Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
www.all-imm.com

ORIGINAL ARTICLE

OPEN ACCESS

CD4+CD25+CD127^{lo}FOXP3+ cell in food allergy: Does it predict anaphylaxis?

Semiha Bahceci Erdem[a], Ferah Genel[b], Hikmet Tekin Nacaroglu[c]*, Sait Karaman[b], Canan Sule Unsal Karkıner[b], Murat Sürücü[b], Demet Can[b]

[a]*Çiğli Training and Research Hospital Pediatric Immunology and Allergy, Izmir Bakırçay University, Izmir, Turkey*
[b]*Department of Pediatric Allergy and Immunology, Dr Behcet Uz Children's Hospital, Izmir, Turkey*
[c]*Department of Pediatric Allergy and Immunology, Istanbul Medipol University, Istanbul, Turkey*

Received 13 November 2022; Accepted 7 February 2023
Available online 1 May 2023

KEYWORDS
FOXP3;
Treg cells;
children;
food allergy;
cow's milk protein allergy;
anaphylaxis

Abstract
Background: Food allergy (FA), hence the incidence of food anaphylaxis, is a public health problem that has increased in recent years. There are still no biomarkers for patients with FA to predict severe allergic reactions such as anaphylaxis.
Objective: There is limited information on whether regulatory T (Treg) cell levels are a biomarker that predicts clinical severity in cases presenting with FA, and which patients are at a greater risk for anaphylaxis.
Methods: A total of 70 children were included in the study: 25 who had IgE-mediated cow's milk protein allergy (CMPA) and presented with non-anaphylactic symptoms (FA/A−), 16 who had IgE-mediated CMPA and presented with anaphylaxis (FA/A+) (a total of 41 FA cases), and a control group consisting of 29 children without FA. The study was conducted by performing CD4+CD25+CD127^{lo}FOXP3+ cell flow cytometric analysis during resting at least 2 weeks after the elimination diet to FA subjects.
Results: When the FA group was compared with healthy control subjects, CD4+CD25+CD127^{lo}FOXP3+ cell rates were found to be significantly lower in the FA group ($p < 0.001$). When the FA/A− and FA/A+ groups and the control group were compared in terms of CD4+CD25+CD127^{lo}FOXP3+ cell ratios, they were significantly lower in the FA/A− and FA/A+ groups compared to the control group ($p < 0.001$).
Conclusions: Although there was no significant difference between the FA/A+ group and the FA/A− group in terms of CD4+CD25+CD127^{lo}FOXP3+ cells, our study is important, as it is the first pediatric study we know to investigate whether CD4+CD25+CD127^{lo}FOXP3+ cells in FA predict anaphylaxis.

***Corresponding author:** Hikmet Tekin Nacaroglu, Department of Pediatric Allergy and Immunology, Istanbul Medipol University, Istanbul, Turkey. *Email address*: tekin212@gmail.com

https://doi.org/10.15586/aei.v51i3.797

Introduction

The frequency of food allergy (FA), hence the frequency of food anaphylaxis, is a public health problem that has increased in recent years. Estimating the risk of a severe reaction is one of the most important knowledge gaps in the management of cases with FA.[1] Although there are many factors that affect the severity of reactions in cases with FA, the presence of biomarkers that predict severe allergic reaction will be useful for identifying high-risk patients, who are more prone to severe allergic reactions and require closer monitoring.[2]

Various factors can affect the severity of allergic reactions, such as allergen-related factors, IgE-mediated immune response-related factors, comorbid diseases, sex, genetic predisposition, co-factors, timely and effective treatment, and the host's ability to compensate for food allergens.[2-4]

In studies, prior anaphylaxis was found to be the best predictor of future anaphylaxis. However, prior anaphylaxis is not a good predictor, perhaps because it depends on various factors such as severity, level of allergen exposure, and the presence of co-factors. In addition, the absence of prior anaphylaxis does not exclude the risk of future anaphylaxis. In some individuals, any active allergic disease may increase the severity of anaphylaxis, however, there is no data indicating that it causes an increased risk of fatal or near-fatal anaphylaxis.[1] The relationship between dose/level of exposure and severity is complex and unclear. Individuals who react to lower amounts of allergens are not necessarily at higher risk of anaphylaxis. There is no solid evidence that individuals with FA who react to very low allergen levels are at higher risk of anaphylaxis.[1] It has been suggested that a lack of baked milk tolerance may be a marker of a more severe allergy to cow's milk; however, it has not been prospectively evaluated.[1,5]

Apart from these, factors related to an IgE-mediated immune response seem more likely to serve as biomarkers of the severity of allergic reactions.[2] Evidence that serum quantitative levels of allergen-specific IgE or skin prick test (SPT) wheal sizes reflect the severity of severe allergic reactions with casein-specific IgE elevation is conflicting.[1,2] Results of cellular tests such as the basophil activation test (BAT) and mast cell activation test (MAT) depend on various factors such as the amount, specificity, diversity, and avidity of IgE for food allergens.[2] In a recently conducted large study, BAT was indeed reported as the best severity biomarker identified, either alone or in combination with other biomarkers.[6] However, it was emphasized that BAT was inferior to peanut-specific IgE, Ara h 2, and even SPT in predicting any anaphylaxis (other than life-threatening anaphylaxis).[6-8]

Elevated baseline mast cell tryptase for FA does not seem to be associated with clinical severity, either.[9-11] Unfortunately, no biomarker has been found to predict a severe reaction in cases with FA.[2] There is limited information on whether Treg levels are a biomarker that predicts clinical severity in cases presenting with FA, and which patients are at greater risk for anaphylaxis. As far as we know, no study has been conducted on how Tregs change in cases presenting with FA.

In this study, we commenced on the assumption that CD4+CD25+CD127^{lo}FOXP3+ Tregs would be lower in IgE-mediated cow's milk protein allergy (CMPA) patients presenting with anaphylaxis (regardless of the severity of the anaphylaxis) (FA/A+) compared to patients presenting with non-anaphylactic symptoms (FA/A−) and the control group. We searched for the answer to the question, "Are CD4+CD25+CD127^{lo}FOXP3+ Tregs lower in the group presenting with anaphylaxis compared to other groups?" We compared the groups in terms of clinical and laboratory parameters.

Material and Methods

Subjects

Cases selected were 53 patients diagnosed with IgE-mediated CMPA, confirmed by specific IgE, SPT, and open oral food challenge (OFC), between the years 2016–2022 at İzmir S.B.U. Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Pediatric Allergy Clinic. Oral steroid users, food protein-induced enterocolitis, and other non-IgE-mediated food allergies were not included in the study. Among the 53 cases selected, 41 agreed to participate in the study. The study was completed with a total of 41 cases. As the control group, 29 non-atopic, healthy children of similar age without chronic disease were included.

Methods

The diagnosis of IgE-mediated CMPA was made by clinical criteria, serum-specific IgE, SPT with specific food allergens, and open OFC. Serum samples were analyzed for serum-specific IgE using an UniCAP 100 system fluorescence enzyme immunoassay (Phadia, Uppsala, Sweden). The lowest limit of detection of the assay was 0.35 kUA/L. SPTs were performed by trained physicians on the volar surface of the forearm of each patient using a commercial extract of milk (ALK Abelló, Horsholm, Denmark). The tests were always performed with a histamine positive control and a saline negative control. A mean wheal diameter greater than 3 mm after 15 min of testing the allergen extracts was accepted as a positive result. The OFC test was an open protocol. OFCs were started using 1 mL of pasteurized cow milk with a 3.3% protein content and continued with increasing amounts of undiluted cow milk at 1, 1.5, 3, 6, 12, 24, 50, and 100 mL until a reaction was noted. The results of the oral challenge were considered positive when objective symptoms occurred. Early and late objective reactions such as urticaria, angioedema, airway obstruction signs, vomiting, and anaphylaxis were assessed. We did not stop the OFCs for subjective reactions such as pruritus, not feeling well, and abdominal pain.

Flow cytometric analysis

Blood samples for the evaluation of transcription factor-forkhead box P3 (FOXP3) were collected from FA patients at least 2 weeks after entering an elimination diet. As the control group, samples were collected similarly to those

from the patient group in 29 patients of the same age who did not have any additional allergic or chronic diseases. Examinations were carried out at the Immunology laboratory of University of Health Sciences, Dr. Behçet Uz Children's Hospital using a device with a FC 500 Flow Cytometer and CXP software. Samples of peripheral blood were collected in EDTA anticoagulant tubes. Freshly collected blood samples were stained within 15 min after arrival at the laboratory with each conjugated monoclonal antibodies (mAb). The following commercial mAbs were used: fluorescein isothiocyanate (FITC) mouse anti-human CD25 (clone: M-A251, BD Pharmingen, USA), peridinin chlorophyll protein complex (PerCP) mouse anti-human CD4 (clone: L200, BD Pharmingen, USA), and phycoerythrin cyanin 7 (PC7) mouse anti-human CD127 (clone: R34.34, Beckman Coulter, France). After surface staining, cells were stained intracellularly for FOXP3 according to the manufacturer's recommendations (Human FOXP3 Buffer Set; BD Pharmingen, USA), and treated with phycoerythrin (PE) mouse anti-human FOXP3 (clone: 259D/C7 BD Pharmingen, USA). Four-color flow cytometry analysis was done with the FC500 Flow Cytometer (Beckman Coulter). Treg cells were evaluated as a percentage of CD4+CD25+FOXP3+CD127^{lo} cells among total CD4+T cells. Low IL-7Rα (CD127^{lo}) expression, high IL-2Rα (CD25hi), and FOXP3 expression were used as they were reported to perfectly identify Tregs in previous studies.[12]

The anaphylaxis diagnosis was made based on anaphylaxis guidelines from the European Academy of Allergy and Clinical Immunology anaphylaxis clinical criteria.[13]

Statistics

IBM SPSS version 22.0 (Armonk, New York, United States) was used for all statistical analyses. The Kolmogorov-Smirnov test was used to test the normality of variables. Parametric methods were used for the analysis of variables with a normal distribution, whereas non-parametric methods were used for the analysis of variables that were not normally distributed. Comparisons of continuous variables were made with the independent-samples t-test and Mann-Whitney U test, as appropriate. Pearson's chi-square and linear-by-linear association tests were used with an exact test for the comparison of categorical data. The categorical data are expressed as a percentage of the number (n) of children evaluated. The level of significance for the analyses was $p < 0.05$.

Ethics

The study was approved by the University of Health Sciences, Dr. Behçet Uz Children's Hospital, Clinical Research Ethics Committee, 2014/16, and written informed consent was obtained from all parents of child subjects.

Results

A total of 70 children were included in the study: 25 who had IgE-mediated CMPA and presented with non-anaphylactic symptoms (FA/A−), 16 who had IgE-mediated CMPA and presented with anaphylaxis (FA/A+) (a total of 41 FA cases), and a control group consisting of 29 children without FA. The study was conducted by performing CD4+CD25+CD127^{lo}FOXP3+ cell flow cytometric analysis during resting at least 2 weeks after the elimination diet in subjects with FA.

In the FA group, 36.6% of subjects were female (n = 15) and 63.4% were male (n = 26), whereas 41.4% of the subjects in the control group were female (n = 12) and 58.6% were male (n = 17). The mean age of the FA group was 25.60 ± 16.59 (6–72) months, and the mean age of the control group was 21.44 ± 13.66 (6–53) months (Table 1).

When the FA group was compared with the control subjects, the FA group CD4+CD25+CD127^{lo}FOXP3+ cell counts and rates were found to be significantly lower ($p < 0.001$ and $p < 0.001$, respectively) (Table 1, Figure 1). In the FA/A− and the FA/A+ groups, CD4+CD25+CD127^{lo}FOXP3+ cell numbers and rates were significantly lower compared to the control group ($p < 0.001$ and $p < 0.001$, respectively) (Table 2). When the FA/A− and the FA/A+ groups were compared within themselves in terms of CD4+CD25+CD127^{lo}FOXP3+ cell numbers and rates, no significant difference was found between the two groups ($p = 0.390$ and $p = 0.614$, respectively) (Table 3). When the FA group and control subjects were compared in terms of mean fluorescence intensity

Table 1 Characteristics of the FA group and the control group.

	FA group	Control group	P
n (%)	41 (58.57)	29 (41.42)	
Age (months)	25.60 ± 16.59	21.44 ± 13.66	
Boys, n (%)	26 (63.41)	17 (58.62)	
Concentration of lymphocytes (cells/μL)	5015.60 ± 1995.05	5104.34 ± 2148.37	0.605
CD4+CD25+CD127$_{lo}$	4.65 ± 1.77	6.5 ± 1.06	<0.001
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (%)*	4.12 ± 1.53	6.43 ± 1.04	<0.001
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (cells/μL)*	4.30 ± 5.15	5.15 ± 2.60	<0.001
MFI of FOXP3 in CD4+ CD25+ CD127low FOXP3+ Treg cells	4.18 ± 1.37	3.76 ± 1.63	0.088

Data is presented as mean ± SD. P < 0.05 was considered statistically significant. (in CD4+ T cells)*
FA, food allergy; MFI, mean fluorescence intensity.

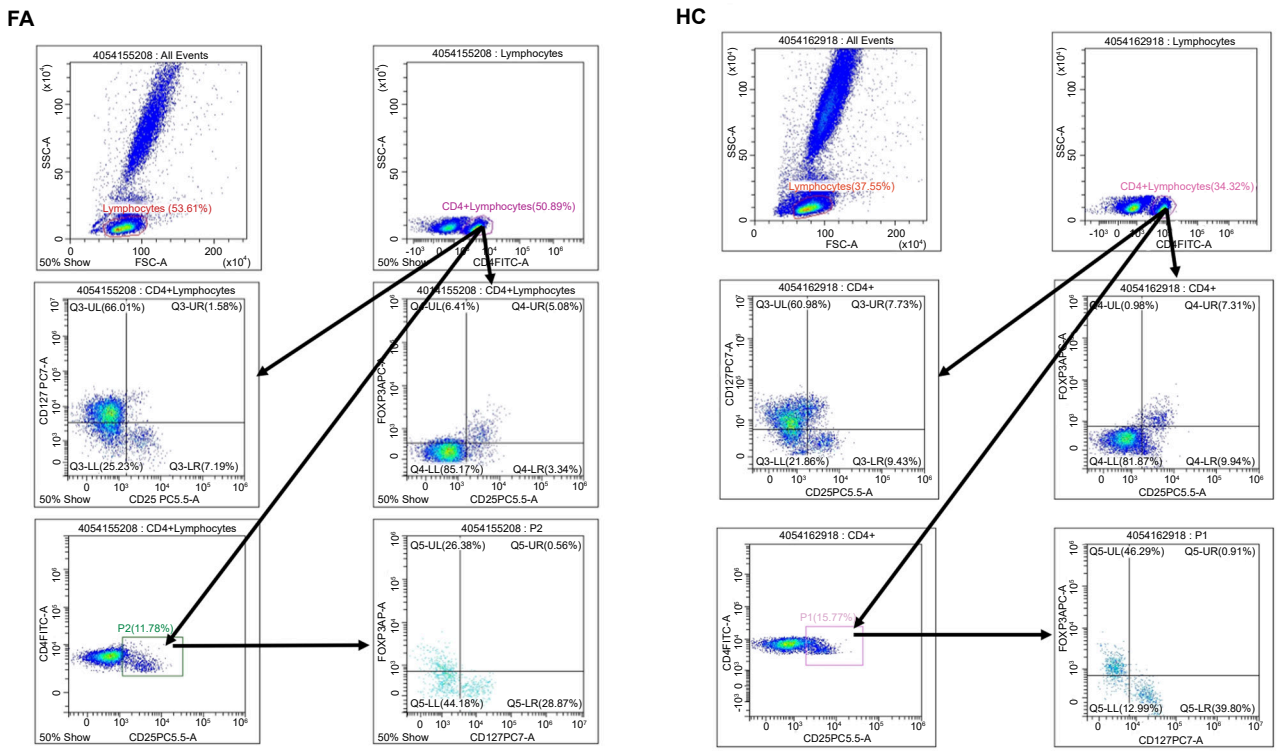

Figure 1 Flow cytometry analysis of Treg cells in patient (FA) and healthy control. FA: Food allergy, HC: Healthy control.

Table 2 FA/A−, FA/A+ group and control group characteristics.

	FA/A− group	FA/A+ group	Control group	P
n (%)	25 (35.71)	16 (22.85)	29 (41.42)	
Concentration of lymphocytes (cells/μL)	5304.40 ± 2135.04	4564.37 ± 1721.70	5104.34 ± 2148.37	0.536
CD4+CD25+CD127^{lo}	4.49 ± 1.53	4.9 ± 2.12	6.5 ± 1.06	<0.001
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (%)*	3.96 ± 1.24	4.38 ± 1.91	6.43 ± 1.04	<0.001
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (cells/μL)*	3.40 ± 4.64	5.70 ± 5.73	5.15 ± 2.60	<0.001
MFI of FOXP3 in CD4+ CD25+ CD127low FOXP3+ Treg cells	4.16 ± 1.38	4.27 ± 1.47	3.76 ± 1.63	0.232

Data is presented as mean ± SD. P < 0.05 was considered statistically significant. (in CD4+ T cells)*
FA/A−, children with food allergy and without anaphylaxis; FA/A+, children with food allergy and with anaphylaxis; MFI, mean fluorescence intensity.

(MFI) of FOXP3 in CD4+ CD25+ CD127low FOXP3+ Treg cells, no significant difference was found between the groups (p = 0.088) (Table 1).

The FA/A− group and the FA/A+ group were compared in terms of clinical and other laboratory characteristics. Anaphylaxis was found to be significantly higher in cases with inhaled allergen sensitivity, cases with atopic dermatitis, and cases with additional FA (p = 0.005, p = 0.008, and p = 0.020, respectively). When both groups were compared in terms of other laboratory findings, it was found that the difference between the milk-specific IgE averages of the two groups at admission was significant, and the milk-specific IgE average at admission was significantly higher in the FA/A+ group (p = 0.006) (Table 3).

Discussion

Dendritic cells (DC), Treg cells, B regulatory cells, intestinal epithelial cells, and microbiome contribute to oral tolerance to food allergens.[14] However, the best marker is FOXP3 Treg lymphocytes.[14–18] Treg cells directly or indirectly suppress allergen-induced degranulation of effector cells and prevent eosinophils and other effector T cells from entering tissues. Treg cells interact with resident tissue cells to contribute to tissue remodeling and promote the formation of tolerogenic DC phenotypes.[18] In addition, Treg cells directly inhibit the activation of allergen-specific Th2 cells, blocking all the effects mediated by these cells during allergic reactions, and inducing allergen-specific

Table 3 FA sub-groups FA/A− and FA/A+ groups' characteristics.

	FA/A−	FA/A+	P
n (%)	25 (60.97)	16 (39)	
Boys, n (%)	15 (57.7)	11 (42.3)	
Atopic disease in the family, n (%)	12 (48.0)	6 (37.5)	0.509
Inhaled allergen sensitivity, n (%)	6 (24.0)	11 (68.8)	0.005
Atopic dermatitis, n (%)	0 (0.0)	4 (25.0)	0.008
Additional food allergy, n (%)	4 (16.0)	8 (50.0)	0.020
Casein-specific IgE	24.08 ± 37.60	44.94 ± 41.57	0.104
Eosinophil (%)	4.99 ± 2.94	6.80 ± 3.97	0.102
CD4+CD25+CD127^{lo} (%)*	4.49 ± 1.53	4.90 ± 2.12	0.484
CD4+CD25+CD127^{lo} (cells/mL)*	4.08 ± 6.36	6.32 ± 6.36	0.277
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (%)*	3.96 ± 1.24	4.38 ± 1.91	0.390
CD4+CD25+CD127^{lo}FOXP3+ Treg cells (cells/mL)*	3.40 ± 4.64	5.70 ± 5.73	0.165
Total IgE (IU/mL)	153.85 ± 185.78	399.61 ± 521.13	0.072
Skin prick test diameter (mm)	4.92 ± 1.97	6.66 ± 3.81	0.063
Milk-specific IgE (kU/L)	21.78 ± 32.32	65.10 ± 50.78	0.002
Baked tolerance, n (%)	7 (28.0)	2 (12.5)	0.242

Data is presented as mean ± SD. P < 0.05 was considered statistically significant. (in CD4+ T cells)*
FA/A−, children with food allergy and without anaphylaxis; FA/A+, children with food allergy and with anaphylaxis.

IgG4 while inhibiting IgE by acting directly on B cells.[18,19] To perform these functions, Treg cells use inhibitory cytokines (e.g., IL-10, TGF-β, and IL-35), cytolysis (granzymes A and B), metabolic degradation mechanisms, and DCs CTLA-4, PD-1, or they use histamine receptor 2 related mechanisms.[18,19]

In cases with CMPA, oral immunotherapy (OIT) is an extremely important method in the prevention of anaphylaxis and severe reaction that may occur because of accidental exposure. The purpose of OIT is to increase the reactive threshold of allergic patients and to ensure that the targeted amount of allergen is administered without any reaction.[20] These important gains through OIT occur with the induction of FOXP3-positive Treg cells and the downregulation of Th2 cytokines including IL-4.[21]

There is limited information on whether Treg levels are a biomarker that predicts clinical severity in cases presenting with food anaphylaxis (regardless of anaphylaxis severity), and which patients are more at risk for anaphylaxis. As far as we know, no children's study has been conducted on how Tregs change in cases presenting with food anaphylaxis.

In our study, when we compared the FA group with healthy control subjects, we found that in the FA group that CD4+CD25+CD127^{lo}FOXP3+ cell ratios were significantly lower (p < 0.001). In the FA/A− and FA/A+ groups, CD4+CD25+CD127^{lo}FOXP3+ cell ratios were also significantly lower compared to the control group (p < 0.001) (Tables 1 and 2). When we compared the FA/A− and the FA/A+ groups in terms of CD4+CD25+CD127^{lo}FOXP3+ cells, we found no significant difference between the two groups (p = 0.390) (Table 3).

Shreffler et al.[22] reported in 2009 that a higher proportion of milk allergen-specific Treg cells is associated with mild clinical disease phenotype and good prognosis. In 2013, mouse experiments showed that constitutive FOXP3+ Tregs can control the symptomatic phase of mast cell and IgE-induced anaphylaxis.[23] Tordesillas et al.[24] reported in 2017 that Tregs directly suppress mast cell activation and inhibit anaphylaxis in response to a food challenge. Satitsuksanoa et al.[14] indicated in 2018 that FOXP3 knockout mice developed multi-organ inflammatory responses associated with allergic inflammation. They also reported that adoptive transfer of Treg cells in a FA model in mice was able to suppress anaphylaxis and control the Th2 immune response. In 2019, Abdeladhim et al.[19] used specially designed Tregs to treat allergy. With this approach, they targeted allergen-specific B cell populations and mast cells using Tregs that express a specific allergen. The OVA-induced allergy model was used in the study. By injecting OVA-specific BAR Tregs prior to systemic allergen challenge, they were able to protect OVA-sensitized mice from anaphylaxis, and the effect lasted for at least 30 days. These researchers indicated that the mediators indicative of anaphylaxis were not increased in the plasma of mice injected with OVA-specific BAR Tregs. Researchers said that "BAR-Tregs therapy" has the potential to alleviate allergies to multiple antigens.[19]

FOXP3 deficiency results in a multi-organ lymphoproliferative autoimmune disease called immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX).[17] It has also been reported that the deficiency or dysfunction of Tregs may lead to FA.[25] Krogulska et al.[16] showed in 2011 that FOXP3 and IL10 gene expression was significantly lower in children with FA compared to control subjects. In 2016, it was reported that milder deficiency of Tregs may contribute to the clinical phenotype of food sensitivity.[25] A study published in 2016 found that Treg defects were more pronounced in case groups with peanut allergy compared to egg allergy. It suggested that the more pronounced Treg defect in cases with peanut allergy may be a contributing factor to the persistent course of peanut allergy. In this study, the researchers also obtained data on Treg depletion

and impaired capacity to renew the Treg pool following allergen exposure in children with FA.[25]

In cases presenting with food anaphylaxis, just as there is little data that Treg cells prevent anaphylaxis, there is very little data on whether low levels of Treg cells increase clinical severity.[14,19,24] In 2015, Krogulska et al.[26] found lower FOXP3 gene expression levels in children with IgE-mediated FA and asthma compared to control subjects, and correlated it with a more severe clinical course.

In our study, when we compared the FA/A− and the FA/A+ groups in terms of CD4+CD25+CD127^{lo}FOXP3+ cells, we found no significant difference between the two groups ($p = 0.390$). Krogulska et al.[26] stated in 2015 that the use of ICS-activated FOXP3 formed Tr1, and it could increase the number of Tregs in the blood of patients with severe asthma. In our study, the FA/A− and the FA/A+ groups did not differ significantly in terms of CD4+CD25+CD127^{lo}FOXP3+ cells, which made us think that 11 of the FA/A+ group might have been affected by the inhaled corticosteroids they used since they had inhaled allergen sensitivity and concomitant respiratory diseases.

There are various co-factors (such as rhinitis, asthma, atopic dermatitis, mastocytosis, cardiovascular disease) that are known to increase the severity of allergic reactions.[3,4] In line with the literature, we found anaphylaxis to be significantly higher in cases with inhaled allergen sensitivity and in cases with atopic dermatitis ($p = 0.005$ and $p = 0.008$, respectively). In our study, anaphylaxis was also found to be significantly higher in cases with additional FA ($p = 0.020$).

Evidence that serum quantitative levels of allergen-specific IgE or SPT wheal sizes reflect the severity of severe allergic reactions with casein-specific IgE elevation is reported to be conflicting.[1,2] When we compared the FA/A and FA/A+ groups in terms of other laboratory findings, we found that the difference between the milk-specific IgE averages at the admission of the two groups was significant, and the milk-specific IgE average at admission was significantly higher in the FA/A+ group ($p = 0.006$).

In our study, we were able to measure Treg levels at rest. We were unable to examine Treg levels after activation. We were unable to examine Treg depletion or the regeneration capacity of the Treg pool. Examining CD4+CD25+CD127^{lo}FOXP3+ cells after allergen exposure or anaphylaxis will provide more information about Treg consumption and the regeneration capacity of the Treg pool. The limitations of our study are the inability to examine activated Tregs and cytokines, and the small number of cases. In addition, the fact that 11 of the FA/A+ group used inhaled corticosteroids due to inhaled allergen sensitivity and concomitant respiratory diseases can be considered a limitation due to the possibility of affecting the results.

Conclusion

In the FA/A− and the FA/A+ groups, CD4+CD25+CD127^{lo}FOXP3+ cell ratios were significantly lower compared to the control group. No significant difference was found between the FA/A+ group and the FA/A− sub-groups in terms of CD4+CD25+CD127^{lo}FOXP3+ cells. In our study, the FA/A− and the FA/A+ groups did not differ significantly in terms of CD4+CD25+CD127^{lo}FOXP3+ cells, which made us think that 11 of the FA/A+ group might have been affected by the inhaled corticosteroids they used since they had inhaled allergen sensitivity and concomitant respiratory diseases.

Severe clinical reactions, such as anaphylaxis, were found to be increased in cases with FA and accompanying inhaled allergen sensitivity, atopic dermatitis, or additional FA, and in cases with high specific IgE at admission. For this reason, in terms of anaphylaxis, it was thought that it was important to monitor more closely the cases with additional inhaled allergen sensitivity to FA, atopic dermatitis, or additional FA, and cases with high specific IgE levels at admission.

However, it is important to interpret all biomarkers in the light of other clinical information available for each patient. The biomarkers to be detected will allow us to identify cases at risk for severe allergic reactions and to closely monitor these cases. Well-designed studies are needed to determine the role of CD4+CD25+CD127^{lo}FOXP3+ cells in severe allergic reaction phenotypes.

Funding Source

No external funding was secured for this study.

Financial Disclosure

The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Trial Registration

Not applicable

References

1. Turner PJ, Arasi S, Ballmer-Weber B, Baseggio Conrado A, Deschildre A, Gerdts J, et al. Risk factors for severe reactions in food allergy: rapid evidence review with meta-analysis. Allergy. 2022;77(9):2634–2652. https://doi.org/10.1111/all.15318
2. Buyuktiryaki B, Santos AF. Food allergy severity predictions based on cellular in vitro tests. Expert Rev Mol Diagn. 2020;20(7):679–692. https://doi.org/10.1080/14737159.2020.1782192
3. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241–1255. https://doi.org/10.1111/all.12924
4. Smith PK, Hourihane JO, Lieberman P. Risk multipliers for severe food anaphylaxis. World Allergy Organ J. 2015;8(1):30. https://doi.org/10.1186/s40413-015-0081-0
5. Nowak-Wegrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin

Immunol. 2008;122(2):342–347. https://doi.org/10.1016/j.jaci.2008.05.043
6. Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, et al. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. J Allergy Clin Immunol. 2020;146(2):344–355. https://doi.org/10.1016/j.jaci.2020.03.035
7. Ballmer-Weber BK, Lidholm J, Lange L, Pascal M, Lang C, Gernert S, et al. Allergen recognition patterns in walnut allergy are age dependent and correlate with the severity of allergic reactions. J Allergy Clin Immunol Pract. 2019;7(5):1560–1567. https://doi.org/10.1016/j.jaip.2019.01.029
8. Kiewiet MBG, Apostolovic D, Starkhammar M, Grundström J, Hamsten C, van Hage M. Clinical and serological characterization of the α- gal syndrome- importance of atopy for symptom severity in a European cohort. J Allergy Clin Immunol Pract. 2020;8(6):2027–2034. https://doi.org/10.1016/j.jaip.2020.02.016
9. De Schryver S, Halbrich M, Clarke A, La Vieille S, Eisman H, Alizadehfar R, et al. Tryptase levels in children presenting with anaphylaxis: temporal trends and associated factors. J Allergy Clin Immunol. 2016;137(4):1138–1142. https://doi.org/10.1016/j.jaci.2015.09.001
10. Dua S, Dowey J, Foley L, Islam S, King Y, Ewan P, et al. Diagnostic value of tryptase in food allergic reactions: a prospective study of 160 adult peanut challenges. J Allergy Clin Immunol Pract. 2018;6(5):1692–1698. https://doi.org/10.1016/j.jaip.2018.01.006
11. Sahiner UM, Yavuz ST, Buyuktiryaki B, Cavkaytar O, Yilmaz EA, Tuncer A, et al. Serum basal tryptase may be a good marker for predicting the risk of anaphylaxis in children with food allergy. Allergy. 2014;69:265–268. https://doi.org/10.1111/all.12317
12. Prince BT, Devonshire AL, Erickson KA, Bergerson J, Fuleihan D, Szychlinski C, et al. Regulatory T-cell populations in children are affected by age and food allergy diagnosis. J Allergy Clin Immunol. 2017;140(4):1194–1196. https://doi.org/10.1016/j.jaci.2017.04.039
13. Muraro A, Roberts G, Worm M, Bilo MB, Brockow K, Fernandez Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy. 2014;69:1026–1045. https://doi.org/10.1111/all.12437
14. Satitsuksanoa P, Jansen K, Głobińska A, van de Veen W, Akdis M. Regulatory immune mechanisms in tolerance to food allergy. Front Immunol. 2018;12(9):2939. https://doi.org/10.3389/fimmu.2018.02939
15. Sardecka-Milewska I, Łoś-Rycharska E, Gawryjołek J, Toporowska-Kowalska E, Krogulska A. Role of FOXP3 expression and serum vitamin D and C concentrations when predicting acquisition of tolerance in infants with cow's milk allergy. Investig Allergol Clin Immunol. 2020;30(3):182–190. https://doi.org/10.18176/jiaci.0422
16. Krogulska A, Borowiec M, Polakowska E, Dynowski J, Młynarski W, Wasowska-Królikowska K. FOXP3, IL-10, and TGF-ß genes expression in children with IgE-dependent food allergy. J Clin Immunol. 2011;31(2):205–215. https://doi.org/10.1007/s10875-010-9487-1
17. Noval Rivas M, Chatila TA. Regulatory T cells in allergic diseases. J Allergy Clin Immunol. 2016;138(3):639–652. https://doi.org/10.1016/j.jaci.2016.06.003
18. Palomares O. The role of regulatory T cells in IgE-mediated food allergy. J Investig Allergol Clin Immunol. 2013;23(6):371–382.
19. Abdeladhim M, Zhang AH, Kropp LE, Lindrose AR, Venkatesha SH, Mitre E, et al. Engineered ovalbumin-expressing regulatory T cells protect against anaphylaxis in ovalbumin-sensitized mice. Clin Immunol. 2019;207:49–54. https://doi.org/10.1016/j.clim.2019.07.009
20. Vandenplas Y, Brough HA, Fiocchi A, Miqdady M, Munasir Z, Salvatore S, et al. Current guidelines and future strategies for the management of cow's milk allergy. J Asthma Allergy. 2021;14:1243–1256. https://doi.org/10.2147/JAA.S276992
21. Schworer SA, Kim EH. Sublingual immunotherapy for food allergy and its future directions. Immunotherapy. 2020;12(12):921–931. https://doi.org/10.2217/imt-2020-0123
22. Shreffler W, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson H. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. J Allergy Clin Immunol. 2009;123:43. https://doi.org/10.1016/j.jaci.2008.09.051
23. Kanjarawi R, Dy M, Bardel E, Sparwasser T, Dubois B, Mecheri S, et al. Regulatory CD4+FOXP3+ T cells control the severity of anaphylaxis. PLoS One. 2013;8(7):e69183. https://doi.org/10.1371/journal.pone.0069183
24. Tordesillas L, Mondoulet L, Blazquez AB, Benhamou PH, Sampson HA, Berin MC. Epicutaneous immunotherapy induces gastrointestinal LAP(+) regulatory T cells and prevents food-induced anaphylaxis. J Allergy Clin Immunol. 2017;139(1):189–201. https://doi.org/10.1016/j.jaci.2016.03.057
25. Dang TD, Allen KJ, Martino DJ, Koplin JJ, Licciardi PV, Tang ML. Food-allergic infants have impaired regulatory T-cell responses following in vivo allergen exposure. Pediatr Allergy Immunol. 2016;27(1):35–43. https://doi.org/10.1111/pai.12498
26. Krogulska A, Polakowska E, Wąsowska-Królikowska K, Małachowska B, Młynarski W, Borowiec M. Decreased FOXP3 mRNA expression in children with atopic asthma and IgE-mediated food allergy. Ann Allergy Asthma Immunol. 2015;115(5):415–421. https://doi.org/10.1016/j.anai.2015.08.015