The relationship between T cell HLA-DR expression and intravenous immunoglobulin treatment response in Kawasaki disease

Running title: HLA-DR and IVIG in Kawasaki disease

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Abstract

Background: Kawasaki disease (KD) is an early childhood febrile illness caused by vasculitis. Administration of intravenous immunoglobulin (IVIG) is now accepted as the standard treatment for KD. However, IVIG treatment is not effective in approximately 15% of KD children. Some reports have presented evidence of immunological responses in IVIG-resistant KD. We assessed the possibility that T cell activation was a contributory mechanism underlying this phenomenon.

Methods: We analyzed HLA-DR expression on peripheral blood $CD4^+$ and $CD8^+$ T cells in 82 children with KD who were admitted to our hospital between October 2007 and February 2012. We compared the percentages of $HLA\text{-}DR^+CD4^+$ T cells among $CD4^+$ T cells and $HLA\text{-}DR^+CD8^+$ T cells among $CD8^+$ T cells between the IVIG-effective and IVIG-resistant groups.

Results: Among 82 subjects, 51 children had IVIG-effective KD, and 31 children had IVIG-resistant KD. The percentages of $HLA\text{-}DR^+CD4^+$ among $CD4^+$ T cells and $HLA\text{-}DR^+CD8^+$ T cells among $CD8^+$ T cells in the IVIG-effective group were significantly lower than those in the IVIG-resistant group.

Conclusion: Our results suggest that decreased T cell HLA-DR expression is associated with IVIG-effective KD. It is likely that T cell HLA-DR expression is useful for predicting IVIG response in the acute phase of KD.

Keywords: Kawasaki disease, intravenous immunoglobulin, T cell, HLA-DR, CD4, CD8

Introduction

Kawasaki disease (KD) is an acute febrile illness associated with the development of vasculitis (1, 2). The most serious complication of KD is coronary arterial lesion (CAL) (3). High-dose intravenous immunoglobulin (IVIG) reduces the incidence of CAL in the majority of KD patients (4-6), but IVIG treatment is not effective in 13–21% of cases (7). The underlying cause of KD remains unknown, and many details of the immune response have not been characterized; however, it is known that all components of the immune system are involved (8). Few previous studies have addressed the role of different peripheral T cell populations in the acute phase of KD (9, 10). With respect to adaptive immunity, autopsy studies of children who died during the acute phase clearly indicate that $CD4^+$ and $CD8^+$ T cells participate in the transmural infiltration of the coronary arterial wall (11). HLA-DR is known as a marker of T cell activation (12-14); therefore, we investigated the relationship between HLA-DR expression on peripheral blood $CD4^+$ and $CD8^+$ T cells and IVIG response in children with KD.

Results

The parents of the patients enrolled in this study provided informed consent. This study included 82 children with KD who were admitted to the Department of Pediatrics at Yamaguchi University Hospital between October 2007 and February 2012. There were 54 boys and 28 girls, aged 2.8 ± 2.6 years (mean, 2.8 years; median, 1.8 years). All

the patients fulfilled the criteria described in the fifth revision of the Diagnostic Guidelines for Kawasaki Disease (15). We administered IVIG (2 g/kg) and oral aspirin (30 mg/kg daily) to all patients. We considered the cases of patients who showed reduced fever within 48 h after the end of IVIG infusion as IVIG-effective cases (Group A, 51 patients, 31 boys, 20 girls; aged 2.5 ± 2.3 years; mean, 2.5 years; median, 1.7 years), and those of patients with continued fever as IVIG-resistant cases (Group B, 31 patients, 23 boys, 8 girls; aged 3.8 ± 3.5 years; mean, 3.8 years; median, 3.1 years). Table 1 lists the clinical features of children in both groups.

The $CD4^+$ T cell and HLA-DR^+CD4^+ T cell results are shown in Table 2 and Figure 1. The percentage of HLA-DR^+CD4^+ T cells among peripheral blood mononuclear cells (PBMCs) was significantly lower in Group A than in Group B ($p = 0.005$). The HLA-DR^+CD4^+ T cell count and the percentage among $CD4^+$ T cells in Group A were significantly lower than those in Group B ($p = 0.004$ and $p = 0.003$, respectively).

The $CD8^+$ T cell and HLA-DR^+CD8^+ T cell results are shown in Table 3 and Figure 2. $CD8^+$ T cells and HLA-DR^+CD8^+ T cells exhibited very similar trends to those observed for $CD4^+$ T cells and HLA-DR^+CD4^+ T cells. The count of HLA-DR^+CD8^+ T cells was significantly lower in Group A than in Group B ($p = 0.019$). The percentage of HLA-DR^+CD8^+ T cells among $CD8^+$ T cells in Group A was significantly less than that in Group B ($p = 0.046$). The relationship of the percentage of HLA-DR^+CD8^+ T cells among $CD8^+$ T cells with the effect of IVIG was the same as that of HLA-DR^+CD4^+ T cells among $CD4^+$ T cells with the effect of IVIG (Figs. 1 and 2).

Discussion

We previously reported on the pathogenesis of KD (16-22, 25, 26). In terms of changes in peripheral blood immunocyte counts in KD patients, increases in white blood cell number correlated with increases in neutrophil count, but there was no great variation in mononuclear cell count (16). However, the numbers of $CD14^+$ monocytes/macrophages and $CD19^+$ B cells was increased among mononuclear cells, whereas those of $CD4^+$ and $CD8^+$ T cells were slightly decreased (16, 17). It has been reported that activation of nuclear factor- κ B (NF- κ B) is evident in peripheral blood $CD14^+$ monocytes/macrophages, and this activation is reduced by IVIG (18-20). The number of peripheral blood $CD14^+CD16^+$ (Fc γ RIII) monocytes/macrophages is also increased in KD, and IVIG strongly suppresses Fc γ RIII expression in monocytes/macrophages (21-22). Many studies have demonstrated the effect of IVIG treatment on KD, but few of these have utilized flow cytometry (23, 24). In this study, we investigated the relationship between HLA-DR expression on peripheral blood $CD4^+$ and $CD8^+$ T cells and IVIG responsiveness.

We previously reported that HLA-DR expression is low in $CD4^+$ and $CD8^+$ T cells during the acute phase of KD (17, 25, 26), and another group corroborated the observation that $HLA\text{-}DR^+CD8^+$ T cell number does not increase during the acute phase of KD (27). An additional study showed that soluble interleukin-2 receptor (a marker of T cell activation) is elevated in IVIG-resistant KD (28). Here, we determined that HLA-DR expression levels on $CD4^+$ and $CD8^+$ T cells are significantly suppressed in IVIG-effective KD patients compared to IVIG-resistant

KD patients. These results support those of previous studies (17, 25, 26) and suggest that both $CD4^+$ and $CD8^+$ T cell activation is generally suppressed during the acute phase of KD. Moreover, they imply that patients with weak suppression of $CD4^+$ and $CD8^+$ T cell activation may not respond to high-dose IVIG therapy. Finally, these results indicate that HLA-DR expression on $CD4^+$ and $CD8^+$ T cells may be used as a predictive marker for the effectiveness of IVIG treatment in KD patients.

Methods

Flow cytometry

We collected heparinized whole blood from children in the acute phase of KD just before IVIG infusion. They were labeled with peridinin chlorophyll protein (PerCP)-conjugated anti-HLA-DR, FITC-conjugated mouse anti-human CD4, or FITC-conjugated mouse anti-human CD8. The samples were also labeled with FITC-conjugated mouse IgG1 and PerCP-conjugated mouse IgG2a as a negative control. Erythrocytes were lysed with fluorescence-activated cell sorting (FACS) Lysing Solution. The cells were centrifuged and resuspended in FACS Flow. For each sample, 5,000 cells were analyzed by a FACScaliber flow cytometer equipped with CellQuest software. All the reagents and equipment used for FACS analysis was purchased from BD Biosciences (Franklin Lakes, NJ, USA).

Statistical analysis

Differences between the two groups were analyzed using Mann-Whitney U tests, Fisher exact test, and p values less than 0.05 were considered statistically significant. The analyses and calculations were performed using the Statistical Package for Social Sciences (SPSS), version 12.0 (SPSS Inc., Chicago, IL, USA).

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Figure legends

Figure 1. The relationship between IVIG effectiveness and HLA-DR$^+$CD4$^+$ T cells. The percentages of peripheral blood HLA-DR$^+$CD4$^+$ T cells among CD4$^+$ T cells in patients with KD were analysed by flow cytometry. Group A: IVIG-effective KD, Group B: IVIG-resistant KD. ** $p < 0.01$. Horizontal lines indicate means.

Figure 2. The relationship between IVIG effectiveness and HLA- DR$^+$CD8$^+$ T cells. The percentages of peripheral blood HLA-DR$^+$CD8$^+$ T cells among CD8$^+$ T cells in patients with KD were analysed by flow cytometry. Group A: IVIG-effective KD, Group B: IVIG-resistant KD. * $p < 0.05$. Horizontal lines indicate means.

Table 1 Clinical features of children with KD

	KD (n = 82)		
	IVIG effective (n = 51) Group A	IVIG resistant (n = 31) Group B	*P* value A vs. B
Median age (years) (range)	2.5 (1.0–7.5)	3.8 (0.2–9.1)	0.257
Male : Female	31 : 20	23 : 8	0.240†
Median starting days of IVIG (range)	5 (3-9)	4 (3-11)	0.317
Coronary arterial leision (n)	1	7	0.004**†

** $P < 0.01$.
† Fisher exact test was used

Table 2 $CD4^+$ and $HLA\text{-}DR^+CD4^+$ T cells in children with KD

	KD (n = 82)		
	IVIG effective (n = 51) Group A	IVIG resistant (n = 31) Group B	*P* value A vs. B
PBMCs (/μl)	4,318 ± 1,829	5,499 ± 4,570	0.339
$CD4^+$ T cells among PBMCs (%)	35.6 ± 10.7	34.4 ± 7.3	0.916
$CD4^+$ T cells (/μl)	1,601 ± 957	1,876 ± 1,323	0.293
$HLA\text{-}DR^+CD4^+$ T cells among PBMCs (%)	1.7 ± 1.1	2.3 ± 1.0	0.005**
$HLA\text{-}DR^+CD4^+$ T cells (/μl)	70 ± 47	131 ± 110	0.004**
$HLA\text{-}DR^+CD4^+$ T cells among $CD4^+$ T cells (%)	5.1 ± 3.4	6.8 ± 2.8	0.003**

** $P < 0.01$.

Table 3 $CD8^+$ and $HLA\text{-}DR^+CD8^+$ T cells in children with KD

	KD (n = 82)		
	IVIG effective (n = 51) Group A	IVIG resistant (n = 31) Group B	*P* value A vs. B
PBMCs (/μl)	4,318 ± 1,829	5,499 ± 4,570	0.339
$CD8^+$ T cells among PBMCs (%)	23.8 ± 7.8	23.8 ± 6.7	0.863
$CD8^+$ T cells (/μl)	1,000 ± 493	1,315 ± 1,116	0.202
$HLA\text{-}DR^+CD8^+$ T cells among PBMCs (%)	2.5 ± 2.2	3.4 ± 2.9	0.071
$HLA\text{-}DR^+CD8^+$ T cells (/μl)	96 ± 81	186 ± 192	0.019*
$HLA\text{-}DR^+CD8^+$ T cells among $CD8^+$ T cells (%)	9.9 ± 7.4	13.7 ± 10.1	0.046*

* $P < 0.05$.

Figure 1

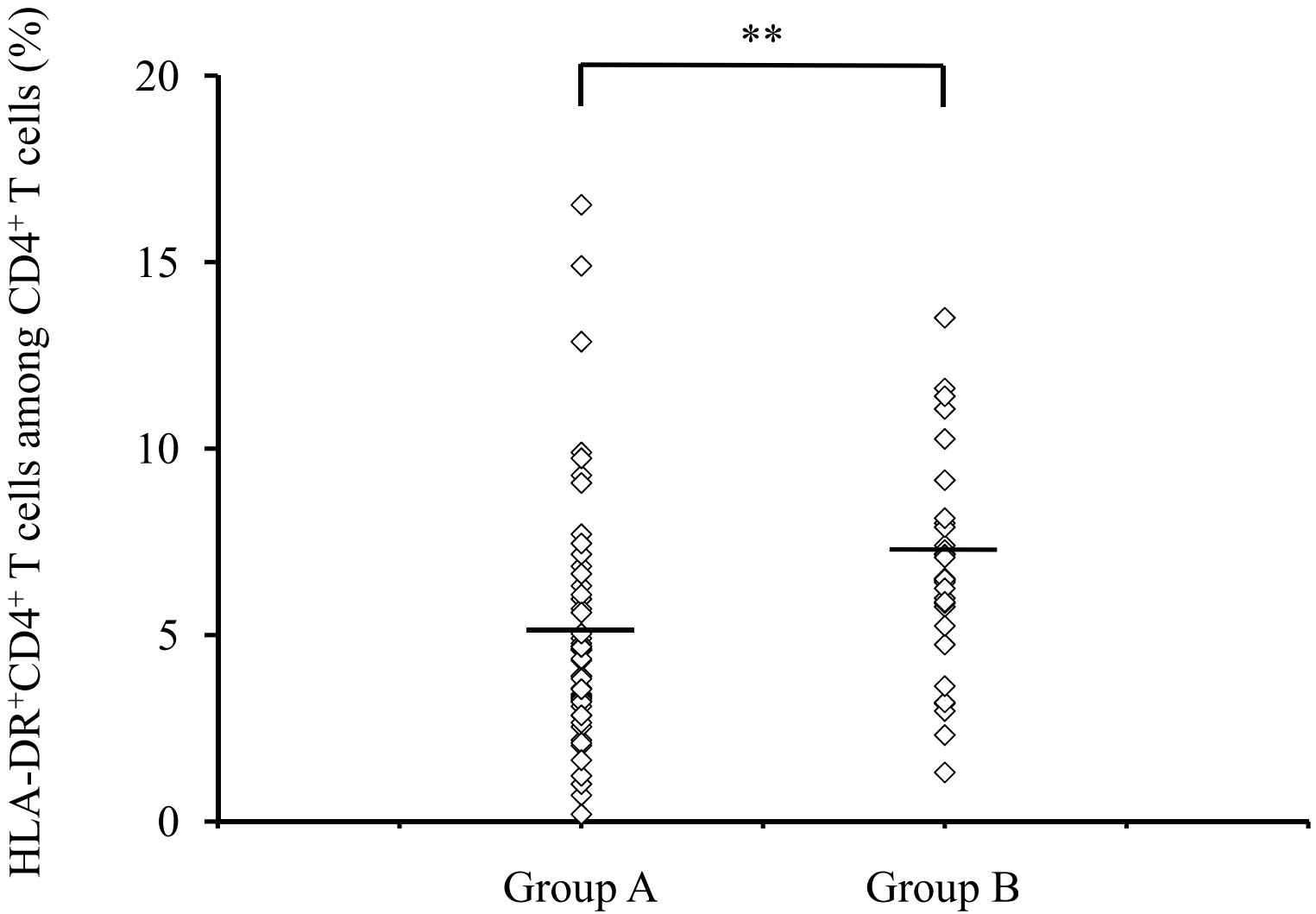
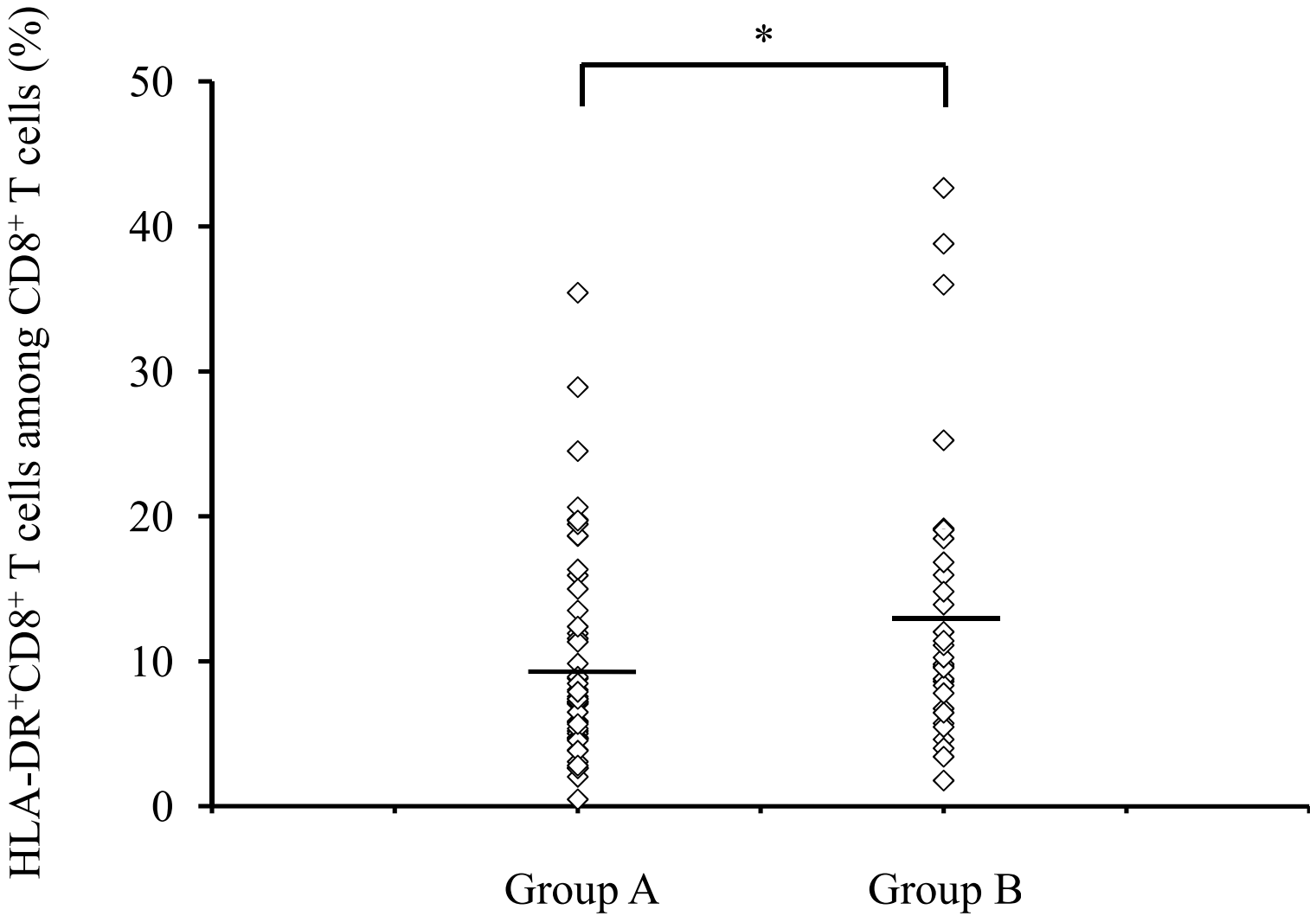


Figure 2


HLA-DR+CD8+ T cells among CD8+ T cells (%)
50
40
30
20
10
0
*
Group A
Group B