

Peripheral follicular cytotoxic T -like cells in Kawasaki disease with coronary artery aneurysms

A case report

Meng Xu, MM, Jinxiang Liu, MM, Lu Pan, MM, Sirui Yang, PhD*

Abstract

Introduction: Kawasaki disease (KD) is the leading cause of acquired heart abnormalities during childhood. The infiltration of CD8+ T cells plays an essential role in the formation of coronary aneurysms. Follicular cytotoxic T (Tfc) cells are a newly defined subset of CD8+ T cells that express CXC-chemokine receptor 5. The role of Tfc cells in KD is unclear. However, in this report, we present 2 KD children with sustained coronary artery aneurysms (CAA), and we found that their peripheral C-X-C Chemokine Receptor 5+ T cells contained quite amounts of CD4 negative cells. Importantly, these cells have never been reported in KD.

Patients concerns: Case 1 was a 3-year-old boy with a complaint of continuous fever for 6 days and conjunctival injection for 3 days. Case 2 was a 6-month-old boy who was hospitalized because of persistent fever for 5 days, rashes and conjunctival injection for 1 day.

Diagnosis: Case 1 was diagnosed with KD according to typical symptoms and signs including fever over 5 days, conjunctival injection, rashes, swelling cervical lymph nodes and a strawberry tongue. Case 2 had atypical symptoms including persistent fever for 5 days, rashes and conjunctival injection, and he was diagnosed with KD based on the echocardiographic findings.

Intervention: Both the 2 patients received intravenous immunoglobulin and oral aspirin. Besides, case 1 was given the second infusion of intravenous immunoglobulin, intravenous prednisolone and low-molecular-weight heparin.

Outcomes: The CAA of case 1 did not regress until the 12th month after disease onset. The CAA of patient 2 began to regress at the third month after disease onset. During the months from disease onset to the recent follow-up, no cardiovascular events had occurred.

Conclusions: We speculate that Tfc cells may be associated with the formation of CAA. Further studies with larger sample size and functional analysis of these cells are needed.

Abbreviations: CAA = coronary artery aneurysm, CXCR5 = C-X-C Chemokine Receptor 5, IVIG = intravenous immunoglobulin, KD = Kawasaki disease, LMCA = left main coronary artery, Tfc = follicular cytotoxic T.

Keywords: coronary artery aneurysm, follicular cytotoxic T cells, Kawasaki disease

1. Introduction

Kawasaki disease (KD) is an acute, systemic vasculitis with special predilection for coronary arteries. The status of coronary artery abnormalities can be assessed with luminal dimensions that are detected by echocardiography and then based on the Z

score system corrected by body surface area (BSA), the severity of coronary involvement can be classified into no involvement, dilation only, small aneurysm, medium aneurysm, and large aneurysm.^[1] In contrast with the patients without coronary involvement, patients with coronary artery aneurysms (CAA)

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MX and JL contributed equally to this work.

The experimental protocols were established following the Declaration of Helsinki and approved by the Human Ethics Committee of Jilin University (Changchun, China). Each patient's father signed an informed consent form stating that he agrees to give the authors full permission to use his child's protected health information for the purposes of clinical research, discussion, presentation, and publication. A copy of each signed informed consent form is available upon request.

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Table 1
The clinical characteristics of the study participants.

| | CASE 1 | CASE 2 | Normal range |
|---------------------------------|----------|----------|--------------|
| WBC, 10 ⁹ /L | 14.46 | 20.38 | 3.5 – 9.5 |
| Neutrophils, 10 ⁹ /L | 11.57 | 12.3 | 1.8 – 6.3 |
| lymphocyte, 10 ⁹ /L | 1.3 | 5.6 | 1.1 – 3.2 |
| Platelet, 10 ⁹ /L | 473 | 328 | 125 – 350 |
| CRP, mg/L | 33 | 145 | 0 – 3 |
| ESR, mm/1h | 84 | 98 | 0 – 20 |
| Procalcitonin, ng/ml | 0.36 | 0.23 | 0 – 0.5 |
| AST, U/L | 38 | 29 | 13 – 35 |
| ALT, U/L | 22 | 46 | 7 – 40 |
| Albumin, g/L | 22.7 | 40.6 | 40 – 55 |
| IgG, g/L | 7.37 | 4.08 | 8.6 – 17.4 |
| IgA, g/L | 0.83 | 0.33 | 1.0 – 4.2 |
| IgM, g/L | 1.23 | 0.28 | 0.5 – 2.8 |
| C3, g/L | 1.61 | 1.09 | 0.7 – 1.4 |
| C4, g/L | 0.48 | 0.22 | 0.1 – 0.4 |
| Blood culture | Negative | Negative | Negative |

ALT=alanine transaminase, AST=aspartate transaminase, C=complement, CRP=C-reactive protein, ESR=erythrocyte sedimentation rate, Ig=immunoglobulin, WBC=white blood cell count.

have a higher risk of cardiovascular events such as arrhythmia, myocardial infarction, and even sudden death.^[2] The formation of CAA is a complex procedure including the activation of both innate and adaptive immunity. To date, histological investigations have demonstrated that the infiltration of CD8+ T cells into the vessel wall play a critical role in the development of CAA.^[3,4] However, the mechanisms have not been well-understood. In this article, we involved 2 KD patients with persistent CAA and found high levels of circulating CXCR5-chemokine receptor 5 (CXCR5) expressing CD4- T cells.

2. Case report

Case 1: A 3-year-old boy was admitted with the complaint of continuous fever for 6 days and conjunctival injection for 3 days on December 4, 2016. Physical examination found diffuse erythematous rashes on the trunk and extremities, bilateral bulbar conjunctival injection without exudate injection, swelling cervical lymph nodes and a strawberry tongue. Laboratory tests showed increased white blood counts and platelets, elevated inflammatory indicators and reduced serum albumin (Table 1). Initial echocardiography indicated the formation of a medium aneurysm in the left main coronary artery (LMCA). Accordingly, the patient was diagnosed with Kawasaki disease. Initial treatment included intravenous immunoglobulins (IVIG) at a dose of 2 g/kg within 1 day, 100 mg/kg/d of oral aspirin in divided doses. However, the patient had persistent fever during the next 36 hours and then he was administrated with the second infusion of IVIG (2 g/kg) and intravenous prednisolone (2 mg/kg/d). After that, his symptoms were improved. Subsequent treatment included low-dose of oral aspirin and a tapered dose of oral prednisolone. One month after the disease onset, echocardiography showed a large aneurysm in LMCA (Z-score<10 but the diameter is 8.1 mm) and thereby low-molecular-weight heparin was administrated additionally. The recent 2 echocardiography indicated that the size of the aneurysm was inclined to regress. The variation of the Z-score was shown in Figure 1. During the months from disease onset to the recent follow-up, no cardiovascular events had occurred.

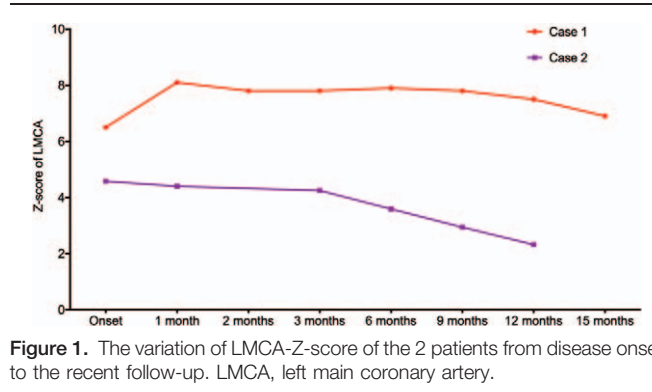


Figure 1. The variation of LMCA-Z-score of the 2 patients from disease onset to the recent follow-up. LMCA, left main coronary artery.

Three milliliters fresh blood were collected at admission and at the time when aneurysm began to regress, respectively. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation, and then stained with fluorescent anti-CD3, anti-CD4, and anti-CXCR5, and analyzed using multicolor flow cytometry. These cells were measured by gating initially live lymphocytes, subsequently CD3+CXCR5+ T cells, and finally CD4 negative population (Fig. 2A). To ensure proper gating strategy, isotype controls were used to determine the gating parameters. As shown in Figure 2B, we found that 41.3% of circulating CXCR5+ T cells were CD4- T cells in the acute stage and 2.47% in the regressive stage.

Case 2: A 6-month-old boy came to our hospital due to persistent fever for 5 days, rashes and conjunctival injection for 1 day on March 17, 2018. There was no definite correlation between fever and rashes. The patient had no medical history from birth. Additional signs necessary for KD diagnosis was not found. The diagnosis was established after the 2-dimensional echocardiography, which showed a small aneurysm in LMCA and another small aneurysm in left anterior descending. Laboratory examination showed significantly increased levels of white blood counts, CRP and ESR, and a slightly elevated level of alanine transaminase. The patient was given only 1 infusion of IVIG (2 g/kg) within 1 day. In addition, 100 mg/kg/d of oral aspirin was given from the establishment of diagnosis to the third day after defervescence and followed by a dose of 5 mg/kg/d. The patient was followed at the 1st, 3rd, 6th, 9th and 12th month after disease onset. At the first follow-up, the echocardiography revealed that the diameter of left anterior descending was normal, whereas the variation of LMCA was not obvious. At the third month after the disease onset, his aneurysm began to regress. The most recent echocardiography performed on April 2, 2019, only showed dilatation of LMCA (Fig. 1). During the past months, no cardiovascular events had occurred. His peripheral blood mononuclear cells were processed in the same method as mentioned in Case 1. We found that 23.6% of CXCR5+ T cells were CD4- T cells in the acute stage and 6.29% in the regressive stage (Fig. 2B).

3. Discussion

In peripheral blood, CD4 negative CXCR5+ T cells that are recorded are within 1 subpopulation of CD8+ T cells. Although it has been more than a decade since the first description of CXCR5 +CD8+ T cells,^[5] till recent years they are investigated as a new subset, which has been named follicular cytotoxic T (T_{fc}) cells.^[6]

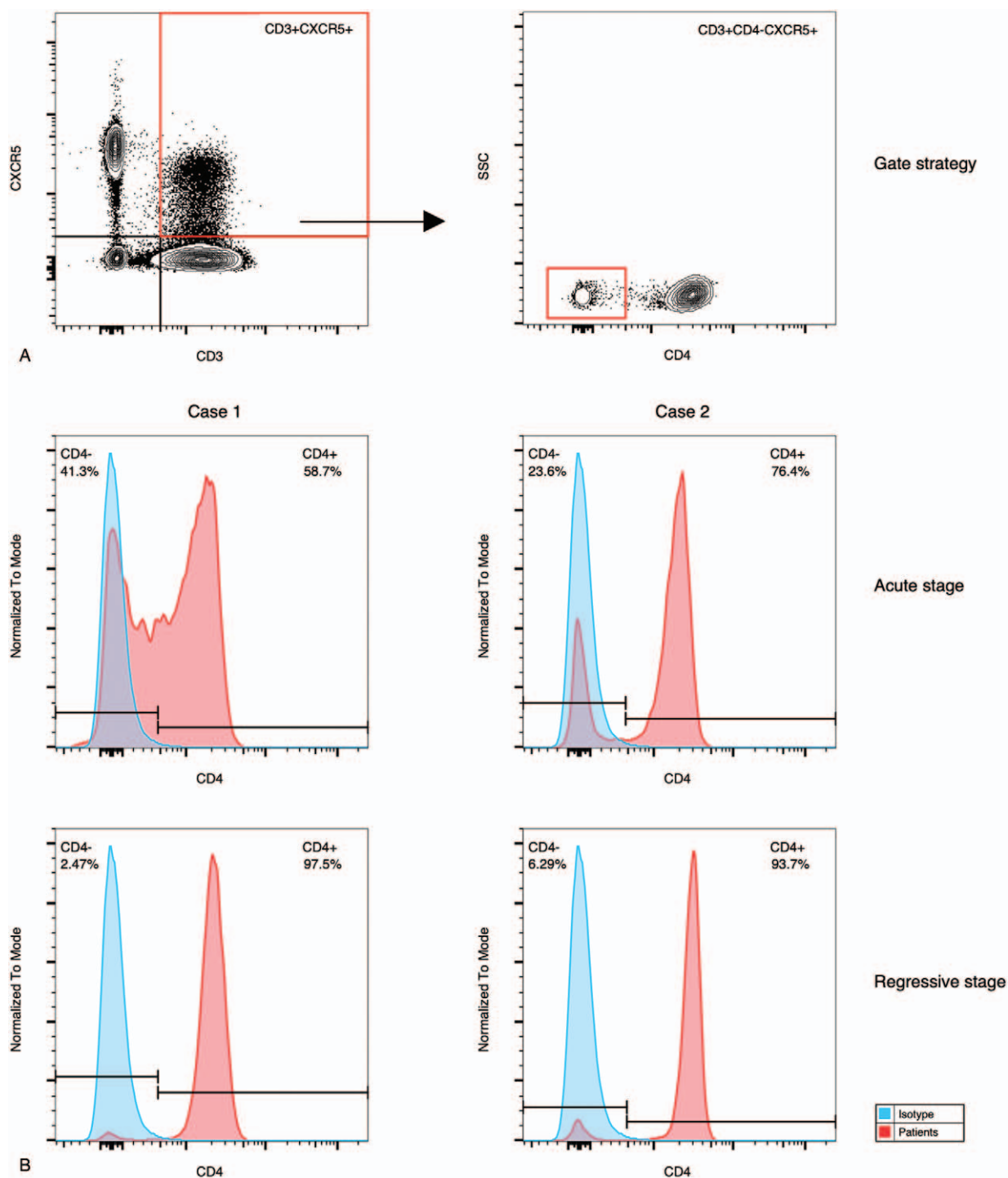


Figure 2. Flow cytometry analysis of the circulating CXCR5+ T cells of the 2 patients. (A) Gate strategy. (B) The percentages of CD4 negative subpopulation of the 2 patients in different stages.

Accordingly, we would like to define those CD4-CXCR5+ T cells as Tfc-like cells. It has been documented that the percentage of circulating CXCR5+ T cells that express CD8 is less than 10% in healthy individuals.^[7] Furthermore, according to our previous studies that included 24 KD patients without coronary involvement and 20 healthy controls, the percentages of CD4 negative subpopulation were 6.45% (2.99–14.5) and 6.87% (2.6–10.3), respectively, of circulating CXCR5+ T cells.^[8] Thus, we believe

that the levels of Tfc-like cells of the 2 patients in the acute stage are affirmatively increased.

Tfc cells that are increased during viral infection present an ability to eliminate infected cells.^[9,10] Furthermore, current understanding insists on the notion that KD is strictly relevant to viral infection.^[11,12] Hence, 1 possibility is that the increase of Tfc-like cells in our patients with CAA may result from a high viral load. However, there is no sufficient evidence to demon-

strate that the formation of CAA is associated with viral load. Another scenario is that the increase of Tfc cells is associated with the function of cytotoxic immunity. At the early stage of infection, Tfc cells predominantly present a memory-like phenotype with a lower capacity to proliferate and perform cytotoxicity in comparison with CXCR5-CD8⁺ T cells.^[10,13] With the persistence of the antigens, CXCR5-CD8⁺ T cells would gradually lose their cytotoxic capacity; in contrast, Tfc cells exhibit an enhanced ability of expansion and cytotoxicity.^[9,10,13,14] In KD, histological biopsy from coronary artery of KD patients with CAA find infiltrated CD8⁺ T cells do not express perforin and granzymes,^[15] whereas the level of perforin+CD8⁺ T cells in the patients without CAA is comparable to that in healthy individuals, suggesting that the function of CD8⁺ T cells is seriously impaired in patients with CAA rather than the patients without CAA.^[16] Coincidentally, the level of Tfc-like cells is obviously increased in patients with CAA but not in those patients without CAA (according to our previous study). Therefore, we speculate that the increase of Tfc-like cells in the 2 patients is a compensatory result of the dysfunction of the cytotoxic immune response.

The invalid expression of the cytotoxic proteins of CD8⁺ T cells can lead to the persistence of causative agents and subsequently result in an uncontrolled inflammation, with finally the formation of CAA.^[15] Although the increase of Tfc-like cells is likely to be contributive for the clearance of pathogens, once increased Tfc-like cells are observed, maybe CAA has already formed, at least in our 2 patients. There have been studies that focus on the strategies to potentiate the function of Tfc cells to be against human immunodeficiency virus and simian immunodeficiency virus.^[17,18] Possibly, it also can be used in KD in advance to prevent the lost function of CD8⁺ T cells and thereby to restrain the formation of CAA. On the other hand, the pro-inflammatory cytokines produced by activated Tfc cells, such as IFN- γ ,^[14] may induce the differentiation of macrophages towards a phagocytic phenotype^[19] and drive the infiltration of CXCR3 expressing cells into the vessel wall and subsequently aggravate the damage of coronary artery.^[20] Thus, the further studies that focus on their exact function in KD is needed. In summary, we first describe an increased level of Tfc-like cells in KD patients with CAA and speculate that Tfc cells are involved in the formation of CAA. The increase of Tfc-like cells is not only supportive of a relation between viral infection and KD, but also suggestive of a cytotoxic dysfunction of CD8⁺ T cells in KD patients with coronary aneurysms.

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References

- [1] TK, SF, NS, et al. A new Z score curve of the coronary arterial internal diameter using the Lambda-Mu-Sigma method in a pediatric population. *Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography* 2016;29:794–801. e729.
- [2] YN, EA, MY, et al. Mortality among Japanese with a history of Kawasaki disease: results at the end of 2009. *Journal of epidemiology* 2013;23:429–34.
- [3] Brown TJ, Crawford SE, Cornwall ML, et al. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *J Infect Dis* 2001;184:940–3.
- [4] Noval Rivas M, Lee Y, Wakita D, et al. CD8⁺ T cells contribute to the development of coronary arteritis in the lactobacillus casei cell wall extract-induced murine model of Kawasaki disease. *Arthritis Rheumatol* 2017;69:410–21.
- [5] Quigley MF, Gonzalez VD, Granath A, et al. CXCR5⁺ CCR7[–] CD8 T cells are early effector memory cells that infiltrate tonsil B cell follicles. *Eur J Immunol* 2007;37:3352–62.
- [6] DY, LY. A Portrait of CXCR5 follicular cytotoxic CD8 T cells. *Trends Immunol* 2018;39:965–79.
- [7] Schaerli P, Willmann K, Lang AB, et al. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *The Journal of experimental medicine* 2000;192:1553–62.
- [8] MX, YJ, JZ, et al. Variation in IL-21-secreting circulating follicular helper T cells in Kawasaki disease. *BMC immunology* 2018;19:43.
- [9] He R, Hou S, Liu C, et al. Follicular CXCR5⁺ expressing CD8⁽⁺⁾ T cells curtail chronic viral infection. *Nature* 2016;537:412–28.
- [10] Leong YA, Chen Y, Ong HS, et al. CXCR5⁽⁺⁾ follicular cytotoxic T cells control viral infection in B cell follicles. *Nat Immunol* 2016;17:1187–96.
- [11] AHR, SCB, STS, et al. Ultrastructural, immunofluorescence, and RNA evidence support the hypothesis of a “new” virus associated with Kawasaki disease. *J Infect Dis* 2011;203:1021–30.
- [12] Rowley AH, Shulman ST. The epidemiology and pathogenesis of Kawasaki disease. *Front Pediatr* 2018;6:374.
- [13] Im SJ, Hashimoto M, Gerner MY, et al. Defining CD8⁺ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 2016;537:417.
- [14] Brummelman J, Mazza EMC, Alvisi G, et al. High-dimensional single cell analysis identifies stem-like cytotoxic CD8⁺ T cells infiltrating human tumors. *The Journal of experimental medicine* 2018;215:2520–35.
- [15] Guzman-Cottrill JA, Garcia FL, Shulman ST, et al. CD8 T lymphocytes do not express cytotoxic proteins in coronary artery aneurysms in acute Kawasaki disease. *Pediatr Infect Dis J* 2005;24:382–4.
- [16] Ehara H, Kiyohara K, Izumisawa Y, et al. Early activation does not translate into effector differentiation of peripheral CD8 T cells during the acute phase of Kawasaki disease. *Cell Immunol* 2010;265:57–64.
- [17] Petrovas C, Ferrando-Martinez S, Gerner MY, et al. Follicular CD8 T cells accumulate in HIV infection and can kill infected cells in vitro via bispecific antibodies. *Sci Transl Med* 2017;9:eaag2285.
- [18] Webb GM, Li S, Mwakalundwa G, et al. The human IL-15 superagonist ALT-803 directs SIV-specific CD8⁺ T cells into B-cell follicles. *Blood Advances* 2018;2:76–84.
- [19] Kang K, Park SH, Chen J, et al. Interferon-gamma represses M2 gene expression in human macrophages by disassembling enhancers bound by the transcription factor MAF. *Immunity* 2017;47:235–50.
- [20] Lacotte S, Brun S, Muller S, et al. CXCR3, inflammation, and autoimmune diseases. *Ann N Y Acad Sci* 2009;1173:310–7.