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Investigation of serum IL-12, IL-16, and IL-17A as diagnostic biomarkers in children with cow's milk protein allergy

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Abstract

Background: Cow's milk protein allergy (CMPA) is an abnormal immune response caused by milk proteins and is most common in infancy and early childhood. Statistics revealed up to 7.5% of children suffered from milk allergy. Its clinical symptoms were characterized by diversity, non-specificity, and can affect multiple systems, including the digestive tract, skin, and respiratory tract. In this study, we aimed to investigate the effects of IL-12, IL-16, and IL-17A on diagnosing and monitoring CMPA in children for clinical treatment.

Methods: A total of 158 infants with CMPA and 89 healthy babies were recruited and evaluated. Demographic and clinical information of all participants were recorded. An extensive analysis of inflammatory cytokine levels, including IL-12, IL-16, and IL-17A, was performed in blood samples from 247 infants younger than 9 months. Meanwhile, the serological specificity immunoglobulin E (sIgE) levels were evaluated. In addition, the area under the curve (AUC) values of IL-12, IL-16, and IL-17A in differentiating CMPA from healthy babies were measured by receiver operating characteristic analysis. Finally, the correlation between sIgE and IL-12, IL-16, and IL-17A levels were detected using Spearman correlation analysis.

Results: Compared with healthy control, infants who developed CMPA had decreased IL-12, increased IL-16, and IL-17A. Moreover, a significant correlation between serum IL-12, IL-16, IL-17A and sIgE levels was observed in the CMPA group. In addition, AUC values of IL-12, IL-16, and IL-17A in discriminating CMPA from healthy infants were 0.8425, 0.9196, and 0.8813, respectively. Finally, IL-12 was increased while IL-16 and IL-17A levels were decreased in the CMPA group after three months of milk avoidance treatment.

Conclusions: We found that IL-12, IL-16, and IL-17A levels in children with CMPA were associated with SCORAD scores, sIgE levels, and disease severity, functioning as valuable disease-monitor markers in CMPA.

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Introduction

Food allergy is an adverse reaction to a specific food mediated by an immune mechanism and repeatedly occurs.¹⁻⁵

Although any food can trigger allergic reactions, common food allergens include eggs, milk, peanut, other nuts, shellfish, wheat, and soy can cause severe allergic reactions,⁶⁻⁸ among them, allergy to milk was defined as cow's milk protein allergy (CMPA), the most common food allergy disease in children.⁹ It has been reported internationally that about 0.6%-0.9% of infants suffered from CMPA,¹⁰ and in some developing countries, the incidence even reached 2-3%.¹¹ Milk was the main source of food and nutrition for infants and young children, and CMPA might affect their dietary sources and growth. The treatment principle for CMPA was to avoid cow's milk protein and switch to a hypoallergenic formula, supporting children's growth while waiting for tolerance to develop.

Up to date, the incidence of CMPA was increasing, which may be due to a decrease in breastfeeding and increase in milk-based formula feeding. Most children with CMPA presented with mild allergic symptoms, including urticaria, vomiting, cough, and dizziness.¹² However, in a few cases, some children had more severe clinical allergic reactions, such as anaphylaxis.¹³ Approximately 60% of milk-induced adverse reactions were caused by immunoglobulin E (IgE), affecting one or more organs.¹⁴ The typical symptoms of IgE-mediated CMPA often appeared within 1-2 h of milk ingestion. Conventional CMPA diagnostic methods included avoidance-provocation test, serological specificity immunoglobulin E (sIgE) testing, skin prick testing (SPT), and patch testing.¹⁵ These tests were mainly based on crude milk extracts as capture antigens. The tests' diagnostic specificity based on crude extracts as capture antigens were insufficient due to the presence of allergenic and non-allergenic components in the crude extracts.¹⁶ Due to the possible absence of allergenic ingredients in the extraction process, the interference of non-allergenic components in the tests, and the possibility that some allergenic components may act as pan-allergens, the accuracy and specificity of diagnosis remained to be promoted.¹⁷

CMPA, as an allergic disease, was often associated with chronic inflammation characterized by infiltration and aggregation of eosinophils, T cells, and mast cells.¹⁸ For example, it has been reported that serum expression of IL-10 were markedly decreased CMPA children in comparison with the healthy controls;¹⁹ Furthermore, in children with CMPA, it was observed that the levels of TNF α , IL-6, IL-12 (Th1), IL-4, IL-10, IL-13 (Th2), and IL-17 were higher in the rectum than duodenum.²⁰ In this study, we aimed to investigate the roles of three inflammatory cytokines, IL-12, IL-16, and IL-17A in CMPA diagnosis and treatment response.

Methods

Participant recruitment and sample processing

The diagnosis of CMPA was based on the positive results of cow's protein avoidance-provocation test as previously

described.²¹ CMPA symptoms included the persistent distress or colic, gastrointestinal disorders, stools blood, chronic cough, wheezing and dermatological disorders. Infants <9 months of age who attended the pediatric gastroenterology outpatient clinic were recruited from the First People's Hospital of Lianyungang, Xuzhou Medical University Affiliated Hospital of Lianyungang, the First Affiliated Hospital of Kangda College of Nanjing Medical University between May 2017 and July 2019. The Ethics Committee of the First People's Hospital of Lianyungang, Xuzhou Medical University Affiliated Hospital of Lianyungang, the First Affiliated Hospital of Kangda College of Nanjing Medical University approved this study. Demographic information and clinical parameters were recorded in Table 1. Healthy controls were included as follows: (1) age <9 months at the time of consultation; (2) full-term parity, singleton, birth weight 2.5-4.0 kg, no maternal risk factors during pregnancy; (3) artificial feeding (regular infant formula); (4) born in the First People's Hospital of Lianyungang, Xuzhou Medical University Affiliated Hospital of Lianyungang, the First Affiliated Hospital of Kangda College of Nanjing Medical University hospital or Lianyungang city; (5) guardians agreed to participate in this study and signed informed consent forms. Exclusion criteria of all subjects included: (1) congenital genetic proneness; (2) congenital malformations, structural abnormalities of the digestive tract; (3) acute infectious diseases in the last 1 month. Blood samples (5 mL/infant) of the CMPA patients and healthy controls were taken from all subjects after receiving informed consent.

sIgE measurement and evaluation of serum cytokines

According to the manufacturer's instructions, serological specificity immunoglobulin E (sIgE) levels were determined by ImmunoCAP100 system (Phadia, Uppsala, Sweden).

The IL-12, IL-16, and IL-17A enzyme-linked immunosorbent assay (ELISA, all purchased from Beyotime, Shanghai, China) kits were used to detect on microtiter plates following the protocol. Blood samples in the CMPA group were collected at the time of CMPA diagnosis and after three months of not drinking milk to determine the response of IL-12, IL-16, and IL-17A to CMPA, respectively.

Table 1 Demographic and clinical information of all subjects

Variables	CMA group (n = 158)	Healthy controls (n = 89)	P value
Age (months)	2.9 \pm 2.4	2.5 \pm 3.1	0.2599
Gender			0.5067
Male	69	35	
Female	89	54	
Weight (kg)	5.5 \pm 1.1	5.8 \pm 1.3	0.0554
Family allergic history	87	10	<0.0001
sIgE (UI/mL)	375.5 \pm 11.6	35.8 \pm 7.5	<0.0001

CMA, cow's milk allergy; sIgE, serum immunoglobulin E

Procedures of CMPA avoidance-provocation test

Cow's protein avoidance-provocation test was performed as previously described.¹⁹ All participants fasted for at least 2 h before the test. One drop of milk was put on the children's mouths and lips, and they observed any allergic symptoms. If there was no reaction after 15 min, the amount was increased every 20 min, gradually increasing the amount to 3, 10, 30, and 100 mL. The children's vital signs, skin, respiratory, and gastrointestinal reactions were observed. If no allergic symptoms occurred, the children could go home and continue to be observed for 1 to 2 weeks, eating at least 250 mL of the milk used in the provocation test daily, without adding new foods or using anti-allergic drugs and having the parents keep dietary records. If any allergy-related symptoms occurred, they should be reported to the hospital promptly so that the doctor can evaluate and determine whether it was CMPA. If the child did not show any allergic symptoms after 2 weeks, CMPA could be ruled out.

Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0; IBM, New York). The Kolmogorov-Smirnov test was performed to determine the distribution of variables. Non-parametric Mann-Whitney test was used for comparison between groups. Data are shown as mean \pm standard error of the mean (SEM). For categorical variables,

chi-square test was applied for the comparison. Spearman correlation test established the correlation between variables. ROC curves analyzed the capacity of discrimination between groups for the different variables. GraphPad PRISM (version 7.0, GraphPad Software, San Diego, CA, USA) was applied for drawing graphs. P-value <0.05 was considered significant.

Results

Demographic and clinical information of all enrolled infants

Basic information from 158 CMPA and 89 healthy control children were recorded and compared in Table 1. As shown in Table 1, there were no significant differences in age, gender, and weight between the two groups. However, allergic family history and sIgE indexed presented significant differences between CMPA and healthy control.

Decreased IL-12 and elevated IL-16 and IL-17A expressions in CMPA group

To measure serum concentrations of IL-12, IL-16, and IL-17A in two groups, ELISA assays were conducted. As demonstrated in Figure 1A-C, IL-12 was reduced whereas IL-16 and IL-17A were significantly elevated in serum samples from CMPA infants compared with healthy control infants.

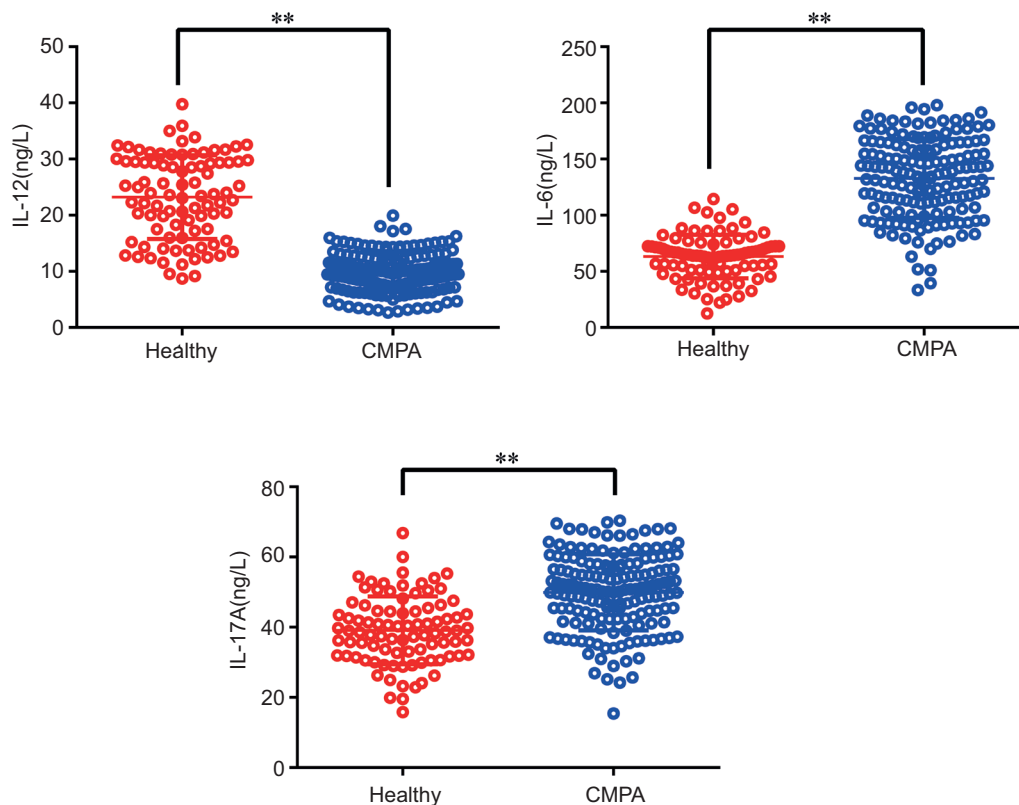


Figure 1 Expressions of IL-12, IL-16, and IL-17A in CMPA infants and healthy children. Levels of IL-12 (A), IL-16 (B), and IL-17A (C) in CMPA infants and healthy children. ** $P < 0.01$.

Association between inflammatory cytokines levels and sIgE

Patients with allergy or hypersensitivity had significantly higher sIgE than normal individuals. Detection of the body's IgE levels can identify the immune function status, help diagnose immune hyperplasia, immunodeficiency, infection, autoimmune, and other immune-related diseases. Results in Figure 2A-C illustrated that IL-12, IL-16, and IL-17A were associated with sIgE levels in CMPA infants.

Aberrant IL-12, IL-16, and IL-17A could discriminate between CMPA infants and healthy controls

To verify IL-12, IL-16, and IL-17A as the definitive diagnosis of CMPA, the receiver operating characteristic (ROC) assays were performed accordingly. As displayed in Figure 3A-C, the area under the curves (AUCs) of IL-12, IL-16, and IL-17A in differentiating CMPA from healthy controls were 0.9442 (95% CI, 0.9155-0.9730), 0.9553 (95% CI, 0.9303-0.9804) and 0.7744 (95% CI, 0.7151-0.8332).

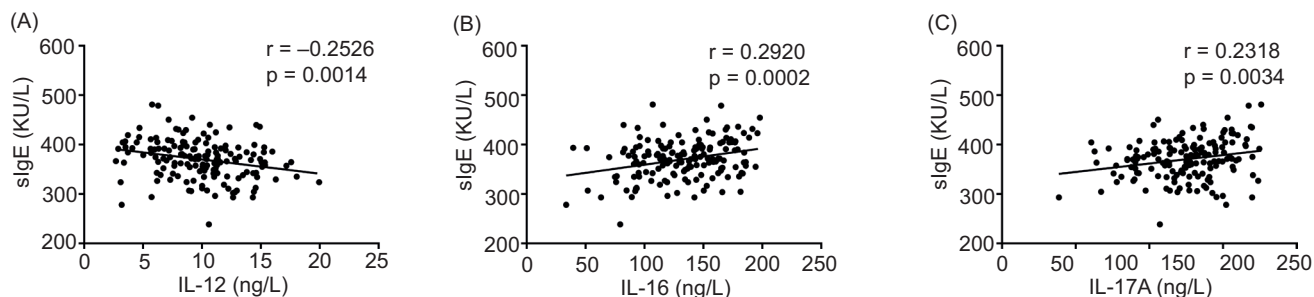


Figure 2 Correlation between inflammatory cytokines levels and sIgE. (A-C) Correlation analysis between IL-12, IL-16, IL-17A, and sIgE levels in CMPA.

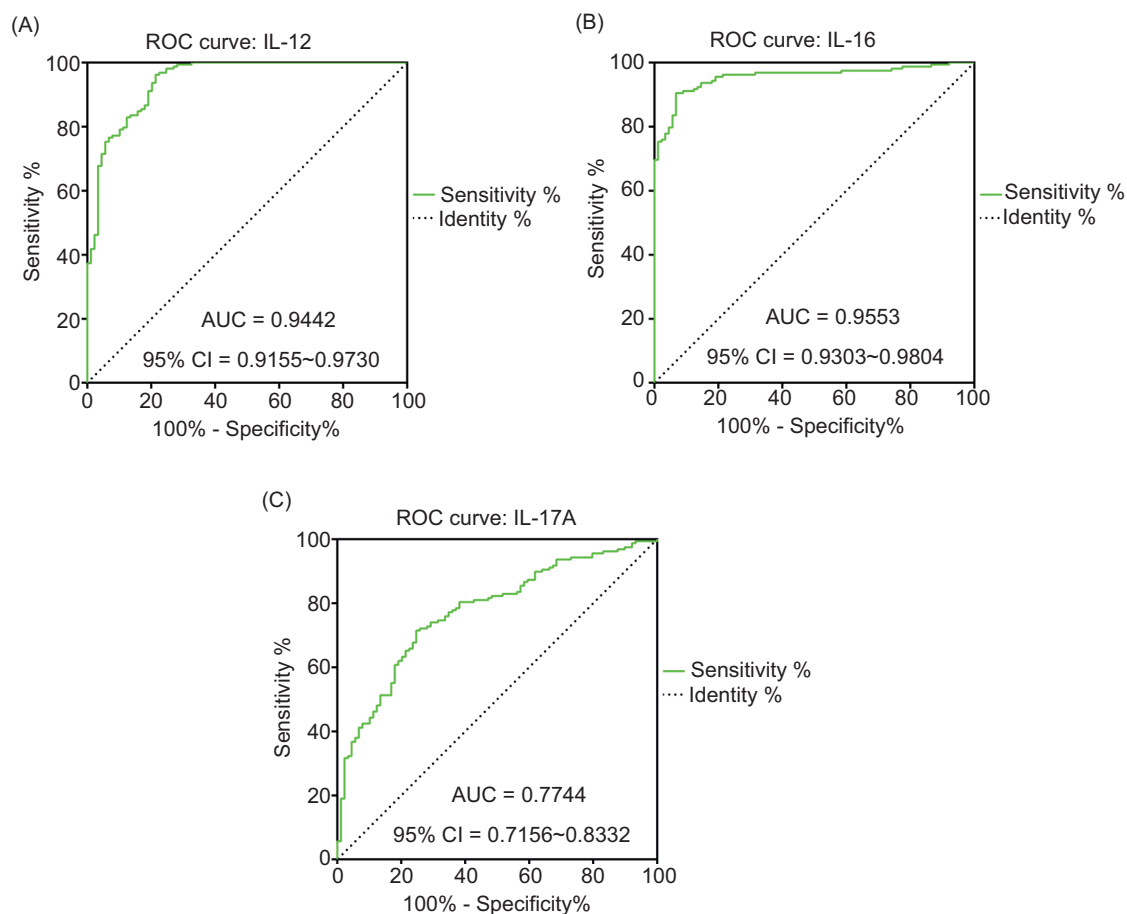


Figure 3 ROC analysis of IL-12, IL-16, and IL-17A in differentiating CMPA from healthy controls. The area under the curves (AUCs) of IL-12 (A), IL-16 (B), and IL-17A (C) in discriminating CMPA from healthy infants.

Variance of IL-12, IL-16, and IL-17A was associated with the progress of CMPA treatment

Finally, we followed CMPA infants for 3 months after avoiding milk treatment. As shown in Figure 4A-C, the expression of IL-12 was increased whereas IL-16 and IL-17A was remarkably decreased after 3 months of treatment compared with the first diagnosis.

Discussion

As one of the most common allergic diseases in infants and children, CMPA was considered the beginning of allergic diseases. The specific pathogenesis of CMPA was currently unknown. Mechanically, Th1/Th2 changes and deficiency of T-cell regulatory factors were the main pathogenesis of CMPA.^{22,23} In IgE-mediated CMPA, Th2 cells stimulated specific IgE production, whereas non-IgE-mediated CMPA may be due to Th1-mediated inflammatory response.²⁴ Interleukins (ILs), a class of cytokines, were produced by various immune cells and might act on many cells. As reported, ILs played crucial roles in activating and regulating immune cells,²⁵ mediating the activation, proliferation, and differentiation of T or B cells in the inflammatory response.²⁶ IL-12 was a determinant of the Th1 cell immune response and can effectively promote the production of Th1-like cytokines such as IFN- γ ,²⁷ contrarily, it can also inhibit the production of

Th2-like factors such as IL-4 and IL-13, thereby suppressing the Th2-type response.^{28,29} IL-17A, a member of the IL-17 family, may induce the expression of inflammatory factors and chemokines, thereby recruiting more immune cells to the site of inflammation and exacerbating the organism's inflammatory response.³⁰ In allergic diseases, IL-12, IL-16, and IL-17A were reported to be dysregulated in allergic contact dermatitis,^{31,32} allergic rhinitis,³³⁻³⁵ and allergic asthma.³⁶⁻³⁸ In this study, for the first time we found that IL-12 was decreased whereas IL-16 and IL-17A were prominently increased in serum samples from CMPA infants compared with healthy controls, high sensitivity, and specificity of discriminating CMPA from healthy controls. Meanwhile, IL-12, IL-16, and IL-17A expressions were also verified to be correlated with sIgE levels, further illustrating the potentials in CMPA diagnosis. Finally, after 3 months of milk protein avoidance treatment, levels of IL-12 were elevated whereas IL-16 and IL-17A were gradually decreased in relation with those at diagnosis.

This study has limitations. First, this work was based on the Chinese Han population, and the results of this work should be confirmed by the children of other races. Second, more samples should be included in future study to further confirm the results.

In conclusion, we reviewed and evaluated the utilities of IL-12, IL-16, and IL-17A, as potential biomarkers for diagnosis, monitoring, and prediction in children with CMPA. However, further studies in larger cohorts of infants must be conducted to confirm the value of these markers.

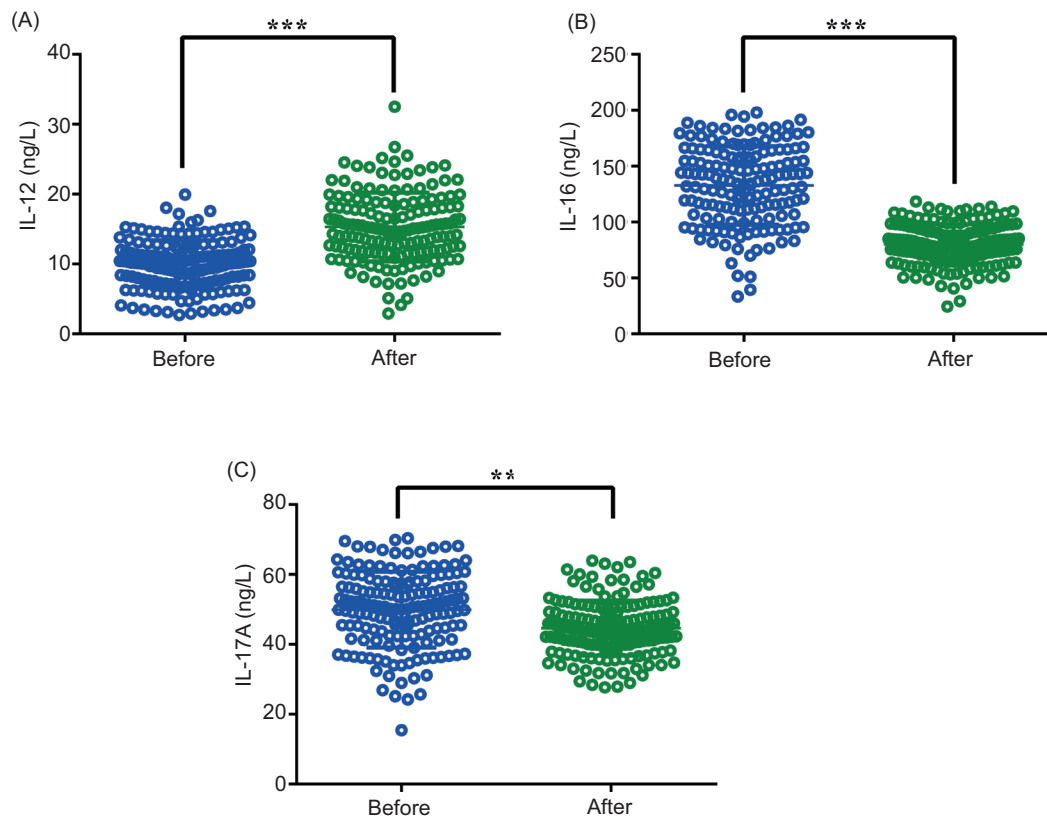


Figure 4 The response of IL-12, IL-16, and IL-17A to CMPA treatment. The variance of IL-12 (A), IL-16 (B), and IL-17A (C) levels in CMPA group at diagnosis and three months after CMPA treatment. ** $P < 0.01$, *** $P < 0.001$.

References

1. Yu W, Freeland DMH, Nadeau K. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol*. 2016;16(12):751-65. <https://doi.org/10.1038/nri.2016.111>
2. Nowak-Węgrzyn A, Katz Y, Mehr SS, Koletzko S. Non-IgE-mediated gastrointestinal food allergy. *J Allergy Clin Immunol*. 2015;135(5):1114-24. <https://doi.org/10.1016/j.jaci.2015.03.025>
3. Burks AW, Sampson HA, Plaut M, Lack G, Ardis CA. Treatment for food allergy. *J Allergy Clin Immunol*. 2018;141(1):1-9. <https://doi.org/10.1016/j.jaci.2017.11.004>
4. Xu GF, Snetselaar LG, Jing J, Liu BY, Strathearn L, Bao W. Association of food allergy and other allergic conditions with autism spectrum disorder in children. *JAMA Netw Open*. 2018;1(2):e180279. <https://doi.org/10.1001/jamanetworkopen.2018.0279>
5. Wang XY, Zhuang Y, Ma TT, Zhang B, Wang XY. Prevalence of self-reported food allergy in six regions of Inner Mongolia, Northern China: a population-based survey. *Med Sci Monit*. 2018;24:1902-11. <https://doi.org/10.12659/msm.908365>
6. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol*. 2010;125(2):S116-25. <https://doi.org/10.1016/j.jaci.2009.08.028>
7. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Daviset MM, et al. Prevalence and severity of food allergies among US adults. *JAMA Netw Open*. 2019;2(1):e185630. <https://doi.org/10.1001/jamanetworkopen.2018.5630>
8. Comberiati P, Costagliola G, D'Elios S, Peroni D. Prevention of food allergy: the significance of early introduction. *Medicine*. 2019;55(7):323. <https://doi.org/10.3390/medicina55070323>
9. Guadamuro L, Diaz M, Jiménez S, Molinos-Norniella C, Pérez-Solis D, Rodríguez JM, et al. Fecal changes following introduction of milk in infants with outgrowing non-IgE CMPA are influenced by previous consumption of the probiotic LGG. *Front Immunol*. 2019;10:1819. <https://doi.org/10.3389/fimmu.2019.01819>
10. Navarrete-Rodríguez EM, Ríos-Villalobos LA, Alcocer-Arreguín CR, Del-Río-Navarro BE, Del Río-Chivardi JM, Saucedo-Ramírez OJ, et al. Cross-over clinical trial for evaluating the safety of camel's milk intake in patients who are allergic to cow's milk protein. *Allergol Immunopathol*. 2018;46(2):149-54. <https://doi.org/10.1016/j.aller.2017.06.005>
11. Ho MHK, Lee SL, Wong WHS, Ip P, Lau YL. Prevalence of self-reported food allergy in Hong Kong children and teens--a population survey. *Asian Pac J Allergy Immunol*. 2012;30(4):275-84.
12. Schichter-Konfino V, Almog M, Bamberger E, Berkowitz D, Kessel A. The significance of allergic contact urticaria to milk in children with cow's milk allergy. *Pediatr Allergy Immunol*. 2015;26(3):218-22. <https://doi.org/10.1111/pai.12375>
13. Teymourpour P, Pourpak Z, Fazlollahi MR, Barzegar S, Shokouhi, Rosita Akramian, et al. Cow's milk anaphylaxis in children first report of Iranian food allergy registry. *Iran J Allergy Asthma Immunol*. 2012;11(1):29-36. <https://doi.org/10.1186/2045-7022-1-S1-P47>
14. Franco JM, Pinheiro AP, SG, Vieira SCF, Barreto IDC, Gurgel RQ, Coccoet RR, et al. Accuracy of serum IgE concentrations and papule diameter in the diagnosis of cow's milk allergy. *J Pediatr*. 2018;94(3):279-85. <https://doi.org/10.1016/j.jpeds.2017.06.022>
15. 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(2):171-7. <https://doi.org/10.12932/AP0319.32.2.2013>
16. Norgaard A, Skov PS, Bindslev-Jensen C. Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils. *Clin Exp Allergy*. 1992;22(10):940-7. <https://doi.org/10.1111/j.1365-2222.1992.tb02068.x>
17. Majamaa H, Moio P, Holm K, Kautiainen H, Turjanmaa K. Cow's milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. *Allergy*. 1999;54(4):346-51. <https://doi.org/10.1034/j.1398-9995.1999.00834.x>
18. Iacono G, Cavataio F, Montalto G, Florena A, Tumminello M, Soresi M, et al. Intolerance of cow's milk and chronic constipation in children. *N Engl J Med*. 1998;339(16):1100-4. <https://doi.org/10.1056/NEJM199810153391602>
19. Manti S, Lougaris V, Cuppari C, Tardino L, Dipasquale V, Arrigo T, et al. Breastfeeding and IL-10 levels in children affected by cow's milk protein allergy: a retrospective study. *Immunobiology*. 2017;222(2):358-62. <https://doi.org/10.1016/j.imbio.2016.09.003>
20. Toro-Monjaraz EM, Fonseca-Camarillo G, Zárate-Mondragón F, Montijo-Barrios E, Cadena-León J, Avelar-Rodríguez D, et al. Differential cytokine expression in the duodenum and rectum of children with non-immunoglobulin e-mediated cow's milk protein allergy. *Dig Dis Sci*. 2021;66(11):3769-75. <https://doi.org/10.1007/s10620-020-06743-z>
21. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. *Clin Exp Allergy*. 2014;44(5):642-72. <https://doi.org/10.1111/cea.12302>
22. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. *Mucosal Immunol*. 2012;5(3):267-76. <https://doi.org/10.1038/mi.2012.5>
23. Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, et al. Lactobacillus GG effect in increasing IFN-gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol*. 2004;114(1):131-6. <https://doi.org/10.1016/j.jaci.2004.03.036>
24. Canani RB, Paparo L, Nocerino R, Cosenza L, Pezzella V, Di Costanzo M, et al. Differences in DNA methylation profile of Th1 and Th2 cytokine genes are associated with tolerance acquisition in children with IgE-mediated cow's milk allergy. *Clin Epigenetics*. 2015;7(1):38. <https://doi.org/10.1186/s13148-015-0070-8>
25. Fonseca-Camarillo G, Furuzawa-Carballeda J, Iturriaga-Goyon E, Yamamoto-Furusho JK. Differential expression of IL-36 family members and IL-38 by immune and non-immune cells in patients with active inflammatory bowel disease. *Biomed Res Int*. 2018;2018:5140691. <https://doi.org/10.1155/2018/5140691>
26. Teng DK, Liu Y, Lv YF, Wang L, Zhange W, Wang J-P, et al. Elevated interleukin-35 suppresses liver inflammation by regulation of T helper 17 cells in acute hepatitis B virus infection. *Int Immunopharmacol*. 2019;70:252-9. <https://doi.org/10.1016/j.intimp.2019.02.048>
27. Libetta C, Rampino T, Canton AD. Polarization of T-helper lymphocytes toward the Th2 phenotype in uremic patients. *Am J Kidney Dis*. 2001;38(2):286-95. <https://doi.org/10.1053/ajkd.2001.26092>
28. Kirmaz C, Bayrak P, Yilmaz O, Yuksel H. Effects of glucan treatment on the Th1/Th2 balance in patients with allergic rhinitis: a double-blind placebo-controlled study. *Eur Cytokine Netw*. 2005;16(2):128-34.
29. Tagoma A, Haller-Kikkatalo K, Roos K, Oras A, Kirss A, Ilonnet J, et al. Interleukin-7, T helper 1, and regulatory T-cell activity-related cytokines are increased during the second trimester of healthy pregnancy

- compared to non-pregnant women. *Am J Reprod Immunol*. 2019;82(6):e13188. <https://doi.org/10.1111/aji.13188>
30. Glatt S, Baeten D, Baker T, Griffiths M, Ionescu L, Lawsonet ADG, et al. Dual IL-17A and IL-17F neutralisation by bimekizumab in psoriatic arthritis: evidence from pre-clinical experiments and a randomised placebo-controlled clinical trial that IL-17F contributes to human chronic tissue inflammation. *Ann Rheum Dis*. 2018;77(4):523-32. <https://doi.org/10.1136/annrheumdis-2017-212127>
 31. Yawalkar N, Brand CU, Braathen LR. Interleukin-12 expression in human afferent lymph derived from the induction phase of allergic contact dermatitis. *Br J Dermatol*. 1998;138(2):297-300. <https://doi.org/10.1046/j.1365-2133.1998.02078.x>
 32. Todberg T, Zachariae C, Krusturp D, Skov L. The effect of anti-IL-17 treatment on the reaction to a nickel patch test in patients with allergic contact dermatitis. *Int J Dermatol*. 2019;58(3):e58-e61. <https://doi.org/10.1111/ijd.14347>
 33. Testa B, Mesolella C, Mosti MR, Mesolella M, Testa D, Motta G. Changes in serum interferon-gamma, interleukin-4, and interleukin-12 cytokine levels in anti-histamine type 2-treated allergic rhinitis patients. *Laryngoscope*. 2001;111(2):236-9. <https://doi.org/10.1097/00005537-200102000-00009>
 34. Pullerits T, Lindén A, Malmhäll C, Lötvalld J. Effect of seasonal allergen exposure on mucosal IL-16 and CD4+ cells in patients with allergic rhinitis. *Allergy*. 2001;56(9):871-7. <https://doi.org/10.1111/j.1398-9995.2001.00916.x>
 35. Abbasi R, Zomorodian K, Shahrabadi ZZ, Saadat F, Nabavizadeh SH, Esmaeilzadeh H, et al. IL-25 and IL-33 serum levels are not associated with the type of allergen causing allergic rhinitis. *Iran J Immunol*. 2019;16(4):321-6. <https://doi.org/10.22034/IJI.2019.80283>
 36. Tazaki T, Minoguchi K, Yokoe T, Oda N, Hashimoto T, Tanaka A, et al. Allergen rush immunotherapy increases interleukin (IL)-12 production and IL-12 receptor beta2 chain expression in patients with allergic asthma. *Cell Immunol*. 2004;228(1):20-6. <https://doi.org/10.1016/j.cellimm.2004.03.008>
 37. Afifi SS, ElArab AE, Mostafa SY. Interleukin 16 (IL-16) in asthma and allergic rhinitis. A comparison between upper and lower airways. *Egypt J Immunol*. 2004;11(2):31-36.
 38. Graser A, Ekici AB, Sopel N, Melichar VO, Zimmermann T, Papadopoulos NG, et al. Rhinovirus inhibits IL-17A and the downstream immune responses in allergic asthma. *Mucosal Immunol*. 2016;9(5):1183-92. <https://doi.org/10.1038/mi.2015.130>