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The CXCL10-CXCR3 axis plays an important role in Kawasaki disease

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Abstract

The precise pathogenesis of Kawasaki disease remains unknown. In an attempt to elucidate the pathogenesis of KD through the analysis of acquired immunity, we comprehensively examined the immunophenotypic changes in immune cells such as lymphocytes and monocytes along with various cytokines, focusing on differences between pre- and post- treatment samples. We found high levels of CXCL9 and CXCL10 chemokines that decreased with treatment, which coincided with a post-treatment expansion of Th1 cells expressing CXCR3. Our results show that the CXCL10-CXCR3 axis plays an important role in the pathogenesis of KD.

Keywords: Kawasaki disease, Th1, CXCR3, CXCL10

Abbreviations: CAL: coronary artery lesion; KD: Kawasaki disease; MDSC: myeloid-derived suppressor cells; TCR: T-cell receptor; Th1: type 1 helper T cell; Treg: regulatory T cell.

Introduction

Kawasaki disease (KD) is an acute vasculitis in children that is accompanied by signs of systemic inflammation such as fever and erythema [1]. Despite extensive research, the cause or precise pathogenesis of KD remains unknown. Many studies have depicted elevated levels of inflammatory cytokines such as TNF-α and IL-6 [1, 2], and other studies have found that innate immune cells, such as neutrophils and monocytes, play a central role in the pathogenesis of KD [3, 4]. However, several studies have depicted the changes in acquired immunity during the course of KD, such as an expansion of certain subsets of follicular helper T cells (Tfh) [5] and an increase in regulatory T cells (Treg) after treatment [6]. In an attempt to elucidate the pathogenesis of KD through the analysis of acquired immunity, we comprehensively examined the immunophenotypic changes in immune cells such as lymphocytes and monocytes along with various cytokines, focusing on differences between pre- and post- treatment. We found that elevated CXCL10 levels decreased with time and coincided with an increase in CXCR3-positive cells, suggesting an important role of the CXCL10-CXCR3 axis in the pathogenesis of KD.

Material and Methods

Subjects

Patients newly diagnosed with KD based on established criteria [7] were enrolled after obtaining informed consent from their guardians. Patients were treated according to the physician's choice, which mainly involved aspirin and intravenous immunoglobulin therapy (IVIG) with or without glucocorticoid steroids [8] (Table 1). Clinical features were collected from medical records. Coronary artery lesions (CAL) were defined as having an internal diameter *Z*-score of > 2.5, measured by echocardiography. Whole blood was collected in ethylenediamine tetra-acetic acid (EDTA) Na2-coated tubes, and 0.5 ml was used for flow-cytometric analysis after red blood cell lysis, while plasma was obtained from the remaining 1.5 ml by centrifugation and stored at −80°C for cytokine analysis.

Blood samples were obtained at several time points; the acute phase prior to first-line treatment (TP1), the convalescent phase, generally 2–3 days after treatment (TP2), and the recovery phase, generally 1 week after treatment (TP3).

This study was approved by the institutional review board of University of Tsukuba Hospital (H29-310).

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Table 1. Characteristics of patients with Kawasaki disease (n = 47)

Age (years, median)	**1.79 (range 0.25-6.42)**
Sex (male/female)	27/20
Number of major symptoms (median ± SD)	5 ± 0.78 (range 3-6)
RAISE score (median ± SD)	3.0 ± 1.77 (range 0-8)
Duration of fever at treatment (days, median ± SD)	5.0 ± 1.58 (range 2-10)
Patients treated with aspirin	45
Patients treated with IVIG	43
Patients treated with PSL	2
Refractory to 1st line treatment	7
Patients with CAL	4

CAL = coronary artery lesions, IVIG = intravenous immunoglobulin, PSL = prednisolone, RAISE = Randomized controlled trial to Assess Immunoglobulin plus Steroid Efficacy for Kawasaki disease.

Cytokine analysis

Plasma cytokines (IL-1β, IL-6, TNFα, MCP-1, IL-4, IL-10, IL-2, IL-12p70, IFNγ, MIG (CXCL9), IP10 (CXCL10), and eotaxin (CCL11)) were measured using the Cytometric Beads Assay (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were processed according to the manufacturer's instructions, acquired with a BD LSR Fortessa flow cytometer (Becton Dickinson), and analyzed using FCAP array software (Becton Dickinson).

Flow cytometric analysis

Whole blood samples were stained with a panel of antibodies described separately (Table S1). After staining for 15 min in room temperature, the samples were lysed and fixed using the TQ-Prep Workstation (Beckman Coulter Inc, Tokyo, Japan). After washing twice, cells were acquired using a BD LSR Fortessa flow cytometer, and data were analyzed using FlowJo software version 10.6.2, (Becton Dickinson). The absolute number of each leukocyte subset was calculated by multiplying the percentages of the designated subsets and the leukocyte or lymphocyte count obtained from medical records. The normal values for each cell population are previously described [9, 10].

T-cell receptor repertoire analysis

T cell receptor (TCR) Vβ repertoire analysis was performed using the IOTest Beta Mark kit (Beckman Coulter Inc., South Kraemer Boulevard, CA, USA) which contains 24 monoclonal antibodies identifying ~70% of the TCR repertoire (Table S1). Whole blood samples were additionally stained with antibodies for surface antigens to analyze the TCR repertoire among specific T-cell subtypes. All samples were processed using the TQ-prep Workstation, acquired with a BD LSR Fortessa flow cytometer, and analyzed with FlowJo software.

Statistical analysis

Data were analyzed statistically using the Mann–Whitney U-test, or the Student's t-test when indicated. Calculations were performed using SPSS software ver 28.0 (IBM Inc, NY, USA) or GraphPad Prism software ver 8.0 (SanDiego, CA, USA).

Results

Clinical features of patients

Forty-seven patients diagnosed with KD were enrolled in the study. The median age for all patients was 1.7 years, and the male/female ratio showed a slight predominance in males. For first-line treatment, 43 patients were treated with IVIG, of which 2 patients were treated with additional prednisolone. Seven patients were refractory to first-line treatment and 4 patients developed CAL (Table 1). TP1 samples were available for 40 patients, TP 2 samples were available for 43 patients, and TP3 samples were available for 36 patients. The median interval between TP1 and TP2 was 2 days, and the interval between TP1 and TP3 was 5 days (Table S1).

High levels of plasma chemokines which decreased with treatment

Cytokine levels were compared between TP1, TP2, and TP3 using the cytometric beads assay method. Pro-inflammatory cytokines such as IL-6 and MCP-1 were elevated at the acute phase (TP1), and decreased after treatment (TP2 and TP3). This trend was also true for Th1-cytokines (IL-12p70, IFNγ) and CXCR3-ligand chemokines (MIG (CXCL9) and IP10 (CXCL10)). As for Th2 cytokines, a similar trend was observed for eotaxin (CCL11) but not for IL-4 (Figure 1).

Flow cytometric analysis revealed expansion of circulating CXCR3-positive T cells

Multiple leukocyte subsets were evaluated by flow cytometry, and statistically analyzed by comparing values from post-treatment samples (TP2, TP3) versus pre-treatment samples (TP1) (Table 2). Five separate panels of antibodies were used.

Panel PBMC focuses on the major leukocyte subsets, such as lymphocytes, monocytes, $CD3^+$ T cells, $CD19^+$ B cells, and $CD56^+$ NK cells. When comparing TP3 with baseline (TP1), a significant recovery of total lymphocytes was observed, but this increase was observed across all lymphocyte subsets without any difference in the ratio of T/B/NK (Table S3). On the contrary, monocytes decreased right after treatment but recovered at TP3. When focusing on monocyte subpopulations after treatment, there was a decrease in $CD14^+CD16^+$ intermediate and $CD14^{dim}CD16^+$ non-classical monocytes, while there was no significant change in $CD14^+CD16^-$ classical monocytes.

Panel T1 focuses on T-cell subsets, such as $CD4^+$ helper T (Th) cells, $CD8^+$ cytotoxic T (Tc) cells, and more detailed subpopulations. There were no significant findings in Th/Tc ratio, naïve/memory T-cell ratio, or $TCR\alpha\beta^+/TCR\gamma\delta^+$ T-cell ratio at baseline (TP1). No expansion of activated Th or Tc cells was observed at baseline. No differences associated with treatment were observed in the rate of naïve/memory T cells, $TCR\alpha\beta^+/TCR\gamma\delta^+$ T cells, or $CD38^+HLA\text{-}DR^+$-activated T cells, with the exception of a slight recovery of activated Tc cells, double-negative T cells, and effector Th cells at TP3.

Panel T2 focuses on memory Th-cell subpopulations, such as $CD25^+CD127^{lo}$ regulatory T (Treg) cells, type 1 helper T (Th1) cells, type 2 helper T (Th2) cells, and type 17 helper T (Th17) cells, each designated as $CXCR3^+CCR6^-CD45RO^+$ Th cells, $CXCR3^-CCR6^-CD45RO^+$ Th cells, and $CXCR3^-CCR6^+CD45RO^+$ Th cells, respectively. A significant expansion of Tregs was observed after treatment at TP3, although there was no change in the composition of

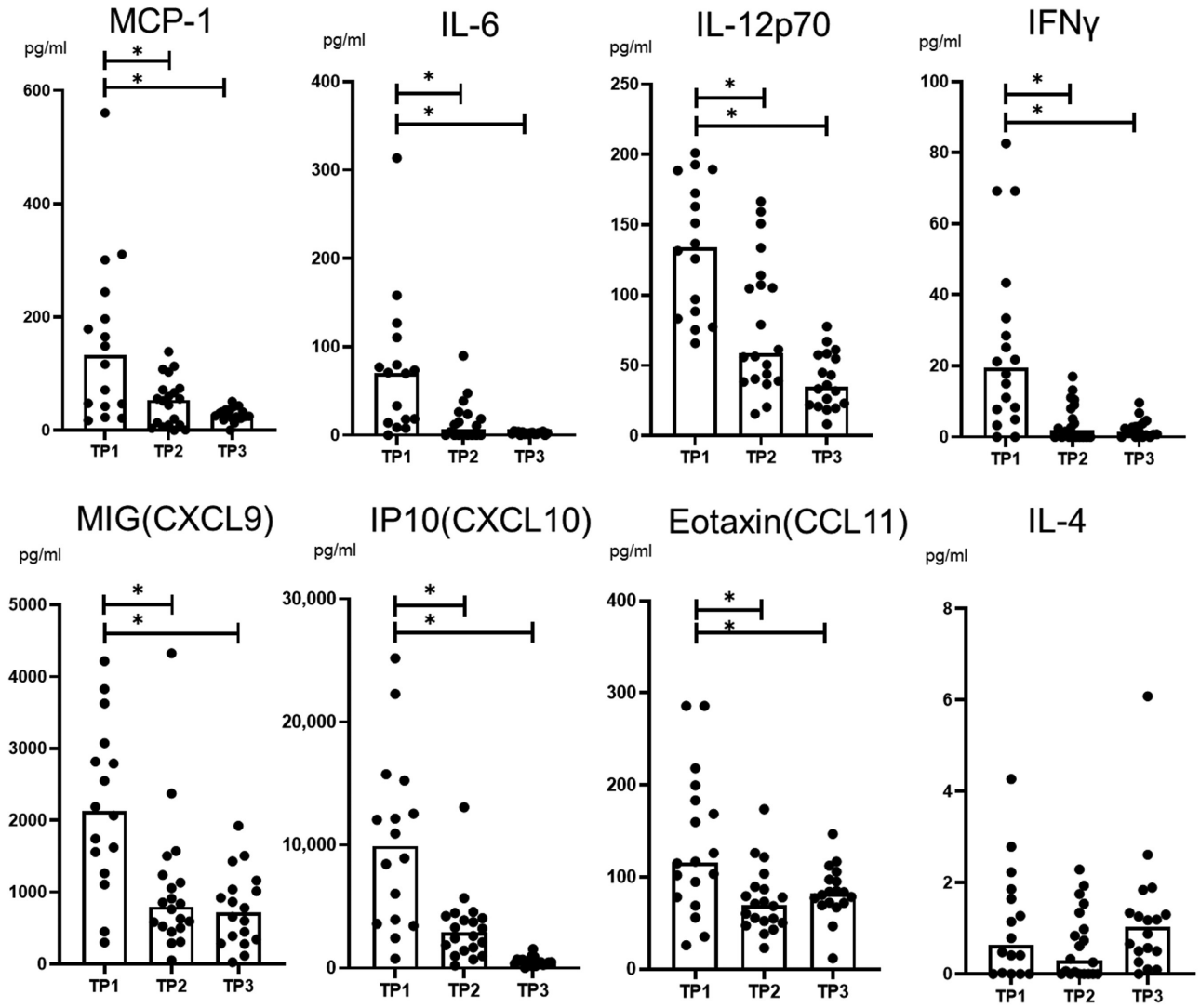

Figure 1. Results of representative plasma cytokines assessed by the cytometric beads assay. The values were compared between each time point. All measurements are in picagram per milliliter. Note that the value ranges differ greatly for each cytokine. The results for all cytokines are shown in Supplementary Table S2. *$P < 0.05$.

type 1-, type 2-, or type 17-Tregs (Supplementary Table S3). Although the proportion of naïve/memory T cells did not change with treatment, within the memory T-cell population, there was a significant expansion of Th1 cells after treatment, along with an expansion of type 1 ($CXCR3^+CCR6^-$) Tc cells, cells both positive for CXCR3 which is a chemokine receptor that binds to CXCL9 and CXCL10 (Figure 2).

Panel B focuses on various B-cell subpopulations. There were no major findings in B-cell subpopulations, with the exception of a slight expansion of IgD^+ memory B cells and naïve B cells after treatment.

Panel MDSC focuses on myeloid-derived suppressor cells ($CD11b^+CD33^+HLA\text{-}DR^-lin^-CD45^+$ cells), which are known to have an immunosuppressive role in various tumors or inflammatory conditions. The number of MDSCs at baseline was comparable to previously published normal controls [11]. There was a decrease in the number of MDSCs at TP2 compared to TP1, but no significant difference was observed at TP3 (Table 2).

No expansion of specific T-cell receptor subpopulation observed by repertoire analysis.

Flow cytometry-based TCR V beta repertoire analysis was available for seven TP1 samples. No TCR repertoire skewing was observed in Th cells, and there was no significant expansion of any specific V beta subpopulation compared to previously published controls [12]. The same trend was observed for repertoire analysis within other Th subpopulations, such as $CD45RO^+$ memory Th cells and Th1 cells (Figure 3).

Discussion

Kawasaki disease is a form of systemic vasculitis characterized by highly elevated levels of inflammatory cytokines, such as TNF-α, IL-6, and MCP-1. The detailed pathogenesis of KD still remains to be revealed, but the mainstream theory is a hyper-inflammatory response evoked by non-specific environmental triggers, attenuated by underlying host factors. Extensive research into the pathogenesis of KD has identified innate cells such as neutrophils and monocytes to

Table 2. Results of flow cytometric analysis

		TP1	TP2	*P*-value (vs TP1)	TP3	*P*-value (vs TP1)
Panel PBMC	Lymphocytes (/μl)	3078.6	2790.2	0.872	4641.0	0.002
	Monocytes (/μl)	392.5	201.0	<0.001	275.9	0.053
	$CD56^+$ cells (/μl)	166.0	117.6	0.333	236.1	0.029
	$CD3^+$ cells (/μl)	1668.7	1870.8	0.375	2559.3	0.013
	$CD19^+$ cells (/μl)	930.2	799.6	0.554	1319.9	0.025
	Classical monocytes (/μl)	118.9	85.5	0.11	138.3	0.857
	Intermediate monocytes (/μl)	25.2	12.3	0.008	13.3	0.149
	Nonclassical monocytes (/μl)	14.3	5.1	0.004	4.5	0.002
Panel T1	$CD4^+$ $CD3^+$(Th) cells (/μl)	982.0	1271.6	0.564	1493.1	0.258
	$CD8^+CD3^+$(Tc) cells (/μl)	479.5	411.4	0.902	545.3	0.081
	$CD38^+HLADR^+$ (activated Th) cells (/μl)	15.8	13.9	0.731	20.5	0.153
	Recent thymic emmigrants ($CD31^+CD45RA^+$Th) (/μl)	692.1	954.3	0.334	1044.8	0.278
	$CD38^+HLADR^+$ (activated Tc) cells (/μl)	7.0	8.2	0.141	18.2	0.007
	$TCR\alpha\beta^+$ cells (/μl)	1643.2	1750.0	0.771	2033.0	0.157
	$TCR\gamma\delta^+$ cells (/μl)	62.1	65.5	0.693	98.0	0.131
	$CD4^-CD8^-$ $TCR\alpha\beta^+$cells (double-negative cells) (/μl)	18.5	17.5	0.683	28.7	0.022
	Central memory Th cells (/μl)	93.2	79.6	0.128	94.7	0.322
	Effector Th cells (/μl)	72.6	82.9	0.202	153.9	0.017
	effector memory Th cells (/μl)	84.7	96.4	0.655	141.8	0.019
	Naive Th cells (/μl)	610.8	799.9	0.771	1398.9	0.018
	Central memory Tc cells (/μl)	10.8	11.7	0.609	14.7	0.172
	Effector Tc cells (/μl)	50.6	41.4	0.851	61.7	0.271
	Effector memory Tc cells (/μl)	31.0	17.7	0.573	43.1	0.096
	Naive Tc cells (/μl)	271.9	280.6	0.861	350.3	0.198
Panel T2	$CD25^+CD127^{lo}$ (Treg) cells (/μl)	44.6	70.5	0.079	80.0	0.012
	$CD45RO^-$ (naive Th) cells (/μl)	711.4	986.1	0.166	1147.7	0.062
	$CD45RO^+$ (memory Th) cells (/μl)	142.2	185.7	0.964	206.9	0.098
	$CXCR3^+CCR6^-CD45RO^+$(Th1) cells (/μl)	28.4	51.0	0.088	68.1	<0.001
	$CXCR3^-CCR6^-CD45RO^+$(Th2) cells (/μl)	103.5	106.5	0.228	113.0	0.955
	$CXCR3^-CCR6^+CD45RO^+$(Th17) cells (/μl)	11.5	14.2	0.406	19.3	0.003
	$CXCR5^+CD45RO^+$(Tfh) cells (/μl)	45.2	44.1	0.881	53.1	0.427
	$PD1^+$ Tfh cells (/μl)	25.1	26.9	0.708	24.8	0.649
	$CXCR3^+CCR6^-$(type 1) Tc cells (/μl)	40.5	116.7	0.073	232.4	<0.001
	$CXCR3^-CCR6^-$(type 2) Tc cells (/μl)	8.4	7.3	0.053	13.1	0.443
	$CXCR3^-CCR6^+$(type 17) Tc cells (/μl)	279.0	228.6	0.214	247.7	0.804
	Naive Tc (/μl)	249.2	335.9	0.908	445.9	0.35
	Memory Tc (/μl)	64.2	51.8	0.386	95.5	0.006
Panel B	IgD^+CD27^+ (IgD memory) cells (/μl)	22.4	26.7	0.139	46.2	<0.001
	IgD^-CD27^+ (switched memory) cells (/μl)	39.1	27.6	0.083	49.4	0.238
	IgD^+CD27^- (naive B) cells (/μl)	695.7	573.7	0.591	997.8	0.009
	$CD38^+CD10^+$ (transitional B) cells (/μl)	34.6	43.8	0.946	71.0	0.049
	$CD21^{hi}$(T1) cells (/μl)	22.2	23.2	0.819	41.6	0.154
	$CD21^{lo}$ (T2) cells (/μl)	7.1	10.4	0.727	18.4	0.015
	$CD38^{hi}CD27^+$ (plasmablasts) (/μl)	2.6	3.3	0.361	5.0	0.032
	$IgM^+IgD^+CD27^+$ (IgM memory) cells (/μl)	5.3	4.9	0.909	5.9	0.3
	$CD38^-CD21^{lo}$ cells (/μl)	24.3	13.6	0.058	35.3	0.355
Panel MDSC	$CD11b^+CD33^+HLA\text{-}DR^-lin^-$ cells (MDSC) (/μl)	125.355	28.4229	<0.001	62.241	0.059

All measurements are in median.

play a central role in KD [1, 13], while the role of acquired immunity remains unknown. Past studies have proposed the role of certain T-cell subsets such as activated Tc cells [14] or Tregs [6], but a comprehensive view including vast immune cell subtypes and cytokines/chemokines remains in need. Thus, in order to elucidate the role of acquired immunity in KD, we performed a comprehensive analysis of various immune cells and cytokines using pre- and post-treatment samples.

A previous study showed that CXCL10, a chemokine which binds to CXCR3 was elevated in patients with KD and could be used to discriminate patients from other febrile conditions with high accuracy [15]. However, in the study by Ko et al., the expression of CXCR3 on circulating $CD3^+$

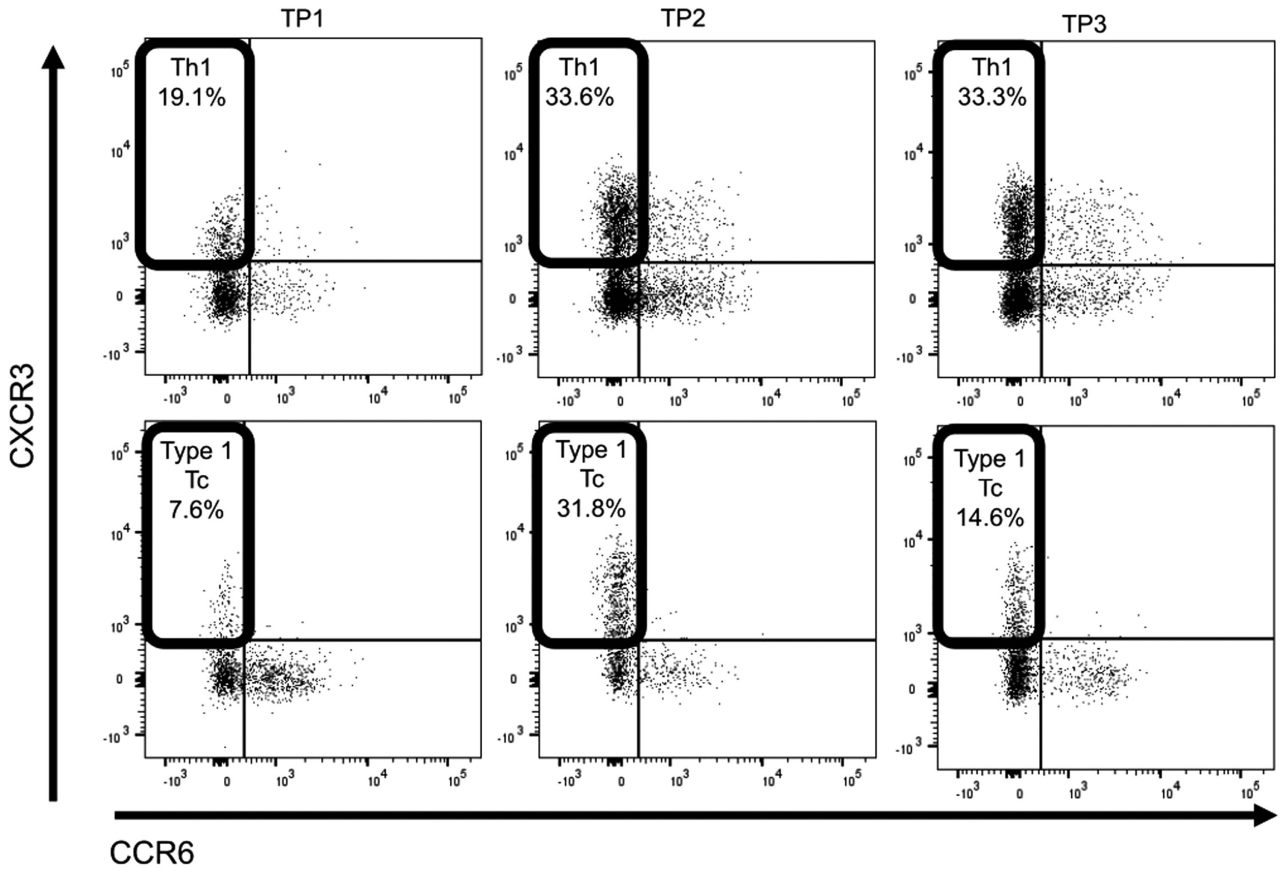

Figure 2. A representative plot for flow cytometric analysis of CXCR3$^+$CCR6$^-$ Th1 cells and CXCR3$^+$ Tc cells from a patient analyzed at TP1, TP2, and TP3. On the upper row, cells are gated on CD45RO$^+$CD4$^+$CD3$^+$ memory Th cells, and on the bottom row, cells are gated on CD3$^+$CD4$^-$ Tc cells.

cells was paradoxically downregulated, which was discussed as recruitment of CXCR3$^+$ cells to the site of inflammation. Our results strongly support this theory, since we observed a large treatment-induced decrease in CXCL10 along with an increase in CXCR3 + cells in post-treatment samples. Another study by Xu et al. observed an increase in Th1-type follicular T cells (Tfh1) and a decrease in Th2-type follicular T cells (Tfh2) after treatment in patients with KD [5]. Although the authors focused on different populations within the Tfh subset, we have reason to believe that their results reflect the changes in overall Th1/Th2 populations, since their definition of Th1/Th2 phenotype was the same as ours. However, we do not have enough pathological evidence to prove that the observed increase in circulating CXCR3$^+$ cells lead to the development of coronary arteritis, and it is important to note that the majority of cells infiltrating the perivascular area in KD are macrophages and neutrophils, with only a few lymphocytes [16]. Furthermore, oxidized LDLs, which are associated with coronary lesions in KD [17, 18], are known to dampen Th1 responses [19]. Therefore, further studies are needed to clarify the accumulation of CXCR3$^+$ cells in the perivascular area and their role using KD models.

In our study, we defined Th1 cells as CXCR3$^+$CCR6$^-$ memory T cells on the basis of surface markers [20], and not the traditional definition of IFNγ positivity by intracellular staining. We observed an increase of Th1 cells from TP1 to TP2 and TP3, along with a decrease in CXCL10 and CXCL9. These cytokines are ligands for CXCR3 and are secreted by several cell types including monocytes, fibroblasts, dendritic cells, and endothelial cells. These kinetics support the theory that the CXCL10-CXCR3 axis plays an important role in KD and that after treatment with IVIG, the decrease in cytokine levels lead to a return of CXCR3$^+$ cells into the circulation (Figure 4). However, it is also possible that the CXCL10-CXCR3 axis functions primarily in endothelial cells rather than circulating lymphocytes, since the CXCR3-B isoform is primarily expressed by endothelial cells in humans and is known for its antiangiogenic properties [21], which may lead to endothelial cell apoptosis and ultimately, coronary artery aneurysms. The notion that chemokines attract certain effector cells, such as Th1 cells and pulls them out of the circulation may explain the paradoxical phenomenon in which Th1 cells contribute to the pathogenesis of a disease, but are decreased in peripheral blood. For example, circulating Th1 cells are decreased in patients with Multisystem Inflammatory Syndrome in Children (MIS-C) [9, 22], although Th1 immunity is thought to play a central role in their pathogenesis [22, 23]. It is interesting to observe that CXCL9 and CXCL10 are also elevated in MIS-C [24], which may serve as a clue to why KD and MIS-C display overlapping symptoms.

Regulatory T cells (Treg) are known to increase in the circulation after IVIG treatment in patients with KD [6], although the mechanism is poorly understood. From our results, we initially speculated that the increased fraction of Tregs would be Th1-type CXCR3$^+$Tregs. However, we did not observe any difference in the proportion of CXCR3$^+$CCR6$^-$Tregs between TP1 and TP2 or TP3 (Table S3), suggesting that the increase in Tregs is mediated by

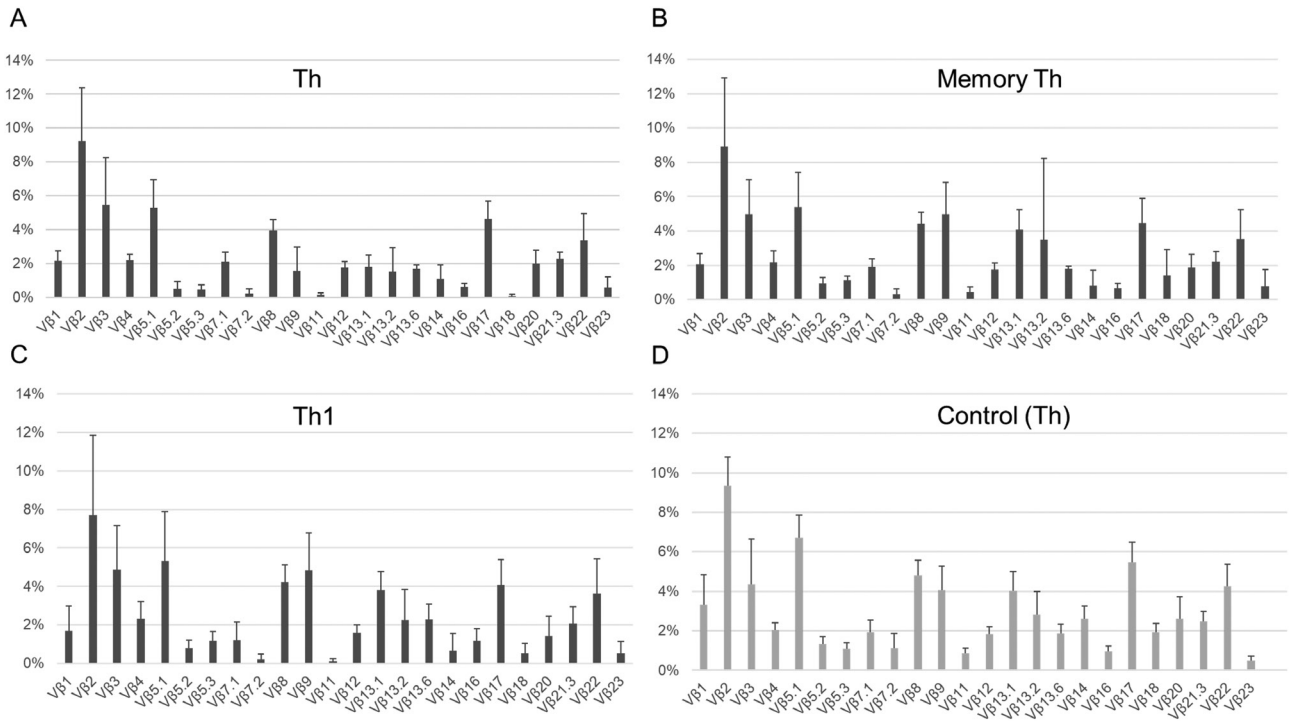

Figure 3. Flow cytometry-based TCR repertoire analysis for Th cells (A), memory Th cells (B), and Th1 cells (C), all from TP1 samples ($n = 7$). For each T-cell population, the mean values for each TCR subtype were compared to previously published control Th cells [12] (D, $n = 46$) using the *t*-test. There were no significantly expanded TCR subtypes compared to controls.

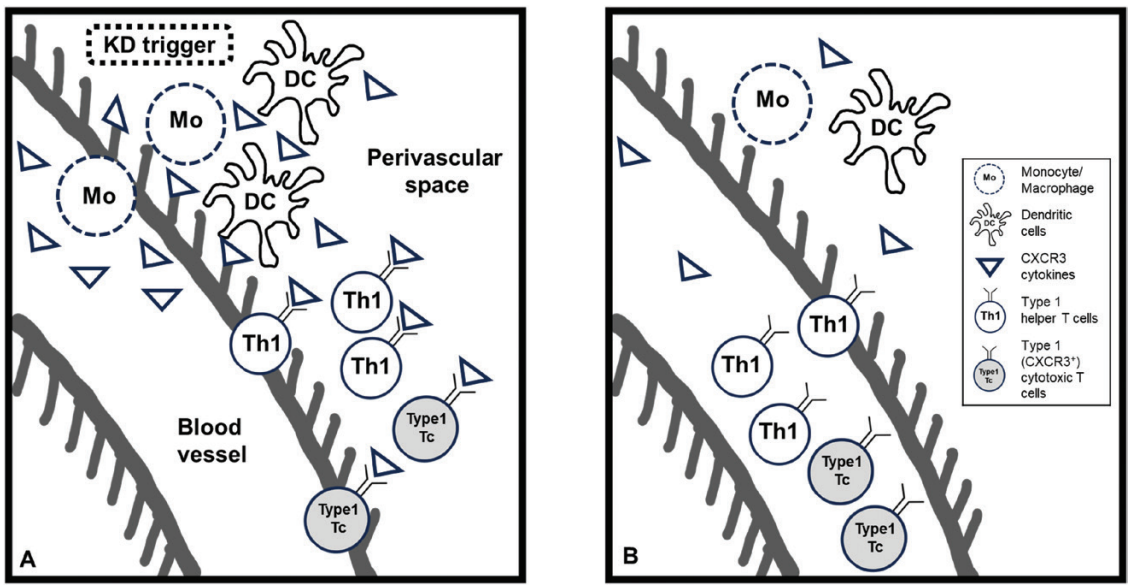

Figure 4. A schematic illustration of the proposed mechanism of the CXCL10-CXCR3 axis in KD. (A) During the acute phase, an unknown environmental trigger stimulates innate cells such as monocytes/macrophages, dendritic cells, and endothelial cells (not shown) to produce CXCL10 and other CXCR3 cytokines, possibly attracting CXCR3-positive Th1 cells and type 1 Tc cells into the perivascular area. (B) After treatment with IVIG with or without steroids, cytokine production decreases and thus the CXCR3-positive cells are released into the circulation.

a CXCR3-independent mechanism, perhaps involving miRNAs [25].

MDSCs are myeloid cells with immunosuppressive potential, and are receiving attention in the field of various pathological conditions such as cancer or infections. Although this was the first study focusing on MDSCs in the setting of KD, we could not find a significant role, since the number of MDSCs at baseline were comparable with previously published normal controls.

Although previous studies have confirmed that there are no specific TCR subtypes that are expanded in KD [26], we initially speculated that we may observe skewing in TCR repertoires if we focused on T-cell subpopulations that may play a central role in KD. However, when focusing on Th

cells, CD45RO$^+$ memory Th cells and Th1 cells, we could not detect any TCR subtype that was significantly expanded compared to previously published controls [12]. These results are in line with previous reports denying the role of superantigens or any specific single antigen in the pathogenesis of KD.

In summary, we performed a comprehensive analysis of plasma cytokines and immune cells in KD pre- and post-treatment samples. We found high levels of CXCL9 and CXCL10 chemokines that decreased with treatment, which coincided with a post-treatment expansion of CXCR3$^+$ cells such as Th1 and type 1 Tc cells. Our results show that the CXCL10-CXCR3 axis plays an important role in the pathogenesis of KD. Further analysis of CXCR3$^+$ cells in coronary artery samples using KD models are warranted.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

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Ethical approval

The protocol for this study was in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects of the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare. The protocol was approved by the ethics committee at University of Tsukuba Hospital (H29-310). Written informed consent was obtained from the parents or guardians of the patients.

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Conflict of interests

All authors have no financial or commercial conflicts of interest to declare.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Author contributions

S.H. performed the experiments and wrote the manuscript with input from A.M. and F.F., S.H., K.I., and H.T. conceived the study. Y.Y., L.L., J.S., M.T.I., D.H., H.I. enrolled the patients and collected the clinical data. H.T revised the manuscript for important intellectual content. All authors commented on the paper and approved the final manuscript as submitted.

References

1. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. Clin Exp Immunol 2016, **186**, 134–43. doi:10.1111/cei.12832.
2. Tamura K, Kanazawa T, Tsukada S, Kobayashi T, Kawamura M, Morikawa A. Increased serum monocyte chemoattractant protein-1, macrophage inflammatory protein-1beta, and interleukin-8 concentrations in hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2008, **51**, 662–8. doi:10.1002/pbc.21660.
3. Furukawa S. Activation of peripheral blood monocytes/macrophages in Kawasaki disease. Pediatr Res 2003, **53**, 160–160. doi:10.1203/00006450-200301000-00042
4. Matsubara T, Ichiyama T, Furukawa S. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. Clin Exp Immunol 2005, **141**, 381–7. doi:10.1111/j.1365-2249.2005.02821.x.
5. Xu M, Jiang Y, Wang J, Liu D, Wang S, Yi H, et al. Distribution of distinct subsets of circulating T follicular helper cells in Kawasaki disease. BMC Pediatr 2019, **19**, 43. doi:10.1186/s12887-019-1412-z
6. Furuno K, Yuge T, Kusuhara K, Takada H, Nishio H, Khajoee V, et al. CD25+CD4+ regulatory T cells in patients with Kawasaki disease. J Pediatr 2004, **145**, 385–90. doi:10.1016/j.jpeds.2004.05.048.
7. Kobayashi T, Ayusawa M, Suzuki H, Abe J, Ito S, Kato T, et al. Revision of diagnostic guidelines for Kawasaki disease (6th revised edition). Pediatr Int 2020, **62**, 1135–8. doi:10.1111/ped.14326.
8. Miura M, Ayusawa M, Fukazawa R, Hamada H, Ikeda S, Ito S, et al. Guidelines for medical treatment of acute Kawasaki disease (2020 Revised Version). J Pediatr Cardiol Cardiac Surg 2021, **5**, 41–73. doi:10.24509/jpccs.0501G1
9. Morita A, Hosaka S, Imagawa K, Ishiodori T, Nozaki Y, Murakami T, et al. Time course of peripheral immunophenotypes of multisystem inflammatory syndrome in children. Clin Immunol 2022, **236**, 108955. doi:10.1016/j.clim.2022.108955.
10. Takashima T, Okamura M, Yeh TW, Okano T, Yamashita M, Tanaka K, et al. Multicolor flow cytometry for the diagnosis of primary immunodeficiency diseases. J Clin Immunol 2017, **37**, 486–95. doi:10.1007/s10875-017-0405-7.
11. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, et al. Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PLoS One 2013, **8**, e57114. doi:10.1371/journal.pone.0057114
12. Tembhare P, Yuan CM, Xi L, Morris JC, Liewehr D, Venzon D, et al. Flow Cytometric Immunophenotypic assessment of T-cell Clonality by Vβ repertoire analysis: detection of T-cell Clonality at diagnosis and monitoring of minimal residual disease following therapy. Am J Clin Pathol 2011, **135**, 890–900. doi:10.1309/AJCPV2D1DDSGJDBW
13. Zhu YP, Shamie I, Lee JC, Nowell CJ, Peng W, Angulo S, et al.; Pediatric Emergency Medicine Kawasaki Disease Research Group Consortium. Immune response to intravenous immunoglobulin in patients with Kawasaki disease and MIS-C. J Clin Invest 2021, **131**, e147076. doi:10.1172/JCI147076
14. Ehara H, Kiyohara K, Izumisawa Y, Ito T. Early activation does not translate into effector differentiation of peripheral CD8T cells during the acute phase of Kawasaki disease. Cell Immunol 2010, **265**, 57–64. doi:10.1016/j.cellimm.2010.07.003
15. Ko TM, Kuo HC, Chang JS, Chen SP, Liu YM, Chen HW, et al. CXCL10/IP-10 is a biomarker and mediator for Kawasaki disease. Circ Res 2015, **116**, 876–83. doi:10.1161/CIRCRESAHA.116.305834.
16. Takahashi K, Oharaseki T, Naoe S, Wakayama M, Yokouchi Y. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. Pediatr Int 2005, **47**, 305–10. doi:10.1111/j.1442-200x.2005.02049.x.
17. He YE, Qiu HX, Wu RZ, Rong X, Xu HT, Xiang RL, et al. Oxidised low-density lipoprotein and its receptor-mediated endothelial dysfunction are associated with coronary artery lesions in Kawasaki disease. J Cardiovasc Transl Res 2020, **13**, 204–14. doi:10.1007/s12265-019-09908-y.

18. van der Valk FM, Bekkering S, Kroon J, Yeang C, Van den Bossche J, van Buul JD, et al. Oxidized phospholipids on lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. Circulation 2016, **134**, 611–24. doi:10.1161/CIRCULATIONAHA.116.020838.
19. Tsilingiri K, Fuente H, Relaño M, Sánchez-Díaz R, Rodríguez C, Crespo J, et al. Oxidized low-density lipoprotein receptor in lymphocytes prevents atherosclerosis and predicts subclinical disease. Circulation 2019, **139**, 243–55. doi:10.1161/CIRCULATIONAHA.118.034326
20. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the human immunology project. Nat Rev Immunol 2012, **12**, 191–200. doi:10.1038/nri3158.
21. van den Borne P, Quax PH, Hoefer IE, Pasterkamp G. The multifaceted functions of CXCL10 in cardiovascular disease. Biomed Res Int 2014, **2014**, 893106. doi:10.1155/2014/893106.
22. Sacco K, Castagnoli R, Vakkilainen S, Liu C, Delmonte OM, Oguz C, et al. Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19. Nat Med 2022, **28**, 1050–62. doi:10.1038/s41591-022-01724-3
23. Pavel AB, Glickman JW, Michels JR, Kim-Schulze S, Miller RL, Guttman-Yassky E. Th2/Th1 cytokine imbalance is associated with higher COVID-19 risk mortality [Original Research]. Front Genet 2021, **12**, 706902. doi:10.3389/fgene.2021.706902.
24. Esteve-Sole A, Anton J, Pino-Ramirez RM, Sanchez-Manubens J, Fumadó V, Fortuny C, et al. Similarities and differences between the immunopathogenesis of COVID-19–related pediatric multisystem inflammatory syndrome and Kawasaki disease. J Clin Invest 2021, **131**, e144554. doi:10.1172/JCI144554
25. Ni FF, Li CR, Li Q, Xia Y, Wang GB, Yang J. Regulatory T cell microRNA expression changes in children with acute Kawasaki disease. Clin Exp Immunol 2014, **178**, 384–93. doi:10.1111/cei.12418
26. Pietra BA, De Inocencio J, Giannini EH, Hirsch R. TCR V beta family repertoire and T cell activation markers in Kawasaki disease. J Immun (Baltimore, Md.: 1950) 1994, **153**, 1881–8.