specific IgE/IgG₄ in food specific atopic dermatitis. *Pediatr Allergy Immunol* 2007;18:63–70.
- [17] Merrett J, Barnetson RS, Burr ML, Merrett TG. Total and specific IgG₄ antibody levels in atopic eczema. *Clin Exp Immunol* 1984;56:645–652.
- [18] Iwata Y, Komura K, Kodera M, Usuda T, Yokoyama Y, Hara T, Muroi E, Ogawa F, Takenaka M, Sato S. Correlation of IgE autoantibody to BP180 with a severe form of bullous pemphigoid. *Arch Dermatol* 2008;144:41–48.
- [19] Nagel A, Lang A, Engel D, Podstawa E, Hunzelmann N, de Pita O, Borradori L, Uter W, Hertl M. Clinical activity of pemphigus vulgaris relates to IgE autoantibodies against desmoglein 3. *Clin Immunol* 2010;134:320–330.
- [20] Muro Yoshinao. Antinuclear antibodies. *Autoimmunity* 2005;38:3–9.
- [21] Shinohara T, Singh DP, Fatma N. LEDGF, a survival factor, activates stress-related genes. *Prog Retin Eye Res* 2002;21:341–358.
- [22] Maertens G, Cherepanov P, Pluymers W, Busschots K, De Clercq E, Debyser Z, Engelborghs Y. LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J Biol Chem* 2003;278:33528–33539.
- [23] Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, Shinn P, Ecker JR, Bushman F. A role for LEDGF/p75 in targeting HIV DNA integration. *Nat Med* 2005;11:1287–1289.
- [24] Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, Tomita Y, Muro Y. Anti-DFS70 antibodies in 597 healthy hospital workers. *Arthritis Rheum* 2004;50:892–900.
- [25] Okamoto M, Ogawa Y, Watanabe A, Sugiura K, Shimomura Y, Aoki N, Nagasaka T, Tomita Y, Muro Y. Autoantibodies to DFS70/LEDGF are increased in alopecia areata patients. *J Autoimmun* 2004;23:257–266.
- [26] Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, Torii H, Asahina A, Onai N, Matsushima K, Tamaki K. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001;107:535–541.
- [27] Saeki H, Tamaki K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J Dermatol Sci* 2006;43:75–84.
- [28] Samochocki Z, Paulochowska E, Zabieiski S. Prognostic value of Hanifin and Rajka's feature sets in adult atopic dermatitis patients. *J Med* 2000;31:117–182.
- [29] Ogawa Y, Sugiura K, Watanabe A, Kunimatsu M, Mishima M, Tomita Y, Muro Y. Autoantigenicity of DFS70 is restricted to the conformational epitope of C-terminal alpha-helical domain. *J Autoimmun* 2004;23:221–231.
- [30] Kikuchi Y, Takai T, Ota M, Kato T, Takeda K, Mitsuishi K, Ikeda S, Okumura K, Ogawa H. Application of immunoreaction enhancer solutions to an enzyme-linked immunosorbent assay for antigen-specific IgE in mice immunized with recombinant major mite allergens or ovalbumin. *Int Arch Allergy Immunol* 2006;141:322–330.
- [31] Kadooka Y, Idota T, Gunji H, Shimatani M, Kawakami H, Dosako S, Samori T. A method for measuring specific IgE in sera by direct ELISA without interference by IgG competition or IgG autoantibodies to IgE. *Int Arch Allergy Immunol* 2000;122:264–269.
- [32] Lehrer SB, Reish R, Fernandes J, Gaudry P, Dai G, Reese G. Enhancement of murine IgE antibody detection by IgG removal. *J Immunol Meth* 2004;284:1–6.
- [33] Matsubara T, Aoki N, Hino S, Okajima T, Nadano D, Matsuda T. Serum and monoclonal immunoglobulin E antibodies from NC/Nga mice with severe atopic-like dermatitis recognize auto-antigen, histone H3. *Clin Exp Allergy* 2009;39:579–590.
- [34] Fujisawa T, Nagao M, Hiraguchi Y, Katsumata H, Nishimori H, Iguchi K. Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: Elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 2009;20:633–641.
- [35] Sugiura K, Muro Y, Nishizawa Y, Okamoto M, Shinohara T, Tomita Y, Usukura J. LEDGF/DFS70, a major autoantigen of atopic dermatitis, is a component of keratohyalin granules. *J Invest Dermatol* 2007;127:75–80.
- [36] Mittermann I, Reininger R, Zimmermann M, Gangl K, Reisinger J, Aichberger KJ, Greisenegger EK, Niederberger V, Seipelt J, Bohle B, Kopp T, Akdis CA, Spitzauer S, Valent P, Valenta R. The IgE-reactive autoantigen Hom s 2 induces damage of respiratory epithelial cells and keratinocytes via

- induction of IFN-gamma. *J Invest Dermatol* 2008;128:1451–1459.
- [37] Aichberger KJ, Mittermann I, Reininger R, Seiberler S, Swoboda I, Spitzauer S. Hom s 4, an IgE-reactive autoantigen belonging to a new subfamily of calcium-binding proteins, can induce Th cell type 1-mediated autoreactivity. *J Immunol* 2005;175:1286–1294.
- [38] Valenta R, Mittermann I, Werfel T, Garn H, Renz H. Linking allergy to autoimmune disease. *Trends Immunol* 2009;30:109–116.