

T-Helper Cytokine Profiles in Patients with Kawasaki Disease

Sang Bum Lee, MD¹, Young Hyun Kim, MD², Myung Chul Hyun, MD¹, Yeo Hyang Kim, MD¹, Hee Sun Kim, MD³, and Young Hwan Lee, MD²

¹Department of Pediatrics, Kyungbook National University School of Medicine, Daegu,

²Department of Pediatrics, ³Department of Microbiology, Yeungnam University College of Medicine, Daegu, Korea

Background and Objectives: Kawasaki disease is an acute systemic vasculitis of which pathogenesis suspected is caused by immune dysregulation. The goal of this study is to evaluate the activation pattern of T helper cell type 1 (Th1) and T helper cell type 2 (Th2) in patients with Kawasaki disease.

Subjects and Methods: Prospective study of 60 patients (male 36, female 24) with diagnosis of Kawasaki disease were enrolled. One hundred and eighty blood samples from these patients were collected according to the different clinical stages {before initial intravenous immunoglobulin (IVIG), 5 days after initial IVIG, 2 months after initial IVIG}. The plasma level of Th1 cytokines; interferon-gamma (IFN- γ) & interleukin (IL)-2 and Th2 cytokines; IL-4 & IL-10 were measured by enzyme-linked immunosorbent assay.

Results: In all patients, the plasma level of Th1 cytokines (IFN- γ , IL-2) and Th2 cytokines (IL-4 and IL-10) were markedly elevated during the acute stage of Kawasaki disease. Since then, the plasma level of all these cytokines decreased significantly along with the process of clinical stages. Regardless of the existence of coronary artery lesion or no response to initial IVIG treatment, there were no significant differences between them.

Conclusion: These data suggest that both Th1 and Th2 cells may be activated simultaneously during the acute stage of Kawasaki disease. Further studies are therefore required to establish the difference of activation pattern of T helper cells between Kawasaki disease and other inflammatory diseases. (**Korean Circ J 2015;45(6):516-521**)

KEY WORDS: Mucocutaneous lymph node syndrome; Cytokine; Th1-Th2 cytokine balance.

Introduction

Kawasaki disease (KD), an acute systemic vasculitis that primarily affects children was first described by Tomisaku Kawasaki in 1967.¹⁾ The diagnosis of KD depends on clinical manifestation. As far as

research is concerned, the clinical and epidemiological features of KD suggest an infectious origin, yet its etiology remains unknown. Although, it has been commonly assumed that KD is probably driven by immune alteration after the infectious effect. Therefore, KD is being considered in such a way that abnormal immune responses to infectious agents play key roles in the initiation of the disease.²⁾

Many studies have been conducted purposely to the discovery of the role of T cells and inflammatory cytokines during the acute stage of KD.³⁻⁶⁾ Cytokine production pattern in immune reactions, that function in two distinct subsets has been identified; T helper cell type 1 (Th1) and T helper cell type 2 (Th2) cells. Th1 cells play an important role in cell-mediated immunity by predominantly producing interferon-gamma (IFN- γ) and interleukin (IL)-2. Th2 cells are responsible for antibody-producing humoral immunity by producing IL-4, IL-5, IL-6 and IL-10.⁷⁾⁸⁾ Matsubara et al.⁷⁾ suggested that there is an imbalance of Th1 and Th2 subsets during the acute stage of KD.

Recently, several epidemiological studies have documented

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Correspondence: Young Hwan Lee, MD, Department of Pediatrics, Yeungnam University College of Medicine, 170 Hyeonchung-ro, Nam-gu, Daegu 42415, Korea

Tel: 82-53-620-4381, Fax: 82-53-629-2252

E-mail: yhlee3535@ynu.ac.kr

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increased cases of atopic dermatitis and allergic rhinitis in children with a history of KD than general population.^{2[8]9)} Based on the 'hygiene hypothesis,' which states that lack of early childhood exposure to infectious agents, symbiotic microorganisms, and parasites increases susceptibility to allergic diseases by suppressing the natural development of the immune system, KD as immunological slant is in the Th1 direction.⁷⁾ However, the role of T cells and the functional state of Th1 and Th2 cells in KD are still controversial.⁷⁻¹¹⁾

The determination of the T helper cell activation patterns may help in predicting the prognosis and designing treatment strategies for KD.²⁾ To clarify the functional state of T cells in KD, the plasma levels of IFN- γ , IL-2 for Th1 cells activation and IL-4, IL-10 for Th2 cell activation in patients with KD were measured over time.

Subjects and Methods

Patients

In total, 60 patients {36 males and 24 females, median age 25.5 (2-130) months} who met the diagnostic guidelines for typical KD were selected based on the criteria published by the American Heart Association in 2004.¹²⁾ All patients were then admitted at three tertiary hospitals in Daegu city (Kyungpook National University Medical Center, Keimyung University Dongsan Medical Center, and Yeungnam University Hospital) from February to September 2012. The patients agreed to participate in this study upon obtaining the approval of the Institutional Review Board.

In the acute phase, a combination of intravenous immunoglobulin (IVIG) (2 g/kg, 12 hours) infusion and oral intake of high-dose (80-100 mg/kg/day, divided three times) aspirin were administered. When an afebrile condition was maintained for 48 hours, low-dose aspirin (3-5 mg/kg/day, once a day) were used to replace the previous high dose for eight weeks.

Febrile patients with persistent fever after completion of the initial IVIG were retreated with a second course of IVIG (2 g/kg, 12 hours). Defervescence (decreasing fever) was defined as a temperature lower than 38°C that last for at least 48 hours after completion of IVIG. The coronary artery lesion (CAL) was defined as it is determined by the Japanese Ministry of Health and Welfare criteria.¹³⁾

Sampling

Blood sampling was conducted three times for each patient according to the clinical stage of KD. The sampling was done as follows: acute stage - prior to the beginning of treatment with IVIG and aspirin, sub acute stage - 5 days after defervescence with acute phase treatment, and convalescent stage - at the eighth week of

Table 1. Baseline characteristics and clinical outcomes of patients (n=60)

| | |
|---|--------------|
| Median age, months (range) | 25.5 (2-130) |
| Gender, n (male:female) | 60 (36:24) |
| CAL, n (male:female) | |
| No | 50 (25:25) |
| Yes | 10 (9:1) |
| Response to initial IVIG treatment, n (male:female) | |
| Responders | 51 (31:20) |
| Non-responders | 9 (5:4) |

CAL: coronary artery lesion, IVIG: intravenous immunoglobulin

outpatient visits. All patients underwent blood sampling of about 5 mL of peripheral blood at each stage, and then the samples were centrifuged at 2000 rpm/min for 30 minutes. Plasma samples were frozen at -70°C for the enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbent assays

ELISAs for IFN- γ , IL-2, IL-4 and IL-10 were performed according to manufacturer's instruction using kits from R&D systems (Minneapolis, MN, USA). Briefly, the 100 mL of standard of patient plasma was incubated in anti-human IFN- γ (or IL-2 or IL-4 or IL-10) well-coated plates at room temperature for 2 hours. After the 200 mL of prepared biotinylated antibody was added, the plates were incubated at room temperature for 1 hour, and then the 200 mL of tetramethylbenzidine substrate solution was added. The plates were then developed in the dark state at room temperature for 20 minutes. The reaction was ceased by adding 50 mL stop solution. Absorbency of plasma was measured at 450 nm for IFN- γ , IL-2, IL-4 and IL-10.

Statistical methods

Results were presented as mean \pm standard deviation. Statistical differences were analyzed by the Mann-Whitney test. Values were considered significant when $p < 0.05$.

Results

Characteristics and clinical outcomes of patients

A total 60 KD patients were analyzed in this study. Table 1 shows baseline characteristics and clinical outcomes of patients. There were a total of 10 patients (9 males, 1 female) who had CAL and 9 patients (5 males, 4 females) who required a second course of IVIG. All of the retreated patients responded well after a second course of IVIG (Table 1).

Serial assay of plasma interferon-gamma levels

The plasma levels of IFN- γ were as follows: acute stage,

Table 2. The plasma levels of Th1 and Th2 cytokines in patients with Kawasaki disease (n=60)

| Cytokines | Plasma levels (pg/mL) | | | p |
|---------------|-----------------------|-------------------|-------------------------------|----------------------|
| | Acute stage | Sub acute stage | Convalescent stage | |
| IFN- γ | 5.96 \pm 0.05 | 5.50 \pm 0.07* | 4.50 \pm 0.07 [†] | <0.001* [†] |
| IL-2 | 7.96 \pm 0.17 | 6.25 \pm 0.14* | 4.24 \pm 0.13 [†] | <0.001* [†] |
| IL-4 | 9.74 \pm 0.09 | 9.31 \pm 0.05* | 9.07 \pm 0.04 [†] | <0.001* [†] |
| IL-10 | 12.46 \pm 0.06 | 11.90 \pm 0.06* | 10.76 \pm 0.12 [†] | <0.001* [†] |

Data are expressed as mean \pm standard deviation. *: between acute stage and sub acute stage, [†]: between sub acute stage and convalescent stage. Th: T helper cell, IFN: interferon, IL: interleukin

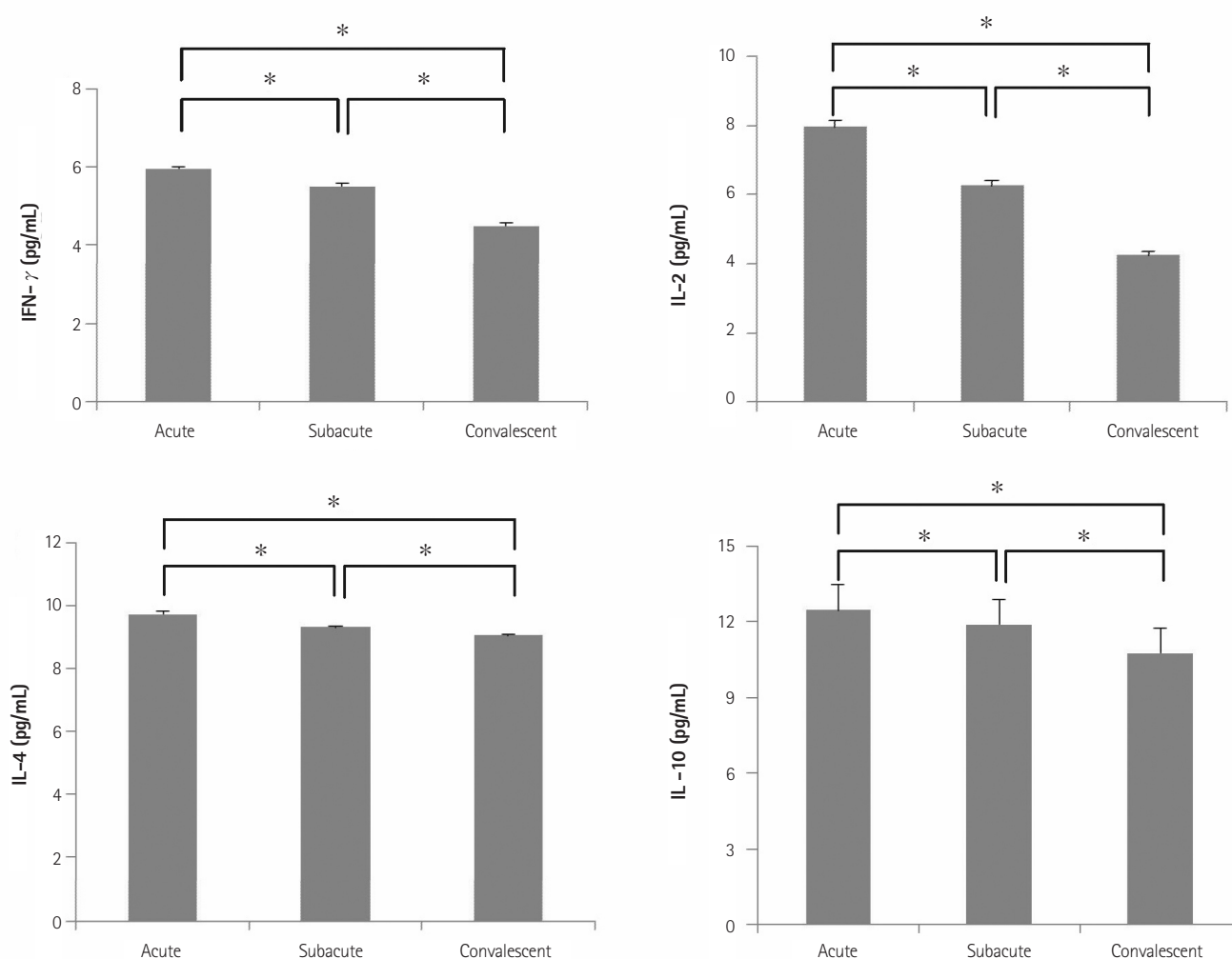


Fig. 1. The changes of plasma cytokines (IFN- γ , IL-2, IL-4, IL-10) level according to clinical stages in patients with Kawasaki disease. *p<0.001. IFN: interferon, IL: interleukin.

5.96 \pm 0.05 pg/mL; sub acute stage, 5.50 \pm 0.07 pg/mL; convalescent stage, 4.50 \pm 0.07 pg/mL, respectively. There was statistically meaningful decrease, according to the clinical stages (p<0.001) (Table 2 and Fig. 1).

Serial assay of plasma interleukin-2 levels

The plasma levels of IL-2 were as follows: acute stage, 7.96 \pm 0.17 pg/mL; sub acute stage, 6.25 \pm 0.14 pg/mL; convalescent stage 4.24 \pm 0.13 pg/mL, respectively. There was a statistically meaningful decrease, by the clinical stages (p<0.001) (Table 2 and Fig. 1).

Table 3. The comparison of plasma levels of Th1 and Th2 cytokines between the patients with coronary artery lesion (n=10) and without coronary artery lesion (n=50) in Kawasaki disease

| Cytokines | Group | Plasma levels (pg/mL) | | | p |
|---------------|--------|-----------------------|-----------------|--------------------|-----|
| | | Acute stage | Sub acute stage | Convalescent stage | |
| IFN- γ | No CAL | 5.93±0.05 | 5.52±0.08 | 4.49±0.07 | N-S |
| | CAL | 6.04±0.04 | 5.39±0.02 | 4.53±0.07 | |
| IL-2 | No CAL | 8.05±0.18 | 6.27±0.18 | 4.17±0.13 | N-S |
| | CAL | 7.48±0.10 | 6.14±0.16 | 4.60±0.15 | |
| IL-4 | No CAL | 9.77±0.09 | 9.32±0.06 | 9.05±0.05 | N-S |
| | CAL | 9.61±0.07 | 9.27±0.04 | 9.17±0.04 | |
| IL-10 | No CAL | 12.46±0.06 | 11.88±0.06 | 10.69±0.12 | N-S |
| | CAL | 12.45±0.05 | 11.97±0.04 | 11.13±0.09 | |

Data are expressed as mean±standard deviation. Th: T helper cell, IFN: interferon, IL: interleukin, CAL: coronary artery lesion, N-S: difference was not significant between the two groups in every stage

Table 4. The comparison of plasma levels of Th1 and Th2 cytokines between the initial IVIG responders (n=51) and non-responders (n=9) in patients with Kawasaki disease

| Cytokines | Group | Plasma levels (pg/mL) | | | p |
|---------------|----------------|-----------------------|-----------------|--------------------|-----|
| | | Acute stage | Sub acute stage | Convalescent stage | |
| IFN- γ | Responders | 5.59±0.05 | 5.45±0.07 | 4.51±0.08 | N-S |
| | Non-responders | 6.34±0.03 | 5.80±0.05 | 4.44±0.03 | |
| IL-2 | Responders | 8.07±0.18 | 6.27±0.14 | 4.23±0.13 | N-S |
| | Non-responders | 7.31±0.11 | 6.15±0.11 | 4.29±0.16 | |
| IL-4 | Responders | 9.75±0.09 | 9.31±0.06 | 9.07±0.05 | N-S |
| | Non-responders | 9.71±0.09 | 9.34±0.04 | 9.09±0.02 | |
| IL-10 | Responders | 12.46±0.05 | 11.88±0.06 | 10.77±0.12 | N-S |
| | Non-responders | 12.41±0.08 | 11.99±0.02 | 10.75±0.09 | |

Data are expressed as mean±standard deviation. Th: T helper cell, IVIG: intravenous immunoglobulin, IFN: interferon, IL: interleukin, N-S: difference was not significant between the two groups in every stage

Serial assay of plasma interleukin-4 levels

The plasma levels of IL-4 were as follows: acute stage, 9.74±0.09 pg/mL; sub acute stage, 9.31±0.05 pg/mL; convalescent stage, 9.07±0.04 pg/mL, respectively. There was a statistically meaningful decrease, according to the clinical stages (p<0.001) (Table 2 and Fig. 1).

Serial assay of plasma interleukin-10 levels

The plasma levels of IL-10 were as follows: acute stage, 12.46±0.06 pg/mL; sub-acute stage, 11.90±0.06 pg/mL; convalescent stage, 10.76±0.12 pg/mL, respectively. There was a statistically meaningful decrease, according to the clinical stages (p<0.001) (Table 2 and Fig. 1).

Comparison of plasma cytokine levels between the sub-groups

There were no significant differences in plasma cytokine levels between patients with and without CAL (Table 3). Same results were found in comparing between initial IVIG responders and non-

responders (Table 4).

Discussion

In the acute stage of Kawasaki disease, T-cells activation is involved in important pathogenesis (the biological manner in which a disease is formed) by the vascular endothelial damage. The role of T cells in KD is still present. The majority of these studies focused on inflammatory cytokines and chemokines.²⁻⁶⁾ Despite the intensive study, analysis of T cell functions in KD has shown variable and often conflicting results. de Inocencio et al.⁴⁾ suggested that the immune activation in KD might be dependent on a number of variables, including the patients' genetic background and the geographical distribution of pathogenic agents that induce the disease.

In addition, several controversial studies have reported on the functional status of Th1 and Th2 cells in KD.⁷⁻¹¹⁾¹⁴⁾¹⁵⁾ Furthermore, Jia

et al.¹⁴) suggested the existence of a Th17/regulatory T (Treg) cell imbalance in KD. Takahashi et al.²¹ suggested that vasculitis in KD may be triggered by aberrant activation of inflammatory cytokines mediated by Th17 cells that have been activated by some infectious agents. The 'hygiene hypothesis' was first proposed by Strachan.¹⁶ According to the 'hygiene hypothesis,' which has been previously defined, the reduced endotoxin exposure and microbial infections in early life lead to decreased Th1 activation and an immunological deviation towards a greater Th2-driven allergy phenotype.¹⁷ Th1 cells produce IFN- γ and IL-2, which promote activating cytotoxic T cells and macrophages. Th2 cells secrete IL-4, IL-5, and IL-10, which initiate humoral immunity and allergic inflammation that stimulate B cells. Usually, Th1 and Th2 cells have reciprocal effect on each.^{18,19}

Th1 and Th2 cytokine profiles are important in such a way that they produce the disease severity and design treatment strategies for KD. Clinically, KD is deemed that a slant is in the Th1 direction.⁸ In fact, marked redness can easily be found with some in duration around the site of bacille de Calmette-Guerin (BCG) vaccination during the acute stage of KD.²⁰ Nowadays, reactivation of the BCG scar is a useful diagnostic tool even for incomplete Kawasaki disease, particularly in communities where BCG vaccination is universal.²¹ The activation of BCG scar is a marker of Th1 mediated delayed hypersensitivity reaction.²² These findings would, therefore, that patients with KD have a lower risk of developing allergic diseases.

However, some questions remain in this hypothesis. There are so much pieces of evidence of imbalance toward Th2 reaction in KD patients.^{7-11,14,15} This is according to Matsubara et al.⁷ first investigative report of T cell activation in KD that is focuses on the Th1 and Th2 imbalance using flow cytometry for detection of intracellular cytokines. They have demonstrated a decrease in the number of Th1 type CD3⁺ T cell in the peripheral blood of patients with acute stage of KD and suggested that there is an imbalance of Th1 and Th2 subsets, a skewed imbalance toward Th2 cell activation, during the acute stage of KD.

In this regard, we assume that KD and other allergic diseases might have some connection. Many cross-sectional studies suggest that KD tends to be associated with allergic diseases through the immunoregulatory dysfunction.^{8,9,23,24} Liew et al.⁸ suggested that KD may be a risk factor for subsequent allergies. They postulate that KD occurs more frequently in children at risk of immune disequilibrium, with an abnormal inflammatory response. This leads to subsequently more allergy manifestations.

Matsuoka et al.⁹ also suggested that a genetic predisposition to atopy (genetic tendency to develop allergic diseases) may be associated with a susceptibility to KD. Furthermore, patients with KD tend to develop atopic dermatitis and allergic rhinitis. Brosius et

al.²³ reported that serum IgE and IL-4 levels (allergy screens) were significantly increased in KD and greater incidence of atopic dermatitis developed in KD patients. Kuo et al.²⁴ suggested that an increased risk of developing allergic diseases following KD may therefore plausibly be related to the effect of regulatory T cells.

The study background was conducted to clarify the functional state of T cells in KD. The plasma levels of IFN- γ , IL-2 for Th1 cells activation and IL-4, IL-10 for Th2 cells activation in patients with KD were then measured over time. In this study, all these cytokines were elevated in the acute stage of KD, and then the level of all these cytokines decreased significantly along with the process of clinical stages (Table 2 and Fig. 1). These results suggest that the immune system is highly activated during the acute stage of KD, which includes both Th1 and Th2 subsets. Although, according to the existence of CAL (Table 3) and response to initial IVIG (Table 4), there were no significant differences between subgroups.

In conclusion, these results suggest that both Th1 and Th2 cells may be activated simultaneously during the acute stage of KD, leaving a certain scope for allergic diseases that have some connection with a susceptibility to KD. Unfortunately, some limitations of the present study are that there we no age-matched control groups, thus missing out the patient data for allergy-related profiles. Further studies are needed to establish the differences between activation pattern of T helper cells between KD and allergic diseases.

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References

1. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* 1967;16:178-222.
2. Takahashi K, Oharaseki T, Yokouchi Y. Update on etio and immunopathogenesis of Kawasaki disease. *Curr Opin Rheumatol* 2014;26:31-6.
3. Leung DY. Immunologic aspects of Kawasaki syndrome. *J Rheumatol Suppl* 1990;24:15-8.
4. de Inocencio J, Hirsch R. The role of T cells in Kawasaki disease. *Crit Rev Immunol* 1995;15:349-57.
5. Jason J, Gregg L, Han A, et al. Immunoregulatory changes in Kawasaki disease. *Clin Immunol Immunopathol* 1997;84:296-306.
6. Brogan PA, Shah V, Clarke LA, Dillon MJ, Klein N. T cell activation profiles in Kawasaki disease. *Clin Exp Immunol* 2008;151:267-74.
7. Matsubara T, Katayama K, Matsuoka T, Fujiwara M, Koga M, Furukawa S. Decreased interferon-gamma(IFN-gamma)-producing T cells in patients with acute Kawasaki disease. *Clin Exp Immunol* 1999;116:554-7.
8. Liew WK, Lim CW, Tan TH, et al. The effect of Kawasaki disease on

- childhood allergies - a sibling control study. *Pediatr Allergy Immunol* 2011;22:488-93.
9. Matsuoka S, Tataru K, Nakagawa R, Mori K, Kuroda Y. Tendency toward atopy in Kawasaki disease. *Eur J Pediatr* 1997;156:30-2.
 10. Kimura J, Takada H, Nomura A, et al. Th1 and Th2 cytokine production is suppressed at the level of transcription regulation in Kawasaki disease. *Clin Exp Immunol* 2004;137:444-9.
 11. Kuo HC, Wang CL, Liang CD, et al. Association of lower eosinophil-related T helper 2 (Th2) cytokines with coronary artery lesions in Kawasaki disease. *Pediatr Allergy Immunol* 2009;20:266-72.
 12. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2004; 110:2747-71.
 13. Japan Kawasaki Disease Research Committee. Report of subcommittee on standardization of diagnostic criteria and reporting of coronary artery lesions in Kawasaki disease. Tokyo: Ministry of Health and Welfare; 1984.
 14. Jia S, Li C, Wang G, Yang J, Zu Y. The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol* 2010;162:131-7.
 15. Wang Y, Wang W, Gong F, et al. Evaluation of intravenous immunoglobulin resistance and coronary artery lesions in relation to Th1/Th2 cytokine profiles in patients with Kawasaki disease. *Arthritis Rheum* 2013;65: 805-14.
 16. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299: 1259-60.
 17. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic disease: an update. *Clin Exp Immunol* 2010;160:1-9.
 18. Del Prete G. The concept of type-1 and type-2 helper T cells and their cytokines in humans. *Int Rev Immunol* 1998;16:427-55.
 19. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136: 2348-57.
 20. Bertotto A, Spinozzi F, Vagliasindi C, Radicioni M, De Rosa O, Vaccaro R. Tuberculin skin test reactivity in Kawasaki disease. *Pediatr Res* 1997;41(4 Pt 1):560-2.
 21. Sinha R, Balakumar T. BCG reactivation: a useful diagnostic tool even for incomplete Kawasaki disease. *Arch Dis Child* 2005;90:891.
 22. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
 23. Brosius CL, Newburger JW, Burns JC, Hojnowski-Diaz P, Zierler S, Leung DY. Increased prevalence of atopic dermatitis in Kawasaki disease. *Pediatr Infect Dis J* 1988;7:863-6.
 24. Kuo HC, Chang WC, Yang KD, et al. Kawasaki disease and subsequent risk of allergic disease: a population-based matched cohort study. *BMC Pediatr* 2013;13:38.