

# Features of the Pattern Recognition Receptor TLR-2 Expression and Cytokines IL-2, IL-4 Profile in Children with Food Hypersensitivity

Olga P. Pakholchuk

Department of Faculty Pediatrics, State Medical University  
26, Mayakovsky av., Zaporizhzhya, Postcode 69035, Ukraine

Copyright © 2018 Olga P. Pakholchuk. This article is distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Abstract

**Aim** of the research was to study features of the pattern recognition receptor TLR-2 expression and cytokine IL-2, IL-4 profile in children with skin symptoms of food hypersensitivity depending on the result of skin-prick test and oral challenge test with food allergens and features of the course of the symptoms.

**Methods.** 424 eligible children 1 - 18 years old were recruited in the study. Skin-prick tests (SPT) and oral challenge test (OCT) with common food allergens were used for hypersensitivity proof, venous blood samples were taken for analysis. ELISA method was used for detection of the IL-2, IL-4, TLR-2 in serum.

**Results.** Mean serum IL-2 level was 9,9 [6,7;16] pg/ml, IL-4 - 4,1 [1,6;18] pg/ml and TLR-2 - 0,154 [0,098;0,19] ng/ml. The next tendency was revealed: children with higher IL-2 and IL-4 levels had lower quantity of the TLR-2. Serum IL-4 level correlated with early skin symptoms manifestation (from birth and up to 1 months of age) ( $r=0,43$ ,  $p<0,05$ ). IL-2 serum level was linked with positive results of the skin prick-test ( $r=0,74$ ,  $p<0,05$ ).

**Conclusion.** Debut of the skin symptoms of FH from birth up to 1 month of age correlates with IL-4 level. Positive result of skin prick-test correlated with IL-2 serum level. The more was age of the symptoms manifestation and duration of the FH symptoms in children, the more were IL-2 levels and the less were TLR-2.

**Keywords:** food hypersensitivity, children, Toll-like receptor, Interleukin

## Introduction

Food hypersensitivity (FH) is known as the pathophysiological process of the intolerance to food allergens which can be driven via Th-2 polarization or have nonimmune mechanisms. IL-4 and IL-2 are well studied cytokines with proven role in that process. In contrast, Dendritic cell (DC) is key cell for processes of oral tolerance formation [3, 9]. Pattern recognition receptors family (Toll-like receptors) has leading role in this signaling transduction [4]. Recent studies showed that children with atopy and atopic eczema have decreased quantity of the TLR-2 on the surface of the keratinocytes as compared with healthy people [5]. Similar data were demonstrated in the serum. As it was found that number of cells with TLR2+ and TLR4+ in patients with atopic dermatitis was less as opposed to the control [7]. Relating to the fact that atopic dermatitis is only one of the forms of the clinical manifestation of the food hypersensitivity on the skin in children, further researches are needed to study profiles of the main Th-2 cytokines and DC receptors in children depending on the mechanism which is on the background of the clinical symptoms.

We **aimed** to study features of the pattern recognition receptor TLR-2 expression and cytokine IL-2, IL-4 profile in children with skin symptoms of food hypersensitivity depending on the result of skin-prick test and oral challenge test with food allergens and features of the course of the symptoms.

**Methods.** 424 eligible children 1 - 18 years old were recruited prospectively from the patients of the Children multiefield children hospital #5, Zaporizhyya and University clinic, Zaporizhyya, Ukraine. Diagnosis of food allergy was made with EAACI criterions in case of FA reproducible occurrence on exposure to the suspected food. Patients with food anaphylaxis were not included. Skin-prick tests (SPT) and oral challenge test (OCT) with common food allergens were used for hypersensitivity proof. Oral challenge test (OCT) was performed in accordance with the EAACI recommendations and local Ethical requirements [1]. Open oral provocation was used for infants up to 1 y.o. Double blind placebo controlled oral challenge was provided in others. Two main products were used for oral challenge: milk and egg. Blood samples were collected after fasting in cooling vacutaner and after that it was immediately centrifugated (4°C for 3.000 × 30 min). After centrifugation serum was blind coded and stored at -25° until used. For levels of IL-2, IL-4 detection ELISA method was used (Human IL-2 Platinum ELISA and Human IL-4 Platinum ELISA, produced by Affymetrix eBioscience, Austria). TLR-2 in serum was detected by ELISA Kit for TLR-2 (Cloud-Clone Corp).

All statistical analyses were performed in commercial software Statistica (Statsoft, USA). All continuous variables were tested for a normal distribution using the Shapiro-Wilk's W test. Continuous variables were presented as median

inter-quartile range because of the non-normal distribution. Linear association between two variables was assessed with Spearman Rank order correlation.

**Results.** 59.4% of patients were children of early age (mean age was 26,3[12,1;54,2] months). Average duration of the disease was 15[3.5;35] months. 9.4% of patients (n=28) reported that FH symptoms on the skin debuted during the 1<sup>st</sup> month of age, 51% - after 1 year of age (figure 1).

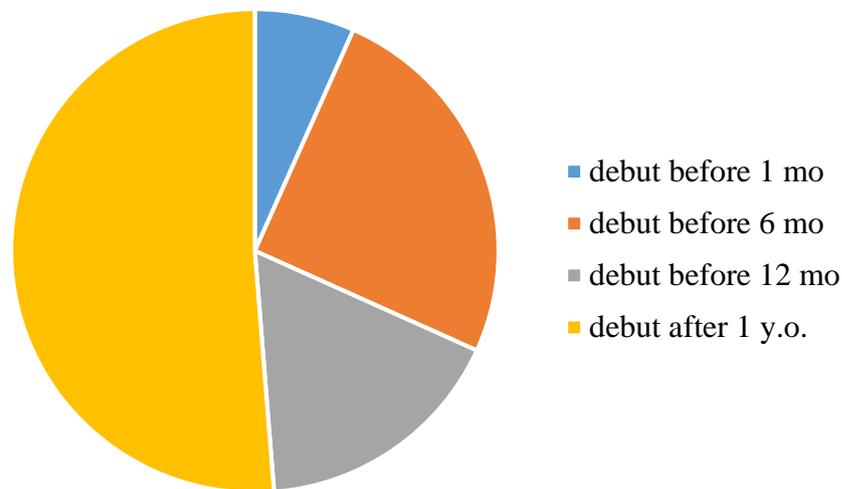


Figure 1. Distribution of the debut age of the skin symptoms of FH.

Only 34,4% (n=146) of patients reported one or two causative products, other 63% - reported allergy to variety of food. Skin prick-test was positive in 18% of patients (n=53/290). Oral challenge test – in 37% (n=128). In 72% of them cow milk was detected as causative product.

Mean serum IL-2 level was 9,9 [6,7;16] pg/ml, IL-4 - 4,1 [1,6;18] pg/ml and TLR-2 - 0,154 [0,098;0,19] ng/ml. The next tendency was revealed: children with higher IL-2 and IL-4 levels had lower quantity of the TLR-2 (figure 2).

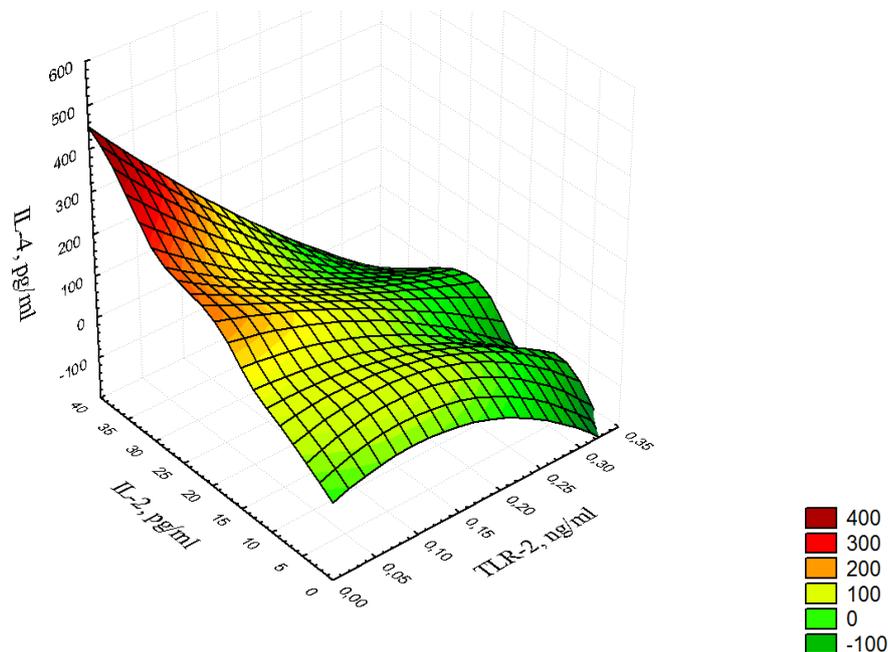


Figure 2. Distribution of the serum IL-2, IL-4 and TLR-2 levels in children with skin symptoms FH.

Correlative analysis revealed no link between serum levels of IL-2, TLR-2 and self-reported causative product, age of the patients and age of the skin symptoms first manifestation ( $p > 0,05$ ). Serum IL-4 level correlated only with early skin symptoms manifestation (from birth and up to 1 months of age) ( $r = 0,43$ ,  $p < 0,05$ ) (table 1).

Table 1.

Coefficients of the paired correlation between biomarkers levels and features of the course of the FH symptoms

Anamnesis data	Biomarker	Serum level		
		IL-2	IL-4	TLR-2
Age		0,16	0,08	0,1
Duration of the disease		0,12	0,09	-0,03
First symptoms debute before 1 month of age		0,03	0,43*	-0,08
First symptoms debute before 6 months of age		-0,15	0,03	0,09
First symptoms debute before 12 months of age		-0,12	0,15	-0,17

\*-  $p < 0,05$ .

Assessment of the graphical data showed the next tendency: the more was age of the manifestation of the first symptoms of FH on the skin and the time of persistence of the symptoms the more was serum IL-4 level and the less serum TLR-2 level (figure 3).

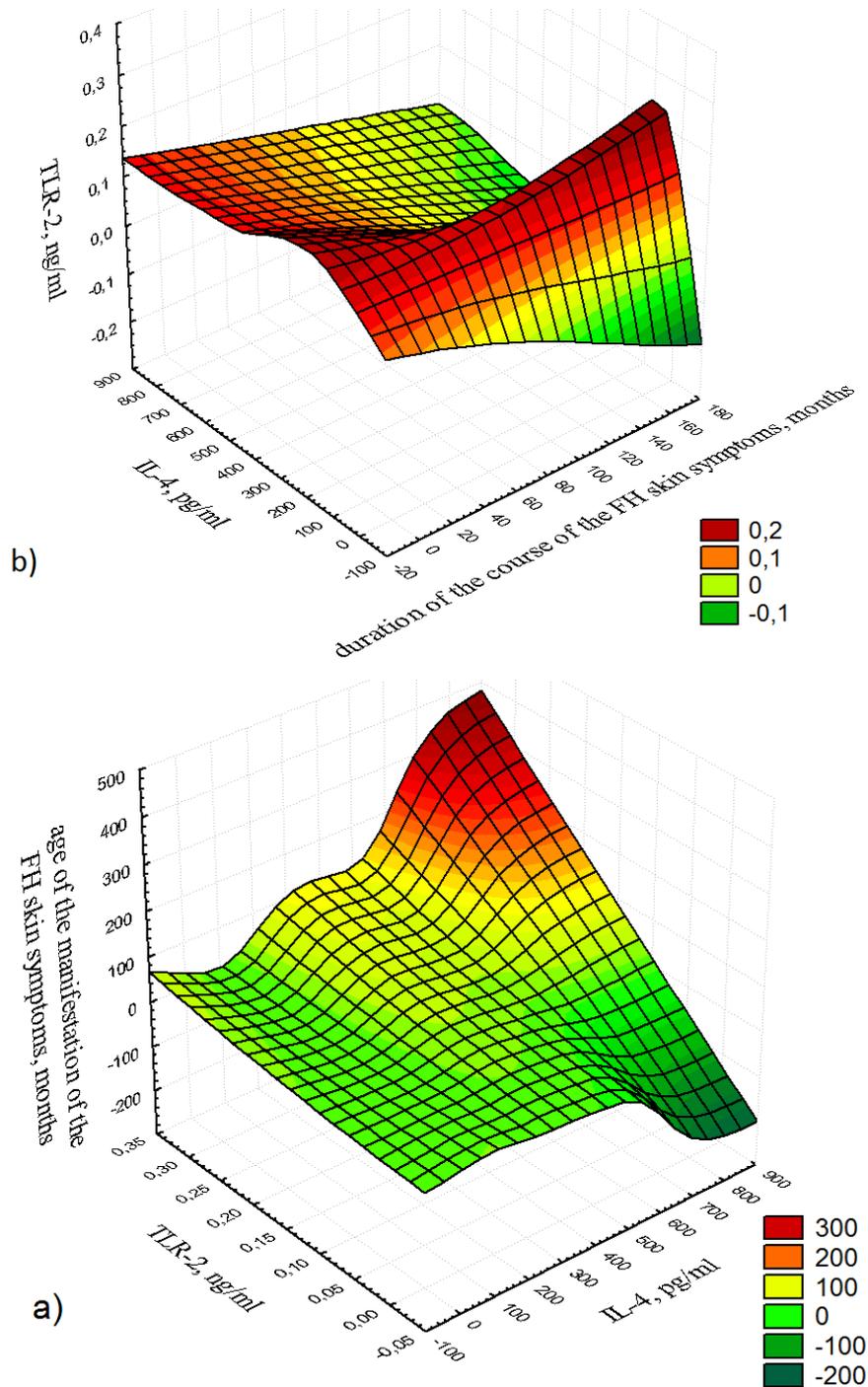


Figure 3. Distribution between serum levels of the IL-4, IL-2 and TLR-2 and age of the manifestation of the first symptoms of FH on the skin (a) and the time of persistence of the symptoms (b).

It was detected that IL-2 serum level correlates with positive results of the skin prick-test with food allergens ( $r=0,74$ ,  $p<0,05$ ) (Table 2).

Table 2.  
Coefficients of the paired correlation between biomarkers levels and features of the course of the FH symptoms

Test result	Biomarker	Serum level		
		IL-2	IL-4	TLR-2
Self-reported food allergen		0,1	0,05	0,03
Positive skin prick-test		0,74*	0,27	0,31
Elevated sIgE to food		0,1	0,009	0,09
Positive oral challenge test		0,12	0,03	0,01

\*-  $p < 0,05$ .

**Discussion and further perspectives.** Retained results of the standard tests (skin prick- and oral challenge test), which are usually used for immune FH diagnostics, corresponded to the recent summarized European data that showed milk and egg as the commonest causes of the food allergy symptoms in children [1]. More than 50% of children with FH symptoms on the skin had negative results of skin prick-test and oral challenge test. This fact demonstrated that in major part of cases patients have nonIgE-dependent mechanisms, which are currently not as well understood as IgE-mediated reactions are [10].

The more was age of the symptoms debut and duration of the FH skin clinical symptoms in children, the more were IL-2 and the less were TLR-2 levels. Link between IL-2 serum level and positive results of the skin prick-test ( $r=0,74$ ,  $p < 0,05$ ) demonstrated IL-2 driven immune mechanisms in the pathogenesis of FH in children. Similar results relating importance of the IL-2 in allergic inflammation were presented by Noma T. et al. (1998) [noma]. Other authors supposed that anti-IL-2 therapy is promising in induction of the tolerance to food allergens [2].

TLR-2 in our study showed no correlation with result of skin-prick test, oral challenge test with food allergens and features of the course of the symptoms. That obviously indicates that this biological marker cannot be used for differential diagnosis of FH symptoms and for prognosis.

Recent studies showed that in presence of IL-4, naïve CD4 T cells differentiate into Th2 cells and produce other type-2 cytokines like IL-5, IL-13, and IL-9 with IL-4 [6]. Our study showed that serum IL-4 level correlated with early skin symptoms manifestation (from birth and up to 1 months of age) ( $r=0,43$ ,  $p < 0,05$ ).

## Conclusions

Debut of the skin symptoms of FH from birth up to 1 month of age correlates with IL-4 level. Positive result of skin prick-test correlated with IL-2 serum level. The more was age of the symptoms manifestation and duration of the FH symptoms in children, the more were IL-2 levels and the less were TLR-2.

**Acknowledgements.** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Ethical Declaration:** The study was approved by the local ethics committee of State Medical University, Zaporozhye, Ukraine. And this study was carried out in conformity with the Declaration of Helsinki.

**Conflict of interest:** The authors declare no conflict of interest

## References

- [1] J. A. Boyce, A. Assa'ad, A. W. Burks et al., Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel, *J. Allergy Clin. Immunol.*, **126** (2010) (60), 1–58. <https://doi.org/10.1016/j.jaci.2010.10.007>
- [2] O. Boyman, C.D. Surh, J. Sprent, Potential use of IL-2/anti-IL-2 antibody immune complexes for the treatment of cancer and autoimmune disease, *Expert Opin Biol. Ther.*, **6** (2006), no. 12, 1323–1331. <https://doi.org/10.1517/14712598.6.12.1323>
- [3] R. Frei, M. Akdis, L. O'Mahony, Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence, *Current Opinion in Gastroenterol.*, **31** (2015), no. 2, 153–158. <https://doi.org/10.1097/mog.0000000000000151>
- [4] M.L. Dongarrà, V. Rizzello, L. Muccio, Walter Fries, Antonio Cascio, Irene Bonaccorsi, Guido Ferlazzo, Mucosal immunology and probiotics, *Curr. Allergy Asthma Rep.*, **13** (2013), no. 1, 19–26. <https://doi.org/10.1007/s11882-012-0313-0>
- [5] I. H. Kuo, A. Carpenter-Mendini, T. Yoshida L.Y. McGirt, A.I. Ivanov, K.C. Barnes, R.L. Gallo, A.W. Borkowski, K. Yamasaki, D.Y. Leung, S.N. Georas, A. De Benedetto, L.A. Beck, Activation of epidermal toll-like receptor 2 enhances tight junction function: implications for atopic dermatitis and skin barrier repair, *J. Invest. Dermatol.*, **133** (2013), no. 4, 988–998. <https://doi.org/10.1038/jid.2012.437>
- [6] M.N. Kweon, M. Yamamoto, M. Kajiki, I. Takahashi, H. Kiyono Systemically derived large intestinal CD4(+) Th2 cells play a central role in STAT6-mediated allergic diarrhea, *J. Clin. Invest.*, **106** (2000), no. 2, 199–206. <https://doi.org/10.1172/jci8490>

- [7] A. Lesiak, P. Smolewski, D. Sobolewska-Sztychny, A. Sysa-Jedrzejowska, J. Narbutt, The role of T-regulatory cells and Toll-like receptors 2 and 4 in atopic dermatitis, *Scand. J. Immunol.*, **76** (2012), no. 4, 405–410.  
<https://doi.org/10.1111/j.1365-3083.2012.02739.x>
- [8] T. Noma, I. Yoshizawa, K. Aoki, Y. Sugawara, H. Odajima, Y. Kabasawa, Matsui, Yata, Yamaguchi, Mukouyama, Baba, Correlation between antigen-specific IL-2 response test and provocation test for egg allergy in atopic dermatitis, *Clin. Exp. Allergy*, **28** (1998), no. 9, 1120-1130.  
<https://doi.org/10.1046/j.1365-2222.1998.00383.x>
- [9] V. Rizzello, I. Bonaccorsi, M.L. Dongarrà, Lisbeth Nielsen Fink, Guido Ferlazzo, Role of natural killer and dendritic cell crosstalk in immunomodulation by commensal bacteria probiotics, *J. Biomed. Biotechnol.*, **2011** (2011), Article ID 473097. <https://doi.org/10.1155/2011/473097>
- [10] J.M. Spergel, Nonimmunoglobulin E-Mediated Immune Reactions to Foods, *Allergy, Asthma, and Clinical Immunology*, **2** (2006), no. 2, 78-85.  
<https://doi.org/10.1186/1710-1492-2-2-78>

**Received: January 7, 2018; Published: January 26, 2018**