

Serum Cytokine Profiles in Infants with Atopic Dermatitis

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ABSTRACT Atopic dermatitis (AD) in infancy is believed to have distinct features as compared to AD in other age groups, and little is known about cytokine production in infants with AD. We aimed to measure the serum cytokine levels of infants with atopic dermatitis and evaluate the association of new anti-inflammatory cytokines with the disease. Eighty-one infant patients with AD and 52 healthy controls were involved in this study. The serum levels of major pro- and anti-inflammatory cytokines of the T-helper (Th) subtypes, as well as more recently defined interleukins (IL-27, IL-35, and IL-37), were measured using the ELISA method. The serum levels of IL-35, IL-5, and interferon (IFN)- γ were found to be significantly higher, while the levels of transforming growth factor (TGF)- β 1 and IL-13 were found to be significantly lower in patients with AD as compared to controls. There was no statistically significant correlation between serum cytokine levels and objective SCORAD index or total immunoglobulin (Ig) E levels. We did not observe prominent serum Th2 polarization in atopic infants. The immunopathogenesis of atopy onset at an early age may be more complicated than that at older ages.

KEY WORDS: atopic dermatitis, cytokines, interleukins, infancy

INTRODUCTION

Atopic dermatitis (AD) is a chronic, intermittent, eczematous form of dermatitis in genetically predisposed individuals that usually starts in infancy or early childhood. Both genetic and environmental factors are important in the disease etiology (1).

A dominant systemic T-helper type 2 (Th2) imbalance and eosinophilia are the major features of atopic diseases. The expression of the Th2-mediated cytokines interleukin (IL)-4, IL-5, and IL-13 in the acute phase of disease is followed by an increase in the Th1-medi-

ated cytokines interferon (IFN)- γ and IL-12, as well as IL-5 (2). Tumor necrosis factor (TNF)- α , IL-9, IL-33, IL-17, and the anti-inflammatory cytokines transforming growth factor (TGF)- β and IL-10 also have roles in the initiation and progression of the disease (3).

Anti-inflammatory cytokines IL-27, IL-35, and IL-37 may have roles in the pathogenesis of the disease. IL-27 and IL-35 have been recently discovered to act as inducible suppressive cytokines, similar to IL-10 (4). IL-27 supports Th1 development and IFN- γ production but inhibits Th2 and Th17 differentiation (5). This cytokine was suggested to play a role in the maintenance of eczema in skin, along with supporting Th1 responses (6). IL-35 is predominantly secreted by regulatory T-cells. IL-35-induced naive T-cells are called iT_h35 cells (inducible regulatory T-cells producing IL-35) and function independently of Foxp3, IL-10, and TGF- β (20). iT_h35 cells are suggested to be potent suppressors of Th2 allergic responses induced by allergic inflammation (7). IL-37 is the most recently identified member of the IL-1 family, which is a fundamental inhibitor of innate immunity (8).

We aimed to evaluate the major serum cytokines of the Th type, pro- and anti-inflammatory cytokines, and the recently defined IL-27, IL-35, and IL-37 in atopic dermatitis in infancy.

PATIENTS AND METHODS

Study population

The study involved 81 atopic children under 1 year of age who had been diagnosed by a dermatologist and 52 sex- and age-matched healthy controls without a family history of atopy. The diagnosis of the patients was performed according to Hanifin and Rajka's criteria for acute dermatitis at the first visit (9). The severity of atopic dermatitis was calculated via the objective SCORAD index (10). Patient family histories of atopy were evaluated.

Total IgE levels were measured via nephelometry (Siemens Healthcare Diagnostics, Deerfield, Germa-

ny). Total IgE levels <15 IU/mL were regarded as normal values. Full blood and eosinophil counts were measured using an automated blood analyzer (ABX Pentra 80; HORIBA Medical, Montpellier, France).

Peripheral venous blood samples taken from patients and healthy controls were centrifuged at 1600 xg for 15 minutes to isolate serum samples, which were then stored at -80°C until the time of analysis. In accordance with the Declaration of Helsinki, the parents of all patients and healthy controls provided written informed consent before sampling. The study was approved by the local ethical committee (approval No. B.10.4.ISM.4.06.68.49).

Measurement of serum cytokines

The serum levels of cytokines were measured via the ELISA method, using commercially available ELISA kits according to the manufacturer instructions: Ready-Set-Go ELISA kits (eBioscience, San Diego, USA) for IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17A, TNF α , TGF β 1, IFN- γ , and IL-27, and ELISA kits (Elabscience, Wuhan, China) for IL-35 and IL-37. IL-27, IL-35, and IL-37 were measured for 81 patients, while the other cytokines were measured for only 72 patients. The detection ranges of the cytokines were as follows: IL-2: 2-250 pg/mL, IL-4: 2-200 pg/mL, IL-5: 4-500 pg/mL, IL-10: 2-300 pg/mL, IL-12p70: 4-500 pg/mL, IL-13: 4-500 pg/mL, IL-17A: 4-500 pg/mL, TNF α : 4-500 pg/mL, TGF β 1: 156.3-10.000 pg/mL, IFN γ : 4-500 pg/mL, IL-27: 64-8000 pg/mL, IL-35: 15.625-1000 pg/mL, and IL-37: 15.625-1000 pg/mL. A sample was reanalyzed at a higher dilution when that sample's value fell outside the reference range for the ELISA kit.

Statistical analysis

Statistical analysis was performed via SPSS 11.5 for Windows. Normality tests of the continuous variables were conducted via the Kolmogorov-Smirnov test, and the homogeneity of variances was explored via Levene's test. Descriptive statistics were given as

Table 1. Demographic and clinical characteristics of patient and control groups

Variables	Controls (n=52)	Patients (n=81)	P-value
Age (month)			
Median (min-max)	4 (2-11)	5 (2-12)	0.767
Gender			0.480
Male	30 (57.7%)	53 (65.4%)	
Female	22 (42.3%)	28 (34.6%)	
History of familial atopy	-	44 (54.3%)	-
Objective SCORAD index	-	18 (10-50)	-
Total IgE (IU/mL)	-	9.2 (5-362)	-
Eosinophil count (μL^{-1})	-	540 (80-3360)	-

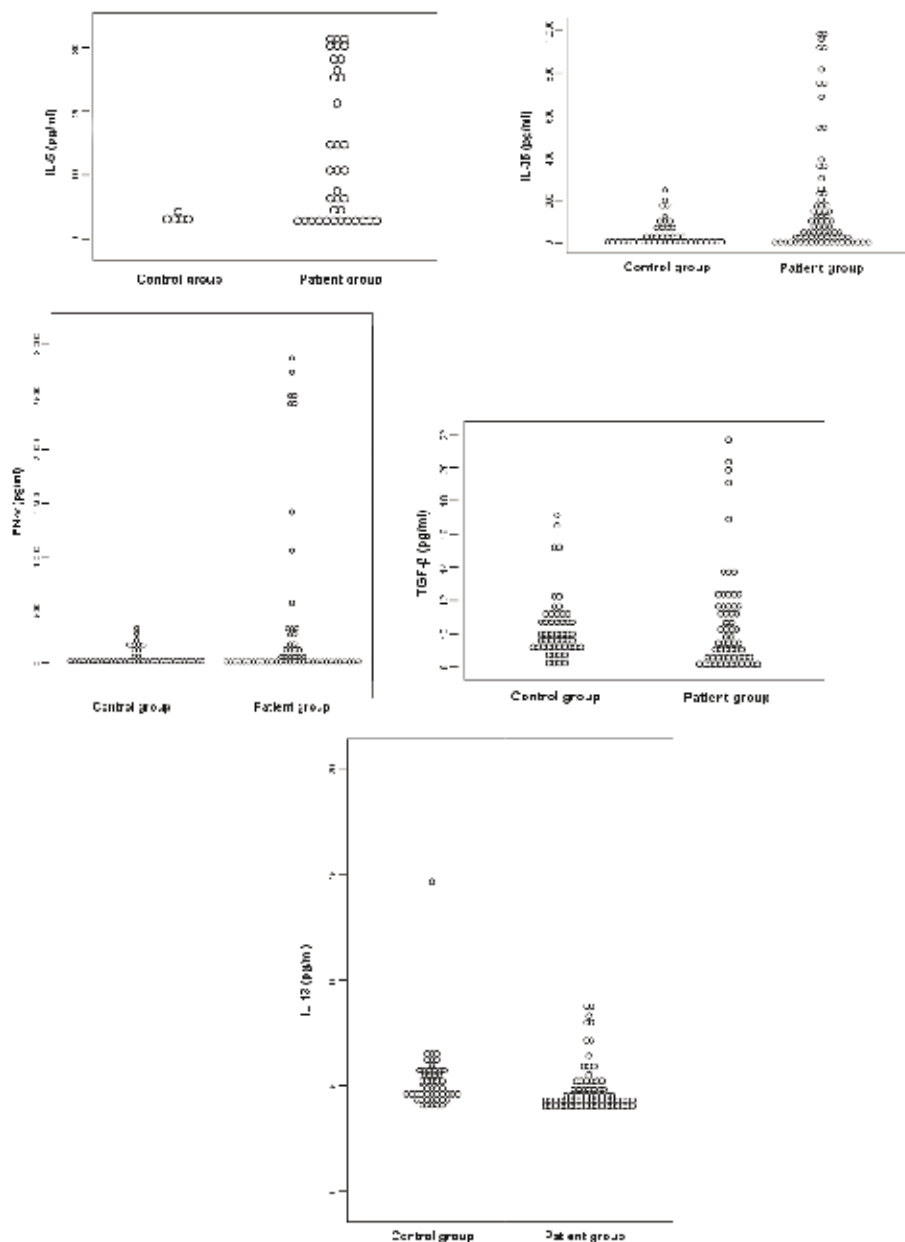


Figure 1. Serum IL-5, IL-35, IFN- γ , TGF- β 1, and IL-13 levels in the patients (n=81 for IL-35 and n=72 for IL-5, IFN- γ , TGF β 1 and IL-13) and control (n=52) groups.

medians and ranges (min-max) or n (%). The statistical significance of the differences between two independent groups was analyzed via the Mann-Whitney U test, and the Kruskal-Wallis test was used when comparing the medians of more than two groups. If the results of the Kruskal-Wallis tests were significant, the Conover non-parametric multiple comparison test was used to determine the groups that caused the exact difference. A Spearman correlation was used to examine the association between continuous variables. Categorical variables were compared

via Pearson's Chi-Square test, Fisher's exact test, or a likelihood-ratio test. The statistical significance level was set at $P < 0.05$.

RESULTS

There were no statistically significant differences between the patient and control groups in terms of gender and age ($P > 0.05$). The demographic and clinical characteristics of the patient and control groups are shown in Table 1.

The serum levels for IL-35, IL-5, and IFN γ were

Table 2. The number of subjects with detectable serum cytokine levels and range of cytokine levels in patient and control groups

Cytokines (pg/mL)	Control group		Patient group		P-value †
	Number (%)	Median (min-max)	Number (%)	Median (min-max)	
IL-35	43/52 (82.7%)	22.9 (0.9-255.3)	76/81 (93.8%)	75.3 (5.3-1000.0)	<0.001
IL-37	52/52 (100.0%)	119.3 (25.9-728.0)	79/81 (97.5%)	114.7 (1.7-497.8)	0.592
IL-27	21/52 (40.4%)	307.8 (65.2-851.6)	23/81 (28.4%)	234.2 (62.5-994.1)	0.851
IL-10	28/52 (53.8%)	3.8 (2.3-11.4)	23/72 (31.9%)	3.8 (2.3-30.9)	0.589
IL-5	5/52 (9.6%)	4.4 (4.2-5.3)	36/72 (50.0%)	10.2 (3.9-33.0)	0.021
IL-12p70	10/52 (19.2%)	2.8 (0.4-14.3)	2/72 (2.8%)	6.3 (5.0-7.6)	0.273
IL-2	10/52 (19.2%)	2.6 (1.9-4.2)	12/72 (16.7%)	3.1 (0.07-4.7)	0.418
IFN- γ	41/52 (78.8%)	15.6 (3.9-326.7)	56/72 (77.8%)	35.5 (3.9-500.0)	0.007
TNF α	47/52 (90.4%)	1.8 (1.0-9.4)	63/72 (87.5%)	2.2 (1.0-132.0)	0.060
IL-4	7/52 (13.5%)	1.8 (1.6-2.9)	16/72 (22.2%)	1.9 (1.6-20.6)	0.341
TGF- β 1	52/52 (100.0%)	10.6 (8.9-17.9)	62/72 (86.1%)	9.6 (8.1-21.8)	0.013
IL-17A	29/52 (55.8%)	5.2 (3.9-29.5)	31/72 (43.1%)	5.2 (4.0-26.0)	0.970
IL-13	52/52 (100.0%)	4.8 (3.99-35.7)	72/72 (100.0%)	4.4 (3.9-50.1)	0.002

† Mann Whitney U test

significantly higher ($P < 0.001$; $P = 0.021$, $P = 0.007$, respectively) and the serum levels for TGF β 1 and IL-13 were significantly lower ($P = 0.013$ and $P = 0.006$, respectively) in patients with AD than in the control group (Figure 1). The range of cytokine levels for the patient and control groups is shown in Table 2.

The objective SCORAD index ranged from 10 to 50 with a median value of 18, and total IgE ranged from 5 to 362 with a median value of 9.2 IU/mL. There was a statistically significant correlation between objective SCORAD index and total IgE levels ($r = 0.300$ and $P = 0.007$). There was no statistically significant correlation between eosinophil counts and serum IL-5 levels ($r = 0.044$ and $P = 0.798$) or the levels of other cytokines ($P > 0.05$).

There was no statistically significant relationship between a family history of atopy and objective SCORAD index, IgE levels, or serum cytokines ($P > 0.05$).

DISCUSSION

AD is considered to be a Th2-driven disease (1). Based on the results of our study, we were unable to show a prominent serum Th2 polarization in infants and children with low detectable levels of serum IL-4 or, conversely, decreased median serum IL-13 levels as compared to controls. The only significantly elevated serum cytokine related to the Th2 type was serum IL-5, which was not correlated with eosinophil count.

According to previous studies, cytokine responses do not show a strict Th1 or Th2 polarization in infantile atopic dermatitis (11, 12). Park *et al.* (13) reported that IL-5 was the prominent cytokine in infantile extrinsic-

type patients with AD, which is related to a higher eosinophil count, and a higher eosinophil count is associated with disease severity. Neaville *et al.* (12) showed a prominent increase in IL-5 levels from birth to 12 months in the secretions of phytohemagglutinin (PHA)-stimulated cord blood cells at birth and peripheral blood mononuclear cells (PBMCs) in the first year of age. In another study, the stimulation of PMBC with ovalbumin induced the increased production of IL-4 and IL-5 in infants as compared to controls and contributed to the development of AD in younger infants by inducing the production of IL-5, not IL-4 (14). Based on these results, we believe that serum IL-5 is the cytokine that is most positively correlated with AD in infants. Importantly, early allergen-specific IL-5 responses were found to increase with age and to be related to the development of asthma (15).

A Th2 bias among cytokines and chemokines in the sera of atopic infants and children also has been shown. Nakazato *et al.* (16) reported that serum levels of Th2 chemokines (CCL17, CCL22, and CCL27) correlated well with the extent and intensity of AD in infants. Plasma IL-4, IL-13, and IL-12p70 levels were found to be significantly higher in food-sensitized infants with lower IL-10 levels as compared with non-sensitized controls (17), and higher serum levels of IL-10 and IL-13, with no significant difference in serum IL-4 levels, were reported in patients with AD as compared to controls (2). In contrast to these results, we found a significantly higher median level of IL-13 in the control group. The elevation of serum IL-2 and IL-17 levels was also reported in adult atopic patients (18); in contrast, we could not find any difference in

IL-2, IL-17A, and TNF- α serum levels between the patient and control groups.

In a recent study, it was shown that the first wave of neonatal naive CD4+ T-cells is poised towards Th2 differentiation unless pro-inflammatory stimuli are present, and this contributes to atopic diseases in infancy in the presence of TGF- β (19). However, in general it is known that the production of immunosuppressive cytokines IL-10 and TGF- β by regulatory T-cells (Tregs) for the suppression of Th2-mediated inflammation is essential in maintaining peripheral tolerance (20). We found lower serum TGF- β 1 levels in atopic children than in controls. Samochocki *et al.* (21) also reported decreased serum TGF- β levels in adult atopic patients as compared to controls.

IFN- γ production was found to be higher in infants with acute disease and lower in children with AD than in non-atopic controls (22). We also found increased IFN- γ serum levels in patients as compared to controls. Increased production of IFN- γ by T-cells could suppress IgE production (23), and decreases in the level of this cytokine by age are consistent with increases in IgE levels (22). Total IgE levels were not found to be associated with serum levels of IFN- γ , as was the case with the other cytokines in our study.

IL-35 suppresses allergic responses, and the neutralization of IL-35 has been shown to exacerbate asthmatic inflammation (7). The inhibition of airway inflammation is reported to be associated with the suppression of inflammatory dendritic cell formation (24). It has also been reported that IL-35 inhibits allergic T-cell response and reduces the level of Th2-type cytokines in allergic rhinitis (25). Wong *et al.* reported elevated plasma concentrations of IL-35 in asthmatic patients and proposed this cytokine as a potential biomarker for disease severity (26). Serum IL-35 levels were higher in our patients as compared to controls, which may be explained as an elevation response secondary to inflammation with the aim of suppressing that inflammation.

Fujita *et al.* found that serum IL-37 levels were significantly higher in adult atopic patients than in healthy controls (8). IL-37 was also reported to be important in suppressing the pro-inflammatory cytokines in asthmatic patients (27). We found no significant difference in serum IL-37 and IL-27 levels between the patient and control groups. IL-27 suppresses Th2 differentiation (28). Interestingly, combined IL-27 and CCL26 (a marker of type-2 activation) expression was reported to be associated with more severe asthma (29), and CD4+ T-cells of asthmatic patients were found to be resistant to IL-27-mediated inhibition (30). The effects of anti-inflammatory cyto-

kines may be influenced by the cytokine milieu and the inflammatory cells at the inflammation site.

AD is a multifactorial disease, and immunopathogenesis in early childhood is more complicated. According to our findings about serum cytokines, there was no distinct polarization towards any Th type, but we can postulate that elevated IL-5 and IFN- γ serum levels and decreased TGF- β and IL-13 serum levels are the prominent cytokine changes associated with AD in infants. The serum levels of anti-inflammatory cytokines IL-27 and IL-37 do not change with the disease, but the elevation of serum IL-35 requires further investigation.

References:

1. Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. *J Dermatol Sci* 2010;58:1-7.
2. Lesiak A, Zakrzewski M, Przybyłowska K, Rogowski-Tylman M, Wozniacka A, Narbutt J. Atopic dermatitis patients carrying G allele in -1082 G/A IL-10 polymorphism are predisposed to higher serum concentration of IL-10. *Arch Med Sci* 2014;10:1239-43.
3. Lee J, Noh G, Lee S, Youn Y, Rhim J. Atopic dermatitis and cytokines: recent patents in immunoregulatory and therapeutic implications of cytokines in atopic dermatitis--part I: cytokines in atopic dermatitis. *Recent Pat Inflamm Allergy Drug Discov* 2012;6: 222-47.
4. Li X, Mai J, Virtue A, Yin Y, Gong R, Sha X, *et al.* IL-35 is a novel responsive anti-inflammatory cytokine-a new system of categorizing anti-inflammatory cytokines. *PLoS One* 2012;7:e33628.
5. Sawant DV, Hamilton K, Vignali DA. Interleukin-35: Expanding Its Job Profile. *J Interferon Cytokine Res* 2015;35:499-512.
6. Wittmann M, Zeitvogel J, Wang D, Werfel T. IL-27 is expressed in chronic human eczematous skin lesions and stimulates human keratinocytes. *J Allergy Clin Immunol* 2009;124:81-9.
7. Sawant D, Corry D, Vignali D. The inhibitory cytokine IL-35 in Treg-mediated control of allergic diseases. *J Immunol Suppl (Pittsburgh)* 2014;192:119.8.
8. Fujita H, Inoue Y, Seto K, Komitsu N, Aihara M. Interleukin-37 is elevated in subjects with atopic dermatitis. *J Dermatol Sci* 2013;69:173-5.
9. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44-7.
10. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on inter-

- pretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. *Br J Dermatol* 2007;157:645-8.
11. Stelmaszczyk-Emmel A, Zawadzka-Krajewska A, Kopatys A, Demkow U. Th1, Th2, Th17, and regulatory cytokines in children with different clinical forms of allergy. *Adv Exp Med Biol* 2013;788:321-8.
 12. Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, *et al.* Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *J Allergy Clin Immunol* 2003;112:740-6.
 13. Park JH, Choi YL, Namkung JH, Kim WS, Lee JH, Park HJ, *et al.* Characteristics of extrinsic vs. intrinsic atopic dermatitis in infancy: correlations with laboratory variables. *Br J Dermatol* 2006;155:778-3.
 14. Kimura M, Obi M. Ovalbumin-induced IL-4, IL-5 and IFN-gamma production in infants with atopic dermatitis. *Int Arch Allergy Immunol* 2005;137:134-40.
 15. Weber-Chrysochoou C, Crisafulli D, Kemp AS, Britton WJ, Marks GB; CAPS Investigators. Allergen-specific IL-5 responses in early childhood predict asthma at age eight. *PLoS One* 2014;9:e97995.
 16. Nakazato J, Kishida M, Kuroiwa R, Fujiwara J, Shimoda M, Shinomiya N. Serum levels of Th2 chemokines, CCL17, CCL22, and CCL27, were the important markers of severity in infantile atopic dermatitis. *Pediatr Allergy Immunol* 2008;19:605-13.
 17. Dang TD, Tang ML, Koplin JJ, Licciardi PV, Eckert JK, Tan T, *et al.* HealthNuts study. Characterization of plasma cytokines in an infant population cohort of challenge-proven food allergy. *Allergy* 2013;68:1233-40.
 18. Batista DI, Perez L, Orfali RL, Zaniboni MC, Samorano LP, Pereira NV, *et al.* Profile of skin barrier proteins (filaggrin, claudins 1 and 4) and Th1/Th2/Th17 cytokines in adults with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2015;29:1091-5.
 19. Hebel K, Weinert S, Kuroopka B, Knolle J, Kosak B, Jorch G, *et al.* CD4+ T cells from human neonates and infants are poised spontaneously to run a non-classical IL-4 program. *J Immunol* 2014;192:5160-70.
 20. Soyer OU, Akdis M, Ring J, Behrendt H, Cramer R, Lauener R, *et al.* Mechanisms of peripheral tolerance to allergens. *Allergy* 2013;68:161-70.
 21. Samochocki Z, Alifier M, Bodera P, Jeziorkowska R, Rosiak E, Jurkiewicz B, *et al.* T-regulatory cells in severe atopic dermatitis: alterations related to cytokines and other lymphocyte subpopulations. *Arch Dermatol Res* 2012;304:795-801.
 22. Vakirlis E, Lazaridou E, Tzellos TG, Gerou S, Chatzidimitriou D, Ioannides D. Investigation of cytokine levels and their association with SCORAD index in adults with acute atopic dermatitis. *J Eur Acad Dermatol Venereol* 2011;25:409-16.
 23. Suárez-Fariñas M, Dhingra N, Gittler J, Shemer A, Cardinale I, de Guzman Strong C, *et al.* Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 2013;132:361-70.
 24. Dong J, Wong CK, Cai Z, Jiao D, Chu M, Lam CW. Amelioration of allergic airway inflammation in mice by regulatory IL-35 through dampening inflammatory dendritic cells. *Allergy* 2015;70:921-32.
 25. Yokota M, Suzuki M, Nakamura Y, Ozaki S, Murakami S. Cytokine modulation by IL-35 in mice with allergic rhinitis. *Am J Rhinol Allergy* 2015;29:251-6.
 26. Wong CK, Leung TF, Chu IM, Dong J, Lam YY, Lam CW. Aberrant expression of regulatory cytokine IL-35 and pattern recognition receptor NOD2 in patients with allergic asthma. *Inflammation* 2015;38:348-60.
 27. Charrad R, Berraïes A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: Correlation with inflammatory cytokines TNF- α , IL- β , IL-6 and IL-17A. *Immunobiology* 2016;221:182-7.
 28. Lucas S, Ghilardi N, Li J, de Sauvage FJ. IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. *Proc Natl Acad Sci USA* 2003;100:15047-52.
 29. Xie M, Mustovich AT, Jiang Y, Trudeau JB, Ray A, Ray P, *et al.* IL-27 and type 2 immunity in asthmatic patients: association with severity, CXCL9, and signal transducer and activator of transcription signaling. *J Allergy Clin Immunol* 2015;135:386-94.
 30. Chen Z, Wang S, Erekosima N, Li Y, Hong J, Qi X, *et al.* IL-4 confers resistance to IL-27-mediated suppression on CD4+ T cells by impairing signal transducer and activator of transcription 1 signaling. *J Allergy Clin Immunol* 2013;132:912-21.