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IgE auto-antibodies to human fatty acid-binding proteins in atopic dermatitis patients

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ABSTRACT

IgE auto-antibodies have been described in some allergic diseases, especially in atopic dermatitis (AD). Clinical implications of these antibodies in allergies are not fully understood, but IgE sensitization to self-proteins may represent an important mechanism involved in the maintenance of severe chronic forms. The aim of this study was to explore the presence of the auto reactive IgE response to endogenous fatty acid-binding proteins (FABPs) from the heart (FABP3) and adipocytes (FABP4), two proteins homologous to group 13 of house dust mite (HDM) allergens, in patients with AD. Seventy-one patients with AD and sixty individuals without AD were recruited. After complete clinical evaluation and informed consent, skin prick test (SPT) and serum IgE, IgG and IgG4 levels were determined using extracts of *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, the recombinants FABP3 and FABP4, and the allergen Blo t 13. The frequency of positive IgE levels to FABP3 and FABP4 in AD group was 61 and 52%, respectively. Patients with positive IgE auto-reactivity to human FABPs were also sensitized to Blo t 13. IgG and IgG4 levels to FABP3 and FABP4 were detected in all AD patients and no-AD subjects, with the antibody levels in no-AD subjects being significantly higher than those in AD patients. In conclusion, patients with AD, who had positive SPT and serum IgE levels to Blo t 13 allergen showed serum IgE and IgG antibodies against FABP3 and FABP4. The molecular mimicry with HDM allergens could explain this finding, which could contribute to the pathomechanism of AD.

KEYWORDS: atopy, auto-reactivity, dermatitis, eczema, FABP, house dust mites.

INTRODUCTION

Some patients with severe and chronic atopic diseases mount an IgE auto-antibody response against self-proteins [1, 2]. In 1991, Valenta *et al.* demonstrated that allergens like plant profilins share IgE epitopes with human proteins [2]. Schmid *et al.* found that human manganese superoxide dismutase (MnSOD) was able to induce *in vitro* T-cell reactivity and eczematous reactions in atopic dermatitis (AD) patients sensitized to fungal MnSOD, probably due to cross reactivity [3]. Several auto-antigens with IgE reactivity have been described, such as the squamous cell carcinoma antigen recognized by T cells [1], cytokeratin type II [4], and the alpha chain of the nascent polypeptide-associated complex (a-NAC) [5]. All of these antigens have been described in patients with AD, which is a chronic pruritic skin condition frequently associated with other allergic disorders, such as allergic rhinitis, conjunctivitis, and asthma [6, 7]. Approximately 5% to 20% of children and 2% to 10% of adults are affected by AD [8, 9]. The causes of this disease are not completely understood, but it is associated with

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elevated total and specific IgE and accompanied by a mixture of Th1 and Th2 cytokine production; therefore, different hypotheses concerning pathogenic mechanisms of AD have been proposed [10-12].

The clinical implications of auto-IgE and auto-antigens in allergic and non-allergic disorders are not fully understood [13, 14], but IgE reactivity to self-proteins may represent an important mechanism involved in the maintenance of chronic inflammation [6]. It is not known why this IgE reaction to self-proteins occurs. One hypothesis is that after chronic recognition and production of IgE to environmental allergens, these immunoglobulins could recognize similar epitopes present in human proteins.

HDMs are the main source of allergens causing IgE sensitization and allergic diseases in worldwide regions [9, 15, 16] and Group 13 of HDM allergens belongs to the family of FABPs [17-19]. FABPs from humans and HDM are structurally and functionally related [20-22]; therefore it is possible that they could have IgE cross-reactivity. Human FABPs are diverse proteins with preponderant localization in different tissues [23]. High serum levels of FABP3 have been associated with the risk of heart attack, and others such as FABP2, FABP4, and FABP5 have been associated with diabetes mellitus, asthma, and psoriasis, suggesting a role of FABPs in the inflammatory response [24-28].

Taking into consideration that self-reactivity to human proteins has been observed in patients with dermatitis, especially severe cases, this study aimed to explore the presence of the self-reactive response to the endogenous proteins FABP3 and FABP4 in AD.

MATERIAL AND METHODS

Population and study design

The population of this study were inhabitants of Medellin, Colombia, a tropical city with an average annual temperature of 24-28 °C and a relative humidity of 66%. Most of the study participants shared poor environmental conditions and their genetic backgrounds resulted from racial admixture between Native Americans, Spaniards, and, at a lower rate (< 10.9%), those of African ancestry [29].

We selected patients with AD from a community-based cohort for a prospective follow-up and collection of epidemiological data and biological samples (TECCEMA cohort: Tropical Environmental Control for Chronic Eczema and Molecular Assessment) [30, 31]. Under the hypothesis that patients with AD were more likely to have autoantibodies, we created two groups: The "AD group" and the "no-AD group". AD was diagnosed using "Hanifin and Rajka" criteria [32-34] by a dermatologist or an allergist. The "AD group" involved patients over 3 years of age with a clinical history of AD for more than 2 years. The severity was ranked according to SCORAD (Scoring Atopic Dermatitis) [32-34] score as severe (over 40 points), moderate (16 to 40), or mild (≤ 15). Allergic comorbidities and environmental stressors were registered. Patients that were using immunosuppressive drugs in the last two months before recruitment were not included. The no-AD group involved subjects without a clinical history of AD. The use of topical drugs was permitted according to the clinical evolution of each patient. Blood samples were taken from each patient and stored at -20 °C until analysis. The Ethics Committee of the University of Antioquia (Medellin, Colombia) approved the study (Record number BE-IIM18, 01 December 2012); a full verbal explanation of the investigation was given and written informed consent was obtained from all participants.

Recombinant Blo t 13, FABP3 and FABP 4

Escherichia coli strains BL21 (DE3) transformed with the expression plasmid pET45b+/Blo t 13.0101, pET45b+/FABP3 or pET45b+/FABP4 were grown in Luria–Bertani medium containing 100 mg/L ampicillin at 37 °C until a 0.4 optical density (OD) at 600 nm. Protein expression was induced by the addition of 1 mM isopropyl-β-D–thio-galacto-pyranoside to the medium and further incubation for 4 hours. Cells were then harvested by centrifugation at 6500 rpm. at 4 °C for 15 min. The cell pellets were solubilized in 8 mol/L urea, 0.1 mol/L NaH_2PO_4 and 0.01 mol/L Tris-HCl at pH 8.0, and the lysate was pulsed by ultrasound several times on ice and then incubated by continuous rotation for 3 hours. Insoluble material was removed by centrifugation. The supernatant

containing the recombinant was applied to nickel–nitrilotriacetic acid–Agarose (Qiagen) and purified in hybrid conditions, as indicated by the supplier. Fractions containing eluted proteins were pooled and dialyzed against 50 mM NaH_2PO_4 and 0.5 M NaCl (pH 8.0). The protein concentration was determined by Bradford assay (Bio-Rad Laboratories). The purity and integrity of all proteins were assessed by Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) and subsequent staining with Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories).

Skin prick test

The SPT was evaluated according to international recommendations [35, 36], using a panel of allergen extracts provided by Inmunotek, SL. (Madrid, Spain): HDM extracts (*B. tropicalis, D. pteronyssinus*, and *D. farinae*), pet's dander (Cat and dog), fungus extracts (*Aspergillus fumigatus, Cladosporium herbarum, Alternaria alternata, Fusarium solani* and *Candida albicans*), insect extracts (Cockroach, mosquito and ant), *Cynodon dactylon*, egg and milk.

For SPT, using the recombinants Blo t 13, FABP3 and FABP4, protein solutions treated with LPS-Free Toxin eraser Kit (Genscript Cat. No. L00338) were used at 25 ng/µL of protein, 50% glycerol, 0.4% phenol and filtered using 0.22 µm filter. This protein concentration was chosen after a dose titration with different concentrations was done in a few patients.

IgG depletion in sera from patients with atopic dermatitis

To avoid the influence of IgG antibodies on IgE reactivity to Blo t 13, FABP3 and FABP4, an IgG antibody depletion assay was performed [37]. G protein coupled to agarose beads (Sigma-P3296, Protein G Sepharose®) was used, following the manufacturer's recommendations. One mL of G protein was re-suspended in 25 mL of distilled water. It was mixed for 30 min at 20 r.p.m at room temperature. G protein was then allowed to precipitate by gravity. The supernatant was discarded and resuspended in 5 ml of 1X PBS.

To deplete the IgG, 500 µL of 1:5 sera was prepared in PBS 1X and incubated with 80 µL of previously prepared G protein. The mixture was mixed for 1 hour at RT. Sera were then centrifuged at 8.000 r.p.m for 5 minutes. The supernatant was rescued and IgE and IgG enzyme-linked immunosorbent assay (ELISA) assays were performed against Blo t 13, FABP4 and FABP3.

Determination of total and specific IgE

Total IgE levels in serum samples were determined using a RIDASCREEN® Total IgE kit (r-biopharm, Cat Nº A0141), following the manufacturer's conditions. For specific IgE, microtiter plate wells ((Immulon-4 Dynatech, Chantilly, VA, USA) were incubated overnight (ON) with 0.5 µg/100 µL of Blo t 13, FABP3, or FABP4. After washing with phosphate-buffered saline (PBS-0.1% Tween 20 (PBS-T), the wells were blocked with 100 µL of blocking buffer (PBS-T, 1% BSA, 0.02% sodium azide) for 3 hours at RT (room temperature) in a wet chamber. After washing again, 100 µL of human sera (diluted 1:5 in PBS-3% BSA) was added to each well with further ON incubation at RT. The wells were then washed several times and incubated for 2 hours at RT with 100 µL of alkaline phosphatase-conjugated anti-IgE (Sigma A3525) diluted 1: 500 in buffer. After a final washing, the wells were incubated with 100 µL of substrate p-nitrophenyl phosphate diluted in 10% diethanolamine and 0.5 mM $MgCl_2$ at RT for 30 min. The OD was measured in an ELISA reader (Spectra MAX 250, Molecular Device, Sunnyvale, CA, USA) at 405 nm. All samples were assayed in duplicate.

Determination of specific IgG and IgG4

Microtiter plate wells (Immulon-4 Dynatech, Chantilly, VA, USA) were incubated with 0.5 µg/100 µL of Blo t 13, FABP3, or FABP4 ON. After washing with PBS-T, the wells were incubated with 100 µL of blocking buffer for 3 hours at RT in a wet chamber. After washing again, 100 µL of serum adsorbed with *E. coli* lysate and diluted 1:100 in PBS-3% BSA was added to each well with further ON incubation at RT. Plate wells were washed several times and incubated with 100 µL of alkaline phosphatase-conjugate anti human IgG (Sigma A3187) diluted 1:10.000 in 0.05 M buffer Tris at pH 8.0,

1% BSA, 1 mM $MgCl_2$, and 0.02% sodium azide for 2 hours at RT. For specific IgG4 measurement, the serum samples adsorbed with *E. coli* lysate were diluted 1:50 in PBS-3% BSA, added to wells and incubated ON. The secondary antibody used in this case was alkaline phosphatase-conjugate anti human IgG4 (Pharmigen. Cat N° 555880) diluted 1:1000 in buffer Tris. After the final washing, the wells were incubated with 100 µL of substrate p-nitrophenyl phosphate diluted in 10% diethanolamine and 0.5 mM $MgCl_2$ at RT for 30 min. The OD was measured as described above. The adsorption of sera with *E. coli* was performed to avoid cross reactivity with antigens from *E. coli* BL21 (DE3 Start), used to express the recombinants.

Statistical analysis

All analyses were performed using IBM® SPSS® Statistics version 21.0 (Chicago, IL, USA). Descriptive information about demographic continuous variables is reported as the mean value and its standard deviation. Differences between proportions in dichotomous variables such as the presence of sensitization or not were analyzed by the Pearson chi-squared test and logistic regression. Univariate and multivariate binary logistic regression were used to analyze the interactions between sensitizations and SCORAD. Gender and age were included in the multivariate models as covariates. Correlation between antibody levels and/or SCORAD score was expressed as Spearman (r) coefficients. Due to the exploratory character of the study, the *p*-value was used to express the difference between the groups [38].

RESULTS

Subject characteristics

The AD group consisted of 71 patients (37 females) and the no-AD group involved 60 subjects (Table 1). According to the SCORAD, most patients in the AD group had moderate or severe dermatitis. Patients in the AD group had higher total IgE levels than subjects in the no-AD group (mean 531 vs. 2.81 UI/mL, $p < 0.0001$) (Figure 1A), and there was a correlation between the total IgE and SCORAD severity ($r = 0.506$, $p = 0.02$) in the AD group.

IgE levels of *B. tropicalis* and *D. pteronyssinus* were higher than those in the no-AD group

Table 1. Sociodemographic and clinical characteristics of AD group and no-AD group. AD: Atopic dermatitis, SCORAD: SCORing Atopic Dermatitis.

Characteristics	AD group (n = 71)	No-AD group (n = 60)
Female	37 (52.1%)	22 (36.6%)
Age	13 years (3 to 41)	14 years (8 to 62)
Allergic diseases	59 (83%)	4 (6.6%)
Asthma	31 (43%)	0
Rhinitis	54 (76%)	4 (6.6%)
Conjunctivitis	44(61%)	0
Atopy (Prick test)	70 (98%)	9 (47%)
SCORAD	30 (6 to 82)	0
Mild (≤ 15 points)	10 (14.0%)	N/A
Moderate (16 to 39)	44 (61.9%)	N/A
Severe (≥ 40)	17 (23.9%)	N/A

(p < 0.001) (Figure 1B). In the AD group, the frequency of positive IgE levels to *B. tropicalis* was 83% and to *D. pteronyssinus*, it was 87%, and in the no-AD group, only 8 subjects (13%) had positive IgE levels to HDM (p < 0.001).

IgE reactivity to FABP3 and FABP4 was observed in the AD group

IgE levels to Blo t 13 in the AD group were higher than those in the no-AD group (p < 0.001) (Figure 1C). The frequency of positive IgE levels to FABP3 and FABP4 was 61 and 52% in the AD group, respectively, and no participants had positive IgE for Blo t 13 or human FABPs in the no-AD group. In the AD group, 46 (63%) patients had serum positive IgE levels to Blo t 13; this reactivity was accompanied by positive IgE to FABP3 in 10 patients and positive IgE to FABP4 in six patients. There was a low correlation between SCORAD and IgE levels to FABP3 (r = 0.347, p = 0.003), but there was no correlation between SCORAD and IgE levels to *B. tropicalis* (r = 0.147, p = 0.12), Blo t 13 (r = 0.158, p = 0.14), or FABP4 (r = 0.242, p = 0.13).

IgG4 levels to Blo t 13 correlated with IgG4 levels to FABP3 and FABP4

Serum levels of IgG and IgG4 to human FABPs in the no-AD group were significantly higher than those in the AD group (Figure 2A). There was no difference in IgG levels to Blo t 13 between groups. IgG4 levels to Blo t 13 were significantly higher in the AD group than those in the no-AD group (Figure 2B). There were positive correlations of IgG4 levels to Blo t 13, FABP3 and FABP4 in the AD group (Figure 3). A correlation was also found in the no-AD group (data not shown).

FABP3 and FABP4 induced positive SPT

SPTs with recombinant FABPs were performed in 71 AD patients and 36 non-allergic subjects. Positive SPT results for FABP3 and FABP4 were observed in 11 (15%) of the AD patients, which were accompanied by positive serum IgE levels to FABP3 and FABP4 in ten and eleven cases, respectively (Table 2). SPT results were positive in 2/36 (5.5%) no-AD subjects. Figure 4 is an example of a positive SPT to human FABPs observed in one AD patient.

In the AD group, a significantly higher frequency of positive SPT results for human FABPs was obtained in patients with SCORAD > 40 compared to those with SCORAD < 40; for FABP3 (6/19 SCORAD > 40 vs. 5/52 SCORAD < 40, p = 0.03) and for FABP4 (5/19 SCORAD > 40 vs. 6/52 SCORAD < 40, p = 0.04).

DISCUSSION

HDMs are a common source of allergens, including Blo t 13 [17-19, 21, 39]. In the present study, we identified FABP3 and FABP4 as targets for IgE and IgG auto-reactive responses in AD patients. Yamane, Y. *et al*. [40] observed a correlation between the expression of epidermal FABP (FABP5) and the severity of skin lesions in patients with AD. However, the role of this protein as a possible auto-allergen is unknown. In a previous study with IgE-sensitized patients and allergy respiratory diseases, we identified cross-reactivity between Blo t 13 and FABPs [41], but the clinical relevance of this cross-reactivity was not explored.

We found a frequency of 61% for serum IgE reactivity to FABP3 and 52% for FABP4 in AD patients, in contrast to 0% in the AD group. Two reflections should be considered for these findings. First, like most auto-allergens (e.g., Hom s 1, Hom s 3, Hom s 4, Hom s 5), the FABPs are intracellular proteins and exposure to these intracellular antigens could be the result of chronic inflammatory diseases, which in the context of a Th2-prone microenvironment like that in AD, would tend to produce de novo IgE antibodies; second, for auto-antigens that share structural and functional similarities with environmental allergens, the cross-reactivity might also boost the production of auto IgE [2, 42]. These hypotheses could even complement each other.

It has been postulated that molecular mimicry between allergens and self-proteins may turn an allergy induced by an environmental allergen into an auto-allergy [3, 5, 42]. Although the origin of this kind of auto-reactive response in AD is not clear, we cannot discard that exposure to environmental allergens of group 13 of HDM allergens, especially Blo t 13, could be a trigger for auto-reactivity in this population.

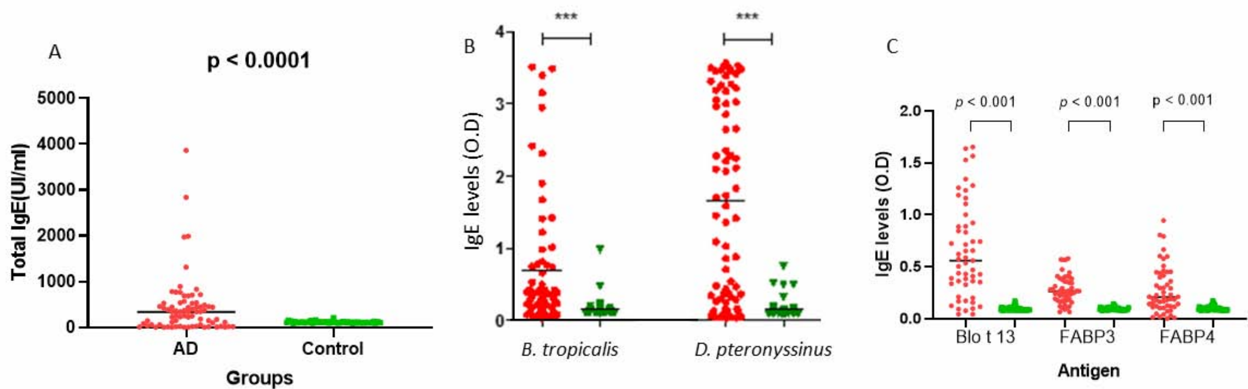

Figure 1. Total and sIgE. (A) Total IgE levels. AD group showed total IgE levels significantly higher than those from no-AD group. **(B)** Specific IgE to HDM extracts, specific IgE levels in AD were significantly higher than those from no-AD. **(C)** Specific IgE levels to different antigens in AD and no-AD group. Red circles represent AD patients and green triangles no-AD subjects. ***p < 0.001.

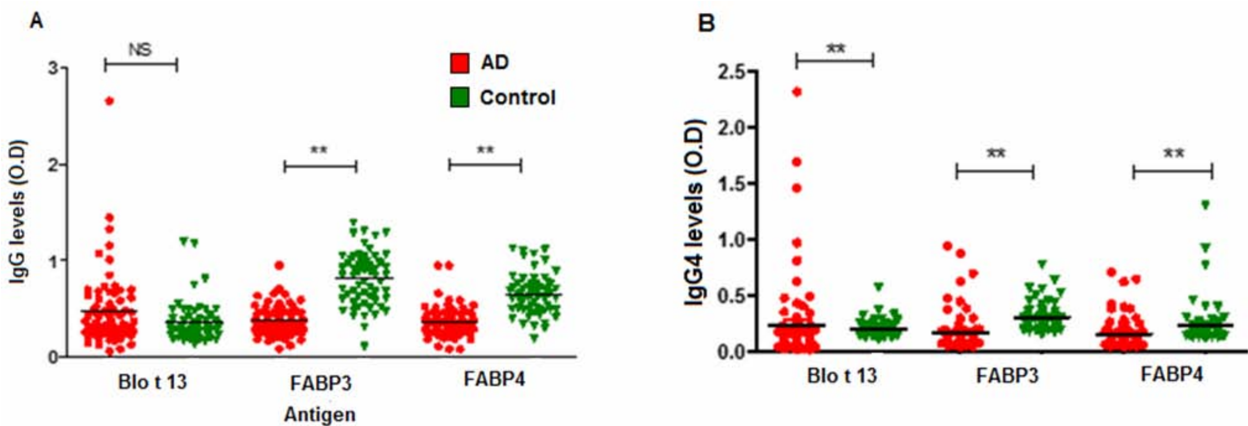

Figure 2. IgG and IgG4 levels to human FABP. (A) IgG levels to human FABPs were higher in no-AD group than those in AD group. **(B)** IgG4 levels to human FABPs were higher in no-AD than AD group. **(p < 0.05). Red circles represent AD patients and green triangles no-AD subjects.

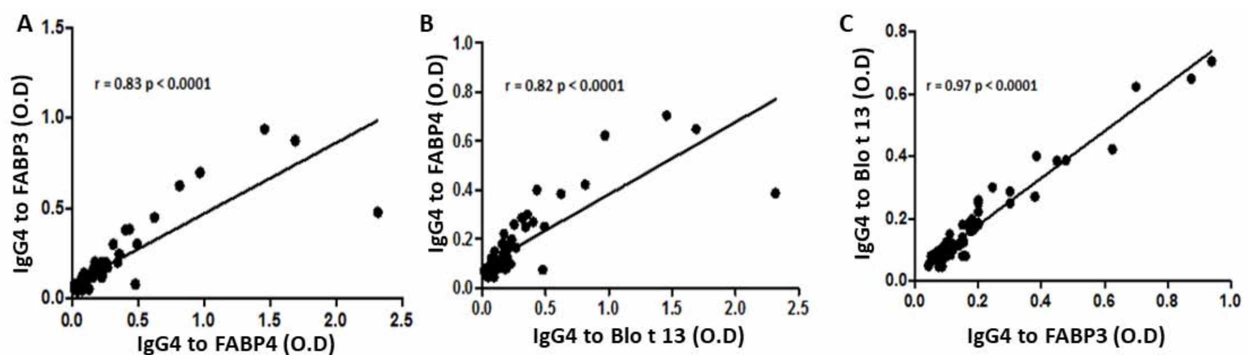

Figure 3. Correlation between IgG4 levels to FABPs. **(A)** FABP3 vs. FABP4. **(B)** FABP4 vs. Blo t 13. **(C)** Blo t 13 vs. FABP3. Statistically significant positive correlation is observed.

Table 2. Relation of subjects with positive SPT (wheal over 3 mm) to at least one of the human FABP. AD, Atopic Dermatitis; A, Asthma; C, Conjunctivitis; F, Female; R, Rhinitis; m, male; mm, millimeter; #, Number of subjects according to serum registration; sIgE, specific IgE.

Subject #	Group	Age	Gender	Dx	SCORAD	Blo t 13	FABP3	FABP4	Blo t 13	FABP3	FABP4
						Wheal (mm)			sIgE		
6	AD	8	F	AD	22	4	3	4	0.248	0.536	0.744
16	AD	13	M	AD / R	50	0	5	5	0.026	0.014	0.3
22	AD	17	M	AD / A / R / C	42	7	4	3	3.464	0.138	0.128
23	AD	12	F	AD / A / R / C	50	4	4	0	0.142	0.136	0.108
24	AD	7	F	AD / A / R / C	40	0	3	0	0.128	0.217	0.118
32	AD	32	F	AD / A / R / C	18	6	4	0	1.185	0.244	0.044
72	AD	17	F	AD / R / C	53	9	6	5	0.172	0.549	0.665
73	AD	21	M	AD / C	82	0	9	5	0.109	0.446	0.203
75	AD	8	F	AD	34	0	3	3	0.087	0.121	0.125
76	AD	12	F	AD / R / C	36	0	4	4	0.051	0.137	0.141
82	AD	11	M	AD	33	4	4	4	0.08	0.18	0.218
10	AD	6	M	AD	78	6	0	4	0.036	0.104	0.131
47	AD	6	M	AD	29	4	0	3	0.03	0.116	0.123
70	AD	15	F	AD / R / C	26	8	0	3	3.509	0.152	0.165
17	Control	56	F	Healthy	not apply	1	2	2	0.007	0.098	0.095
14	Control	32	F	Healthy	not apply	1	1	1	0.015	0.108	0.099
19	Control	50	F	Healthy	not apply	2	2	2	0.015	0.097	0.098

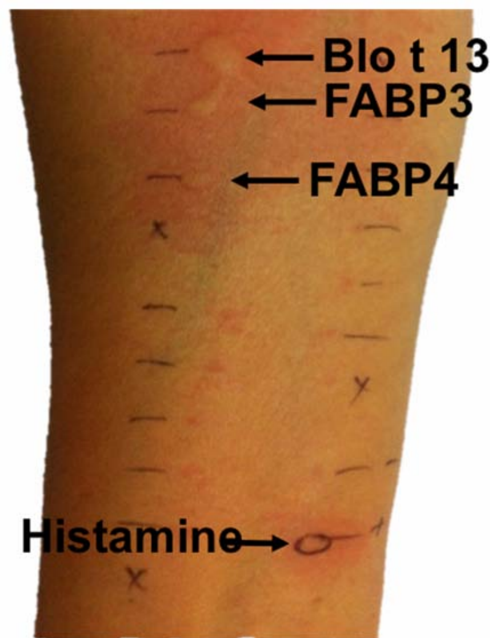

Figure 4. Skin prick test. Image from a patient with AD showing positive SPT to Blo t 13 (9 mm), FABP3 (6 mm) and FABP4 (5 mm).

The correlation between antibody levels to Blo t 13 and human FABPs supports the hypothesis that reactivity to FABP4 and FABP3 in the AD patients is probably due to molecular mimicry, in which antibodies induced by Blo t 13 allergen react against the homologous FABPs. In fact, a murine model of allergic asthma demonstrated that sensitization with a foreign antigen mimicking self can induce an allergic immune response of a mixed Th1 and Th2 profile that is associated with autoreactivity [43]. Taking into consideration that Blo t 13 belongs to FABPs [17, 20] and that sensitization to *B. tropicalis* is high in the study population, we have the hypothesis that at least in part, the auto-reactivity to human FABP in AD group could be the result of molecular mimicry between Blo t 13 and homologous self-proteins. However, the levels of IgG and IgG4 for human FABPs were higher in the non-AD group and there was no correlation with IgG or IgG4 levels to Blo t 13, suggesting that in this group, the production of IgGs is secondary to an immunoregulation mechanism rather than secondary to the recognition of exogenous proteins with cross-reactivity.

Detailed characterization of auto-antigens may lead to more beneficial diagnostic and treatment approaches. The presence of some auto-antigens like Hom s 1, Hom s 2, and Hom s 3 has been proposed as an indicator of poor prognosis and severity in dermatitis [44, 45]. Nevertheless, taking into consideration that human FABPs, like many of those auto-antigens, are cytoplasmic proteins, it is not clear how these proteins could participate in cutaneous inflammation. Some studies suggest that the chronicity of the skin disease may facilitate the release of intracellular antigens from epithelial cells and perpetuate the inflammatory response. It has been reported that FABP4 and FABP5 may contribute to inflammatory responses in the airway and skin [28, 40, 46]. In this context, taking account of this pro-inflammatory role, it is possible that some patients with severe dermatitis may have an increased expression of human FABPs and the tissue damage would facilitate the exposure to these intracellular endogenous proteins and their recognition by self-IgE. The higher frequency of positive SPT to FABP3 and FABP4 in AD patients with SCORAD > 40 compared to those in patients with SCORAD < 40 supports a probable role of human FABPs in the severity of AD, and suggests that in severe forms of AD there is a stronger affinity of IgE to human FABPs, leading to a higher degranulation of mast cells in the skin. In addition, the AD patients with IgE auto-reactivity showed high levels of IgG and IgG4 for Blo t 13, FABP3, and FABP4. Taking into consideration that auto-IgE has been demonstrated in Th1 diseases like lupus [13], in a pilot study, we explored the presence of IgE anti-FABPs in four patients with psoriasis and three with contact dermatitis, finding that one patient in each Th1 disease had IgE against FABPs. However, the levels of IgG and IgG4 were higher than those in the AD group (data not shown).

CONCLUSION

We identified antibody auto-reactivity to FABP3 and FABP4 in some AD patients. The serum IgE reactivity to these FABPs was accompanied by the capacity to produce mast cell degranulation in the skin. Whether this auto-reactivity is caused by molecular mimicry between group 13 of HDM

allergens and human FABPs or by direct neo-sensitization to human FABPs needs further study.

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AUTHORS' CONTRIBUTIONS

JS and LP designed the study; JS and JA recruited and monitored the patients. JS and MM performed experimental analysis and wrote the original draft manuscript. LP, JS and RC; interpretation of data and founding acquisition. All authors critically revised and approved the final manuscript.

FUNDING

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the University of Antioquia (Medellin, Colombia) approved the study (Record number BE-IIM18, 01 December 2012); a full verbal explanation of the investigation was given and written informed consent was obtained from all participants. The survey was performed in accordance with the Helsinki Declaration of 1964.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

ABBREVIATIONS

AD	:	Atopic dermatitis
FABP	:	Fatty acid binding proteins
HDM	:	House dust mite
SPT	:	Skin prick test
MnSOD	:	manganese superoxide dismutase
OD	:	Optical density
SDS-PAGE	:	Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis
RT	:	room temperature

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