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Original Article

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Immune Profile After Oral Immunotherapy in Children With Persistent Cow's Milk Protein Allergy

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ABSTRACT

Purpose: Cow's milk protein allergy (CMPA) is the most common food allergy in childhood. Oral immunotherapy (OIT) has proven effective in achieving tolerance in children with persistent CMPA (pCMPA), although the underlying immunological mechanisms that allow for tolerance remain incompletely understood. We aimed to characterize the immune profile of pCMPA children following successful OIT and compare it to healthy controls (HC).
Methods: Thirty pCMPA children and 33 HC were recruited. T and B cell populations were assessed by flow cytometry, and specific immunoglobulins (sIgs) to milk allergens were measured by fluorometric enzyme-immunoassay.
Results: Compared to HC, pCMPA children showed reduced B cells and activated T cells. Activated $CD4^+$ T cells negatively correlated with OIT duration. Although sIgEs to casein remained ≥ class 3 in some patients, overall post-OIT sIgEs were lower compared to pre-OIT levels. Additionally, sIgG4 increased after OIT, particularly in patients with detectable sIgA. After OIT, pCMPA patients with sIgEs ≥ class 3 presented shorter relapse periods and fewer B cells and T cells compared to those with sIgEs < class 3. Interestingly, sIgEs correlated positively with regulatory T cells, though different sIgEs specificities showed distinct relationships with immune parameters. Age-related differences were observed: only children > 10 years showed significant reductions in T and B cells compared to their respective age-matched controls.
Conclusions: OIT modulates immune activation in pCMPA, reducing sIgE and increasing sIgG4 levels. Different relations between distinct sIgs and immune parameters may denote different stimulating and tolerance-inducing capacities for distinct cow's milk components. Age and time since OIT completion influence immune recovery, highlighting the complexity of tolerance mechanisms.

Keywords: Children; milk hypersensitivity; immunotherapy; flow cytometry; regulatory T cells; B cells

Disclosure

There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

The development of allergic diseases is a complex and multifactorial process conditioned by genetic factors, including family history of allergic disease, as well as environmental factors that actively modulate gene expression.[1]

Food allergy is recognized when there is an exaggerated immune response with clinically reproducible signs and symptoms associated with the ingestion of food antigens.[2] Among these, cow's milk protein allergy (CMPA) represents the most common form in childhood and has a significant impact on the daily lives of affected children and their caregivers. It affects around 2%–4.5% of infants in developed countries,[3] and results from an aberrant immunological reaction against cow's milk proteins, mainly casein and β-lactoglobulin.[4]

Most children with CMPA overcome the allergy spontaneously during early childhood.[3,5] In fact, by the age of five, about 80% of affected children have developed tolerance.[3,6]

If tolerance is not achieved, CMPA may persist over time and is then referred to as persistent CMPA (pCMPA). This condition is often severe and potentially life-threatening, requiring strict avoidance of cow's milk proteins.[3,4] In patients with pCMPA, tolerance induction protocols can be lifesaving, particularly in severe cases, by enabling the development of tolerance to small amounts of milk, thereby reducing the risk of severe reactions due to accidental ingestions.

Successful food tolerance induction through oral immunotherapy (OIT) has been documented in several children with long-term CMPA.[7-12] OIT consists of the oral administration of cow's milk proteins, starting with very low dosages and gradually increasing to either the standard age-appropriate dose or the maximum tolerated dose, facilitating the development of oral tolerance.[13-15] However, most patients remain on maintenance doses without experiencing reactions, and it is uncertain whether OIT leads to effective oral tolerance (also known as sustained unresponsiveness) or to temporary desensitization only.[16]

The mechanisms underlying oral tolerance induction include T cell deletion and/or anergy, and the induction of regulatory T cells (Tregs).[17,18] Changes in these mechanisms, particularly impaired Treg responses, have been linked to the persistence and severity of CMPA, making them highly relevant in the context of OIT.[19] Specifically, the induction of oral tolerance has been associated with increased numbers of Tregs.[20,21] And children who outgrew CMPA and reintroduced cow's milk into their diet exhibited higher levels of circulating $CD4^+CD25^+$ T cells compared to those who remained clinically symptomatic.[21] However, Tiemessen *et al.*[22] reported phenotypical or functional differences in Tregs, based on their *in vitro* suppressive capacity, between adults with CMPA and healthy controls.

As recognized for T cells, subgroups of B cells with regulatory functions (Bregs) also play important roles in maintaining immune tolerance. These cells act through cell-to-cell interactions and the secretion of inhibitory cytokines such as interleukin-10 and transforming growth factor-β.[23] In fact, several studies support the clinical relevance of B cells in pCMPA patients undergoing OIT, although their exact role in tolerance acquisition remains to be clarified.[24-26]

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Early immunological changes during OIT have also been associated with decreased reactivity of mast cells and basophils,[27] increased levels of food-specific serum and salivary immunoglobulin (Ig) G4 and IgA, and an initial rise followed by a decrease in serum food-specific immunoglobulin (sIg) E.[28-30] However, the underlying immunological mechanisms allowing the development of tolerance remain to be clarified.

The investigation of immune cell populations with regulatory functions has provided valuable insights into the immunological basis of tolerance. Nevertheless, the intricate relationship between allergenic stimuli, regulatory immune cells, cytokines, and the development of immune tolerance requires further elucidation. Therefore, this study aimed to evaluate the immune profile of children with pCMPA following OIT and compare it with the immune profile of healthy children.

MATERIALS AND METHODS

Study participants

In 2016, we recruited 30 children with pCMPA who had successfully completed milk-OIT from at the Immunoallergy outpatient clinic of a hospital in Lisbon, Portugal.[11] Additionally, 33 healthy sex- and age-matched controls were selected from the database of the Immunology Laboratory at Nova Medical School, where data on healthy children had been collected, to establish normative immunological parameters by age. All participants and/or their parents signed an informed consent form before enrolling in the study.

The pCMPA had been diagnosed based on clinical history, positive cow's milk-specific serum IgE levels and positive skin prick tests. Relevant clinical data from the children with pCMPA were assessed and analyzed, including age at diagnosis, duration of cow's milk protein avoidance, age at OIT initiation, OIT duration, and post-OIT follow-up period.

Before OIT initiation, all participants either had a positive oral food challenge (OFC) following a period of milk avoidance or had a reproducible history of allergic reaction to accidental exposure to cow's milk proteins within the previous year. OIT protocols ranged in duration from 2 to 15 months. The first immunological evaluation was performed within 8 months after OIT completion, and planned annually thereafter, until reaching 8 years old. A complete immunological evaluation was further performed on all children who had completed a successful milk-OIT protocol, as described in **Table 1**. No further assessments

Table 1. Demographic and clinical data comparison between pCMPA and healthy group

Characteristics	pCMPA group (n = 30)	Healthy group (n = 33)	*P* value
Age (yr)	10.9 ± 4.5	9.7 ± 2.9	0.213*
Sex			1.000†
Male	14 (46.7)	15 (45.5)	
Female	16 (53.3)	18 (54.5)	
Age at diagnosis (mon)	4.1 ± 1.5	-	-
Age at OIT start (yr)	6.9 ± 3.7	-	-
OIT duration (mon)	5.5 [3.8–8.0]	-	-
Age at OIT end (yr)	7.3 ± 3.6	-	-
Years of CMP avoidance (yr)	6.9 ± 3.7	-	-
Post-OIT time (yr)	3.2 ± 2.2 (0–8)‡	-	-

Values are presented as mean ± standard deviation, median [interquartile range], or number (%).
pCMPA, persistent cow's milk protein Allergy; OIT, oral immunotherapy; CMP, cow's milk proteins.
*Unpaired *t*-test with Welch's correction; †Fisher's exact test; ‡Range.

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were performed during the OIT protocol. Except for two patients who followed an adapted protocol,[31] all pCMPA patients underwent a published OIT protocol.[32] Following completion of OIT, all patients had a regular daily consumption of 200 mL of cow's milk and maintained a milk-inclusive diet. Exclusion criteria for both groups included autoimmune diseases, immunodeficiency, other ongoing immunotherapy, or treatment with immunosuppressive drugs. Healthy controls had no history of allergic diseases, including rhinitis, asthma, food allergy, eczema or drug allergy.

All parents completed a detailed questionnaire on pregnancy and personal medical history, family history of allergy, and exposure to tobacco smoke. The study was approved by the ethics committee of NOVA Medical School and was conducted in accordance with the principles of the Declaration of Helsinki.

Blood samples

Peripheral blood samples from patients and controls were collected using standard aseptic venepuncture techniques. For cell immunophenotyping, blood was collected in ethylenediaminetetraacetic acid-containing tubes and processed within 24 hours of collection. Tubes without anticoagulation agents were used for serum collection to evaluate sIg levels. After clot retraction, samples were centrifuged for 10 minutes at 943 g. Serum was then separated, aliquoted, and stored at −20°C until analysis.

Characterization of the circulating T and B cell compartments

For lymphocyte subset evaluation (T, B, and natural killer [NK] cells), a lyse-no-wash protocol was employed using the BD Multitest IMK Kit (BD Biosciences, San Diego, CA, USA), according to the manufacturer's instructions.

For the evaluation of activated and Tregs, a lyse-wash protocol was used. Briefly, cells were incubated with monoclonal antibodies (mAbs) for 15 minutes, followed by lysis using BD FACS Lysing Solution (BD Biosciences). After a wash step with phosphate-buffered saline, samples were acquired.

The mAbs panel for Treg evaluation included anti-CD3 FITC (clone SK7; BD Biosciences), anti-CD25 PE (clone BC96; BioLegend, San Diego, CA, USA), anti-CD4 PerCP Cy5.5 (clone SK3; BioLegend), and anti-CD127 Alexa647 (clone A019D5; BioLegend). A minimum of 15,000 $CD4^+$ T cells was acquired.

Multicolour flow cytometry and data analysis

Flow cytometry was performed using a 4-color BD FACSCalibur (BD Biosciences) equipped with a 488-nm blue laser and a 635-nm red laser. Cell Quest software (BD Biosciences) was used for data acquisition and analysis. Equipment setup, calibration, and quality control protocols were followed to ensure measurement stability over time. A minimum of 10,000 $CD4^+$ T cells and 2,000 B cells were acquired. **Figs. 1** and **2** illustrate the gating strategy used for the assessment of T and B cell populations.

sIgs

Serum levels of sIgE, sIgA and sIgG4 to cow's milk, α-lactalbumin, β-lactoglobulin, and casein were determined by fluoroenzyme immunoassay, using the UniCAP® 100 system (Thermo Fisher Scientific, Waltham, MA, USA). According to the manufacturer's instructions, sIgE results were reported in kilounits of allergen-sIgE per liter (kU_A/L), and

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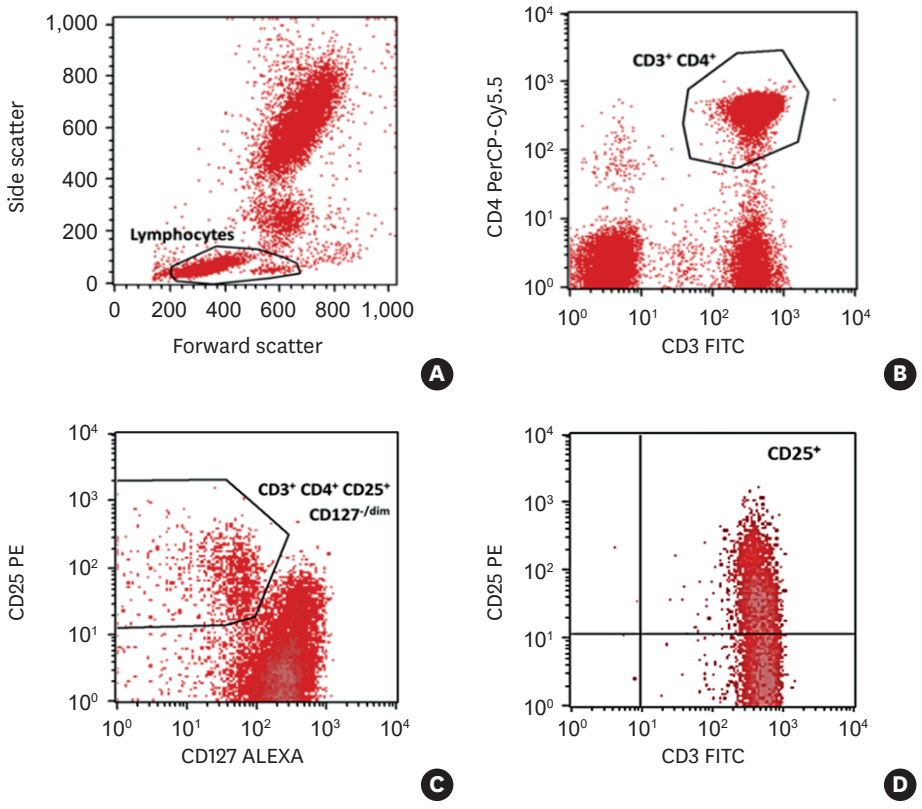

Fig. 1. Gating strategies for the identification of distinct regulatory T cell subsets. (A, B) Identification of $CD3^+$ $CD4^+$ T cells, recognized within the lymphocyte gate. (C) Identification of Tregs according to the expression of CD25 and CD127 ($CD3^+CD4^+CD25^+CD127^{-/dim}$). (D) Identification of $CD25^+$ activated T cells.
Treg, regulatory T cell.

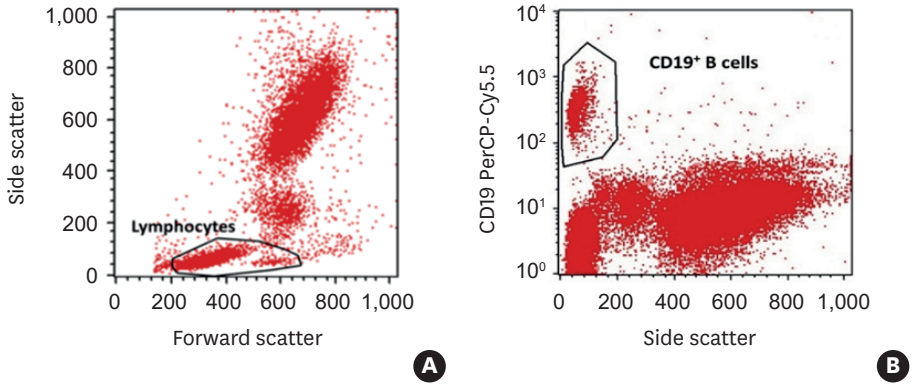

Fig. 2. Gating strategies for the identification of B cells. B cells were identified as the $CD19^+$ population within the lymphocyte gate, as displayed in dot plots (A) and (B).

sIgG4 results in mg_A/L. sIgA values were expressed in both mg_A/L and arbitrary units (AU), derived from fluorescence intensity.

Statistical analysis

Categorical variables were reported as absolute frequencies and percentages, and analyzed using Fisher's exact or the χ^2 test. For continuous variables, normality of distribution was assessed using the D'Agostino and Pearson test. Normally distributed data are presented

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as mean ± standard deviation and non-normally distributed data as median (interquartile range, IQR). The Unpaired *t*-test or the Mann-Whitney *U* test was used to compare each 2 independent groups, as appropriate. The Wilcoxon matched-pairs signed-rank test was used to compare sIgE and sIgG4 levels in patients before and after OIT.

Statistical significance was defined as a *P* value < 0.05. All data were analyzed using GraphPad Prism® software, version 6.01 for Windows™ (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com). Tukey box-and-whiskers plots, also obtained with GraphPad Prism®, were used to present results.

RESULTS

Demographic and clinical characterization of pCMPA patients after OIT

The demographic and anthropometric characteristics of both groups are summarized in **Table 1**. At the start of OIT, the mean age of pCMPA was 6.9 ± 3.7 years, with a similar time interval between diagnosis and the initiation of OIT. The median duration of OIT was 5.5 (3.8–8.0) months, and the mean follow-up time after OIT completion was 3.2 ± 2.2 years. Nearly all the patients (86.7%) had accidental exposure to cow's milk before beginning the protocol. No significant differences in age or sex were observed between the patient and control groups.

After OIT, pCMPA children presented alterations in circulating lymphocyte distribution with lower levels of activated T cells

As shown in **Table 2**, the pCMPA-OIT group presented significantly lower levels of circulating B cells and higher levels of NK cells compared to the healthy controls, both in percentage and absolute counts. No significant differences were observed in the levels of $CD4^+CD25^+CD127^{low}$ Tregs between the two groups. However, the pCMPA-OIT group presented significantly lower levels of $CD4^+CD25^+$ and $CD8^+CD25^+$ T cells (**Fig. 3**). Within the pCMPA-OIT group, the duration of cow's milk protein avoidance positively correlated with the percentage of $CD4^+CD25^+$ T cells ($r = 0.57$, $P = 0.001$), whereas OIT duration was negatively correlated with the absolute counts of this subset ($r = -0.44$, $P = 0.016$; **Fig. 4**).

Additionally, a trend toward higher eosinophil counts was observed among patients, although no significant differences were found in other leukocyte subsets between pCMPA patients and healthy controls.

Cow's milk protein sIgEs go below class 3 after OIT, though higher classes may persist, particularly sIgEs to casein

Within the pCMPA group, we have analyzed the serum levels of sIgE, sIgA, and sIgG4 to milk, α-lactalbumin, β-lactoglobulin, and casein allergens both before and after OIT, when available (**Supplementary Table S1**). The sIgE median (IQR) values obtained before OIT were: 14 kU_A/L (2.3–39.4) for milk, 3.1 kU_A/L (0.8–19.7) for α-lactalbumin, 1.7 kU_A/L (0.4–14.2) for β-lactoglobulin, and 4.3 kU_A/L (0.8–33.5) for casein. After OIT, pCMPA children presented median (IQR) values of 1.0 kU_A/L (0.4–4.8) for milk, 0.6 kU_A/L (0.1–1.1) for α-lactalbumin, 0.2 kU_A/L (0.1–0.5) for β-lactoglobulin, and 0.4 kU_A/L (0.2–2.9) for casein. Paired analysis revealed a statistically significant decrease in sIgE levels for all allergens studied following OIT ($P < 0.0001$).

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Table 2. Cellular immune profile comparison between pCMPA and healthy group

Cell subsets	pCMPA group (n = 30)	Healthy group (n = 33)	*P* value
Leukocytes (cells/μL)	7,708 [6,542–8,579]	7,117 [5,924–8,092]	0.266†
Monocytes (%)	7.7 [5.7–9.0]	7.5 [6.8–9.6]	0.294†
Monocytes (cells/μL)	532 [447–660]	532 [477–627]	0.880†
Basophils (%)	0.89 [0.63–1.01]	0.9 [0.7–1.4]	0.485†
Basophils (cells/μL)	64 [55–76]	61 [52–91]	0.793†
Eosinophils (%)	5.7 (3.0–10.5)	4.5 [2.7–6.4]	0.118†
Eosinophils (cells/μL)	389 [243–768]	293 [174–468]	**0.048**†
Neutrophils (%)	46.5 ± 1.1	44.8 ± 11.2	0.605*
Neutrophils (cells/μL)	3,511 [2,704–4,630]	2,995 [2,563–4,087]	0.298†
Lymphocytes (%)	37.9 ± 13.3	40.8 ± 10.3	0.345*
Lymphocytes (cells/μL)	2,893 ± 966	2,859 ± 751	0.879*
T lymphocytes	1,965 ± 694	1,986 ± 535	0.895*
$CD3^+CD4^-CD8^+$ (%)	28.69 ± 6.03	30.2 ± 5.7	0.326*
$CD3^+CD4^-CD8^+$ (cells/μL)	838 ± 338	869 ± 286	0.703*
$CD3^+CD4^+CD8^-$ (%)	39.3 ± 5.2	39.3 ± 6.3	0.993*
$CD3^+CD4^+CD8^-$ (cells/μL)	1,019 [857–1,327]	1,136 [894–1,279]	0.597†
$CD4^+CD25^+$ (%)	17.4 [15.3–21.7]	24.5 [19.6–33.1]	< **0.001**†
$CD4^+CD25^+$ (cells/μL)	205 ± 61	284 ± 87	< **0.001***
$CD8^+CD25^+$ (%)	1.4 [1.1–1.8]	2.4 [1.8–3.3]	< **0.001**†
$CD8^+CD25^+$ (cells/μL)	13 (6–18)	20 [15–31]	< **0.001**†
$CD4^+CD25^+CD127^-$ (%)	8.5 ± 1.6	9.0 ± 2.0	0.321*
$CD4^+CD25^+CD127^-$ (cells/μL)	93 ± 27	98 ± 31	0.530*
B lymphocytes (%)	12.6 [11.2–14.9]	17 [14–20]	< **0.001**†
B lymphocytes (cells/μL)	380 ± 148	471 ± 148	**0.019***
NK cells (%)	17.7 [12.8–24.6]	11 [8.5–17.5]	**0.002**†
NK cells (cells/μL)	473 [373–660]	300 [214–540]	**0.008**†

Values are presented as mean ± standard deviation or median [interquartile range]. All significant results are indicated in bold.
pCMPA, persistent cow's milk protein allergy; NK, natural killer.
*Unpaired *t* test with Welch's correction; †Mann-Whitney *U* test.

Despite this overall reduction, some patients continued to present sIgE levels ≥ class 3 (i.e., > 3.5 kU_A/L), particularly in response to casein. Specifically, 8 out of 30 patients (27%) had sIgE ≥ class 3 for milk, 2/30 for α-lactalbumin (7%), 2/30 for β-lactoglobulin (7%), and 7/30 for casein (23%).

sIgG4 values increase after OIT, and pCMPA patients with detectable sIgA also present increased levels of sIgG4

Regarding sIgG4 levels, after OIT, median (IQR) concentrations for α-lactalbumin, β-lactoglobulin, and casein were 4.3 (1.9–28.7), 3.4 (0.8–14.6), and 4.2 (1.3–13.7) mg_A/L, respectively (**Supplementary Table S1**). Retrospective analysis of pre-OIT clinical records revealed sIgG4 data for casein in 10 pCMPA patients, showing a median (IQR) concentration of 1.0 (0.6–2.0) mg_A/L. Post-OIT levels were significantly higher compared to pre-OIT values ($P = 0.001$).

Specific IgA levels were generally below the limit of detection (< 0.01 mg/L) for all tested cow's milk proteins. However, 8 pCMPA patients exhibited detectable sIgA for at least one component. Compared to pCMPA patients without detectable sIgA, these individuals had significantly higher sIgG4 levels for α-lactalbumin ($P = 0.007$) and β-lactoglobulin ($P = 0.013$), but not for casein. No differences were observed considering the levels of sIgE or their respective classes between the two groups (25% [2/8] of sIgA-positive patients had sIgE class ≥ 3 vs. 22% [4/18] in IgA-negative patients).

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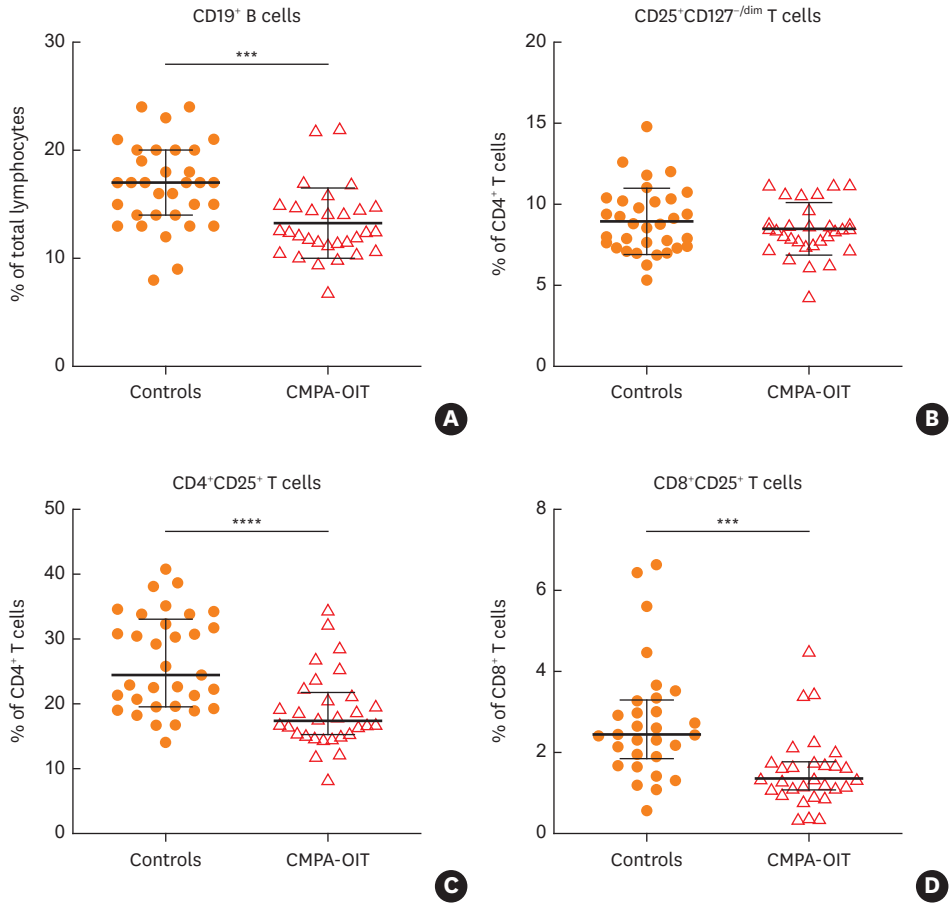

Fig. 3. Cellular immune parameters in pCMPA-OIT and healthy group. (A) Representative scatter dot-plot of $CD19^+$ B cells % of total lymphocytes. (B) Representative scatter dot-plot of $CD4^+CD25^+CD127^{-/dim}$ Tregs % of $CD4^+$ T cells. (C, D) Representative scatter-dot-plots of $CD4^+CD25^+$ T cell and $CD8^+CD25^+$ T cell % of total $CD4^+$ and $CD8^+$ T cells, respective. All comparisons were performed with the Mann-Whitney *U* test (median with interquartile range), except for $CD4^+CD25^+CD127^{-/dim}$ Treg, which were performed using Unpaired *t*-test with Welch's correction (mean with standard deviation).
pCMPA, persistent cow's milk protein allergy; OIT, oral immunotherapy; Treg, regulatory T cell.
$^{***}P < 0.001$, $^{****}P < 0.0001$.

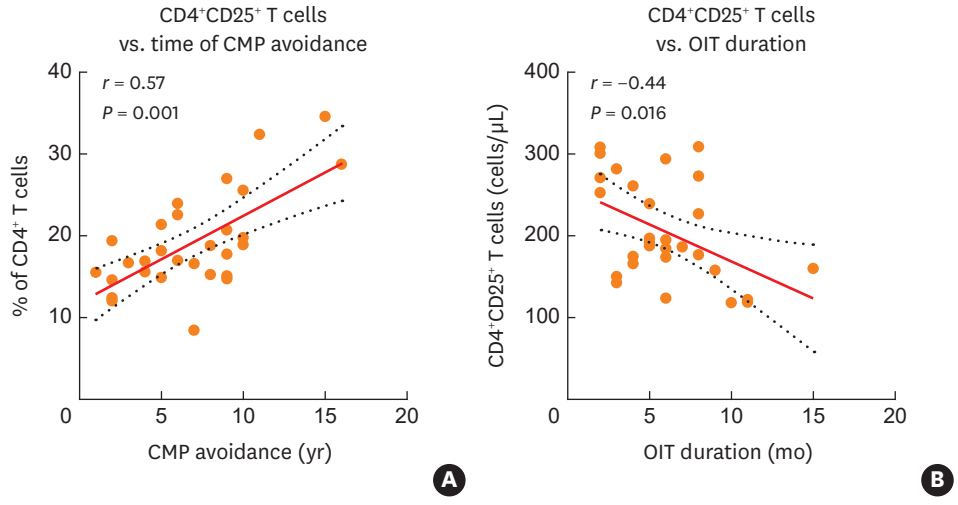

Fig. 4. Correlations between $CD4^+CD25^+$ T cell levels and clinical data parameters within pCMPA-OIT group. Spearman correlation coefficients, 95% confidence interval, and *P* values are indicated (A, n = 29; B, n = 28).
pCMPA, persistent cow's milk protein allergy; OIT, oral immunotherapy; CMP, cow's milk proteins.

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sIgE levels decrease over time after OIT, but distinct sIgEs relate differently with immune parameters

After characterizing the pCMPA-OIT population, we aimed to investigate the interaction between clinical and immune parameters in this population. A significant negative correlation was observed between post-OIT duration and milk-specific sIgE levels ($r = -0.50$, $P = 0.005$) (**Fig. 5A**). Conversely, sIgA levels (measured in fluorescence arbitrary units) for α-lactalbumin ($r = 0.68$, $P < 0.0001$), β-lactoglobulin ($r = 0.69$, $P < 0.001$), and casein ($r = 0.49$, $P = 0.006$) were positively correlated with cow's milk protein avoidance (**Fig. 5B-D**).

Taking into consideration the immune cell parameters, Treg percentages (within $CD4^+$ T cell compartment) showed positive correlations with sIgEs levels to all tested allergens: milk ($r = 0.49$, $P = 0.006$), α-lactalbumin ($r = 0.57$, $P = 0.001$), β-lactoglobulin ($r = 0.45$, $P = 0.013$), and casein ($r = 0.58$, $P = 0.001$). However, sIgEs to individual milk proteins showed distinct associations with other immune cell markers. sIgE and sIgA levels specific to α-lactalbumin and β-lactoglobulin were negatively correlated with B cell counts (**Fig. 5E-H**), while only sIgE levels to casein positively correlated with activated T cells and $CD4^+$ T cells, which may denote different stimulating and tolerance-inducing capacities for distinct allergens.

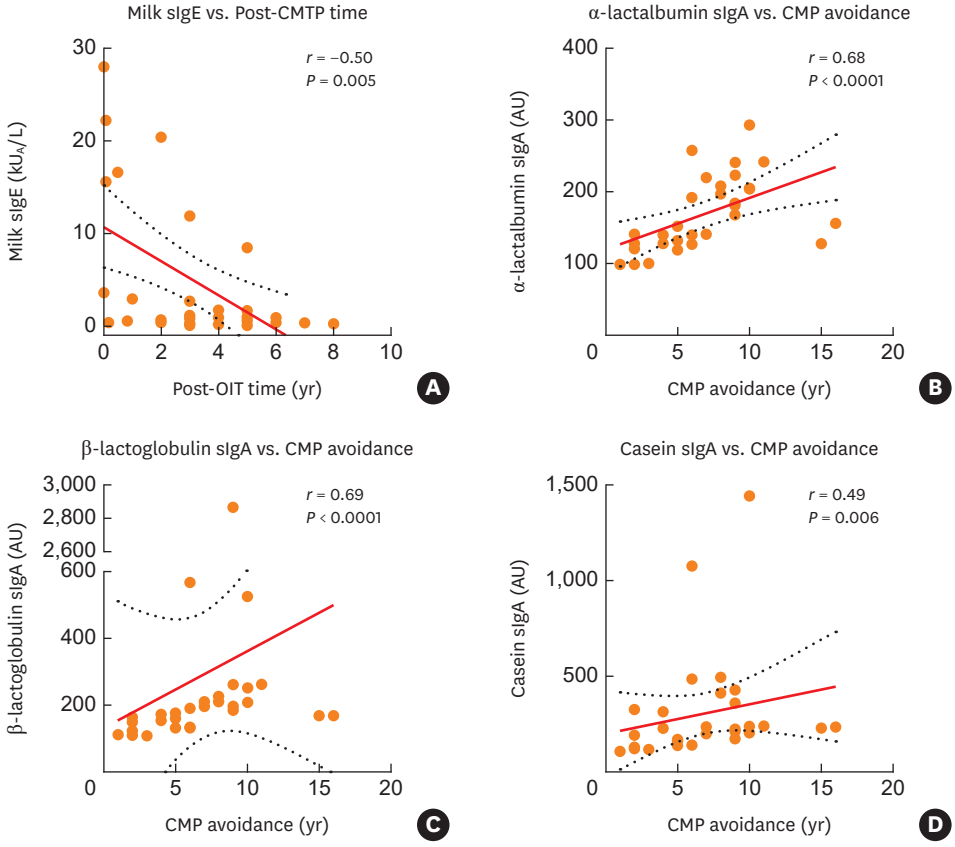

Fig. 5. Correlations between different immune and clinical parameters of pCMPA-OIT group. (A) Correlation between milk sIgE concentration and post-OIP time. (B-D) Correlations between CMP avoidance and levels of sIgA to α-lactalbumin (B), β-lactoglobulin (C), and casein (D) allergens. (E-H) Correlations between B cells counts with levels of sIgEs and sIgAs to α-lactalbumin (E, G), and β-lactoglobulin (F, H) allergens. Respective Spearman correlation coefficients, 95% confidence interval, and *P* values are indicated.
pCMPA, persistent cow's milk protein allergy; OIT, oral immunotherapy; sIg, specific immunoglobulin; CMP, cow's milk proteins; AU, arbitrary units.

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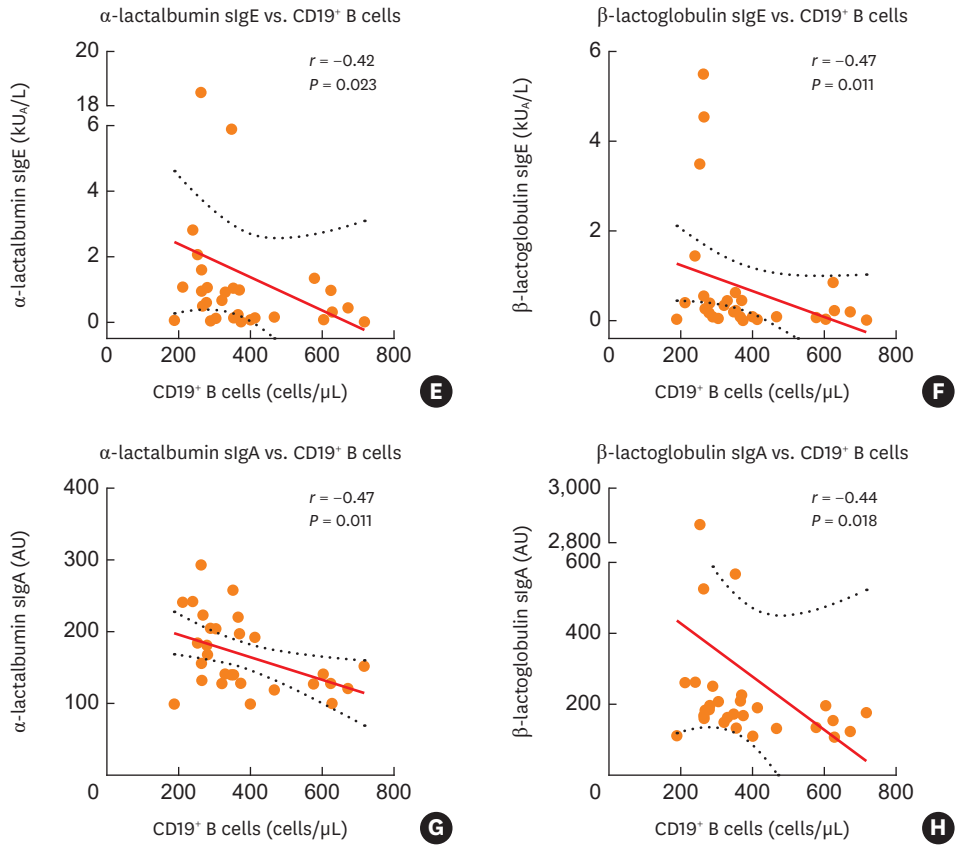

Fig. 5. (Continued) Correlations between different immune and clinical parameters of pCMPA-OIT group. (A) Correlation between milk sIgE concentration and post-OIP time. (B-D) Correlations between CMP avoidance and levels of sIgA to α-lactalbumin (B), β-lactoglobulin (C), and casein (D) allergens. (E-H) Correlations between B cells counts with levels of sIgEs and sIgAs to α-lactalbumin (E, G), and β-lactoglobulin (F, H) allergens. Respective Spearman correlation coefficients, 95% confidence interval, and *P* values are indicated.
pCMPA, persistent cow's milk protein allergy; OIT, oral immunotherapy; sIg, specific immunoglobulin; CMP, cow's milk proteins; AU, arbitrary units.

pCMPA-OIT patients with sIgE class ≥ 3, with shorter post-OIT duration, present decreased B and T cells counts

Given that some patients still presented sIgE levels > 3.5 kU_A/L after OIT, we assessed whether these patients presented distinctive clinical or immune features. Therefore, we divided pCMPA-OIT patients into two subgroups: those with at least one sIgE ≥ class 3 (n = 8) after OIT and those with all sIgE < class 3 (n = 22). No differences were found between subgroups in terms of age, sex, or clinical history (including age at diagnosis, OIT start/end, OIT duration, or cow's milk protein avoidance time). The only clinical difference was post-OIT duration, which was significantly longer in the group with sIgE < class 3 ($P = 0.006$; **Fig. 6A**), in agreement with the previously described negative correlation between post-OIT time and milk sIgE (**Fig. 5A**).

As for the immune cell profile, patients with persistent sIgE ≥ class 3 exhibited significantly reduced total lymphocyte counts ($P = 0.008$), $CD4^+$ T cells ($P = 0,013$), $CD8^+$ T cells ($P = 0.034$), and $CD19^+$ B cells ($P = 0.013$) compared to the subgroup with all sIgEs < class 3 (**Fig. 6B**). No further significant differences were observed in other immune cell populations or immunoglobulin levels.

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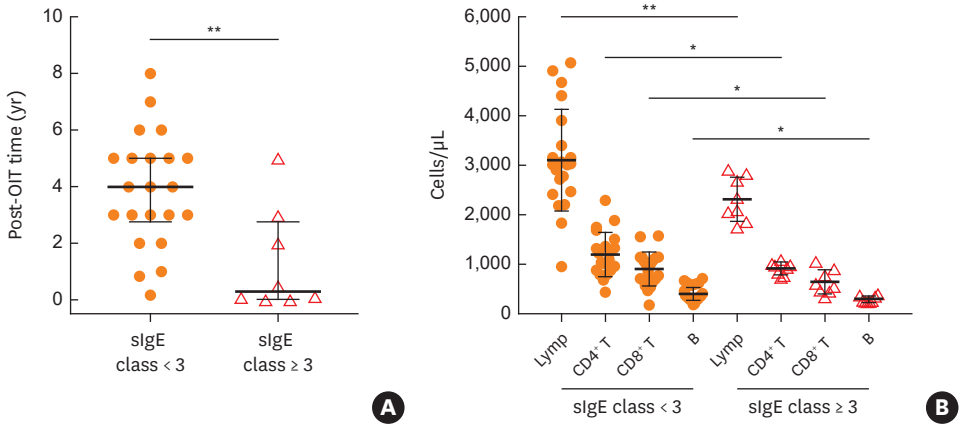

Fig. 6. Comparison of immune and clinical parameters according to sIgE class after OIT. (A) Representative scatter dot-plot of lymphocytes, $CD4^+$ and $CD8^+$ T cell, and B cell counts. (B) Representative scatter dot-plot of post-OIT time. sIgE class < 3 group indicates patients (n = 22) with all sIgE below class 3 (< 3.5 kU_A/L), while sIgE class ≥ 3 group indicates patients (n = 22) with at least one sIgE class ≥ 3 (≥ 3.5 kU_A/L). All comparisons were performed with unpaired *t*-test (mean with standard deviation), except for post-OIT time, which was performed using Mann-Whitney *U* test (median with interquartile range).
sIg, specific immunoglobulin; OIT, oral immunotherapy.
$^*P < 0.05$, $^{**}P < 0.01$.

The impact of age: immune parameters behave differently after OIT in pCMPA patients with ≤ 10 and > 10 years

Given the dynamic nature of immune development, particularly the circulating lymphoid compartment during childhood and adolescence, we assessed age-related influences by comparing pCMPA-OIT patients ≤ 10 years and > 10 years of age (**Fig. 7**). Younger patients presented significantly higher absolute counts of lymphocytes, $CD4^+$ and $CD8^+$ T cells, and B cells (**Fig. 7A**), along with lower percentages of Tregs and activated $CD4^+$ T cells (**Fig. 7B**).

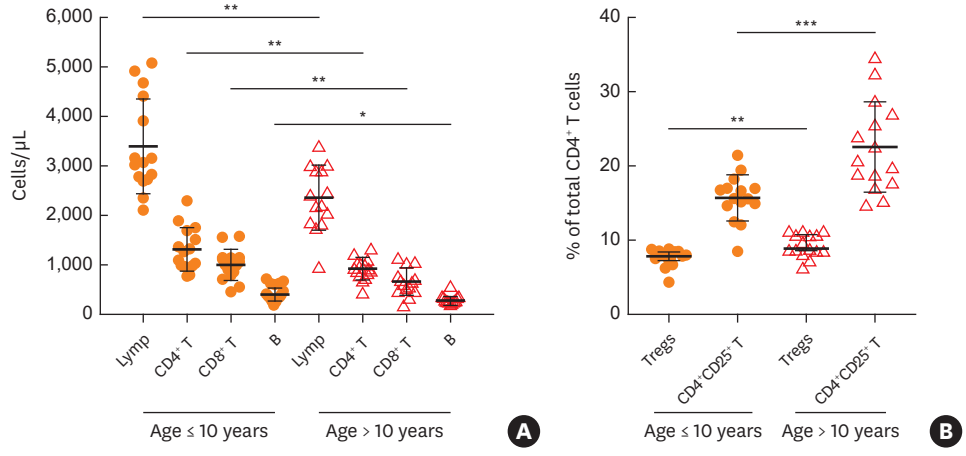

Fig. 7. Comparison of immune parameters according to patients age after OIT. (A) Representative scatter dot-plot of lymphocytes, $CD4^+$ and $CD8^+$ T cells, and B cell counts. (B) Representative scatter dot-plot of $CD4^+CD25^+CD127^{-/dim}$ Tregs and $CD4^+CD25^+$ T cell percentages within total $CD4^+$ T cells. Age ≤ 10 years group indicates patients (n = 15) with age equal or below 10 years old, while age > 10 years group indicates patients (n = 15) with age above 10 years. All comparisons were performed with unpaired *t*-test with Welch's correction (mean with standard deviation), except for B and Tregs which were performed with Mann-Whitney *U* test (median with interquartile range).
OIT, oral immunotherapy; Treg, regulatory T cell.
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

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When stratified by age and compared to respective age-matched healthy controls, both age groups of pCMPA-OIT patients showed decreased percentages and absolute counts of activated $CD4^+$ ($P \leq 0.023$) and $CD8^+$ ($P \leq 0.020$) T cells. However, only pCMPA-OIT patients > 10 years showed significantly lower absolute counts of total T cells ($P = 0.032$), $CD4^+$ T cells ($P = 0.035$), and B cells ($P = 0.020$) compared to their respective age-matched healthy control subgroup, consistent with trends observed in the overall cohort. In contrast, only pCMPA-OIT patients ≤ 10 years exhibited decreased B cell percentages ($P < 0.001$) alongside increased NK cell percentages ($P = 0.017$) and absolute counts ($P = 0.003$), suggesting age-dependent modulation of immune responses following OIT.

DISCUSSION

This study assessed the immune profile of 30 children with pCMPA who successfully completed a cow's milk-OIT protocol, in comparison to 33 healthy controls. OIT has been increasingly adopted in clinical practice for the long-term management of food allergies, supported by international guidelines.[3,14,15,33,34]

Our data revealed significant lymphocyte alterations following OIT, including lower circulating B cell counts and lower levels of sIgE and sIgA, but not sIgG4. One possible explanation is that OIT may impact B cell production in the bone marrow or influence B cell differentiation, thereby limiting plasma cell differentiation and the production of sIgE and sIgA. In both scenario, decreased immunoglobulin levels, particularly sIgEs levels, could represent a mechanism through which OIT facilitates tolerance induction.[28-30]

Interestingly, we observed a negative correlation between B cell counts and the levels of sIgE and sIgA. To our knowledge, this is the first study reporting these findings. These results support the hypothesis that OIT interferes with B cell maturation and differentiation. Future studies should focus on characterizing B cell subsets, including surface immunoglobulin expression before, during, and after OIT, which may clarify the role of B cells, and possibly Bregs, in tolerance induction.[23]

Regarding humoral responses, a reduction in allergen-specific IgE has been widely associated with tolerance.[28-30] In fact, although some studies have reported no changes in sIgE after OIT,[13,35] most studies, including ours, show a decrease in cow's milk-specific sIgE levels after OIT.[9,11,12,36] Notably, our data also demonstrated a significant increase in sIgG4 after OIT, which reinforces our results.[36] Moreover, the inverse correlation between post-OIT time and cow's milk-sIgE levels suggests increased efficacy of treatment throughout time. Similar results were reported by other authors, who found a significant decrease of sIgE levels to cow's milk proteins years after initiating OIT.[31,37] Interestingly, this time-dependent decline in sIgE levels suggests that shorter OIT protocols may be less effective in inducing lasting tolerance. Indeed, this could potentially explain the similar pre- and post-OIT levels of sIgE observed in some studies using shorter protocols.[38]

In addition to humoral changes, our data also support the notion that tolerance achievement is a dynamic immunological process involving distinct cellular populations. In fact, despite the importance of sIgE in allergy, tolerance generation and preservation, regulatory cells and cytokines may play more central roles. In our study, the frequency of Tregs did not differ between pCMPA-OIT and healthy controls. Previous work by Akdis *et al.*[39] demonstrated

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that healthy adult individuals exhibit higher frequencies of allergen-specific Tregs, whereas allergic individuals display elevated frequencies of T helper type 2 (Th2) effector cells. In fact, our data align with those of Syed *et al.*,[40] who reported an increase in allergen-specific $CD4^+CD25^{hi}FOXP3^+$ suppressive cells after peanut OIT. On the other hand, Tregs isolated from the blood of allergic patients proved to be less functional than Tregs purified from healthy subjects.[39] However, due to practical and financial limitations, we could not assess Treg function, which constitutes one limitation of this study. Functional studies are essential to clarify whether OIT interferes with both the generation and function of Tregs and Bregs.

The main effector phases of allergy are primarily driven by antigen-specific activated Th2 cells, which promote eosinophil activation and recruitment, and B cell production of allergen-sIgE. In our cohort, we observed lower levels of activated T cells in pCMPA-OIT compared to healthy controls, suggesting that OIT may promote a reduction in T cell activation and consequently influencing B cell humoral responses and eosinophil compartment, further supported by the lack of differences in these cells between the groups.

We also observed that children with longer OIT duration presented reduced $CD4^+CD25^+$ activated T cell levels. Several studies have documented that prolonged OIT protocols enhance the desensitization effect, suggesting that longer treatment courses are more effective and possibly safer.[41-44] Our findings possibly indicate that extended treatment may be more effective in inhibiting T cell activation, therefore supporting sustained tolerance. Notably, children with prolonged periods of cow's milk avoidance showed increased activated $CD4^+$ T cells percentages (data not shown), emphasizing that early OIT initiation may provide clinical and immunological benefits, allowing better quality of life. In addition, the avoidance of cow's milk protein intake also seems to promote the generation of sIgA, though its role is still unclear.[43]

Interestingly, 8 patients still exhibited at least one sIgE level ≥ class 3 after OIT. While no differences in immunoglobulin levels were observed between groups, these patients had significantly lower lymphocytes, B cells, $CD4^+$ and $CD8^+$ T cell counts compared to those with all sIgEs < class 3. Whether any special subset could be secondarily altered or have an impaired function that could justify a more evident humoral response in these patients remains to be investigated. Importantly, children with sIgE < class 3 had longer post-OIT durations, again pointing to time as a factor in tolerance achievement and potentially serving as a marker for treatment efficacy that should be further investigated and validated.

Moreover, we observed different immune responses to distinct milk proteins. For example, casein-specific sIgEs tended to remain elevated post-OIT, and immune parameters correlated differently with sIgEs to α-lactalbumin, β-lactoglobulin, and casein. These variations may denote different stimulating and tolerance-inducing capacities of individual components of cow's milk. It should be noted that the post-OIT follow-up time varied widely (up to 8 years), which may have introduced heterogeneity in the immune profiles observed. Nevertheless, the negative correlation between post-OIT time and milk-sIgE levels suggests that longer follow-up was associated with more pronounced immunological changes.

We also investigated whether age would have any influence on the cellular immune profile in pCMPA children after OIT. As expected, younger children (≤ 10 years) exhibited increased absolute counts of lymphocytes, $CD4^+$ and $CD8^+$ T cells, and B cells, while older children (> 10 years) had increased percentages of activated $CD4^+$ T cells and Tregs. Age-matched analysis

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further revealed distinct immunologic alterations across age groups, indicating that age can also be a modulating factor for immune responses during tolerance induction.

We are aware of the limitations of our study, mainly marked by the lack of longitudinal immune cell data before and during OIT protocols, which prevents us from assessing these kinetics within the patient group. Such data would have contributed to a better understanding of the dynamics of immune modulation. Additionally, we did not investigate whether permanent oral tolerance was achieved, which is established when ingested food does not trigger any allergic symptoms, despite prolonged periods of avoidance.[16] However, despite the relatively small sample size, statistically significant differences were identified. In fact, regarding our complete study population, we acknowledge that 30 patients are a small cohort. Nevertheless, we managed to assess our patients with age-matched controls and therefore ameliorate this possible limitation. Moreover, other studies that seek to characterize the immune profile in the context of OIT have used a similar number of patients, and our findings are consistent with those from previous reports.[36,43]

Quantification of sIgA was particularly difficult due to its low serum concentrations (< 0.01 mg/L). To overcome this, we analyzed both fluorescence levels obtained in each sample and the presence/absence of detectable sIgA. Patients with detectable sIgA had higher sIgG4 levels to α-lactalbumin and β-lactoglobulin, suggesting a possible relation between these two immunoglobulin classes. Previous studies identifying sIgG4 and sIgA as relevant immune players in both natural and induced tolerance processes support our findings.[45-48] Moreover, we may advocate that those patients with higher levels of both cow's milk proteins sIgG4 and sIgA may have undergone a more effective tolerance induction with lower risk for allergic reactions, as the binding of allergens to sIgE may be compromised. Future studies should investigate whether these serum levels could serve as biomarkers, both of successful tolerance achievement and of an increased risk of unwanted reactions when their levels remain low after OIT. In addition, assessing salivary sIgA levels specific to cow's milk proteins may provide a more reliable biomarker, given the low concentrations typically detected in serum. Lastly, the use of healthy controls without any atopic condition constitutes another limitation, as some immunologic differences might reflect atopy rather than OIT effects. Future studies should include allergic or atopic controls to better isolate the immunological impact of OIT.

Additional studies should aim to evaluate larger cohorts with more appropriate controls and include detailed characterization of circulating T and B cell compartments, including regulatory subsets, surface immunoglobulin expression, and functional assays. Correlation of these parameters with sIgE, sIgA and sIgG4 levels across multiple time points (before, during, and after OIT protocols) will be essential. Moreover, comparisons with pCMPA patients who do not undergo OIT could provide crucial insights into OIT-specific immune modulation.

In summary, beyond the well-recognized reduction of sIgE and increase of sIgG4 levels following OIT, our study identifies several novel immunological features that may contribute to a deeper understanding of tolerance induction. First, the observed negative correlation between B cell counts and sIgE/sIgA levels suggests that B cell maturation and differentiation could represent key mechanisms through which OIT modulates humoral immunity. Second, patients who maintained high sIgE levels (≥ class 3) after OIT exhibited a distinct immune profile, characterized by lower total lymphocytes, with lower $CD4^+$ and

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CD8⁺ T cell and B cell counts, pointing to potential cellular determinants of incomplete desensitization. Third, the heterogeneous associations of immune parameters with specific milk proteins, particularly the distinct behaviour of casein compared to α-lactalbumin and β-lactoglobulin, highlight that not all milk allergens drive tolerance in the same way and may carry different immunomodulatory potential. Finally, the age-stratified analyses revealed that immune modulation after OIT differs between younger and older children, underscoring the importance of age as a factor influencing both clinical and immunologic outcomes. Collectively, these findings provide novel insights into the interplay between humoral and cellular immunity, the relevance of treatment duration, and the heterogeneity of allergen-specific responses, and may ultimately inform the identification of biomarkers and the refinement of OIT protocols in clinical practice.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1

Immunoglobulin characterization of the pCMPA group before and after OIT

REFERENCES

1. Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, et al. Food allergy: a practice parameter update-2014. J Allergy Clin Immunol 2014;134:1016-1025.e43. PUBMED | CROSSREF
2. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;56:813-24. PUBMED | CROSSREF
3. Brozek JL, Firmino RT, Bognanni A, Arasi S, Ansotegui I, Assa'ad AH, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guideline update - XIV - recommendations on CMA immunotherapy. World Allergy Organ J 2022;15:100646. PUBMED | CROSSREF
4. Jo J, Garssen J, Knippels L, Sandalova E. Role of cellular immunity in cow's milk allergy: pathogenesis, tolerance induction, and beyond. Mediators Inflamm 2014;2014:249784. PUBMED | CROSSREF
5. Savilahti EM, Savilahti E. Development of natural tolerance and induced desensitization in cow's milk allergy. Pediatr Allergy Immunol 2013;24:114-21. PUBMED | CROSSREF
6. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. J Allergy Clin Immunol 1999;103:717-28. PUBMED | CROSSREF
7. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 2004;59:980-7. PUBMED | CROSSREF
8. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. J Allergy Clin Immunol 2008;122:1154-60. PUBMED | CROSSREF
9. Martorell A, De la Hoz B, Ibáñez MD, Bone J, Terrados MS, Michavila A, et al. Oral desensitization as a useful treatment in 2-year-old children with cow's milk allergy. Clin Exp Allergy 2011;41:1297-304. PUBMED | CROSSREF
10. Calligaris L, Longo G, Badina L, Berti I, Barbi E. Cow's milk allergy in children, from avoidance to tolerance. Endocr Metab Immune Disord Drug Targets 2014;14:47-53. PUBMED | CROSSREF

11. Mota I, Piedade S, Gaspar Â, Benito-Garcia F, Sampaio G, Borrego LM, et al. Cow's milk oral immunotherapy in real life: 8-year long-term follow-up study. Asia Pac Allergy 2018;8:e28. PUBMED | CROSSREF
12. Alves-Correia M, Gaspar Â, Borrego LM, Azevedo J, Martins C, Morais-Almeida M. Successful oral desensitization in children with cow's milk anaphylaxis: clinical and laboratory evaluation up to nine-years follow-up. Allergol Immunopathol (Madr) 2019;47:133-40. PUBMED | CROSSREF
13. García-Ara C, Pedrosa M, Belver MT, Martín-Muñoz MF, Quirce S, Boyano-Martínez T. Efficacy and safety of oral desensitization in children with cow's milk allergy according to their serum specific IgE level. Ann Allergy Asthma Immunol 2013;110:290-4. PUBMED | CROSSREF
14. Muraro A, de Silva D, Halken S, Worm M, Khaleva E, Arasi S, et al. Managing food allergy: GA²LEN guideline 2022. World Allergy Organ J 2022;15:100687. PUBMED | CROSSREF
15. Bognanni A, Chu DK, Firmino RT, Arasi S, Waffenschmidt S, Agarwal A, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guideline update - XIII - Oral immunotherapy for CMA - systematic review. World Allergy Organ J 2022;15:100682. PUBMED | CROSSREF
16. Wambre E, Jeong D. Oral tolerance development and maintenance. Immunol Allergy Clin North Am 2018;38:27-37. PUBMED | CROSSREF
17. Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004.113:805-19. PUBMED
18. Smith KM, Eaton AD, Finlayson LM, Garside P. Oral tolerance. Am J Respir Crit Care Med 2000;162:S175-8. PUBMED | CROSSREF
19. Turner JA, Stephen-Victor E, Wang S, Rivas MN, Abdel-Gadir A, Harb H, et al. Regulatory T cell-derived TGF-β1 controls multiple checkpoints governing allergy and autoimmunity. Immunity 2020;53:1202-1214.e6. PUBMED | CROSSREF
20. Zhang X, Izikson L, Liu L, Weiner HL. Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. J Immunol 2001;167:4245-53. PUBMED | CROSSREF
21. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 2004;199:1679-88. PUBMED | CROSSREF
22. Tiemessen MM, Van Hoffen E, Knulst AC, Van Der Zee JA, Knol EF, Taams LS. CD4 CD25 regulatory T cells are not functionally impaired in adult patients with IgE-mediated cow's milk allergy. J Allergy Clin Immunol 2002;110:934-6. PUBMED | CROSSREF
23. Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol 2012;30:221-41. PUBMED | CROSSREF
24. Noh J, Noh G, Lee SJ, Lee JH, Kim A, Kim HS, et al. Tolerogenic effects of interferon-gamma with induction of allergen-specific interleukin-10-producing regulatory B cell (Br1) changes in non-IgE-mediated food allergy. Cell Immunol 2012;273:140-9. PUBMED | CROSSREF
25. Noh J, Lee JH, Noh G, Bang SY, Kim HS, Choi WS, et al. Characterisation of allergen-specific responses of IL-10-producing regulatory B cells (Br1) in Cow Milk Allergy. Cell Immunol 2010;264:143-9. PUBMED | CROSSREF
26. Lee JH, Noh J, Noh G, Choi WS, Cho S, Lee SS. Allergen-specific transforming growth factor-β-producing CD19+CD5+ regulatory B-cell (Br3) responses in human late eczematous allergic reactions to cow's milk. J Interferon Cytokine Res 2011;31:441-9. PUBMED | CROSSREF
27. Thyagarajan A, Jones SM, Calatroni A, Pons L, Kulis M, Woo CS, et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. Clin Exp Allergy 2012;42:1197-205. PUBMED | CROSSREF
28. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. J Allergy Clin Immunol 2009;124:292-300. PUBMED | CROSSREF
29. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med 2012;367:233-43. PUBMED | CROSSREF
30. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. J Allergy Clin Immunol 2013;131:128-134.e1-3. PUBMED | CROSSREF
31. Martorell Aragonés A, Félix Toledo R, Cerdá Mir JC, Martorell Calatayud A. Oral rush desensitization to cow milk. Following of desensitized patients during three years. Allergol Immunopathol (Madr) 2007;35:174-6. PUBMED | CROSSREF
32. Morais-Almeida M, Piedade S, Couto M, Sampaio G, Santa-Marta C, Gaspar Â. Innovation in specific oral tolerance induction in children with anaphylaxis to cow's milk proteins. Rev Port Imunoalergol 2011;19:161-9.

33. Martorell A, Alonso E, Echeverría L, Escudero C, García-Rodríguez R, Blasco C, et al. Oral immunotherapy for food allergy: a Spanish guideline. J Investig Allergol Clin Immunol 2017;27:225-37. PUBMED | CROSSREF

34. Pajno GB, Fernandez-Rivas M, Arasi S, Roberts G, Akdis CA, Alvaro-Lozano M, et al. EAACI Guidelines on allergen immunotherapy: IgE-mediated food allergy. Allergy 2018;73:799-815. PUBMED | CROSSREF

35. Nurmatov U, Devereux G, Worth A, Healy L, Sheikh A. Effectiveness and safety of orally administered immunotherapy for food allergies: a systematic review and meta-analysis. Br J Nutr 2014;111:12-22. PUBMED | CROSSREF

36. Perezábad L, Reche M, Valbuena T, López-Fandiño R, Molina E, López-Expósito I. Oral food desensitization in children with IgE-mediated cow's milk allergy: immunological changes underlying desensitization. Allergy Asthma Immunol Res 2017;9:35-42. PUBMED | CROSSREF

37. Salguero CAS, Chacón ÁIS. Immunological changes in specific oral tolerance induction for cow's milk allergy. Int J Allergy Medicat 2016;2:018.

38. Pajno GB, Caminiti L, Ruggeri P, De Luca R, Vita D, La Rosa M, et al. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. Ann Allergy Asthma Immunol 2010;105:376-81. PUBMED | CROSSREF

39. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004;199:1567-75. PUBMED | CROSSREF

40. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). J Allergy Clin Immunol 2014;133:500-10. PUBMED | CROSSREF

41. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. Allergy 2007;62:1261-9. PUBMED | CROSSREF

42. Narisety SD, Skripak JM, Steele P, Hamilton RG, Matsui EC, Burks AW, et al. Open-label maintenance after milk oral immunotherapy for IgE-mediated cow's milk allergy. J Allergy Clin Immunol 2009;124:610-2. PUBMED | CROSSREF

43. Savilahti EM, Kuitunen M, Savilahti E, Mäkelä MJ. Specific antibodies in oral immunotherapy for cow's milk allergy: kinetics and prediction of clinical outcome. Int Arch Allergy Immunol 2014;164:32-9. PUBMED | CROSSREF

44. Meglio P, Giampietro PG, Carello R, Gabriele I, Avitabile S, Galli E. Oral food desensitization in children with IgE-mediated hen's egg allergy: a new protocol with raw hen's egg. Pediatr Allergy Immunol 2013;24:75-83. PUBMED | CROSSREF

45. Sackesen C, Altintas DU, Bingol A, Bingol G, Buyuktiryaki B, Demir E, et al. Current trends in tolerance induction in cow's milk allergy: from passive to proactive strategies. Front Pediatr 2019;7:372. PUBMED | CROSSREF

46. Sommanus S, Kerddonfak S, Kamchaisatian W, Vilaiyuk S, Sasisakulporn C, Teawsomboonkit W, et al. Cow's milk protein allergy: immunological response in children with cow's milk protein tolerance. Asian Pac J Allergy Immunol 2014;32:171-7. PUBMED | CROSSREF

47. Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clin Immunol 1999;104:1251-7. PUBMED | CROSSREF

48. Orivuori L, Loss G, Roduit C, Dalphin JC, Depner M, Genuneit J, et al. Soluble immunoglobulin A in breast milk is inversely associated with atopic dermatitis at early age: the PASTURE cohort study. Clin Exp Allergy 2014;44:102-12. PUBMED | CROSSREF