Transcriptional Analysis of Intravenous Immunoglobulin Resistance in Kawasaki Disease Using an Induced Pluripotent Stem Cell Disease Model

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Background: Approximately 10–20% of Kawasaki disease (KD) patients are resistant to intravenous immunoglobulin (IVIG) treatment. Further, these patients are at a particularly high risk of having coronary artery abnormalities. The mechanisms of IVIG resistance in KD have been analyzed using patient leukocytes, but not patient vascular endothelial cells (ECs). The present study clarifies the mechanisms of IVIG resistance in KD using an induced pluripotent stem cell (iPSC) disease model.

Methods and Results: Dermal fibroblasts or peripheral blood mononuclear cells from 2 IVIG-resistant and 2 IVIG-responsive KD patients were reprogrammed by the episomal vector-mediated transduction of 6 reprogramming factors. KD patient-derived iPSCs were differentiated into ECs (iPSC-ECs). The gene expression profiles of iPSC-ECs generated from IVIG-resistant and IVIG-responsive KD patients were compared by RNA-sequencing analyses. We found that the expression of CXCL12 was significantly upregulated in iPSC-ECs from IVIG-resistant KD patients. Additionally, Gene Set Enrichment Analysis (GSEA) revealed that gene sets involved in interleukin (IL)-6 signaling were also upregulated.

Conclusions: The first iPSC-based model for KD is reported here. Our mechanistic analyses suggest that CXCL12, which plays a role in leukocyte transmigration, is a key molecule candidate for IVIG resistance and KD severity. They also indicate that an upregulation of IL-6-related genes may be involved in this pathogenesis.

Key Words: Endothelial cells differentiated from induced pluripotent stem cell (iPSC-ECs); Induced pluripotent stem cell (iPSC); Intravenous immunoglobulin (IVIG) resistance; Kawasaki disease
speculated that other cell types, such as vascular endothelial cells (ECs), might produce these chemical mediators.

Evidence has suggested that coronary artery ECs are a candidate. It was reported that γ-D-glutamylmesodiaminopimelic acid (γ-E-DAP), a ligand of Nucleotide-binding oligomerization domain protein 1 (Nod1) known as an inflammation initiator in response to peptidoglycan fragments,7 induces intercellular adhesion molecule (ICAM)-1 expression and IL-8 secretion in human coronary artery ECs (HCAECs).8 Furthermore, FK565, a pure synthetic Nod1 ligand, stimulates the production of C-C motif chemokine ligand 2 (CCL2) and IL-8 much more in HCAECs than in human pulmonary artery ECs (HPAECs).9 It was therefore speculated that vascular ECs, especially those of coronary arteries, might produce the chemical mediators, such as IL-6, IL-8 and TNF-α.

Because of its seasonality and epidemicity, it has been suggested that the cause of KD might include some bacterial or viral infection.9,10 Although studies on bacterial or viral agents have been conducted, the lack of reproducibility has left the etiology of KD unknown.13,14 Considering that the incidence of KD is 10–20-fold higher in Japan than in Western countries,15 it has been additionally speculated that genetic factors might influence the onset of the disease. Using the Egami scoring system, Ogata et al predicted IVIG responsiveness in KD patients.16 They found the transcripts for IL-1R2, IL-18R1 and S100A12 protein, which are related to IVIG resistance and the development of coronary artery lesions (CAL), had significantly upregulated mRNA expression levels in the whole blood samples of IVIG-resistant KD patients.

Mouse models of KD, such as coronary arteritis models induced by the subcutaneous administration of a pure synthetic NOD1 ligand,8 the single intraperitoneal injection of Lactobacillus casei cell wall extract (LCWE)7 or the intraperitoneal injection of Candida albicans water-soluble fraction (CAWS),18 have been established, and the pathological findings of coronary arteritis in these models are consistent with those of human CAL in the acute phase of KD. However, these mouse models, as well as commercially available HCAECs from healthy subjects, are inadequate for the mechanistic analysis of KD, because they lack the related genetic factors. Patient-derived induced pluripotent stem cells (iPSCs),19,20 in contrast, can be used as in vitro disease models by differentiating them into the affected cell types to analyze disease mechanisms and search for therapeutic drugs.21

In an attempt to clarify the mechanisms of IVIG resistance in KD, we generated iPSCs from KD patients, differentiated them into vascular ECs and examined susceptible molecules related to the pathophysiology by transcriptome analysis.

### Methods

#### Patients

Cells were taken from 4 KD patients; 2 males (12 and 14 years old) and 2 females (both 16 years old), and all patients met the criteria for the Diagnostic Guidelines of KD (Table 1).22 Patients were recruited with informed consent and approval from the Ethics Committees of Kyoto University and Kyoto Prefectural University of Medicine was obtained. Although all 4 patients were treated with high-dose IVIG, 2 KD patients were IVIG resistant and both suffered from giant aneurysms. The remaining 2 patients were IVIG responders and had no coronary sequelae.

A 4-mm skin punch biopsy was performed under local anesthesia at the medial side of the upper arm, and the wound was thereafter sutured. After 1 week, the suture was removed. The procedures were conducted at Kyoto University Hospital. Dermal fibroblasts from the skin biopsy explants were expanded in Dulbecco’s modified Eagle’s medium (DMEM; Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (Japan Bioserum, Hiroshima, Japan). PBMCNs were purified by density gradient centrifugation with Ficoll-paque Plus (GE Healthcare, Tokyo, Japan), as previously reported.23

#### Generation of iPSCs From KD Patients

Induced pluripotent stem cells were generated from skin fibroblasts using a combination of episomal plasmid vectors encoding OCT4, SOX2, KLF4, L-MYC, LIN28 and p53shRNA.24 Episomal plasmid vectors were transduced into the dermal fibroblasts by electroporation. After 7 days, transfected cells were trypsinized and reseeded onto SNL feeder layers.25 The cells were maintained in Primate ES cell medium (ReproCELL, Kanagawa, Japan), and small cell colonies could be observed about 2 weeks after transfection. Around day 30, the colonies with a human embryonic stem cell (ESC)-like morphology were picked up.

The generation of iPSCs from PBMCNs was also performed using episomal vectors. The plasmid mixture was electroporated into PBMCNs using the Nucleofector 2b Device (Lonza, Basel, Switzerland) with an Amaxa Human T-cell Nucleofector kit.25 For the generation from γβT cells, the transfected cells were incubated in X-vivo10 (Lonza) supplemented with 30 U/mL IL-2 (PeproTech, NJ, USA) and 5 μg/well of Dynabeads Human T-activator CD3/CD28. An equal volume of Primate ES cell medium containing recombinant human basic fibroblast growth factor (bFGF; Wako, Osaka, Japan) and 10 μmol/L Y-27632 (Wako) was added 2 days after the transfection into each well without aspiration of the previous medium. Then, the culture medium was replaced 4 days after the transfection, and the colonies that were morphologically similar to human ESCs were selected for subsequent culture.

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**Table 1. Characteristics of KD Patients Enrolled in This Study and Types of Somatic Tissue Taken From Them**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Tissue</th>
<th>Responsiveness for IVIG, coronary artery sequela</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td>12</td>
<td>M</td>
<td>Dermal tissue</td>
<td>IVIG non-responder, CAL +</td>
</tr>
<tr>
<td>P 2</td>
<td>14</td>
<td>M</td>
<td>Dermal tissue</td>
<td>IVIG non-responder, CAL +</td>
</tr>
<tr>
<td>P 3</td>
<td>16</td>
<td>F</td>
<td>Peripheral blood</td>
<td>IVIG responder, CAL –</td>
</tr>
<tr>
<td>P 4</td>
<td>16</td>
<td>F</td>
<td>Dermal tissue</td>
<td>IVIG responder, CAL –</td>
</tr>
</tbody>
</table>

IVIG, intravenous immunoglobulin; KD, Kawasaki disease; M, male; F, female; CAL, coronary artery lesion.
A total of 7 control iPSC lines previously established from 6 healthy subjects at the Center for iPSC Cell Research and Application (CiRA), Kyoto University, were used as the control (Table 2). The 6 healthy subjects did not have any past history of vasculitis syndromes, including KD.

### Table 2. Profiles of the Seven Control iPSC Lines

<table>
<thead>
<tr>
<th>Clones number</th>
<th>Age</th>
<th>Sex</th>
<th>Original cells</th>
<th>Reference number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4 (TIG114 4F1)</td>
<td>36 y.o.</td>
<td>M</td>
<td>Fibroblasts</td>
<td>25, 26</td>
</tr>
<tr>
<td>M5 (TIG118)</td>
<td>12 y.o.</td>
<td>F</td>
<td>Fibroblasts</td>
<td>25, 26</td>
</tr>
<tr>
<td>M6 (TIG119)</td>
<td>6 y.o.</td>
<td>M</td>
<td>Fibroblasts</td>
<td></td>
</tr>
<tr>
<td>M7 (TIG121)</td>
<td>6 m.o.</td>
<td>M</td>
<td>Fibroblasts</td>
<td></td>
</tr>
<tr>
<td>M8 (TIG975 e4)</td>
<td>6 y.o.</td>
<td>F</td>
<td>Fibroblasts</td>
<td></td>
</tr>
<tr>
<td>M9 (585A1)</td>
<td>30 s</td>
<td>M</td>
<td>T cells</td>
<td>23</td>
</tr>
<tr>
<td>M10 (585B1)</td>
<td>30 s</td>
<td>M</td>
<td>T cells</td>
<td>23</td>
</tr>
</tbody>
</table>

**iPSC:** induced pluripotent stem cell; y.o., years old; m.o., months old; 30 s, thirties.

### Cell Culture

Human iPSCs were cultured on mitomycin C-treated SNL feeder cells in Primate ES cell medium supplemented with 500 U/mL penicillin/streptomycin (Thermo Fisher Scientific, MA, USA) and 4 ng/mL bFGF. For passaging, human iPSC colonies were dissociated with CTK (Collagenase Type IV, Trypsin, KSR) dissociation solution consisting of 0.25% trypsin (Thermo Fisher Scientific), 0.1% collagenase IV (Thermo Fisher Scientific), 20% knockout serum replacement (KSR; Thermo Fisher Scientific) and 1 mmol/L CaCl$_2$ (Thermo Fisher Scientific). Purification of RNA by the removal of genomic DNA was performed by using a RNeasy Plus Mini Kit (Qiagen). The concentration of total RNA was determined from the absorbance ratio 260/280 nm in a UV spectrophotometer. Illumina sequencing libraries were prepared with TruSeq Stranded Total RNA with Ribo-Zero Gold LT Sample Prep kit (Illumina, CA, USA) and then sequenced in 100 cycle Single-Read mode of HiSeq2500. Sequenced

### Formation of Embryoid Bodies

Undifferentiated iPSCs cultured on a 10-cm dish were harvested using CTK solution, and the cells were resuspended in DMEM/F12 (Thermo Fisher Scientific) containing 20% KSR, 0.55 mmol/L 2-mercaptoethanol (Thermo Fisher Scientific), 0.1 mmol/L non-essential amino acid (NEA; Thermo Fisher Scientific), 2 mmol/L glutamine (Thermo Fisher Scientific) and 50 U/mL penicillin/streptomycin, and split into low-attachment 6-well plates (Corning, MA, USA) for 3 days. The sorted cells were transferred to gelatin-coated plates and cultured in the same medium for an additional 14 days. Then, the cells were subjected to immunostaining analyses.

### Flow Cytometry and Cell Sorting

The cells were treated with 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) for 3 min, dissociated by pipetting in DMEM (Nacalai Tesque) containing 10% FBS and centrifuged. For passaging, human iPSC colonies were dissociated with CTK (Collagenase Type IV, Trypsin, KSR) dissociation solution consisting of 0.25% trypsin (Thermo Fisher Scientific), 0.1% collagenase IV (Thermo Fisher Scientific), 20% knockout serum replacement (KSR; Thermo Fisher Scientific) and 1 mmol/L CaCl$_2$ (Thermo Fisher Scientific) and 500 U/mL penicillin/streptomycin (Thermo Fisher Scientific, MA, USA) and 4 ng/mL bFGF. For passaging, human iPSC colonies were dissociated with CTK (Collagenase Type IV, Trypsin, KSR) dissociation solution consisting of 0.25% trypsin (Thermo Fisher Scientific), 0.1% collagenase IV (Thermo Fisher Scientific), 20% knockout serum replacement (KSR; Thermo Fisher Scientific) and 1 mmol/L CaCl$_2$ (Thermo Fisher Scientific). On day 2, the cells were treated with 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) for 3 min, dissociated by pipetting in DMEM (Nacalai Tesque) containing 10% FBS and centrifuged. The pellets were dissociated by pipetting in Hanks’ Balanced Salt Solution (HBSS; Thermo Fisher Scientific), and the cells were analyzed and sorted using a fluorescence-activated cell sorting (FACS) Aria II cell sorter, as previously reported. Dead cells stained with 7-AAD (PerCP-Cy5.5; BD Biosciences) were excluded from the analysis. Antibodies used for sorting were Flk-1 (Alexa Fluor 647; BioLegend, CA, USA), VE-cadherin (PE; BD Biosciences) and TRA1-60 (Alexa Fluor 488; BD Biosciences). The isolated cells were collected in an EGM-2 BulletKit for vascular ECs or in a SmGM-2 BulletKit (#CC-3182; LONZA) for smooth muscle cells (SMCs).

### Immunocytochemistry

The cells were fixed with 4% paraformaldehyde (Nacalai Tesque)/PBS for 20 min at 4°C. Then, the cells were washed with PBS and blocked with 5% normal donkey serum (Merck Millipore) diluted with Phsophate Buffered Saline (PBS) and incubated with samples overnight at 4°C. The cells were washed twice with PBST, and secondary antibodies were diluted at 1:200 in blocking solutions and incubated for 1 h at room temperature. The cells were then washed threefold with PBST. Finally, the cells were observed under an inverted type fluorescence phase-contrast microscope (BZ-9000E; Keyence, Osaka, Japan).

### RNA-Sequencing

ECs that were sorted and incubated for 5 days were washed with PBS and harvested using buffer RLT (Qiagen, Hilden, Germany) containing 2-mercaptoethanol (Thermo Fisher Scientific). Purification of RNA by the removal of genomic DNA was performed by using a RNeasy Plus Mini Kit (Qiagen). The concentration of total RNA was determined from the absorbance ratio 260/280 nm in a UV spectrophotometer. Illumina sequencing libraries were prepared with TruSeq Stranded Total RNA with Ribo-Zero Gold LT Sample Prep kit (Illumina, CA, USA) and then sequenced in 100 cycle Single-Read mode of HiSeq2500.
Data Analysis
Principal component analysis (PCA) was conducted using the standard pipelines of GeneSpring v.12.6 (Agilent technologies, CA, USA). Differentially expressed genes were defined by a threshold of the fold change (≥2). Gene Ontology (GO) analysis was performed by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID; http://www.david.niaid.nih.gov), and P<0.05 was assessed as being statistically significant. Pathway analysis for differentially expressed genes was performed by using iPathwayGuide (Advaita Bioinformatics: http://www.advaitabio.com/ipathwayguide.html). Gene Set Enrichment Analysis (GSEA) was performed with GSEA v2.1.0 according to the provided procedures (http://www.broadinstitute.org/gsea/index.jsp). We used the c2.all.v4.0 symbols. gmt gene set corresponding to MSigDB v4.0. The parameters of GSEA were as follows: number of permutations, 1,000; permutation type, gene_set; enrichment statistic, weighted; metric for ranking genes, Diff_of_Clases; max size, 6,000; and min size, 1.

Results
Generation of iPSCs From KD Patients
In total, 4 KD patients were enrolled for the experiments, with 2 KD patients being IVIG responders and the other 2 patients being IVIG non-responders. Fibroblasts from the KD patients were converted into iPSCs using a combination reads were aligned in the hg19 genome with TopHat2. The expression values were calculated with rpkmforgenes and converted into log2 (RPKM+1).
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KD-iPSCs Can Be Differentiated Into ECs

We next examined whether KD-iPSCs could be differentiated into ECs, which play a crucial role in the vasculitis seen in KD patients. KD-iPSCs were differentiated into ECs using a previously reported protocol. After 9 days of differentiation, TRA1–60+/VE-cadherin+ cells accounted for 5–12% of the total cell population and were isolated using FACS Aria II. Immunofluorescence analysis revealed that these cells were positively stained for an EC marker, CD31. Together, the results indicate that KD-iPSCs can be differentiated into ECs.

IPSC-ECs From IVIG Non-Responders and IVIG Responders Have Distinct Global Gene Expression Patterns

We then examined the gene expression profiles of ECs differentiated from iPSCs (iPSC-ECs). We performed RNA-sequencing of iPSC-ECs derived from the IVIG-responsive KD patients (IVIG responder group), the IVIG-non-responsive KD patients (IVIG non-responder group) and 7 healthy control subjects (control group). A PCA plot showed that the 3 groups had a tendency to separate, and each group had distinctive gene expression profiles.

CXCL12 Is a Candidate Molecule Associated With IVIG Resistance in KD

To identify which gene expressions were specific to IVIG non-responders, we performed a series of comparisons, including gene expressions between: (1) iPSC-ECs from all 4 KD patients (KD-iPSCs) and the control group; (2) the IVIG non-responder group and control group; and (3) the IVIG responder group and IVIG non-responder group. Genes with an average fold change in expression of more than 2 were selected using RNA sequencing (RNA-seq) data. We found 58 and 139 genes that had expressions upregulated and downregulated, respectively, in the KD group vs. the control group. Similarly, we found 127 and 112 genes were upregulated and downregulated in the IVIG non-responsive KD patients (IVIG non-responder group) and 7 healthy control subjects (control group). A PCA plot showed that the 3 groups had a tendency to separate, and each group had distinctive gene expression profiles.
(comparison ii) showed that 9 out of 12 GO terms with statistically significant differences contained the gene for CXCL12, a chemoattractant that is active on leukocytes (Tables 3 and S1).\(^1\) In contrast, no significantly different GO terms found between the KD and control groups contained CXCL12 (Table S2), suggesting an association of CXCL12 with IVIG resistance in KD.

In addition, a pathway analysis of the IVIG non-responder and IVIG responder groups was performed (Table S3). By using the 101 upregulated genes and the 107 downregulated genes in the IVIG non-responder group, we identified 15 pathways as having significant association with IVIG resistance (P<0.05). We focused on gene sets in the extracted pathways and found that only those in the cytokine-cytokine receptor interaction pathway were significantly related to CXCL12 (Table S4). These results also support the involvement of CXCL12 in IVIG resistance in KD patients.

### Gene Sets Related to IL-6 Are Upregulated in iPSC-ECs From IVIG Non-Responders

We further performed GSEA using RNA-seq data of the iPSC-ECs from the IVIG responder, IVIG non-responders and control groups. Gene sets related to IL-6 were upregulated in the iPSC-ECs of the IVIG non-responder group compared with the IVIG responder group (Figure 4A). We also found that gene sets related to breast cancer, NRAS (a member of the RAS oncogene family) and cervical carcinoma were upregulated (Figure 4B). These results indicate that gene sets related to IL-6 are associated with IVIG resistance in the ECs of KD patients.

### Discussion

Kawasaki disease patients with IVIG resistance frequently suffer from coronary artery complications.\(^30\) Although the IVIG resistance has been analyzed using leukocytes and serum samples from acute-phase KD patients, the mechanisms of its manifestation remain unknown.\(^16,31-34\) As an alternative model, we generated iPSCs from the skin fibroblasts or PBMCNCs of KD patients for the first time and differentiated them into ECs.

By using RNA-seq data on ECs from KD- and control-iPSCs, PCA resulted in 3 differential groups: IVIG responder, IVIG non-responder and control group. However, previous studies have indicated that the development of KD and IVIG resistance is stipulated by various factors, including genetic and environmental factors.\(^9,12\) suggesting it is possible that the healthy control group could be further divided into 2 groups based on the genetic predisposition to develop IVIG resistance. Future studies should address this point by using assays other than PCA.

Ogata et al performed microarray analyses of whole blood samples from KD patients and found that the expression levels of IL-1R2, IL18R1 and S100A12 are higher in IVIG-resistant KD patients than IVIG responders.\(^16\) In contrast, our data suggest that the expression levels of IL-1R2, IL18R1 and S100A12 in iPSC-ECs were not significantly different between the IVIG responder and IVIG non-responder groups (data not shown). We assume that the discrepancy of these findings may result from differences in the tissue types analyzed.

GO analysis of the RNA-seq data showed that the expression level of CXCL12 was significantly higher in iPSC-ECs derived from KD patients with IVIG resistance than in those without. CXCL12 is constitutively expressed on various cell types, including vascular ECs,\(^35,36\) and acts as a potent chemoattractant for lymphocytes, monocytes, dendritic cells and hematopoietic stem cells.\(^37\) Furthermore, CXCL12, in combination with its receptor, CXCR4, plays a central role in leukocyte recruitment and transmigration through the ECs during immune or inflammatory responses.\(^37\) One study has shown that 4 chemokines, including CXCL12, have the ability to induce the adhesion of T cells to intercellular adhesion molecule 1 (ICAM-1) and arrest the rolling of T cells within 1 s under flow conditions that mimic the blood stream.\(^38\) CXCL12 is also thought to influence the state of inflammation and autoimmunity. For example, in an in vivo SCID mouse model, CXCL12 could induce the migration of U937 monocyte cells into the rheumatoid arthritis synovium in response to TNF-α stimulation.\(^39\) Furthermore, it has been suggested that CXCL12 might play some role in the recruitment of inflammatory cells.\(^40,41\) In the central nervous system, the expression of CXCL12 was detected in the microvessel ECs.
suggest that it might be a key molecule in IVIG resistance and the severity of vasculitis in KD patients. The results of GSEA further indicated that gene sets related to IL-6 were upregulated in iPSC-ECs from IVIG-resistant KD patients compared with those from IVIG-responsive KD patients. A previous study found an elevation in serum IL-6 concentration in the acute phase of IVIG-resistant KD cases. It has also been reported that the serum levels of IL-6 were significantly higher in IVIG-resistant KD patients than in IVIG-responsive KD patients. Another study reported that the responsiveness to IVIG treatment in acute phase KD may depend on the serum levels of IL-6, CRP and neutrophil count. The current study is the first to report RNA-seq analysis of iPSC-ECs and astrocytes of chronic multiple sclerosis (MS) cases. Consistently, the concentration gradient of CXCL12 was observed to facilitate the adhesion of T lymphocytes to human brain microvessel ECs (HBMECs) pre-treated with TNF-α and interferon (IFN)-γ and the migration of T lymphocytes across a monolayer of HBMECs. These findings suggest that CXCL12 may play a critical role in lymphocyte recruitment across the blood-brain barrier in central nervous system inflammation.

In severe KD cases with IVIG resistance, numerous leukocytes, including monocytes and macrophages, infiltrate the vascular wall to completely destroy the coronary arteries. Considering the role of CXCL12 in the recruitment, adhesion and migration of inflammatory cells, we suggest that it might be a key molecule in IVIG resistance and the severity of vasculitis in KD patients.

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from KD patients, especially IVIG-resistant patients. Like in the aforementioned studies, our data suggest that IL-6 may be associated with IVIG responsiveness in KD patients.

In our study, both CXCL12 and IL-6 were closely associated with IVIG-resistance in KD patients. Noting this point, we hypothesize a developmental mechanism for vascular wall destruction and abdominal aortic aneurysms, which could destroy the coronary artery walls.

The results of the current study indicate the involvement of upregulated CXCL12 and IL-6 expressions by the vascular endothelia in the development of IVIG resistance in KD, while commercially prepared IVIG may contain antibodies to CXCL12 and IL-6, findings that may seem inconsistent. However, it could be that the amount of antibodies to CXCL12 and IL-6 contained in IVIG is insufficient to completely block the upregulated CXCL12 and IL-6, thus resulting in IVIG resistance. From this point of view, specific blockade of CXCL12 and IL-6 might be effective for IVIG-resistant KD.

Treatment with AMD3100 (plerixafor), an antagonist for CXCL12/CXCR4 signaling, was shown to prevent aortic wall destruction and abdominal aortic aneurysms (AAA) formation in CaCl2-induced mouse AAA models. AMD3100 has already been approved by the Food and Drug Administration (FDA) and is used to mobilize stem cells for transplantation in cancer patients.

Migrating inflammatory cells to the vascular walls secrete pro-inflammatory cytokines and matrix metalloproteinases, which could destroy the coronary artery walls.

The anti-IL-6 receptor monoclonal antibody, tocilizumab, was developed in Japan and administered to patients with systemic juvenile idiopathic arthritis (JIA). Later, a randomized trial of tocilizumab revealed efficacy for severe systemic JIA cases. Both systemic JIA and KD share clinical manifestations and laboratory findings, therefore, CXCL12 antagonists could be an effective therapy for KD patients resistant to first-line IVIG treatment.

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Conclusions

By using the first reported iPSC model for KD, we show that CXCL12, which is related to the transmigration of leukocytes, may be a crucial factor in both the resistance to IVIG treatment and severity of KD. In addition, our results indicate that the upregulation of IL-6-related genes in ECs may support a role for IL-6 in the pathogenesis. These new mechanistic findings demonstrate the value of iPSCs for the study of IVIG resistance and KD disease pathogenesis.

Disclosure

K. Osafune is a founder and a member, without salary, of the Scientific Advisory Boards of iPS Portal, Japan.

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