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Peripheral glucocorticoid signaling in Kawasaki disease

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To the editor

Kawasaki disease (KD) is an acute self-limiting inflammatory disorder, associated with systemic vasculitis. It is the leading cause of childhood-acquired heart disease in the developed world.^{1,2} The most important complication of KD is coronary artery vasculitis. Without treatment, this causes coronary artery aneurysms (CAA) in 15–25% of KD patients, while a further 2–3% develop myocardial infarction, ischemic heart disease, or sudden death.¹ Treatment with intravenous immunoglobulin (IVIG) plus aspirin can resolve inflammation and reduce the occurrence of CAA.^{1,2} However, in about 20% of cases IVIG fails to resolve the inflammation, leading to persistent vasculitis: these patients have increased risk of developing CAA unless they receive additional treatment.^{1,2} Recently, IVIG plus adjuvant glucocorticoid hormones (GCs) has been shown to be an effective therapy for these patients,^{3,4} suggesting that GCs may complement IVIG treatment in severe KD. However, this remains controversial with others reporting that additional GCs did not improve coronary artery outcomes and may even cause CAA due to impaired vascular remodeling.^{5,6} An insight into how endogenous glucocorticoids might contribute to disease resolution could be useful for consideration of glucocorticoid therapy. Here we hypothesized that failure to appropriately regulate endogenous GC signaling in inflammatory cells may contribute to persistent inflammation in KD. Intracellular GC levels in peripheral tissues are controlled by pre-receptor metabolism by 11 β -hydroxysteroid dehydrogenase (11 β -HSD). 11 β -HSD1 converts intrinsically inert cortisone to active cortisol, increasing intracellular GC levels available to activate glucocorticoid receptor (GR).⁷ We have previously shown that the direction of regulation of 11 β -HSD1 by dexamethasone in peripheral blood mononuclear cells (PBMC) is associated with the cytotoxic effects of GCs in acute lymphoblastic leukemia, with upregulation in glucocorticoid-sensitive cells but downregulation or no change in patient cells that are resistant to the pro-apoptotic effects of GC.⁸ Therefore, the aim of this study was to establish whether 11 β -HSD1 in PBMC is associated with acute inflammation in KD.

Levels of 11 β -HSD1 and GR mRNA were measured in PBMC isolated from all children diagnosed with KD at Teine-Keijinkai Hospital, Sapporo, Japan between April 2015 and January 2018 (a total of 31). Diagnosis of KD was made by pediatricians on the basis of clinical and laboratory findings according to clinical guideline.¹ IVIG treatment (2 g/kg over 24 h) was initiated in all patients on day 5 or 6 after the onset of high fever (>38 °C). IVIG sensitivity/resistance was determined from the initial response to IVIG treatment according to resolution of fever (<38 °C). Nineteen patients were classified as IVIG-sensitive patients, who showed complete resolution of high fever within 48 h after commencing IVIG treatment and remained afebrile. Twelve patients were classified as IVIG-resistant due to persistent fever >38 °C following IVIG treatment. There was no significant difference in age or gender between patients who responded to IVIG (IVIG-sensitive)

and those who were unresponsive (IVIG-resistant) (Table 1). Adjuvant GC therapy was not used in this study, but additional IVIG treatment (2 g/kg) was administered for patients with high fever >38 °C for >60 h after commencement of the initial IVIG treatment. None of the patients developed CAA. Peripheral blood samples (3 ml) were obtained before (day 4 after the onset of high fever) and after IVIG treatment (day 8) with informed consent. Sampling was also carried out on day 10 for representative patients (Case 1 and Case 2). PBMC isolation, RNA extraction, and quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) were performed as previously described.^{8,9} Values for RNA are mean \pm S.E.M. and were analyzed using a paired Student's *t* test (two-tailed). GAPDH served as internal control. Patient characteristics and laboratory data were compared using Mann–Whitney *U* test for age, body temperature, white blood cell (WBC) counts, and C-reactive protein (CRP) and chi-squared test for gender. Significance was set at $p < 0.05$.

The clinical courses for patients representative of IVIG-sensitive (Case 1) and IVIG-resistant (Case 2) patients are shown in Fig. 1a, c, respectively. In Case 1, an 8-month-old boy was diagnosed as having KD because of 5 days of fever and other clinical symptoms. Inflammatory markers (WBC and CRP) were elevated at diagnosis (Fig. 1a) and treatment with 2 g/kg IVIG and aspirin was initiated. The following day, the fever had resolved and inflammatory markers were decreased (Fig. 1a). In Case 2, a 3-year-old boy was diagnosed with KD on day 5 of high fever (>38 °C). Two g/kg IVIG and aspirin were administered but were ineffective, nor was there a response to additional IVIG treatment given on day 7 (Fig. 1c). On day 9 after onset of high fever, therapeutic plasma exchange (TPE) was carried out in Intensive Care Unit for 3 consecutive days. Following TPE, resolution of fever was achieved and other symptoms of KD gradually resolved (Fig. 1c). Neither boy developed CAA. To investigate whether *HSD11B1* (encoding 11 β -HSD1) mRNA in PBMC was associated with resolution of inflammation, qRT-PCR was used to measure the levels. The expression of *HSD11B1* mRNA in PBMC increased over two-fold following IVIG treatment in Case 1, with a four-fold increase at resolution of inflammation and discharge of the patient (Fig. 1b). In contrast, levels of *HSD11B1* mRNA in Case 2 were unchanged by IVIG treatment and showed only a modest increase (two-fold) following commencement of TPE (Fig. 1d).

These case studies suggested an association between *HSD11B1* mRNA levels in PBMC and resolution of inflammation in KD. Accordingly, we measured the markers of glucocorticoid sensitivity, *HSD11B1* and *NR3C1* (nuclear receptor subfamily 3 group C member 1), encoding GR mRNA levels in PBMC isolated from all patients prior to IVIG treatment (day 4) and following IVIG treatment (day 8). Patient characteristics are shown in Table 1. Following IVIG treatment, body temperature and WBC were significantly lower in IVIG-sensitive than in IVIG-resistant group. None of the patients developed CAA. There was no significant difference in the basal levels of *HSD11B1* and *NR3C1* mRNA between IVIG-sensitive and IVIG-resistant KD (Table 1). However, following IVIG treatment, *HSD11B1* mRNA levels were significantly increased in IVIG-sensitive KD but not in IVIG-resistant KD (Table 1). There was no significant effect of IVIG treatment on *NR3C1* mRNA levels in either group (Table 1). Thus increased expression of

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Table 1. Summary of Kawasaki disease patient characteristics

N = 31		IVIG-sensitive (n = 19)	IVIG-resistant (n = 12)	p (sensitive vs. resistant)
Age (years), mean (range)		2.89 (0.7–6.1)	3.07 (1.3–5.2)	0.715
Gender (%)	Boys	53%	50%	
	Girls	47%	50%	
Body temperature (°C), mean ± S.E.M.				
	Before IVIG	39.57 ± 0.13	39.68 ± 0.21	0.7141
	After IVIG	36.78 ± 0.08	38.86 ± 0.25	<0.0001
Laboratory data, mean ± S.E.M.				
WBC (per mm ³)	Before IVIG	12986 ± 820	15073 ± 1124	0.0777
	After IVIG	6415 ± 570	10324 ± 1138	0.0057
CRP (mg/dl)	Before IVIG	8.71 ± 1.27	9.72 ± 1.55	0.5034
	After IVIG	2.56 ± 0.63	5.03 ± 1.70	0.2068
mRNA levels, mean ± S.E.M.				
<i>HSD11B1</i> mRNA	Before IVIG	1.849 ± 0.392	1.749 ± 0.765	0.5039
	After IVIG	4.330 ± 1.146*	1.361 ± 0.237	0.3307
<i>NR3C1</i> mRNA	Before IVIG	5.679 ± 0.870	3.860 ± 0.497	0.1246
	After IVIG	7.423 ± 1.847	4.336 ± 0.613	0.4548

CRP C-reactive protein, IVIG intravenous immunoglobulin, WBC white blood cell

*p = 0.0386

Bold values indicate statistical significance at p < 0.05

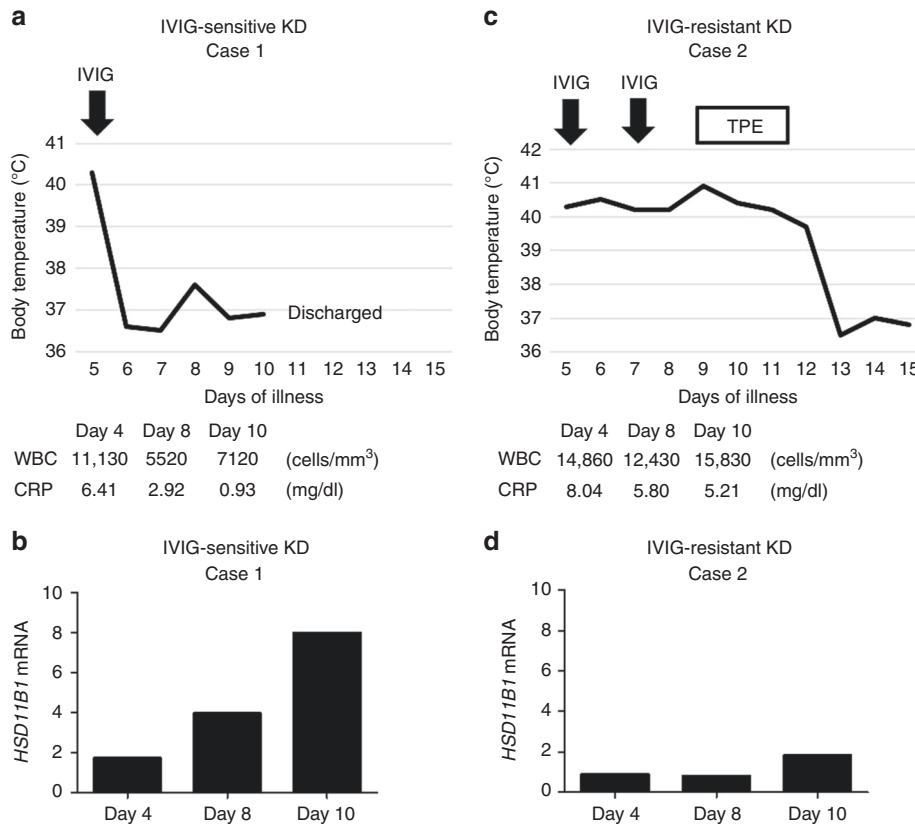


Fig. 1 Clinical courses and peripheral glucocorticoid hormone signaling in representative patients. **a, b** Body temperature, white blood cell (WBC), C-reactive protein (CRP), and *HSD11B1* mRNA levels in intravenous immunoglobulin (IVIG)-sensitive Case 1. **c, d** Body temperature, WBC, CRP, and *HSD11B1* mRNA levels in IVIG-resistant Case 2. *HSD11B1* mRNA levels were measured relative to GAPDH mRNA levels and are expressed in arbitrary units

HSD11B1 in PBMC following IVIG treatment is associated with resolution of inflammation following IVIG treatment in KD. This could reflect induction of *HSD11B1* in a stable population of circulating PBMC or may reflect a gradual change in the population of PBMC themselves as the inflammation resolves with disappearance of lower *HSD11B1*-expressing cells from the circulation and replacement with a higher expressing cell population. Different immune cell populations express *HSD11B1* at different levels and it varies with immune cell activation state.^{7,10} Pro-inflammatory cytokines can induce *HSD11B1*,⁷ which may differ between IVIG-sensitive and IVIG-resistant KD. It is also possible that fever itself affected *HSD11B1* mRNA levels. It is intriguing to speculate that genetic variation in the *HSD11B1* gene could contribute to the differential responses of KD patients.

The significance of increased PBMC *HSD11B1* expression for KD is currently unclear. However, it will be important to establish whether this association between an increase in the levels of *HSD11B1* expression in PBMC and the resolution of inflammation is generalizable to other acute and life-threatening inflammatory disorders, including sepsis. It is interesting to speculate that an increase in the *HSD11B1* expression in PBMC may be a mechanism to promote and assist the resolution of inflammation. Increased 11 β -HSD1 activity in PBMC is predicted to increase the intracellular levels of cortisol generated from cortisone, thereby amplifying intracellular GC-mediated attenuation of proinflammatory cytokine action in PBMC of IVIG-sensitive KD. The failure to upregulate *HSD11B1* in PBMC in IVIG-resistant KD patients might contribute to the persistence of inflammation in these patients. If this is the case for IVIG-resistant patients, then additional GC therapy might be of therapeutic benefit to restore GC action as has been suggested.³ However, an early report showed a high incidence of CAA in patients who received a prolonged course of oral prednisolone.¹¹ Further trials are urgently needed to establish whether IVIG-resistant patients can be safely treated by GCs without occurrence of CAA. Understanding the role of endogenous glucocorticoid signaling in immune cells during the course of KD may highlight future possible therapeutic avenues to treat IVIG-resistant KD. We evaluated only Japanese patients in a single center, who may not be representative of KD in other ethnic groups. However, our findings may reflect the course of acute inflammation and its resolution and could be useful to understand other acute inflammatory conditions as well as the response to IVIG therapy in KD.

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AUTHOR CONTRIBUTIONS

S.S. conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. T.T. carried out the initial analyses and approved the final manuscript as submitted. K.N. conceptualized and designed the study and approved the final manuscript as submitted. K.E.C. helped conceptualize the study, reviewed the manuscript, and approved the final manuscript as submitted.

ADDITIONAL INFORMATION

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