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High-avidity IgG Autoantibodies against DFS70/LEDGF in Atopic Dermatitis

Kanako Watanabe, Yoshinao Muro*, Kazumitsu Sugiura and Masashi Akiyama

Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550, Japan

Abstract

Objective: Dense fine speckles 70 kDa protein (DFS70)/lens epithelium-derived growth factor (LEDGF) autoantibodies have been reported to be present in serum samples from patients with atopic dermatitis (AD) as well as from healthy individuals (HI). We previously revealed that IgE- and IgG4-anti-DFS70 autoantibodies were found in patients with AD and that their presence was associated with high levels of thymus-and activation-regulated chemokine. Our aim was to determine the avidity of IgG-anti-DFS70 antibodies in patients with AD relative to that avidity in HI.

Patients and methods: This study was undertaken to measure the avidity of IgG-anti-DFS70 autoantibodies in AD and HI groups, in comparison with the major recall antigen diphtheria toxoid (DT) and to evaluate the relevance of IgG-anti-DFS70 autoantibodies to the disease. We measured the avidity of IgG-anti-DFS70 autoantibodies and anti-DT antibodies in sera that were positive for IgG-anti-DFS70 autoantibodies, obtained from 20 AD patients and 20 HI using enzyme-linked immunosorbent assay.

Results: The avidity of anti-DFS70 autoantibodies was significantly higher (p<0.01) in the AD patients than in the HI, whereas there was no difference in the avidity of anti-DT between the two groups. There was also no positive correlation between the avidity and the titer of anti-DFS70 in the both groups. In contrast, positive correlation was shown between the serum levels and the avidities of anti-DT.

Conclusion: Our findings indicate that AD patients might have significantly high-avidity IgG-anti-DFS70 autoantibodies and that this avidity might be a novel serological marker for a certain AD subpopulation.

Keywords: Atopic dermatitis (AD); Autoantibody; Avidity; Dense fine speckles 70 kDa (DFS70); Enzyme-linked immunosorbent assay (ELISA); Lens epithelium-derived growth factor (LEDGF)

Introduction

Dense fine speckles 70 kDa protein (DFS70)/lens epithelium-derived growth factor (LEDGF) is a nuclear antigen with a characteristic pattern in immunofluorescence assay that was originally identified from the serum of a patient with interstitial cystitis [1]. It was also identified as a growth factor that stimulates cell growth and activates the expression of heat shock and stress-related genes [2], and was shown to interact with HIV-1 integrase and to help target this protein to chromatin [3,4]. Successive studies showed IgG autoantibodies against DFS70 to be associated with various disease conditions [5], including atopic dermatitis (AD) [6], although the actual involvement of these autoantibodies in these diseases has not been confirmed. Recently, we showed that IgE- and IgG4-anti-DFS70 autoantibodies are found in a certain subpopulation of AD patients and that the serum levels of thymus and activation-regulated chemokine (TARC), which reflect the severity of AD [7,8], were significantly higher in the groups that were positive for either autoantibody [9].

IgG-anti-DFS70 is also known to be present in sera from healthy individuals (HI) [10], and it has been proposed that they are a natural autoantibody [11]. In the present study, we established an enzyme-linked immunosorbent assay (ELISA) that measures the avidity of anti-DFS70, in order to test the hypothesis that IgG-anti-DFS70 have higher avidity in AD patients than in HI.

Patients and Methods

All sera used in this study were previously identified as IgG-DFS70-positive by Western blotting with DFS70 recombinant protein and HeLa cell extract [9,10]. Sera from 20 AD patients (4 men and 16 women) were obtained from the Department of Dermatology, Nagoya University Hospital. The patients fulfilled the criteria of Hanifin and Rajka [12]. Sera from 20 HI (2 men and 18 women) were obtained from healthy volunteers. The ages ranged from 17-45 (mean age: 25 ± 8) for the AD group and 7-60 (mean age: 28 ± 11) for the HI group (p=0.214). 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.

ELISAs for the avidity measurement of anti-DFS70 autoantibodies and anti-diphtheria toxoid antibodies

The ELISAs were performed as previously described [13-16], with modifications. Microtiter wells (Medisorp; Nunc, Roskilde, Denmark) were coated with recombinant DFS70 [9] or diphtheria toxoid (DT; List Biological Laboratories, Inc., Campbell, CA) in PBS (1 and 2 μg/ml, respectively). Uncoated wells were used to measure the background levels for each sample. After blocking with 5% bovine serum albumin in PBS containing 0.05% Tween 20 (TPBS), serum samples diluted to 1:100 in TPBS were applied to two wells with and without the antigens and incubated for 1 h at room temperature (RT). Plates were washed 3 times with TPBS; for the avidity assays, low-avidity antibodies were

***Corresponding author:** Yoshinao Muro, MD, PhD, Division of Connective Tissue Disease & 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

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stripped by incubating the wells with 100 µl of TPBS containing various concentrations of urea (0, 2, 4, 6, 8 M) for 0.5 h at RT. After 5 washes, horseradish-peroxidase-conjugated anti-human IgG (Dako, Glostrup, Denmark) diluted to 1:10,000 in TPBS was added to the wells. After incubation for 0.5 h at RT, the wells were washed, incubated with Ultra TMB (Pierce, Rockford, IL) as the substrate and measured for optical density (OD) at 450 nm. All sera were analyzed in duplicate in the same ELISA run, and the OD of the uncoated well was subtracted from the OD of the coated well for each sample. Avidity of antibodies was measured by the following formula: 100×corrected OD with urea treatment/corrected OD without urea treatment.

Measuring serum TARC concentrations

Commercial kits were used to measure the TARC concentration (R&D Systems, Inc. Mineapolis, NE, USA) following the manufacturer's protocol [9].

Statistical analysis

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL). Differences were analyzed with Mann-Whitney U-test, and correlations between two parameters were analyzed by Spearman's correlation coefficients. A P-value of less than 0.05 was considered statistically significant.

Results

Comparison of antibody avidity in atopic dermatitis patients and healthy individuals

First, we performed ELISAs using 20 sera (10 from AD and 10 from HI) under treatments with 2 M urea, but the serum levels of anti-DFS70 were not reduced to less than 50% in all 20 samples. Then, we tried 4 M urea treatment using the same 20 sera. The avidity of anti-DFS70 in AD turned out to be higher than in HI, however, avidity of less than 50% was observed in only 6 subjects (2 from AD and 4 from HI). Consequently, we measured the avidity of anti-DFS70 in the 40 serum samples under 6 M and 8 M urea treatments, and the avidity of anti-DFS70 in most of the sera was shown to be less than 50% (14 in AD and 19 in HI under 6 M urea treatment, and 17 in AD and 19 in HI under 8 M urea treatment). 6 M and 8 M were considered to be the ideal urea concentrations for this assay. The mean avidity ± SD (%) of anti-DFS70 in AD and HI under each urea treatment is summarized in Table 1. The avidities of anti-DFS70 in AD under 4 M, 6 M and 8 M urea treatments were significantly higher than in HI.

We also measured the avidity of anti-DT under 6 M and 8 M urea treatments to compare the avidity of anti-DFS70 with the avidity of antibodies against a major recall antigen. The mean avidity of anti-DFS70 of the 40 subjects under the 6 M urea treatment was lower than that of anti-DT under the same treatment (37.0 ± 11.8% vs. 63.9 ± 17.1%, $p<0.01$, Figures 1A and 1C). The mean avidity of anti-DFS70 of the 40 subjects under the 8 M urea treatment was also lower than that of anti-DT (30.3 ± 10.0% vs. 49.8 ± 14.6%, $p<0.01$, Figures 1B and 1D). Excluding children under age 18 (two 17-year-olds in the AD group and one 7-year-old in the HI group) did not affect these results.

Urea concentration	2 M	4 M	6 M	8 M
Atopic dermatitis	70.6 ± 7.8 (n=10)	61.8 ± 13.0 (n=10)	40.7 ± 11.3 (n=20)	33.7 ± 9.5 (n=20)
Healthy individuals	70.4 ± 9.9 (n=10)	49.9 ± 6.8 (n=10)	33.5 ± 9.7 (n=20)	27.9 ± 8.9 (n=20)
***p*-Value**	0.42	0.035*	<0.01*	0.022*

Avidities are shown as the mean ± standard deviation (%).
*The avidity of anti-DFS70 is significantly higher in atopic dermatitis than in healthy individuals.

Table 1: Avidities of anti-DFS70 antibodies in atopic dermatitis and healthy individuals under urea treatment of various concentrations.

Correlation of the titers and the avidities of the antibodies

We used scatter plots to compare the avidities of anti-DFS70 and their serum levels, which were determined by OD, in the AD and the HI groups (Figure 2). The avidities were independent of their serum levels in both groups when the 6 M urea solution was used in the ELISA (Figure 2A). Figure 2B shows that there was also no correlation between the avidity and the titer of anti-DFS70 in the AD group when the 8 M urea solution was used in the assay. There was a negative correlation between them in HI (r=-0.54, $p=0.014$). In contrast, positive correlation was shown between the serum levels and the avidities of anti-DT (Figures 2C and 2D).

The serum levels of anti-DFS70 were significantly lower in HI than AD ($p=0.029$, Figure 3A), whereas there was no difference in the titer of anti-DT between HI and AD (Figure 3B). We were concerned that this low titer of anti-DFS70 in HI might affect the analysis of the difference in the avidity between the two groups. However in HI, the titer and the avidity had no correlation under the 6 M urea treatment and had negative correlation under the 8 M urea treatment. Therefore we considered that low avidity of anti-DFS70 in HI was not due to the low titer.

We also examined the correlation between serum TARC levels and avidity of antibodies, and serum TARC levels and titers of antibodies in AD patients. We were unable to find any statistically significant association between them ($p=0.129$ in the experiment with 6 M urea treatment and $p=0.130$ in the experiment with 8 M urea treatment, data not shown). The titers of anti-DFS70 did not correlate with the TARC levels, either ($p=0.286$, data not shown).

Discussion

Antibody avidities are used widely for discrimination between the acute and chronic phases of infectious diseases [13,14]. High-avidity autoantibodies against double-strand DNA are associated with renal damage in systemic lupus erythematosus [15], and high-affinity immune responses to insulin are associated with high risk for developing type 1 diabetes [16,17]. In addition, Xu et al. recently showed that natural autoantibodies against myeloperoxidase (MPO) in normal human plasma had lower avidity than anti-neutrophil cytoplasm antibodies in patients with vasculitis [18].

The clinical importance of avidity of autoantibodies in allergic diseases has not been investigated. To measure the avidity of anti-DFS70 in AD, we established an ELISA using urea as a denaturing agent to dissociate the low-avidity antibodies after antigen-antibody interaction. Our findings support the hypothesis that anti-DFS70 antibodies have higher avidity in AD patients than in HI. However, there was no difference between AD patients and HI in the avidity of antibodies against the well-known recall antigen DT. These findings are consistent with the report that the avidity of anti-DT is significantly higher than the avidity of autoantibodies to anti-citrullinated protein antibodies (ACPAs), which are a well-known serological marker of rheumatoid arthritis (RA) [19]. We also tried the 8 M urea treatment of antigen-coated plates before the incubation of 8 different IgG-anti-DFS70-positive sera and found little decrease in the titers (data not shown). According to this result, we assume that the DFS70 antigens

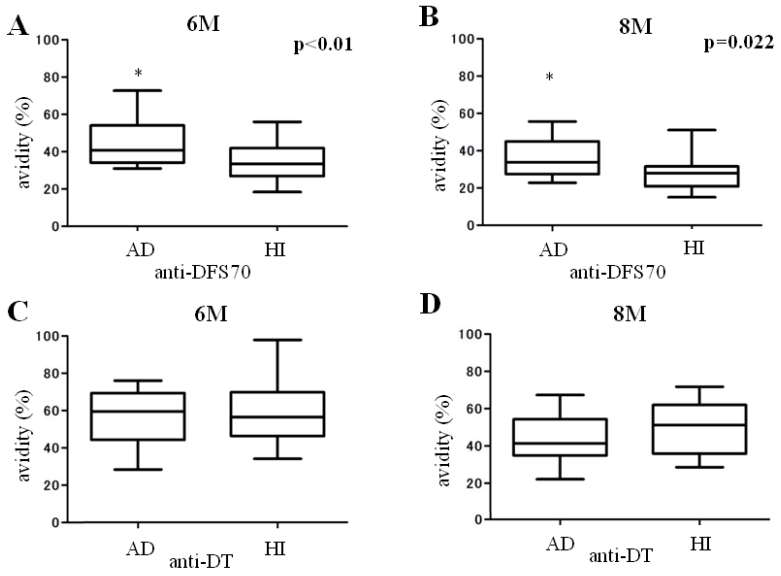

Figure 1: The avidity of anti-DFS70 autoantibodies in atopic dermatitis compared to healthy controls: Avidity of antibodies (%) was measured by the following formula: 100×corrected OD with urea treatment/corrected OD without urea treatment. The avidity of anti-DFS70 (A and B) and anti-DT (C and D) in patients with AD and healthy individuals (HI) when 6 M (A and C) and 8 M (B and D) urea treatment was performed in the ELISAs. 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 maximum and minimum values. Differences were analyzed by Mann-Whitney *U*-test, and statistically significant differences are indicated (**P*<0.05).

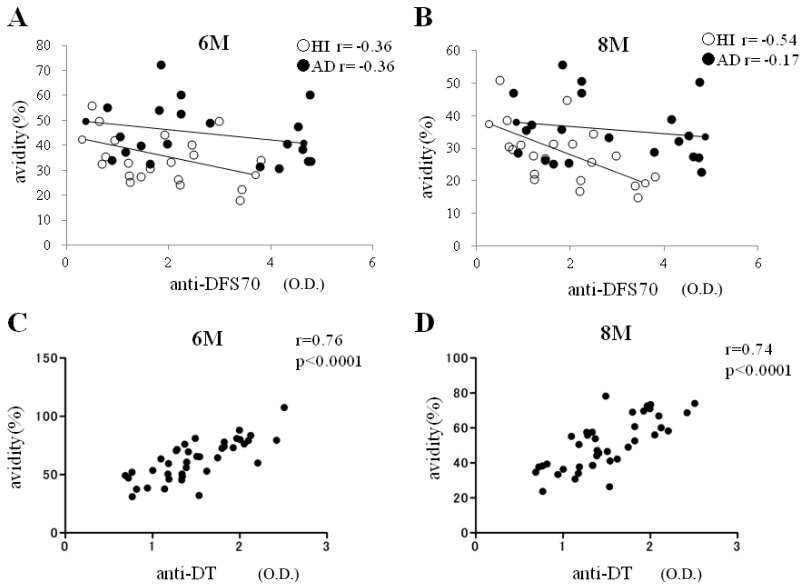

Figure 2: There is no correlation between the serum level and the avidity of anti-DFS70 in AD: The serum level and avidity of anti-DFS70 (A and B) and anti-DT (C and D) are plotted for 6 M (A and C) and 8 M (B and D) urea treatment in the ELISAs. In (A) and (B), open and filled circles represents HI and AD patients, respectively.

O.D.: Optical Density

are not stripped by urea treatment. Overall, our ELISA procedure seems appropriate.

Suwannalai et al. recently reported, regarding RA, that higher-avidity ACPAs are observed in symptomatic patients only, while low-avidity ACPAs are observed in both HI and patients with RA [20]. Another study showed MPO-ANCA as having a high affinity that correlates with disease activity, irrespective of antibody titers, in some cases of vasculitis [21]. Anti-DFS70 with higher avidity was also observed in AD, and it might be involved in onset or severity of the disease. As to the antibody titers, the serum level of anti-DFS70 was shown to be higher in AD than in HI, whereas our previous report found no difference between AD and HI [11]. We attribute this data inconsistency to the limited number of sera from patients with AD in the previous study (6 sera from AD and 37 sera from HI). Another

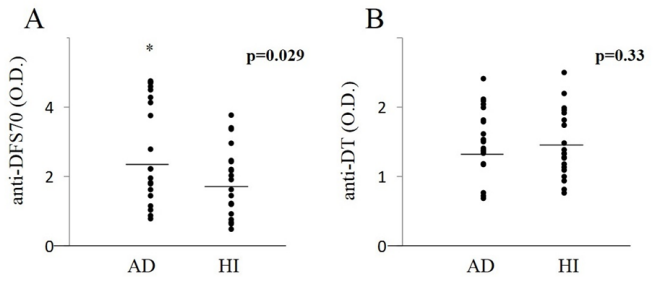

Figure 3: Antibody levels of and-DFS70 and anti-DT in the AD and HI groups: The anti-DFS70 level is significantly lower (p=0.029) in the HI. The horizontal line represents the mean. Differences were analyzed by Mann-Whitney U-test, and statistically significant differences are indicated (*p<0.05).

O.D.: Optical Density

possibility is our previous study's use of recombinant DFS70 protein produced by a bacterial expression system, which is sometimes unstable in ELISA, in contrast to the eukaryotic expression system used in this study (our unpublished observation). Here, we consider that the serum levels of anti-DFS70 in AD are possibly elevated in reflection of the disease as well as the antibody avidities.

We previously showed that DFS70 is present in epidermal cells and infiltrating monocytes in skin sections from patients with AD and from HI [9]. Although the pathological role of IgG autoantibodies against DFS70 in AD is still largely unknown, production of IgG-anti-DFS70 antibodies with high avidity might be driven by DFS70 released from damaged skin and may trigger autoimmune responses. In contrast, IgG-anti-DFS70 antibodies with low avidity in HI might have been produced by cross-reaction with exogenous antigens and might not be involved in the pathogenesis of AD.

In conclusion, our findings indicate that AD patients might have significantly high-avidity IgG-anti-DFS70 autoantibodies and that the avidity might be a novel serological marker for a certain AD subpopulation, in addition to IgE- and IgG4-anti-DFS70 autoantibodies. Whether our findings might have greater clinical importance, such as in predicting the onset or sudden exacerbation of AD, remains to be evaluated by analyzing larger populations and sequential serum samples.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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