Table 1. The results of the pathway impact analysis for a set of genes associated with acute phase of Kawasaki disease

Pathway name	Input Genes in Pathway			Impact factor (IF)	corrected gamma *p*-value
	Total	Up	Down		
Antigen processing and presentation	7	0	7	51.621	2.01E-21
Phosphatidylinositol signaling system	2	0	2	35.807	1.04E-14
Circadian rhythm	3	0	3	22.942	2.60E-09
T cell receptor signaling pathway	14	0	14	18.903	1.23E-07
Toll-like receptor signaling pathway	14	6	8	18.526	1.76E-07
Natural killer cell mediated cytotoxicity	14	4	10	14.664	6.71E-06
Ribosome	11	0	11	13.743	1.59E-05
Apoptosis	10	3	7	13.426	2.13E-05
MAPK signaling pathway	17	4	13	10.964	2.07E-04
Cytokine-cytokine receptor interaction	16	7	9	9.511	7.78E-04
Fc epsilon RI signaling pathway	8	3	5	9.323	9.22E-04
B cell receptor signaling pathway	7	0	7	8.69	0.00163044

Pathway-Express was used for the pathway impact analysis in order to build a list of all associated pathways. An impact factor (IF) is calculated for each pathway incorporating parameters such as the normalized fold change of the differentially expressed genes, the statistical significance of the set of pathway genes, and the topology of the signaling pathway. The corrected gamma *p*-value is the *p*-value provided by the impact analysis. 36 pathways were significant at the 5% levels on corrected *p*-values, and the top 12 pathways were selected. Up-regulated genes were as follows: (1) Toll-like receptor signaling pathway; ERK, CD14, TLR8, MKK6, MD2, and TLR5. (2) Natural killer cell mediated cytotoxicity; TRAIL, ERK, FCER1G, and TRAILR3. (3) Apoptosis; TRAIL, PRKAR1A, and TRAILR3. (4) MAPK signaling pathway; ERK, CD14, IL1R2, and MKK6. (5) Cytokine-cytokine receptor interaction; TRAIL, TNFRSF17, IL18RAP, IL1R2, TNFSF13B, TRAILR3, and HGF. (6) Fc epsilon RI signaling pathway; ERK, FCER1G, and MKK6.

Table 2. Microarray analysis of PBMNC between KD patients and healthy controls

Gene name	Gene Ontology	Synonyms	GeneBank	*Fold difference
NLR family, apoptosis inhibitory protein	nucleotide binding	NAIP	NM_004536	7.2
Fc fragment of IgG, high affinity Ia, receptor (CD64)	immune response	FCGR1A	NM_000566	5.6
hemoglobin, gamma A	oxygen transport	HBG1	NM_000559	5.3
hemoglobin, alpha 1	oxygen transport	HBA1	NM_000558	5.1
grancalcin, EF-hand calcium binding protein	calcium ion binding	GCA	NM_012198	4.5
fibrinogen-like 2 (constitutively expressed in cytotoxic T-cells)	signal transduction	FGL2	NM_006682	4.4
Ice protease-activating factor	defense response to bacterium	NLRC4 (IPAF)	NM_021209	4.2
placenta-specific 8		PLAC8	NM_016619	4.1
immunoglobulin superfamily, member 6	immune response	IGSF6	NM_005849	4.1
S100 calcium binding protein A9 (calgranulin B)	inflammatory response	S100A9	NM_002965	3.9

NLR, nucleotide-binding domain, leucine-rich repeat containing. *The difference of mean gene expression levels between 3 KD patients and controls (healthy donors) in microarray analysis is given. Genes that showed more than 3 fold expressional differences between KD patients and healthy controls were selected and top 10 genes were listed. Gene Ontology was not applied in PLAC8. Hypothetical proteins were excluded.

Table 3. Cykokine- and chemokine-related genes expressed in PBMNCs of acute-phase KD patients

Gene name	Gene Ontology	Synonyms	GeneBank	*Fold difference
interleukin 1, beta	immune response	IL1B	NM_000576	0.3
interleukin 2	immune response	IL2	NM_000586	0.7
interleukin 4	regulation of immune response	IL4	NM_000589	0.4
interleukin 6	inflammatory response	IL6	NM_000600	0.5
interleukin 8	immune response	IL8	NM_000584	0.2
interleukin 10	immune response	IL10	NM_000572	0.8
tumor necrosis factor	inflammatory response	TNF	NM_000594	0.9
interferon, gamma	regulation of immune response	IFNG	NM_000619	0.9
chemokine (C-C motif) ligand 2	inflammatory response	CCL2 (MCP1)	NM_002982	1.1
chemokine (C-C motif) ligand 4	immune response	CCL4 (MIP1B)	NM_002984	0.6
chemokine (C-C motif) ligand 5	immune response	CCL5 (RANTES)	NM_002985	0.4
colony stimulating factor 3 (granulocyte)	immune response	CSF3	NM_172220	1.0
vascular endothelial growth factor A	cytokine activity	VEGFA	NM_001025366	0.4
hepatocyte growth factor	protein binding	HGF	NM_000601	2.8
S100 calcium binding protein A9 (calgranulin B)	inflammatory response	S100A9	NM_002965	3.9
S100 calcium binding protein A12	inflammatory response	S100A12	NM_005621	3.5

Sixteen genes that have been reported to have a role in the pathophysiology of KD were selected from the microarray data.

*The difference of mean gene expression levels between 3 KD patients and controls (healthy donors) in microarray analysis is given.

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Lack of association between E148Q *MEFV* variant and Kawasaki disease

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ABSTRACT

We investigated a possible association between Kawasaki disease (KD), a systemic vasculitis of unknown etiology, or its coronary artery lesions (CAL) and *MEFV* gene variants including E148Q, the most common and mild mutation in the *MEFV* gene for familial Mediterranean fever or vasculitis-related disorders. The study population comprised a total of 138 Japanese patients with KD, including 45 patients with CAL and 93 patients without CAL and 170 normal controls. Sequence variations for the *MEFV* gene were detected by direct sequencing, followed by the TaqMan SNP genotyping assay. The genotype and allele frequencies of *MEFV* gene variants (E148Q, L110P, R202Q, P369S, R408Q) were compared between KD patients with and without CAL or between KD patients with CAL and controls. E148Q heterozygotes and homozygotes were observed in 37.1 and 5.5% of healthy controls, 33.3 and 5.1% of KD patients, and 37.8 and 4.4% of KD patients with CAL. No significant differences were observed in the genotype and allele frequencies of other *MEFV* gene variants (L110P, R202Q, P369S, R408Q) between KD patients with and without CAL or between KD patients with CAL and controls. No associations were detected between the *MEFV* gene variants and the development of KD or CAL formation in KD.

1. Introduction

Kawasaki disease (KD) is an acute, self-limited systemic vasculitis that occurs predominantly in infants and young children. Coronary artery aneurysm or ectasia develops in <5 to 25% of untreated children with the disease [1,2]. Its etiology remains unknown; however, clinical and epidemiological features strongly indicate that it is caused by one or several widely distributed infectious agents [2]. It is likely that KD results from an abnormal immunologic response to certain microbial agents in genetically susceptible individuals. The higher rate of KD in the siblings of KD patients and the racial difference in its incidence support this consideration [2]. Recently, several host genetic factors have been identified in the development of KD and coronary artery lesions (CAL) [3–6].

Familial Mediterranean fever (FMF) is an inherited inflammatory disease that is common in Arabs, non-Ashkenazi Jews, Armenians, and Turks, whereas it is uncommon in east Asia, including Japan. FMF is characterized by self-limited periodic fever and various symptoms such as peritonitis, arthritis, rash, pleurisy, and pericarditis. The *MEFV* gene is responsible for FMF [7,8]. Among the *MEFV* mutations, the role of E148Q (c.442 G>C) is still controversial. Although some reports indicated that E148Q was only one of the gene polymorphisms, other reports indicated that E148Q was associated with the mildest disease with a low penetrance or usually required another additional *MEFV* mutation to cause the classical manifestation of FMF [9,10]. Although FMF is an uncommon disorder in Japan, the frequency of E148Q is higher in Japanese than in European or Arab populations [11–13]. *MEFV* was predominantly expressed in granulocytes and monocytes [7], both of which play major roles in the pathophysiology of KD at the acute phase [2]. Several reports revealed that *MEFV* mutations were associated with vasculitis-related disorders such as Behçet's disease, Henoch–Schönlein purpura, and polyarteritis nodosa [14–16], suggesting that *MEFV* gene mutations contribute to the development of a broader spectrum of vasculitis. Furthermore, it was reported that *MEFV* mutations might increase the baseline of inflammation, induce the development of rheumatic diseases, and affect the clinical course of inflammatory disorders [17].

To clarify the role of the *MEFV* gene in the development of KD as one of the host genetic factors, we investigated the associations between KD and *MEFV* gene variants, particularly E148Q, which is common in Japanese populations.

2. Subjects and methods

One hundred thirty-eight KD patients who were treated with oral aspirin plus intravenous immunoglobulin (IVIG:1–2 g/kg/total in CAL^- patients and 3–4 g/kg/total in CAL^+ patients) at Kyushu University Hospital or its affiliated hospitals from 1991 through 2003 were enrolled. Informed consent was obtained from their parents, and the Ethical Committees of Kyushu University approved the study. All patients were Japanese and met the appropriate diagnostic criteria for KD [18]. The study population consisted of 92 boys and 46 girls; the median age at diagnosis was 19 months

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Table 1
Clinical and laboratory data of KD patients

Variables	With CAL (n = 45) Median (range)	Without CAL (n = 93) Median (range)	p value*
Age (months)	19 (1–151)	19 (2–105)	0.614
Admission (day of illness)	4 (1–13)	4 (1–9)	0.552
Start of IVIG (day of illness)	5 (2–15)	5 (1–9)	0.993
Duration of fever (days)	10 (4–27)	7 (3–15)	7.60×10^{-7}†
Peak of white blood cell ($\times10^3/\mu l$)	16.7 (7.3–35.9)	15.6 (7.9–31.0)	0.357
Peak of C-reactive protein (mg/dl)	15.5 (3.3–32.4)	9.6 (2.0–33.3)	0.00534†

IVIG = intravenous immunoglobulin therapy.
*Mann–Whitney U test.
†Significant difference.

(range: 1 to 151 months). Forty-five patients developed CAL and 93 patients did not. According to the criteria of the Japanese Ministry of Health, Labour, and Welfare, the coronary artery was considered abnormal if the diameter of the initial lumen was >3 mm in a child younger than 5 years or >4 mm in a child at or over 5 years of age or if the initial diameter of a segment was at least 1.5 times larger than that of an adjacent segment [19]. Clinical and laboratory data are shown in Table 1.

Peripheral blood was collected from KD patients and 170 randomly selected healthy Japanese volunteers. The donors and their families had no episodes of periodic fever similar to FMF. Genomic DNA was extracted from whole-blood leukocytes with a QIAamp blood kit (Qiagen GmbH, Hilden, Germany).

We screened coding regions of the *MEFV* gene for polymorphisms from genomic DNA of KD patients by direct sequencing with an ABI Prism 3100 Genetic Analyzer (PerkinElmer, Foster City, CA), as described previously [7,20]. The forward and reverse oligonucleotide primers used to amplify each exon were as follows (all oligonucleotide sequences are given 5′ to 3′): exon 1 forward, AACCTGCCTTTTCTTGCTCA; exon 1 reverse, CACTCAGCACTGGATGAGGA; exon 2a forward, ATCATTTTGCATCTGGTTGTCCTTCC; exon 2a reverse, TCCCCTGTAGAAATGGTGACCTCAAG; exon 2b forward, GGCCGGGAGGGGGCTGTCGAGGAAGC; exon 2b reverse, TCGTGCCCGGCCAGCCATTCTTTCTC; exon 3 forward, GAACTCGCACATCTCAGGC; exon 3 reverse, AAGGCCCAGTGTGTCCAAGTGC; exon 4 forward, TTGGCACCAGCTAAAGATGGC; exon 4 reverse, TCTCCCTCTACAGGGATGAGC; exon 5 forward, TATCGCCTCCTGCTCTGGAATC; exon 5 reverse, CACTGTGGGTCACCAAGACCAAG; exon 6 forward, TCCAGGAGCCCAGAAGTAGAG; exon 6 reverse, TTCTCCCTATCAAATCCAGAG; exon 7 forward, AGAATGTAGTTCATTTCCAGC; exon 7 reverse, CATTTCTGAACGCAGGGTTT; exon 8 forward, GCATGCTCACTTCCTCCCTA; exon 8 reverse CTTTGCTCCAGGTGTTTGGT; exon 9 forward, TTAGACCACAGTCCCCAACA; exon 9 reverse, CAGGAAACAGGGACAGGGTA; exon 10a forward, CCAGAAGAACTACCCTGTCCC; exon 10a reverse, AGAGCAGCTGGCGAATGTAT; exon 10b forward, GAGGTGGAGGTTGGAGACAA; exon 10b reverse, TCCTCCTCTGAAATCCATGG. Exons 2 and 10 were amplified in two overlapping polymerase chain reaction (PCR) fragments. The PCR conditions were as follows: initial denaturation at 94°C for 1 minute, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for exons 1, 3, 4, and 5 and each part of exon 10, 58°C for exons 6, 7, 8, and 9, 55°C for each part of exon 2 for 30 seconds and extension at 72°C for 30 second, followed by a final extension step at 72°C for 5 minutes.

Genotyping of each variant was carried out using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA): *MEFV* L110P (rs11466018, Applied Biosystems code c_11186727_10), *MEFV* R202Q (rs224222, c_2394721_10), *MEFV* P369S (rs11466023, c_2394737_10), and *MEFV* R408Q (rs11466023, c_45171223_10). Detection of probes and primers for *MEFV* E148Q (rs3743930) was performed using Custom TaqMan SNP genotyping assays, and each oligonucleotide sequence was as follows (given 5′ to 3′); forward primer, CCAGCCTGCGGTGCA; reverse, GCCTTCTCTCTGCGTTTGCT;

Table 2
Polymorphisms of the *MEFV* genes in KD patients with and without CAL and control subjects

Gene	Genotype/ allele	Ctrl (n = 170)	KD total (n = 138)	KD CAL (+) (n = 45)	KD CAL (−) (n = 93)	Ctrl vs KD total			KD CAL (+) vs Ctrl			KD CAL (+) vs CAL (−)		
						p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI
E148Q	GG	98 (0.58)	85 (0.62)	26 (0.58)	59 (0.63)									
	GC	63 (0.37)	46 (0.33)	17 (0.38)	29 (0.31)	0.496			0.824			0.512		
	CC	9 (0.05)	7 (0.05)	2 (0.04)	5 (0.05)									
	Allele G	259 (0.76)	216 (0.78)	69 (0.77)	147 (0.79)									
	Allele C	81 (0.24)	60 (0.22)	21 (0.23)	39 (0.21)	0.540	0.89	0.608–1.30	0.923	0.97	0.562–1.68	0.655	1.15	0.628–2.10
L110P	TT	139 (0.82)	115 (0.83)	36 (0.80)	79 (0.85)									
	TC	29 (0.17)	22 (0.16)	9 (0.20)	13 (0.14)	0.598			0.445			0.321		
	CC	2 (0.01)	1 (0.01)	0	1 (0.01)									
	Allele T	307 (0.90)	252 (0.91)	81 (0.90)	171 (0.92)									
	Allele C	33 (0.10)	24 (0.09)	9 (0.10)	15 (0.08)	0.667	0.89	0.510–1.54	0.933	1.03	0.475–2.25	0.593	1.27	0.532–3.02
R202Q	GG	163 (0.96)	132 (0.96)	43 (0.96)	89 (0.96)									
	GA	7 (0.04)	6 (0.04)	2 (0.04)	4 (0.04)									
	AA	0	0	0	0									
	Allele G	333 (0.98)	270 (0.98)	88 (0.98)	182 (0.98)									
	Allele A	7 (0.02)	6 (0.02)	2 (0.02)	4 (0.02)	0.855	1.05	0.351–3.18	0.751	1.08	0.221–5.30	0.688	1.03	0.186–5.75
P369S	CC	153 (0.90)	120 (0.87)	38 (0.84)	82 (0.88)									
	CT	17 (0.10)	18 (0.13)	7 (0.16)	11 (0.12)									
	TT	0	0	0	0									
	Allele C	323 (0.95)	258 (0.93)	83 (0.84)	175 (0.94)									
	Allele T	17 (0.05)	18 (0.07)	7 (0.16)	11 (0.06)	0.417	1.33	0.670–2.62	0.307	1.60	0.643–3.99	0.557	1.34	0.502–3.59
R408Q	GG	157 (0.92)	123 (0.89)	40 (0.89)	83 (0.89)									
	GA	13 (0.08)	15 (0.11)	5 (0.11)	10 (0.11)									
	AA	0	0	0	0									
	Allele G	327 (0.96)	261 (0.95)	85 (0.94)	176 (0.95)									
	Allele A	13 (0.04)	15 (0.05)	5 (0.06)	10 (0.05)	0.340	1.45	0.676–3.09	0.466	1.48	0.513–4.27	0.825	1.04	0.343–3.12

CI = confidence interval; Ctrl = Control subjects; OR = odds ratio.
Numbers in parentheses indicate the percentages of the genotype or allele frequencies. After Bonferroni's correction of multiple comparison, $p < 0.0166$ was considered statistically significant.
All evaluated SNPs in controls were under Hardy–Weinberg disequilibrium.

reporter 1 (VIC), CAGCCCGAGGCCG; reporter 2 (FAM), CAGCCCCAGGCCG. Genotyping using the TaqMan method was performed with an ABI Prism 7700 sequence detection system.

A χ^2 test was used to compare the genotype and allele frequency distributions of each variant between the KD patients and controls, between the KD patients with and without CAL, and between KD patients with CAL and controls. We also used the Mann–Whitney *U* test to compare the clinical and laboratory data between patients with and without minor alleles of E148Q or other *MEFV* variants.

3. Results

We first analyzed the allelic frequency of E148Q (c.442 G>C), which is a common SNP in Japanese populations [21] compared with other populations, in 138 KD patients and 170 controls by Taqman genotyping assay. E148Q heterozygotes and homozygotes were observed in 37.1 and 5.5% of healthy controls, 33.3 and 5.1% of KD patients, and 37.8 and 4.4% of KD patients with CAL (Table 2). The genotype and allele frequencies of E148Q variant between KD patients and healthy controls, KD patients with and without CAL, or KD patients with CAL and controls did not differ.

Because it might be still possible that other *MEFV* gene variants were associated with the development of KD in combination with E148Q, we investigated other *MEFV* gene variants by direct sequencing of PCR-amplified genomic DNA from 53 KD patients who had at least one Q148 allele. We found four types of nonsynonymous variants, L110P (c.329T>C), R202Q (c.606G>A), P369S (c.1105C>T), and R408Q (c.1223G>A), in 23, 1, 9, and 6 of the 53 patients, respectively. Then, we performed genotyping of the four gene variants for the 138 KD patients and 170 controls using TaqMan SNP genotyping assays. As a result, the genotype and allele frequencies of each variant indicated no significant differences between all KD patients and healthy controls or between KD patients with and without CAL (Table 2). We performed haplotype analysis of the five *MEFV* variants, but the assembly of these alleles indicated no significant differences between KD and healthy controls (data not shown). Clinical and laboratory data were analyzed by the comparison of subgroups with E148/E148, Q148/Q148, E148/Q148 or Q148/Q148, (E148/Q148 or Q148/Q148) and (L110/P110 or P110/P110), (E148/Q148 or Q148/Q148), and (P369/S369) (Table 3). The median serum C-reactive protein concentration (18.2 mg/dl) tended to be higher in Q148 homozygous or heterozygous groups (E/Q or Q/Q) than that in wild type group (9.3 mg/dl; E/E) among KD patients with CAL ($p = 0.059$), but there were no significant differences between the other groups in other variables.

Several limitations must be acknowledged and addressed regarding the present study. The sample sizes in this study did not have a sufficient power of statistical analysis, especially for the L110P, R202Q, P369S, and R408Q, because their minor allele frequencies in the controls were as low as 0.10 or less. Based on the minor allele frequencies of E148Q (0.24), L110P (0.10), R202Q (0.02), P369S (0.05), R408Q (0.04), or the cohort size, the estimated smallest detectable risks of their variants were calculated to 1.45, 1.67, 2.54, 1.89, and 2.07 for KD development (control: $2N = 340$ vs. KD: $2N = 276$); 1.68, 2.01, 3.40, 2.34, and 2.45 for CAL formations (control: $2N = 340$ vs. KD CAL(+): $2N = 90$), respectively. Alternatively, the study would require 81, 432, >1000, >1000, and >1000 KD patients to reach statistical significance ($p < 0.05$), respectively, if the relative risks for KD development were set to 1.50.

4. Discussion

Ozen *et al.* [17] reported that among 70 individuals with *MEFV* gene mutations, 28 (40.0%) had some form of rheumatic complaints and 15 (21.4%) developed rheumatic diseases or vasculitis, including Behçet's disease. They also reported that 30.5% of the children with rheumatic diseases and 25.4% of patients with juvenile idiopathic arthritis had mutations of the *MEFV* gene [17]. FMF and Behçet's disease are not rare disorders in the eastern Mediterranean and it was suggested that the *MEFV* mutation played a role in

Table 3
Clinical and laboratory data of all KD patients or KD patients with CAL, subgrouped by combined genotypes of MEFV gene

Population	All KD patients								
Subgroup	(1)	(2)	(3)	(4)	(5)	Comparison (*p* values)			
Genotype									
E148Q	E/E	Q/Q	E/Q or Q/Q	E/Q or Q/Q	E/Q or Q/Q				
L110P	–	–	–	L/P or P/P	–				
P369S	–	–	–	–	P/S				
Comparison						(1) vs (2)	(1) vs (3)	(1) vs (4)	(1) vs (5)
Number	85	7	53	23	9				
Variables									
Age (months)	18 (2–151)	22 (7–66)	22 (1–96)	20 (2–88)	40 (2–96)	0.473	0.38	0.669	0.338
Admission (day of illness)	4 (1–13)	3 (2–8)	4 (1–10)	4 (2–10)	4 (1–6)	0.348	0.382	0.243	0.870
Start of IVIG (day of illness)	5 (2–10)	4 (3–8)	5 (1–15)	4 (1–15)	4 (3–6)	0.552	0.518	0.485	0.218
Duration of fever (days)	7 (3–27)	6 (5–12)	7 (4–20)	7 (4–17)	7 (5–20)	0.781	0.574	0.451	0.55
Peak of white blood cell ($\times 10^3/\mu l$)	16.2 (7.3–35.9)	12.5 (10.8–21.3)	15.7 (8.8–31.0)	15.4 (8.8–22.6)	15.6 (12.5–19.1)	0.381	0.992	0.328	0.931
Peak of C-reactive protein (mg/dl)	9.3 (2.5–33.3)	12.2 (3.5–23.9)	13.4 (2.0–32.4)	5.4 (2.0–22.6)	10.7 (9.2–13.3)	0.664	0.157	0.518	0.495

	KD patients with CAL								
Subgroup	(1)	(2)	(3)	(4)	(5)	Comparison (*p* values)			
Genotype									
E148Q	E/E	Q/Q	E/Q or Q/Q	E/Q or Q/Q	E/Q or Q/Q				
L110P	–	–	–	L/P or P/P	–				
P369S	–	–	–	–	P/S				
Comparison						(1) vs	(1)	(1)	(1) vs (5)
Number	26	2	19	9	2				
Variables									
Age (months)	17 (2–151)	(13, 66)	38 (1–96)	17 (5–77)	(40, 96)	0.459	0.186	0.752	0.152
Admission (day of illness)	4 (2–13)	(3, 3)	4 (1–10)	4 (3–10)	(3, 4)	0.345	0.600	0.779	0.629
Start of IVIG (day of illness)	5 (2–10)	(3, 6)	5 (3–15)	5 (3–15)	(3, 5)	0.694	0.757	0.779	0.459
Duration of fever (days)	10 (4–27)	(6, 12)	11 (5–20)	12 (5–17)	(11, 20)	0.629	0.990	0.690	0.247
Peak of white blood cell ($\times 10^3/\mu L$)	16.2 (7.3–35.9)	(10.8, 21.3)	16.8 (10.8–22.6)	16.9 (9.9–22.6)	(16.7, 19.1)	0.636	0.843	0.607	0.776
Peak of C-reactive protein (mg/dl)	9.3 (3.9–29.3)	(11.8, 12.7)	18.2 (3.3–32.4)	10.7 (9.2–13.3)	(12.5, 13.7)	0.784	0.059	0.836	0.717

Patients who had Q148 + Q202 and Q148 + Q408 were so rare ($n = 1$ and 6) that we did not analyze this group.

the pathogenesis of Behçet's disease [16]. Furthermore, Rozenbaum and Rosner reported that the prognosis of 3 juvenile idiopathic arthritis patients who had a M694V mutation of the *MEFV* gene was extremely poor [22]. In addition, Tunca *et al.* demonstrated an increased acute phase response in carriers for the *MEFV* gene [23]. These reports indicated that *MEFV* gene mutation was also related to enhanced inflammatory responses and severity in these disorders. On the other hand, we reported that *MEFV* gene variants in Japanese subjects were not related to the development of KD or CAL formation in KD and played no major role in the inflammatory responses in KD.

Most Japanese FMF patients have compound heterozygous *MEFV* mutations [21,24,25]. Among them, E148Q is the most common and is reported to have a weak effect that serves only to enhance the development and severity of FMF that was primarily induced by another mutation [25]. We reported that Q148 was not associated with the development of KD that occurs in a higher frequency in Japanese populations. It is possible that the functional effect of Q148 might be too small to accelerate the inflammation for the development of KD or to exacerbate vasculitis in KD. Because Q148 was not significantly associated with enhanced inflammatory responses in KD patients, it is less likely that these Q148 homozygotes will develop FMF, rheumatic disease, or vasculitis in the future. Rather, the high frequency of Q148 variant and the low incidence of FMF, as well as the presence of many asymptomatic Q148 homozygotes in Japanese populations strongly suggest that E148Q is one of the genetic polymorphisms of the *MEFV* gene with little functional significance. Further long-term evaluation of Q148 homozygotes would be necessary to clarify a role of Q148 in the pathogenesis of rheumatic or vasculitis disorders.

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研究分担報告書

難治性川崎病における血管内皮細胞特異的のサイトカインの動態

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研究要旨： 【背景】川崎病は小中動脈を中心とした全身の血管炎であり、特に冠動脈が傷害される。免疫グロブリン大量療法（IVIG）は、最も有効な川崎病の治療法であり、冠動脈病変（CAL）の合併率を減少させたが、冠動脈病変は川崎病を発症した10%に認められ、15%はIVIGに抵抗性の難治の川崎病であり、新たな治療法の確立が望まれている。川崎病急性期には様々なサイトカインの活性化が報告され、なかでも炎症性サイトカインであるTumor necrosis factor-alpha（TNF-α）は発症早期に高値を示し、炎症機転の中心的な役割を果たすと考えられている。一方、川崎病は、自然免疫の爆発的な活動を特徴とし、中でも、Pathogen Associated Molecular Pattern(PAMP)の一つであるMRP8/MRP14やS100A12は、血管内皮細胞のパターン認識受容体により認識され、シグナル経路から、様々な炎症性サイトカインを誘導し、川崎病では、冠動脈病変を含めた血管炎の重要なマーカーであることを我々は明らかにしてきた。インフリキシマブはTNF-αモノクローナル抗体であり、TNF-αを介して引き起こされる慢性関節リウマチやクローン病といった炎症性疾患において、有効な治療薬であることが報告されてきている。近年、川崎病においても、IVIG無効、メチルプレドニンパルス療法（IVMP）の患者に対するインフリキシマブの投与例の報告が散見されてきている。

【目的】IVIG無効、IVMP無効の難治川崎病において、インフリキシマブを投与し、炎症性サイトカインとDAMPの動態の解析を行い、インフリキシマブの血管炎に対する作用機序を明らかにした。

【方法】対象は、平成15年から平成18年に発症した11人の川崎病患者（男6人、5人）で、年齢は3ヶ月から7歳5ヶ月（中央値3.8歳）であった。対象の患者は、すべて診断基準を満たし、IVIG 療法またはIVMP療法が無効であった。インフリキシマブ投与前後の血液を採取し、ELISA法を用いて、soluble TNF-α receptor（sTNFR）、interleukin(IL)-6、vascular endothelial growth factor (VEGF)、myeloid-related protein (MRP) 8/MRP14、S100A12、soluble receptor for advanced glycation end products (sRAGE)の動態を検討した。また健常群33例および川崎病IVIG反応群18例とIVIG不応群14例についても動態を解析し、インフリキシマブ投与群と比較検討を行った。

【結果】インフリキシマブ投与例11例中8例が、投与後に臨床症状の改善が認められた。3

例はインフリキシマブ投与後も臨床症状が改善せず、炎症反応が遷延し、IVMPの再投与およびサイクロフォスファマイドの投与を余儀なくされた。11例中4例において、冠動脈病変を認めたが、インフリキシマブ投与前より冠動脈病変が認められていた。IVIG反応群ではIVIG投与前において、STNFR、IL-6、VEGF、 MRP8/MRP14、S100A12は健常群との比較では有意に高値であったが、IVIG投与後に有意に低下した。IVIG不応群ではIVIG投与前後において、STNFR、IL-6、VEGF、 MRP8/MRP14、S100A12は有意に高値を示し続けた。インフリキシマブ投与例ではSTNFR、IL-6はインフリキシマブ投与前に有意に高値を示し、投与後に低下したが、MRP8/MRP14およびS100A12はむしろインフリキシマブ投与後に増加し、VEGFは変化なく高値を示し続けた。

【結論】インフリキシマブは難治川崎病における有効な治療法のひとつであることが示された。またインフリキシマブ治療によりMRP8/MRP14やS100A12といったPAMPおよびVEGFが抑制されなかったことは、全身の炎症機転はインフリキシマブにより抑制されても、局所の血管炎は抑制されないことが示唆され、冠動脈病変の悪化を十分に阻止できない可能性が示された。そのため、難治川崎病においては、インフリキシマブを早期投与することで、単球・マクロファージの活性化を抑え、血管炎の進展を防止することが望ましいと思われた。

A. 研究目的

川崎病は、乳幼児に発症する全身の中小動脈を主体とした血管炎であり、特に冠動脈を侵し、種々の心合併症を引き起こす。1983 年に Lancet に古庄先生が川崎病の治療として、免疫グロブリン大量療法を発表されて以来、予後は劇的に改善されてきているが、今尚約 10%の治療不応例が存在し、冠動脈に後遺症を残す例も少なくない。その病態および冠動脈病変進展の機序にはいまだ不明な点が多く、根本的な治療法がない。

近年、種々のサイトカインと川崎病の冠動脈病変の関連が報告されてきている。これまで我々は、サイトカインと血管内皮細胞障害に関する研究を続け、血管内皮の障害に焦点を当て、①VEGF、iNOs の冠動脈瘤形成への関与を明らかにした(Pediatr Res 2001,2004)。②血管内皮から血栓形成および炎症反応を誘導する S100 蛋白に着目し、川崎病の病態解明の研究を進めた(Lancet 2003, Blood 2005)。③S100 蛋白の一つである S100A12 は、川崎病急性期において、冠動脈瘤形成期である発症 2 週をピークに好中球から大量に遊離し、Receptor for Advanced Glycation End Products(RAGE)を介して血管内皮細胞に影響を及ぼすことで、冠動脈病変の進行の一助となり、免疫グロブリン大量療法に対する好中球の反応性を反映している可能性を指摘した(Am J Cardiol 2004)。④S100 蛋白である、MRP8/MRP14(S100A8/S100A9)はプロテオグリカンやカルボキシ N グリカンを介してヒト微小血管内皮細胞に特異的に結合することが報告されており、in vitro のヒト微小血管内皮細胞において MRP8/MRP14 が特異的な炎症性の反応を示すことが明らかにし、MRP8/MRP14 の発現が血管炎の炎症の活動性と相関する

ことを実証した(Blood 2005)。⑤MRP8/MRP14 の川崎病における動態解析では、川崎病急性期では、顆粒球が主体となって産生された MRP8/MRP14 が、血管内皮細胞に結合し、血栓形成および炎症反応が惹起され、血管炎が生じることを示唆した。また流血中の血管内皮細胞の増加は、冠動脈病変群において明らかであり、MRP8/MRP14 の冠動脈病変の進展に重要な役割を担っている可能性を示唆した(J Am Coll Cardiol 2006)。

川崎病は、自然免疫の爆発的な活動を特徴とし、中でも、Pathogen Associated Molecular Pattern(PAMP)は、パターン認識受容体により認識され、シグナル経路から、様々な炎症性サイトカインを誘導する。Receptor for Advanced Glycation End Products (RAGE)は炎症、特に血管炎を惹起する内因性のリガンドに対するパターン認識受容体として作用し、PAMP として、S100A12 が報告されているが、川崎病においてはその作用機序は十分に明らかにされていない。

一方、炎症性サイトカインである Tumor necrosis factor-alpha (TNF-α) は川崎病の発症早期に高値を示し、川崎病において炎症機転の中心的な役割を果たすと考えられている。インフリキシマブはマウス・ヒトのキメラの IgG1 の TNF-α モノクローナル抗体であり、TNF-α と結合し、可溶性 TNF-α の中和、TNF-α の解離、TNF-α 産生細胞を傷害することで、抗炎症作用を発揮し、経静的に投与することで、TNF-α を介して引き起こされる、慢性関節リウマチやクローン病等の炎症性疾患において、有効な治療薬であると報告されている。近年、川崎病の IVIG 無効、メチルプレドニンパルス療法 (IVMP) の患者においても有効性が報告されてきている。

PAMP の動態を明らかすることで、川崎病の炎症反応の促進を抑制し、冠動脈病変の予後改善にもつながる端緒となる可能性があると考えた。そこで我々の目的は、RAGE とそのリガンドである S100A12 に焦点をあて、PAMP の動態および血管炎における作用機序に関して検討を行うことである。

B. 研究方法

対象は、当該大学倫理委員会の承認を得て、文書による同意が得られた患者のうち、川崎病の診断基準を満たし、免疫グロブリン大量療法およびアスピリンの経口投与が行われた、平成15年から平成18年に発症した11人の川崎病患者(男6人、女5人)で、年齢は3ヶ月から7歳5ヶ月 (中央値3歳5ヶ月) である (Table 1)。対象の患者は、IVIG 療法またはIVMP 療法が無効であった。インフリキシマブ投与前後の血液を採取し、sandwich ELISA法を用いて、soluble TNF-α receptor (sTNFR)、interleukin(IL)-6、vascular endothelial growth factor (VEGF)、myeloid-related protein (MRP) 8/MRP14、S100A12、soluble receptor for advanced glycation end products (sRAGE)の動態を検討した。また健常群33例および川崎病IVIG反応群18例とIVIG不応群14例についても動態を解析し、インフリキシマブ投与群と比較検討を行った。なおIVIG反応群は投与後48時間以内に解熱するものとする。発症から1ヶ月後において、心エコーより冠動脈が3mm以上拡張

をきたすものを、冠動脈病変群とする。

C. 研究結果

インフリキシマブ投与例11例中8例が、投与後にすみやかに解熱し、臨床症状の改善が認められた（Table 2)。1例はインフリキシマブ投与後も発熱が遷延し、臨床症状および炎症反応が遷延し、IVMPの再投与を行ったが、改善が認められず、サイクロフォスファマイドの投与を余儀なくされた（Table 2)。11例中4例において、冠動脈病変を認めたが、インフリキシマブ投与前より冠動脈病変が認められ、インフリキシマブの投与が10病日以後と、投与の遅い症例であった。また全例において、インフリキシマブによる有害事象は認めなかった（Table 1、2)。

川崎病急性期における各種サイトカインの検討では、IVIG反応群ではIVIG投与前において、IL-6、sTNFR、MRP8/MRP14、S100A12、VEGFは健常群と比較して、有意に高値であったが、IVIG投与後に有意に低下した（sTNFR前0.580±0.180ng/ml後0.326±0.147ng/ml；P<0.05、IL-6前245±321pg/ml後69±217pg/ml；P<0.05、MRP8/MRP14前3261±1724ng/ml後2063±1499ng/ml；P<0.01、S100A12前412±315ng/ml後244±286ng/ml；P<0.01、VEGF前525±607pg/ml、後425±426pg/ml；P<0.05、sRAGE前1495±834ng/ml後3212±1597ng/ml；P<0.01）（Table 3、Figure 1-3）。

IVIG不応群ではIVIG投与前において、IL-6、STNFR、MRP8/MRP14、S100A12、VEGFは健常群と比較して、有意に高値であり、IVIG投与後も変化に乏しかった（sTNFR前0.600±0.241ng/ml後0.474±0.220ng/ml；N.S.、IL-6前276±167pg/ml後182±387pg/ml；N.S.、MRP8/MRP14前4818±3983ng/ml後4588±4397ng/ml；N.S.、S100A12前1148±1837ng/ml後651±574ng/ml；N.S.、VEGF前790±674pg/ml、後975±636pg/ml；N.S.、sRAGE前637±404ng/ml後864±553ng/ml;N.S.（Table 3、Figure 1-3))。

インフリキシマブ投与例ではsTNFR、IL-6は投与前に健常群に比べて、有意に高値を示し、投与後に低下したが、MRP8/MRP14およびS100A12はむしろ増加した。VEGFは投与前後で高値のままで遷延した（sTNFR前0.714±0.161ng/ml後0.391±0.161ng/ml；P<0.01、IL-6前1013±1386pg/ml後233±561pg/ml;P<0.01、MRP8/MRP14前4859±2997ng/ml後5860±5468ng/ml；N.S.、S100A12前1027±615ng/ml後1180±1201ng/ml；N.S.、VEGF前970±1030pg/ml、後814±946pg/ml；N.S.、sRAGE前868±613ng/ml後1224±497ng/ml；P<0.05）（Table 3、Figure 1-3）。

D. 考案

IVIG治療は川崎病の炎症反応をすみやかに改善し、CALの発生率を減少させる有効な治療である。本研究においても、川崎病の急性期において、種々の炎症性サイトカインの血清中濃度は上昇し、IVIG治療に反応すれば減少することが示された。IVIG治療反応群において、炎症性サイトカインであるIL-6およびTNF-αの受容体であるsTNFRは治療後速やかに、減少を認め、PAMPであり、川崎病において血管内皮細

胞に特異的に作用し、血管炎において中心的な役割を果たすサイトカインであるMRP8/MRP14およびS100A12も、VEGFに加えて速やかに低下した。対照的に、インフリキシマブを投与した難治川崎病例ではIL-6およびsTNFRは低下したが、PAMPであるMRP8/MRP14およびS100A12はむしろ増加し、VEGFは高値のまま遷延した。このことから、インフリキシマブではTNF-αを系とした炎症の進展は抑制できても、局所の血管炎を十分に抑制されない可能性があることが示唆された。またインフリキシマブは川崎病の病初期に出現することが考えられている単球・マクロファージから放出されるTNF-αを拮抗すると考えられる。本研究での治療の無効の3例および冠動脈病変を合併した4例は投与病日が遅いことから、早期にインフリキシマブを投与することで、治療効果の改善が得られる可能性もあることが示唆された。

E. 結論

本研究により、インフリキシマブは難治川崎病における有効な治療法のひとつであることが示された。またインフリキシマブ治療によりMRP8/MRP14やS100A12といったDAMPおよびVEGFが抑制されなかったことは、全身の炎症機転はインフリキシマブにより抑制されても、局所の血管炎は抑制されないことが示唆され、冠動脈病変の悪化を十分に阻止できない可能性が示された。そのため、難治川崎病においては、インフリキシマブを早期投与することで、単球・マクロファージの活性化を抑え、血管炎の進展を防止することが望ましいと思われた。

また今後の計画として、川崎病急性期における自然免疫応答による炎症の制御機構の解明を明らかにし、川崎病の原因を究明する予定である。 川崎病の発症にはNF-kB 活性化に基づく高炎症性サイトカイン血症が極めて重要である。 近年、NF-kB を調節する miRNA について、解明が進められ、なかでも、miR-146 が NF-kB を介して、自然免疫応答の調節を行っており、Toll-like receptor およびサイトカインのネガティブフィードバックを調節していると考えられているが、川崎病急性期において、miRNA の遺伝子発現の調節は明らかではない。 これらの観点から、川崎病急性期における miRNA による NF-kB を中心とした自然免疫応答による炎症の制御機構の解明を試みる予定である。

F. 健康危険情報

特になし

G. 研究発表

1. 論文発表

1) Hirono K, Saito K, Ichida F, Saji T et al. The efficacy of infliximab treatment and dynamic changes of inflammatory cytokines in patients with refractory Kawasaki disease. **Peditaric Res** 2009 Jun;65(6):696-701.

2) 佐地　勉、鈴木啓之、市田蕗子、小林徹。川崎病急性期治療の最前線。Pharma Medica, 2009;27:3:167-175

3) 廣野恵一、市田蕗子：『川崎病のすべて』インフリキシマブ療法　石井正浩他編　アクチュアル小児科　中山書店　東京、2009:112-115

2. 学会発表

国際会議

1) Kazuyoshi Saito, Keiichi Hirono, Keijirou Ibuki, Hirokazu Kanegane, Fukiko Ichida, and Toshio Miyawaki. S100 proteins could not be suppressed by infliximab treatment in refractory Kawasaki disease. 5th World Congress of Pediatric Cardiology and Cardiac Surgery. 22.Jun 2009, Cairns, Australia
2) Keiichi Hirono, Yasushi Kemmotsu, Toho, Kazuyoshi Saito, Keijirou Ibuki, Hirokazu Kanegane, Fukiko Ichida, Tsutomu Saji, Toshio Miyawaki. S100 Proteins Could Not Be Suppressed By Infliximab Treatment In Refractory Kawasaki Disease. American Heart Association, Basic Cardiovascular Sciences Conference. 2009. 20. July 2009. Las Vegas. USA

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1) 齋藤和由、伊吹圭二郎、小澤綾佳、渡辺一洋、廣野恵一、上勢敬一郎、金兼弘和、市田蕗子、宮脇利男. 「難治性川崎病に対してシクロスポリン A が奏効した 3 症例」第 23 回日本小児科学会富山地方会、2009 年 6 月 28 日、富山
2) 中村常之、秋田千里、太田邦雄、廣野恵一、田村知史、西尾夏人、畑崎喜芳、上勢敬一郎、西田公一、石原靖紀、酒詰忍. 北陸地区の川崎病急性期治療の現状. 第 45 回日本小児循環器学会総会・学術集会、2009 年 7 月 15 日、神戸
3) 斎藤和由、廣野恵一、伊吹圭二郎、小澤綾佳、渡辺一洋、上勢敬一郎、市田蕗子、宮脇利男. 「シクロスポリン A を投与した難治性川崎病 3 症例におけるサイトカインの検討」第 29 回日本川崎病学会　2009 年 10 月 17 日、名古屋

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得
   特になし
2. 実用新案登録
   特になし
3. その他
   特になし

Figure Legends

Figure 1. Sequential changes of pro-inflammatory cytokines, CRP (left panel), soluble tumor necrosis factor-alpha receptor (sTNFR) I (middle panel) and IL-6 (right panel), in refractory KD patients who were treated with infliximab (closed circle), compared with responders (open square).
Blood samples were collected from patients before and within 48 hours after infliximab treatment in refractory KD, and before and within 48 hours after the first IVIG treatment in responders to IVIG.
P value shows changes between before and after treatment in each group. **P*<0.05, †*P*<0.01.

Figure 2. Sequential changes of endothelial cell specific cytokines and markers of local tissue damage, myeloid-related protein (MRP8/MRP14) (left panel), S100A12 (left middle panel), soluble receptor for advanced glycation end product (sRAGE) (right middle panel) and VEGF (right panel), in refractory KD patients who were treated with infliximab (closed circle), compared with responders (open square).
P value shows changes between before and after treatment in each group. **P*<0.05, †*P*<0.01.

Figure 3. Tumor necrosis factor (TNF)-alpha and other proinflammatory cytokines activate endothelium and lead to the expression of carboxylated *N*-glycans. MRP8/MRP14 are released in high amounts at local sites of inflammation and have been recently described as novel members of the DAMP-family acting as endogenous ligands of Toll-like receptor 4 (TLR4). Activated neutrophils and monocytes secrete MRP-8/MRP-14 heterodimers, which bind to the carboxylated *N*-glycans and heparin sulfate on the endothelial cell surface. Leukocytes also secrete S100A12, which binds to the receptor for advanced glycation end products (RAGE) expressed on endothelial cells, lymphocytes, and macrophages. This receptor signals through the nuclear factor-kappa-B pathway and induces expression of many proinflammatory molecules. The net result of S100 protein binding is platelet aggregation and adherence to endothelium, increased expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, adhesion of neutrophils and monocytes, loosening of endothelial cell junctions, and trafficking of inflammatory cells across the endothelial cell barrier. Adapted from Burns et al with permission (Ref 23).

Table 1. Clinical demographic data of refractory KD Patients treated with infliximab, comparing with patients responding to IVIG treatment and those who did not

	Healthy controls	Refractory patients treated with infliximab	Responders to IVIG	Non-responders to IVIG	*p* value
Number of patients	31	11	18	14	
Coronary artery lesion	0	4 (37%)	4 (9%)	6 (40%)	0.0984
Sex (male)	16 (52%)	6 (55%)	9 (50%)	10 (69%)	0.6833
Age in years (median, range)	3.8 (0.3-7.5)	4.0 (1.0-7.1)	2.6 (0.4-7.2)	2.8 (0.2-6)	0.6890
Max. CRP, mg/dl		14.3±9.1	9.9±5.2	15.5±7.4	0.0068
Max. WBC, $\times 10^3/mm^3$		24.6±4.7	15.4±4.1	14.6±8.7	0.0001
Duration of fever (days)		13.4±6.8	6.7±1.5	10.3±3.1	0.0002

KD, Kawasaki disease; IVIG, intravenous immune globulin; CRP, C-reactive protein; WBC, white blood cells; Data are mean ± SD.
P value is derived from comparison of refractory patients and responders.

Table 2. Clinical baseline characteristics and outcome of refractory KD Patients treated with infliximab

Patient #	Age	Sex	Illness day of 1st IVIG	IVIG dose	Other treatment	Infliximab dose	Illness day of Infliximab	Fever duration	Efficacy	CAL	Adverse effect
1	1.2	M	4	4 g/kg	UTI	5 mg/kg	9	9	Yes	No	No
2	7.1	M	3	4	UTI	5	8	8	Yes	No	No
3	2	F		0 *	IVMP, UTI	5	12	12	Yes	Yes *	No
4	2	F	5	4	IVMP, CyA, UTI	10 *	8	32	No	No	No
5	4	M	3	3	UTI	5	8	8	Yes	No	No
6	4	F	5	5	IVMP	5	12	12	Yes	No	No
7	1	M	3	3	IVMP	5	9	9	Yes	Yes *	No
8	2	M	6	6	IVMP	5	12	14	Yes	No	No
9	1	M	3	4	IVMP	5	12	16	No	Yes *	No
10	1	F	7	4	IVMP	5	12	15	No	No	No
11	3	F	4	4	IVMP	5	11	12	Yes	Yes *	No

KD, Kawasaki disease; IVIG, intravenous immune globulin; UTI, ulinastatin; IVMP, intravenous methylpredonisolone pulse; CyA, cyclophosphamide A; CAL, coronary artery lesion. *Patient #3 did not use IVIG because she had a history of allergic reaction to immunoglobulin. †Patient #4 received 5mg/kg infliximab twice.

Table 3. Serum Concentrations of pro-inflammatory cytokines and endothelial cell specific cytokines in refractory KD patients treated with infliximab, comparing with responders and non-responders to IVIG

	Healthy	Refractory	Responders	Non-responders	*p* value
Number of patients	33	11	18	14	
pro-inflammatory					
CRP mg/dL)					
before		13.9±9.5	7.1±3.8	12.3±5.6	0.00590
after		10.6±10.3	4.5±4.2	14.6±8.8	0.01595
STNFR (ng/mL)					
before	0.217±0.080	0.714±0.161	0.580±0.180	0.600±0.241	0.2155
after		0.391±0.161	0.326±0.147	0.474±0.220	0.0402
IL-6 (pg/mL)					
before	20±10	1013±1386	245±321	276±167	0.01786
after		233±561	69±217	182±387	0.14883
endothelial cell specific cytokines					
MRP8/MRP14					
before	220±40	4859±2997	3261±1724	4818±3983	0.03907
after		5860±5468	2063±1499	4588±4397	0.00459
S100A12					
before	52 ±32	1027±615	412±315	1148±1837	0.00165
after		1180±1201	244±286	651±574	0.00201
sRAGE (pg/mL)					
before	1794±368	868±613	1495±834	637±404	0.00210
after		1224±497	3212±1597	864±553	0.00023
VEGF (pg/mL)					
before	92±12	970±1030	525±607	790±674	0.00809
after		814±946	425±426	975±636	0.07437

KD, Kawasaki disease; IVIG, intravenous immune globulin; CRP, C-reactive protein; STNFR, soluble tumor necrosis alpha receptor; IL-6, interleukin 6; MRP, myeloid-related protein; sRAGE, soluble receptor for advanced glycation end products; VEGF, vascular endothelial growth factor.

Data are mean ± SD. *P* value is derived from comparison of refractory patients and IVIG responders.

Figure 1

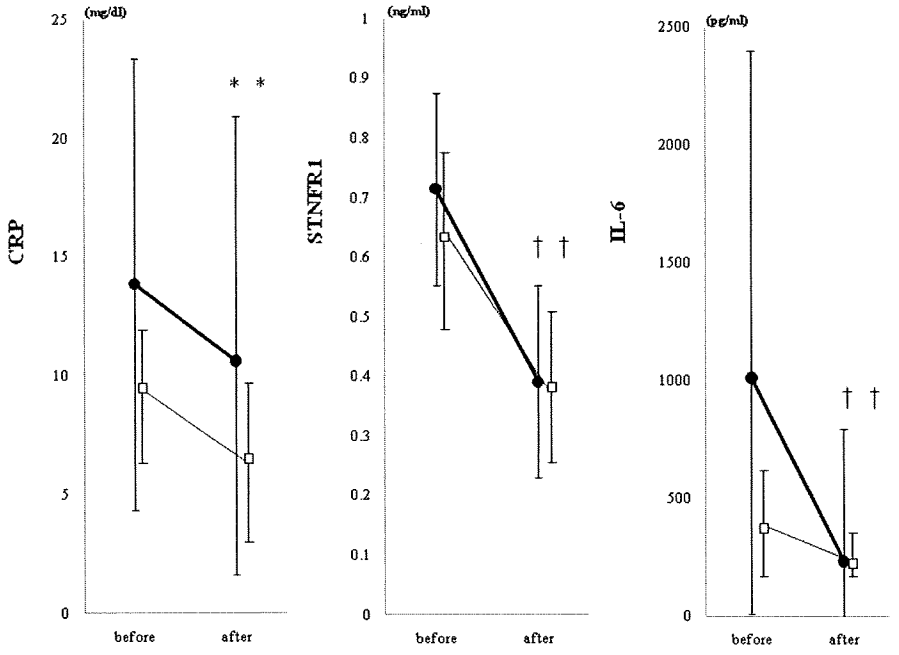
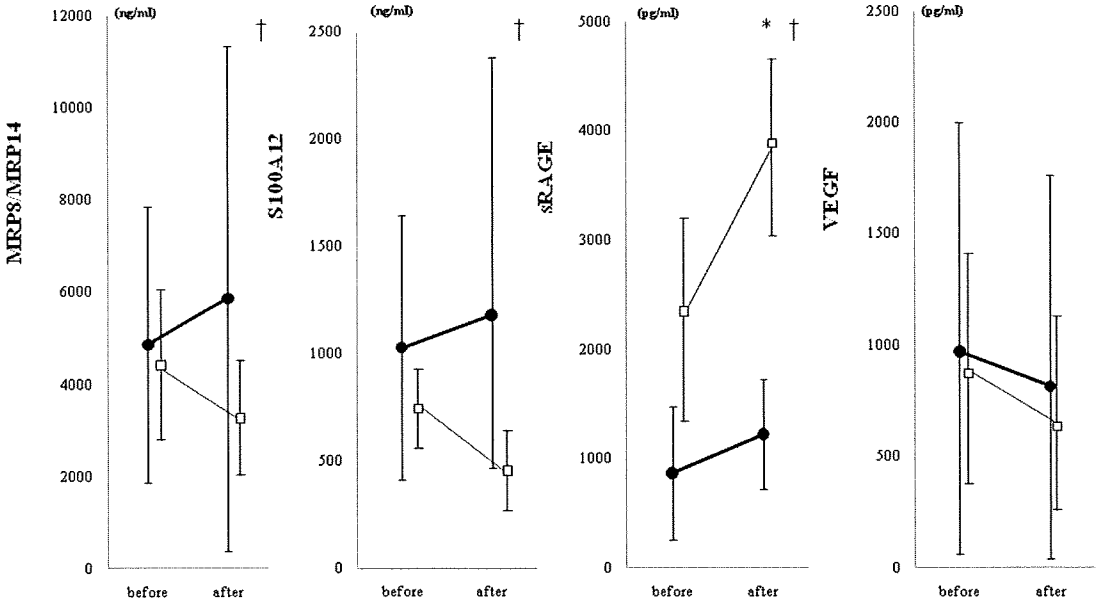


Figure 2



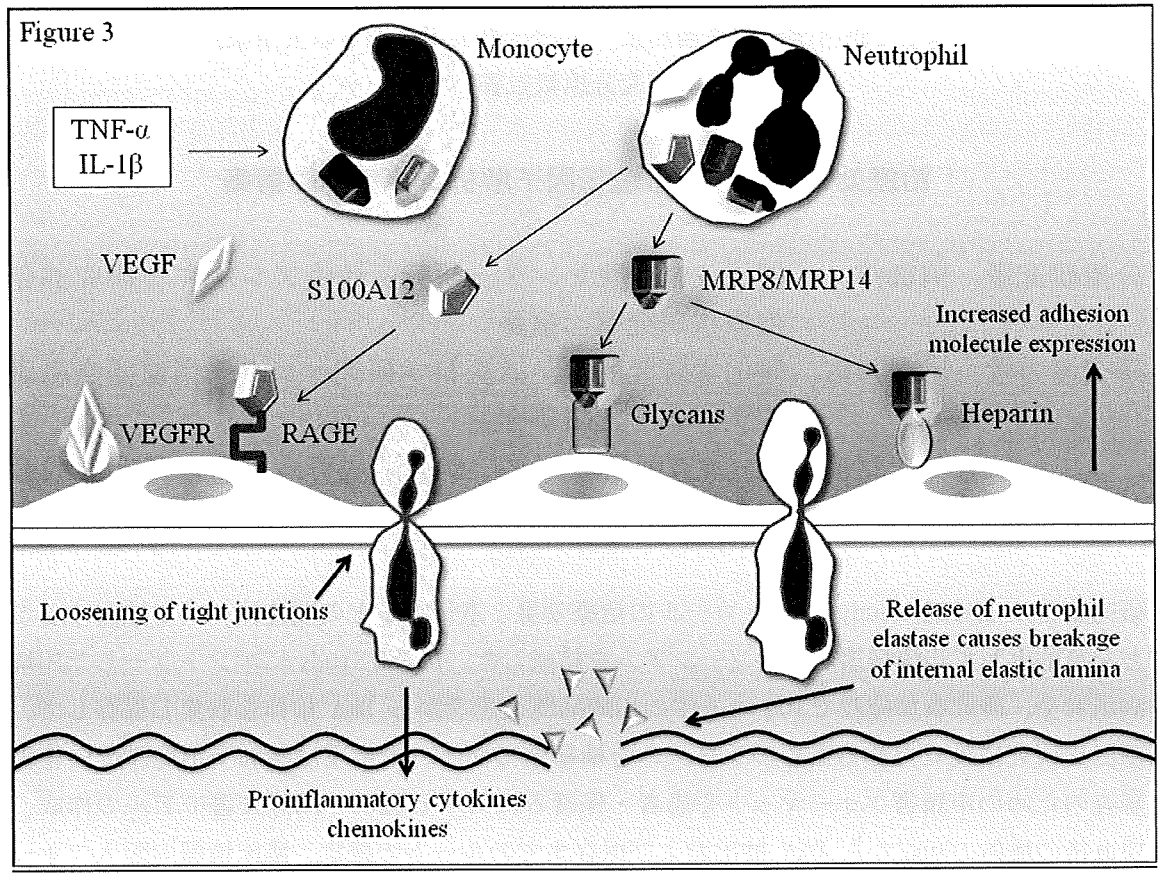


Figure 3
Monocyte
Neutrophil
TNF-α
IL-1β
VEGF
S100A12
MRP8/MRP14
Increased adhesion
molecule expression
VEGFR
RAGE
Glycans
Heparin
Loosening of tight junctions
Release of neutrophil
elastase causes breakage
of internal elastic lamina
Proinflammatory cytokines
chemokines