Bull Yamaguchi Med School 55(3-4):27-33, 2008

Immunoglobulin G1 Allotypes in Intravenous Immunoglobulin Non-responders of Kawasaki Disease Patients

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(Received September 10, 2008, accepted October 10, 2008)

Abstract The standard therapy for Kawasaki disease (KD) consists of intravenous immunoglobulin (IVIG) treatment. However, at least 10% of KD patients fail to exhibit defervescence with initial IVIG therapy, and they are defined as IVIG non-responders. We investigated the prevalence of certain GM allotypes in IVIG responder and non-responder KD patients. Sera obtained from 83 KD patients and 34 controls were tested for G1M(1), G1M(2), G1M(3), and G1M(17) by using the standard agglutination-inhibition method. All the patients were treated with IVIG, and 14 patients were identified as IVIG non-responders.

All samples tested were positive for the G1M(1) and G1M(17). With regard to G1M(2), 8 of 14 (57.1%) IVIG non-responders, 19 of 69 (27.5%) IVIG responders, and 7 of 34 (20.6%) controls tested were positive for this allotype. G1M(3) was detected in 9 (13%) responders, 3 (21.4%) non-responders. A significant difference in the G1M(2) distribution was noted between the non-responders and controls ($p = 0.019$). The G1M(2) was found to be significantly associated with the IVIG non-responders after adjustments for age, sex, and each allotype (odds ratio, 3.59; 95% confidence interval, 1.03-12.46; $p = 0.040$). IVIG treatment was 8.97 times more likely to fail in patients with the G1M phenotype (1,2,3,17) (95% confidence interval 1.41- 57.21, $p = 0.020$).

In conclusions, the G1M(2) allotype may be associated with KD patients who were IVIG non-responders.

Key words: Kawasaki disease, intravenous immunoglobulin, G1M allotype, IVIG non-responder

Introduction

Kawasaki disease (KD) is an acute febrile illness of early childhood.[1] The histopathological findings of KD comprise panvasculitis with endothelial necrosis and infiltration of mononuclear cells into small- and medium-sized blood vessels.[2] Vascular inflammation results in damage to the coronary arteries and the development of aneurysms and stenosis, leading to ischemic heart disease.[3] We previously reported that KD is one of the diseases associated with inflammatory cytokines, such as tumor necrosis factor-a wherein peripheral blood monocytes/macrophages are activated.[4-6] The standard therapy for KD is the administration of intravenous immunoglobulin (IVIG) along with oral aspirin.[7-8] Without IVIG treatment, coronary artery ectasia or aneurysms develop in 15-25%

of affected children.[3] IVIG administration during the acute phase reduces the prevalence of coronary dilation to <5% and that of giant coronary aneurysms to <1%. However, at least 10% of KD patients fail to exhibit defervescence with initial IVIG therapy.[9–11]

GM allotypes are genetic variants of the immunoglobulin heavy chain of IgG molecules and are coded by genes on chromosome 14q32.[12] These genetic variations in GM allotypes have been associated with some infectious diseases,[13–16] and autoimmune diseases.[17,18] In KD, it was reported that the haplotype G1M(1) with G3M(16) was related to susceptibility.[19] However, few reports are available on GM allotypes and the responses to the treatment, in particular IVIG, of conditions caused by them. Since GM allotypes might influence the effect of IVIG treatment, we investigated the GM allotypes in KD patients in this study, distinguishing between IVIG responders and non-responders. Regarding GM allotypes, we focused on IgG1 which is the major component of human IVIG preparations.

Study subjects

Patients

The study involved KD patients who were referred to Yamaguchi University Hospital between 1998 and 2006. All the KD patients and controls were Japanese. We studied 83 patients who met the diagnostic criteria for KD,[1] including 42 boys and 41 girls (0.5-9 years old; median age, 2 years). KD patients received 1 $g \cdot kg^{-1} \cdot day^{-1}$ IVIG, for 2 consecutive days together with oral aspirin (30 $mg \cdot kg^{-1} \cdot day^{-1}$). IVIG non-responders were defined as those with persistent or recrudescent fever at more than 36 h after the completion of the initial IVIG infusion, as reported previously.[20] Fourteen patients (16.9%) were defined as IVIG non-responders, and 69 patients (83.1%) as IVIG responders. We retreated all IVIG non-responders with a second IVIG infusion (2 $g \cdot kg^{-1}$). Of these patients, 2 were additionally treated with corticosteroids and cyclosporine. As estimated using Japanese Kawasaki Disease Research Committee guidelines, 2 of the 83 IVIG-treated KD patients developed coronary artery lesions (CALs). Blood samples were collected prior to treatment and during the convalescent stage at more than 1 year after the treatment.

Controls

The control population comprised 34 healthy adult volunteers with no history of KD, autoimmune diseases, or chronic treatment with anti-inflammatory agents.

Methods

Detection of G1M allotypes

Serum samples were maintained at −20℃ until typing. As described previously,[12,21] all the samples were tested for G1M(1), G1M(2), G1M(3), and G1M(17) by the standard agglutination-inhibition method using antibodies purchased from Dr. Jean-Michel Dugoujon (Centre d'Anthropologie, Toulouse, France).

Statistical analyses

Phenotype frequencies among the IVIG responder, and IVIG non-responder KD patients and the controls were compared using the chi-square test. The ages of the IVIG responders and non-responders were com pared using the t-test after log-transformation, and the gender ratio was compared using the chi-square test. The percentage of IVIG non-responders for each allotype, and the differences in the phenotypic frequencies exhibited by the IVIG responders and non-responders were analyzed using chi-square test. Odds ratios were obtained using a logistic regression model, with adjustments for confounders. The odds ratios of IVIG non-responders for each allotype were adjusted for age, gender, and each allotype. The odds ratio of each phenotype with a reference phenotype was adjusted for age and gender. All statistical analyses were performed using SAS (ver. 9.1, SAS Institute Japan, Tokyo, Japan). The results were considered significant when the p values, as determined by the exact method, were less than 0.05.

This study was approved by the Institutional review board of Yamaguchi University Hospital.

Results

No significant differences were noted between the IVIG responders and non-responders with regard to the patient characteristics such as age, gender, illness day at the time of initial IVIG treatment, and laboratory data obtained prior to the treatment (e.g., white blood cell count and C-reactive protein level; Table 1).

All the KD patients and controls tested were positive for G1M(1) and G1M(17). With regard to G1M(2), 8 of the 14 IVIG non-responders (57.1%), 19 of the 69 IVIG responders (27.5%), and 7 of the 34 controls (20.6%) tested were positive for this allotype. G1M(3) was detected in 9 (13%) responders, 3 (21.4%) non-responders, and 5 (14.7%) controls, as shown in Table 2. A significant difference was noted in the G1M(2) distribution between the non-responders and the controls ($p = 0.019$).

Although no significant differences were observed in the G1M(2) distribution between the responders and the non-responders ($p = 0.057$), as determined using the chi-square test, the G1M (2) allotype was found to be significantly associated with the IVIG non-responders following adjustments for age, sex, and each allotype (odds ratio, 3.59; 95% confidence interval, 1.03-12.46; $p = 0.040$), as shown in Table 3. The odds ratio determined for patients exhibiting the G1M(3) allotype was not significant ($p = 0.549$).

The phenotypic frequencies exhibited by the IVIG non-responders differed significantly from those exhibited by the responders ($p = 0.047$; Table 4). Since no IVIG failures occurred among patients exhibiting the phenotype G1M(1, 3, 17), the sum of the G1M(1, 17) values added to that of the G1M(1, 3, 17) values was set as the reference point for the odds ratios. Although only a few patients exhibited the G1M(1, 2, 3, 17) phenotypes, IVIG treatment was 8.97 times more likely to fail in the case of these patients than in the case of the other patients (95% confidence

Table 1 Charactoristics of KD patients, illness day of intial IVIG, and laboratory data obtained prior to treatment

	n	Gender male (%)	Age (years)	Illness day of initial IVIG	WBC (/mm^3)	CRP (mg/dl)
Responders	69	37 (53.6%)	2.8 ± 2.4	4.5 ± 1.2	14,208 ± 8,817	7.6 ± 4.6
Non-responders	14	5 (35.7%)	2.7 ± 1.3	3.9 ± 0.9	17,497 ± 7.539	7.6 ± 3.6

No significant differences were observed between the IVIG responders and non-responders.

KD: Kawasaki disease IVIG: intravenous immunoglobulin WBC: white blood cell

Table 2 Prevalence of G1M(2) and G1M(3) among IVIG responders, non-responders, and controls

		n	G1M(2) (%)	G1M(3) (%)
KD patients	Responders	69	19(27.5)	9(13)
	Non-responders	14	8(57.1)*	3(21.4)
Controls		34	7(20.6)	5(14.7)

The ratio of IVIG responders, non-responders, and controls was determined for each allotype using Pearson chi-square test.

*IVIG non-responders tested were positive for G1M(2) more frequently than the controls did ($p=0.019$).

Table 3 G1M allotypes in IVIG non-responders

	n	IVIG non-responders (%)	p	Odds ratio	95% CI	p
G1M(2)	27	8 (29.6)	0.057	3.59	1.03 - 12.46	0.040*
G1M(3)	12	3 (25.0)	0.681	1.57	1.03 - 12.46	0.549
Overall	83	14 (16.9)				

The odds ratio among IVIG non-responders was tested for each allotype using the Pearson chi-square test.
A logistic regression model was used to perform adjustments for each allotype, age, and sex.

Table 4 G1M phenotypes exhibited by IVIG responders and non-esponders

G1M phenotype	Responders(n=69) n(%)	Non-responders(n=14) n(%)	p	Odds ratio	95% CI
1, 17 + 1. 3. 17	50 (72.5)	6 (42.9)		ref	
1, 2, 17	15 (21.7)	5 (35.7)	0.047*	2.85	0.74 - 11.04
1, 2, 3, 17	4 (5.8)	3 (21.4)		8.97**	1.41 - 57.21

The phenotypic frequencies were tested using the Pearson chi-square test.
A logistic regression model was used to perform adjustments for age and sex.
*The phenotypic frequencies among IVIG non-responders differed significantly from that among IVIG responders.
**The patients exhibiting the G1M phenotype(1, 2, 3, 17) were 8.97 times more likely to respond to IVIG treatment than the other patients.

interval 1.41-57.21, p = 0.020).

All 14 IVIG non-responders were re-treated with a second IVIG infusion. IVIG re-treatment was effective in 12 patients (85.7%); of these, 6 tested were negative for G1M(2) while 6 tested were positive. The remaining 2 IVIG non-responders, who were administered other treatment, in addition to repeat IVIG infusion, were G1M(2) positive. The complication of transient coronary dilatation was observed in 1 G1M(2)-positive and G1M(2)-negative patient.

Discussion

In this study, we investigated the prevalence of the G1M(1), G1M(2), G1M(3), and G1M(17) allotypes among Japanese children with KD and controls. Dugoujon et al. investigated the GM genetic diversity of different populations over the world.[22] G1M(1) was found to be positive in 40-70% of Caucasians and 100% of Asians. G1M(2) occurred at a frequency of 17-25% in Europeans and 30% in the Japanese. In our study, the frequency of G1M allotypes among IVIG responder KD patients and controls was similar to that reported previously. Shulman et.al. reported that the frequency of GM heterozygous with

KM1 positive increased in white KD patients, while those of G1M(1) with G3M(16) increased in Japanese patients.[19] They investigated the susceptibility, but did not stratify the patients with IVIG responders and non-responders.

We found that G1M (2) was more prevalent among Japanese KD patients in whom IVIG treatment had failed than among patients in whom it had succeeded and controls. No other allotypic difference was observed among IVIG non-responders, responders, and controls in the present study. In addition, the incidence of the G1M(1, 2, 3, 17) phenotypes was higher among the IVIG non-responders than among the responders. G1M is the antigenic determinant of the immunoglobulin heavy chain. G1M(2) epitopes are present on the Fc portion of the IgG molecule. Thus, the Fc of a particular GM allotype could preferentially associate with the Fc receptor of a particular allotype. The exact function of G1M(2) has not been clarified. We previously reported the effects of IVIG in acute KD.[23-26] IVIG reduced nuclear factor kappa B (NF-kB) activation in monocytes/macrophages[23] and the number of $CD14^+CD16^+$ (FcgRIII) monocytes/macrophages in acute KD.[24] The inhibition of NF-kB activation and FcgRIII expression achieved by IVIG in acute KD may be influenced by the G1M(2) allotype. Further investigations are required to elucidate the functions of G1M(2). In this study, the G1M(2) allotype was associated with KD patients who were IVIG non-responders. The results of our study raise the really intriguing question of whether these patients show similar responses to other diseases treated with high-dose IVIG, such as Guillain-Barre' syndrome.

Re-treatment with IVIG has been recommended,[9-11,27] although the corresponding protocol is not clearly defined. In our study, IVIG re-treatment was effective in 12 patients, including 6 who tested positive for G1M(2). These patients may respond to larger than usual doses of IVIG. The remaining 2 patients, in whom IVIG re-treatment had failed, received additional treatment and tested positive for G1M(2). Further studies are required to determine whether the G1M(2) allotype is a risk factor for repeated treatment. Since only a few KD patients developed CAL, we could not clarify the exact influence of G1M(2) expression on CAL development.

In conclusion, the G1M(2) allotype may be associated with IVIG non-responders among Japanese KD patients. The results presented here provide an impetus for large-scale studies to explore the role of G1M(2) allotypes in the pathogenesis of KD with IVIG failure.

Acknowledgements

This work was supported by a grant (1999-2004) from the Japan Kawasaki Disease Research Center (Director: Tomisaku Kawasaki). We wish to thank Dr. Takuma Iwaki (Department of Pediatrics, Kagawa University School of Medicine), Dr. Yuichi Ishikawa (Yamaguchi University School of Medicine) and Yoshiko Ueno (Yamaguchi University School of Medicine) for obtaining samples and assisting with the experiments. We also thank Prof JM Dugoujon (Laboratoire d'An thropobiologie) for his excellent advice with regard to our research.

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