Kemmotsu et al. Pediatric Rheumatology 2011, 9:28
http://www.ped-rheum.com/content/9/1/28

PEDIATRIC
RHEUMATOLOGY

RESEARCH Open Access

Clinical characteristics of aseptic meningitis induced by intravenous immunoglobulin in patients with Kawasaki disease

Yasushi Kemmotsu*, Tomotaka Nakayama, Hiroyuki Matsuura and Tsutomu Saji

Abstract

Background: Aseptic meningitis is a serious adverse reaction to intravenous immunoglobulin (IVIG) therapy. We studied the clinical characteristics of patients with acute Kawasaki disease (KD) who developed IVIG-induced aseptic meningitis.

Methods: A retrospective analysis of the medical records of patients with KD who developed aseptic meningitis after IVIG treatment was performed.

Results: During the 10-year period from 2000 through 2009, among a total of 384 patients with Kawasaki disease, 4 (3 females and 1 male; age range, 19-120 months) developed aseptic meningitis after IVIG. All 4 developed aseptic meningitis within 48 hours (range, 25-40 hours) of initiation of IVIG. The analyses of cerebrospinal fluid (CSF) revealed elevated white blood cell counts (22-1,248/μL) in all 4 patients; a predominance of polynuclear cells (65%-89%) was noted in 3. The CSF protein level was elevated in only 1 patient (59 mg/dL), and the glucose levels were normal in all 4 patients. Two patients were treated with intravenous methylprednisolone; the other 2 children were observed carefully without any special therapy. All patients recovered without neurological complications.

Conclusions: In our patients with Kawasaki disease, aseptic meningitis induced by IVIG occurred within 48 hours after initiation of IVIG, resolved within a few days, and resulted in no neurological complications, even in patients who did not receive medical treatment.

Keywords: Kawasaki disease, intravenous immunoglobulin, aseptic meningitis

Background

Intravenous immunoglobulin (IVIG) is a blood product that is widely used in the treatment of a number of medical conditions, including immunodeficiency disorders, inflammatory diseases, and autoimmune diseases.

Kawasaki disease (KD) is a self-limited systemic vasculitis syndrome of childhood that was first reported by Tomisaku Kawasaki in 1967 [1]. Patients typically develop a fever, bulbar conjunctival injection, changes in the oropharyngeal mucosa and peripheral extremities, cervical lymphadenopathy, and a polymorphous rash. Coronary aneurysm and myocardial infarction are the most serious complications of this disease. In Japan, there are approximately 10,000 incident cases per year [2]. The etiology of the disease is not well understood, but high-dose IVIG is known to prevent the coronary complications [3,4].

There have been a number of reports regarding IVIG-induced adverse reactions, including mild reactions such as tachycardia, headache, facial flushing, nausea, diarrhea, and rash, as well as serious adverse reactions such as anaphylaxis, acute renal failure, and thromboembolic events [5]. Aseptic meningitis is a neurologic adverse event that can be caused by IVIG. Although there have been case reports describing IVIG-induced aseptic meningitis, few studies have described the characteristics of a group of such patients. In this study, we describe the clinical and laboratory characteristics of IVIG-induced aseptic meningitis in 4 patients with KD.

* Correspondence: kemmotsuyasushi@yahoo.co.jp
Department of Pediatrics, School of Medicine, Faculty of Medicine, Toho University, Tokyo 143-8541, Japan

Patients and methods

Patients

To investigate the clinical characteristics of IVIG-induced meningitis in KD patients, we retrospectively reviewed the medical records of patients who were admitted to our university hospital during the 10-year period from 2000 through 2009. All patients met the Japanese criteria for typical KD on admission. They were treated with oral aspirin and 1 or 2 g/kg of IVIG, the latter of which was administered over 12 or 24 hours, respectively. The IVIG products were freeze-dried sulfonated (Kenketsu Venilon®-I, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) and freeze-dried, polyethylene glycol (PEG) -treated (Kenketsu Glovenin®-I, Nihon Pharmaceutical Co, Ltd, Tokyo, Japan) human normal immunoglobulin. Testing of the CSF was done soon after the diagnosis of suspected IVIG-induced meningitis, and a diagnosis of meningitis was made on the basis of clinical symptoms such as fever and headache, meningeal irritation signs, and CSF pleocytosis. A final diagnosis of aseptic meningitis was made by negative bacterial culture results.

Results

Characteristics of the study population and IVIG products

A total of 384 patients with KD were admitted to our hospital; 4 developed aseptic meningitis after IVIG. Table 1 shows the background characteristics of these 4 patients. Three were females older than 5 years. The other patient was a 1-year-old male. Their serum C-reactive protein (CRP) levels and white blood cell counts before IVIG treatment were 3.3-5.5 mg/dL and 6,500-27,100/μL, respectively. Sulfonated immunoglobulin was given to 2 patients, and a polyethylene glycol-treated product was given to the other 2 patients. Two patients were treated with 1 g/kg IVIG, and the other 2 received 2 g/kg IVIG. There were no adverse reactions during the IVIG administration in any of the patients.

Clinical course and laboratory findings

All 4 patients responded well to initial IVIG: their fevers ceased and the clinical symptoms of KD improved. Table 2 shows the clinical course of the patients. Aseptic meningitis developed within 48 hours (range, 25-40 hours) after initiation of IVIG. All 4 patients developed a sudden, severe fever. Their recorded highest body temperatures were 38.0, 38.7, 38.8, and 39.1°C. The 3 females complained of headache, and the 1-year-old male was irritable and vomited frequently. On physical examination, there were typical signs of meningeal irritation, including neck rigidity, Kernig's sign, and Brudzinski's sign. Table 3 shows the CSF findings of the 4 patients. The initial pressure was recorded in 1 patient and was mildly elevated (24 cm H_2O). The analyses of the CSF revealed elevated white blood cell counts (22-1,248/μL) in all 4 patients, 3 of whom were neutrophil-predominant (65%-89%). The CSF protein level was elevated in only 1 patient (59 mg/dL), and the glucose levels were normal in all 4 patients (51-77 mg/dL). The CSF chloride and lactate dehydrogenase (LDH) levels were measured in 3 patients and were normal (123-128 mEq/L and 33-40 U/L, respectively). In addition, the results of CSF bacterial culture were negative in all patients. There was no worsening of inflammatory markers, ie, serum CRP and peripheral white blood cell counts, at the onset of meningitis (mean ± SD CRP: 4.3 ± 4.1 mg/dL, WBC: 9,300 ± 7,700/μL), as compared with the levels at admission (mean ± SD CRP: 5.9 ± 2.0 mg/dL, WBC: 14,800 ± 9,000/μL). Two patients were treated with a single dose of 15 mg/kg of intravenous methylprednisolone; the other 2 patients recovered without medical treatment. Fever and signs of meningeal irritation disappeared in 1 or 2 days, and no patient developed any neurological complications such as seizures or disturbances in consciousness. There was no recurrence of KD in any of the patients, and all four patients were discharged without coronary artery aneurysms.

Discussion

Aseptic meningitis after IVIG was first reported in 1988 [6]. Since then, there have been similar case reports of IVIG-induced meningitis in patients with medical conditions such as idiopathic thrombocytopenic purpura

Table 1 Background characteristics of the patients

Age	Sex	KD criteria	CRP(mg/dL)/WBC(/μL) on admission	IVIG product and dose	Day on IVIG
1 y	male	5/6	5.5/6,500	PEG-treated 2 g/kg	8
6 y	female	5/6	7.1/15,600	Sulfonated 1 g/kg	5
7 y	female	6/6	7.8/27,100	Sulfonated 2 g/kg	5
10 y	female	6/6	3.3/9,900	PEG-treated 1 g/kg	4

KD = Kawasaki disease; CRP = C-reactive protein; WBC = white blood cell; IVIG = intravenous immunoglobulin; PEG = polyethylene glycol.

Table 2 The clinical course of the patients

Patient	Time from start of IVIG to onset, hrs	Treatment	Time to recovery
1 y male	33	15 mg/kg mPSL	1 day
6 y female	40	15 mg/kg mPSL	2 days
7 y female	25	None	2 days
10 y female	31	None	1 day

IVIG = intravenous immunoglobulin; mPSL = methylprednisolone.

(ITP), myasthenia gravis, and inflammatory demyelinating neuropathy [7-9]. There has previously been only 1 case report describing this complication in a patient with KD [10].

The rate of aseptic meningitis after IVIG was 1% (4 of 384) in this study, but the frequency varies widely, from 0% to 11%, in reports of patients with different underlying diseases [11,12]. It was also reported that the development of aseptic meningitis was not correlated with the patient age or the type of underlying neuromuscular disease [12].

Hamrock reported that most patients who developed aseptic meningitis received 2 g/kg of IVIG, and that meningitis did not occur in any of their patients receiving a standard replacement dose of IVIG for a congenital immunodeficiency [5]. All of our patients received high-dose IVIG at a dose of 1 or 2 g/kg. Our patients almost equally received sulfonated IVIG or PEG-treated IVIG, and 2 patients in each group (total 4) developed meningitis, thus indicating that there are no apparent differences in the effects of sulfaonated or PEG-treated IVIG with regard to the development of meningitis. In this study, patients were exposed to either sulfonated IVIG or PEG-treated IVIG, but not to products manufactured by other processes such as cold ethanol Cohn fractionation/ultrafiltration, ion exchange, or low-PH treatment. The inability to further explore the possible etiological factors related to specific IVIG brand or manufacturing lots may be a limitation of this study. There were no obvious differences of clinical and laboratory data, including the severity of KD on admission, day of initiating IVIG, or changes of inflammatory markers after IVIG between patients who developed meningitis and those who did not.

In the present study, aseptic meningitis developed within 25 to 40 hours after initiation of IVIG. In previous case reports, most patients also developed meningitis within 48 hours of beginning IVIG. Although all of our patients developed a fever and typical meningeal irritation signs, it may be possible that milder cases of aseptic meningitis could be misdiagnosed as IVIG-refractory KD, since the onset of fever after completion of IVIG therapy is often interpreted as recrudescence of KD. It is important to consider the possibility of IVIG-induced meningitis with careful physical examinations to avoid unnecessary therapies, such as additional IVIG, steroids, and infliximab.

CSF examinations revealed neutrophilic pleocytosis in 3 of our 4 patients, slight elevation of the protein level in 1 patient, and normal glucose levels in all 4 patients. These findings were similar to those of previous reports. The analysis of the CSF in patients with aseptic meningitis usually shows pleocytosis with neutrophil predominance, normal or slightly elevated protein, and normal glucose levels. It may therefore be difficult to differentiate IVIG-induced meningitis from viral meningitis by the CSF findings, as it has been reported that the CSF protein levels are normal to mildly elevated, glucose levels are normal to slightly depressed, and neutrophil predominance is also seen in pediatric patients with viral meningitis [13,14].

All of our patients recovered without developing any neurological complications. Two were treated with intravenous methylprednisolone, and the other 2 were monitored without medical treatment. Jayabose et al. reported that children with ITP who were given prednisone had a lower risk of neurological complications after IVIG [15]. However, it has also been reported that such symptoms are self-limiting, and that there is no specific therapy that shortens the duration of symptoms. Thus, it may be advisable to carefully observe such patients and avoid systemic therapy [5]. In our study, there were no obvious differences in the clinical courses between patients treated with intravenous methylprednisolone and those who received no medical treatment,

Table 3 Cerebrospinal fluid findings

Patient	Cells (/μL)	Glucose (mg/dL)	Protein (mg/dL)	LDH (U/L)
1 y male	1,248 (P 89%)	51	59	39
6 y female	120 (P 13%)	54	23	33
7 y female	648 (P 83%)	77	30	40
10 y female	21 (P 65%)	52	37	NT

LDH = lactate dehydrogenase; P = polynuclear cells; NT = Not tested.

which suggests that systemic steroid administration is not beneficial for IVIG-induced meningitis.

The mechanisms underlying IVIG-induced meningitis are not clear. One possible cause is an allergic hypersensitivity reaction caused by direct entry of the IVIG preparation into the CSF compartment. This is supported by the fact that CSF eosinophilia has been observed in some patients [11]. In our study, one patient exhibited peripheral eosinophilia (11% of the total 5,800/μL white blood cells) but CSF eosinophilia was not observed in any of our patients. None of our patients developed exanthema after IVIG. Although our patients received no pre-treatment, it may be useful to give antihistamines prior to IVIG if allergic reaction is one of the mechanisms responsible for IVIG-induced meningitis. Recently, it was reported that there were increased levels of CSF monocyte chemoattractant protein-1 (MCP-1) in ITP patients with IVIG-induced meningitis, which suggests a role for monocytes in the inflammation of the meninges [16]. On the other hand, Jarius et al. reported that aseptic meningitis was frequently associated with neutrophillic pleocytosis in the CSF and *in vivo* activation of TNF-α-primed neutrophils by atypical antineutrophil cytoplasmic antibodies in IVIG might contribute to aseptic meningitis [17]. In our present study, the CSF cytokines or chemokines were not measured.

Meningitis is also a known complication of KD. Dengler et al reported that one-third of patients with KD who underwent a lumbar puncture had CSF pleocytosis with mononuclear cell predominance [18], which is in contrast to the polynuclear cell predominance observed in IVIG-induced meningitis. Meningitis as a complication of KD usually occurs early in the course of the disease and improves after KD treatment, which is mainly IVIG therapy [19]. Table 4 shows a comparison between IVIG- and KD-induced meningitis. It is not difficult to differentiate IVIG-induced meningitis from aseptic meningitis complicating KD, as both the time of onset and CSF findings differ.

Conclusions

In conclusion, IVIG-induced meningitis developed within 48 hours of initiating IVIG and resolved in a few days, without neurological complications, and systemic steroid administration was not beneficial in our patients. Further investigations of the pathophysiology of IVIG-induced meningitis, including a detailed analysis of the underlying mechanisms, are needed.

Table 4 A comparison between IVIG- and KD-induced meningitis

	Meningitis due to IVIG	Meningitis due to KD
Appearance	Within 48 hrs after IVIG	Early in the stage, before IVIG
Clinical findings	Typical meningeal signs	Can lack meningeal signs
CSF findings	Polynuclear cell predominance	Mononuclear cell predominance
Effective therapy	No special therapy	Therapy for KD

IVIG = intravenous immunoglobulin; KD = Kawasaki disease; CSF = Cerebrospinal fluid.

Authors' contributions

YK contributed by taking care of the patients. All authors contributed to the analysis and interpretation of the data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 20 May 2011 Accepted: 14 September 2011
Published: 14 September 2011

References

1. Kawasaki T: **Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children.** *Arerugi* 1967, **16(3)**:178-222.
2. Nakamura Y, Yashiro M, Uehara R, Oki I, Watanabe M, Yanagawa H: **Epidemiologic Features of Kawasaki Disease in Japan: Results from the Nationwide Survey in 2005-2006.** *J Epidemiol* 2008, **18**:167-172.
3. Newburger JW, Takahashi M, Burns JC, *et al*: **The treatment of Kawasaki syndrome with intravenous gamma globulin.** *N Engl J Med* 1986, **315**:341-347.
4. Newburger JW, Takahashi M, Beiser AS, *et al*: **A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome.** *N Engl J Med* 1991, **324**:1633-1639.
5. Hamrock DJ: **Adverse events associated with intravenous immunoglobulin therapy.** *Intl Immunopharmacol* 2006, **6**:535-542.
6. Kato E, Shindo S, Eto Y, Hashimoto N, Yamamoto M, Sakata Y, *et al*: **Administration of immune globulin associated with aseptic meningitis.** *JAMA* 1988, **259**:3269-3271.
7. Jayabose S, Roseman B, Gupta A: **Aseptic meningitis syndrome (AMS) after IV gammaglobulin (I.V. Gg) therapy for ITP.** *Am J Pediatr Hematolo Oncol* 1990, **12**:117.
8. Meiner Z, Ben-Hur T, River Y, Reches A: **Aseptic meningitis as complication of intravenous immunoglobulin therapy for myasthenia gravis.** *J Neurol Neurosurg Psychiatry* 1993, **56**:830-831.
9. Vera-Ramirez M, Charlet M, Parry GJ: **Recurrent aseptic meningitis complicating intravenous immunoglobulin therapy for chronic inflammatory demyelinating polyradiculoneuropathy.** *Neurology* 1992, **42**:1636-1637.
10. Boyce TG, Spearman P: **Acute aseptic meningitis secondary to intravenous immunoglobulin in a patient with Kawasaki syndrome.** *Pediatr Infect Dis J* 1998, **17**:1054-1056.
11. Orbach H, Katz U, Sherer Y, Shoenfeld Y: **Intravenous immunoglobulin: adverse effects and safe administration.** *Clin Rev Allergy Immunol* 2005, **29**:173-184.
12. Sekul EA, Cupler EJ, Dalakas MC: **Aseptic Meningitis Associated with High-Dose Intravenous Immunoglobulin Therapy: Frequency and Risk Factors.** *Ann Intern Med* 1994, **121**:259-262.
13. Irani DN: **Aseptic Meningitis and Viral Myelitis.** *Neurol Clin* 2008, **26(3)**:635-655.
14. Negrini B, Kelleher KJ, Wald ER: **Cerebrospinal fluid findings in aseptic versus bacterial meningitis.** *Pediatrics* 2000, **105(2)**:316-319.
15. Jayabose S, Mahmoud M, Levendoglu-Tuga O, *et al*: **Corticosteroid prophylaxis for neurologic complications of intravenous immunoglobulin G therapy in childhood immune thrombocytopenic purpura.** *J Pediatr Hematol Oncol* 1999, **21**:514-517.
16. Asano T, Koizumi S, Mishina-Ikegami K, Hatori T, Miyasho T, Fujino O: **Increased levels of Monocyte Chemoattractant Protein-1 in cerebrospinal fluid with gamma globulin induced meningitis.** *Acta Paediatr* 2010, **99**:164-165.
17. Jarius S, Eichhorn P, Albert MH, Wagenpfeil S, Wick M, Belohradsky BH, Hohlfeld R, Jenne DE, Voltz R: **Intravenous immunoglobulins contain**

naturally occurring antibodies that mimic antineutrophil cytoplasmic antibodies and activate neutrophils in a TNFα-dependent and Fc-receptor-independent way. *Blood* 2007, **109**:4376-4382.

18. Dengler LD, Capparelli EV, Bastian JF, Bradley DJ, Glode MP, Santa S, Newburger JW, Baker AL, Matsubara T, Burns JC: **Cerebrospinal fluid profile in patients with acute Kawasaki disease.** *Pediatr Infect Dis J* 1998, **17**:478-481.
19. Takagi K, Umezawa T, Saji T, Morooka K, Matsuo N: **Meningoencephalitis in Kawasaki disease.** *No To Hattatsu (in Japanese)* 1990, **22**:429-435.

doi:10.1186/1546-0096-9-28
Cite this article as: Kemmotsu *et al.*: Clinical characteristics of aseptic meningitis induced by intravenous immunoglobulin in patients with Kawasaki disease. *Pediatric Rheumatology* 2011 **9**:28.

Takahashi et al. Pediatric Rheumatology 2011, 9:30
http://www.ped-rheum.com/content/9/1/30

PEDIATRIC RHEUMATOLOGY

SHORT REPORT Open Access

Mizoribine provides effective treatment of sequential histological change of arteritis and reduction of inflammatory cytokines and chemokines in an animal model of Kawasaki disease

Kei Takahashi[1], Toshiaki Oharaseki[1], Tomokazu Nagao[2], Yuki Yokouchi[1], Hitomi Yamada[1], Noriko Nagi-Miura[3], Naohito Ohno[3], Tsutomu Saji[4], Tomio Okazaki[5] and Kazuo Suzuki[2*]

Abstract

Background: Intravenous immunoglobulin (IVIg) treatment results in an effective response from patients with acute-phase Kawasaki disease (KD), but 16.5% of them remain nonresponsive to IVIg. To address this therapeutic challenge, we tried a new therapeutic drug, mizoribine (MZR), in a mouse model of KD, which we have established using injections of *Candida albicans* water-soluble fractions (CAWS).

Methods: CAWS (4 mg/mouse) were injected intraperitoneally into C57BL/6N mice for 5 consecutive days. MZR or IgG was administered for 5 days. After 4 weeks, the mice were sacrificed and autopsied, the hearts were fixed in 10% neutral formalin, and plasma was taken to measure cytokines and chemokines using the Bio-Plex system. The incidence of panvasculitis in the coronary arteries and aortic root was 100% in the control group. The incidence of panvasculitis in the MZR group decreased to 50%. Moreover, the scope and severity of the inflammation of those sites were significantly reduced in the MZR group as well as the IgG group. On the other hand, increased cytokines and chemokines, such as IL-1α, TNF-α, KC, MIP-1α, GM-CSF, and IL-13, in the nontreatment group were significantly suppressed by treatment with MZR, but the MCP-1 level increased. In addition, IL-1α, TNF-α, IL-10, IL-13, and MIP-1α were suppressed by treatment in the IgG group.

Results: The incidence of panvasculitis in the coronary arteries and aortic root was 100% in the control group. The incidence of panvasculitis in the MZR group decreased to 50%. Moreover, the scope and severity of the inflammation of those sites were significantly reduced in the MZR group as well as the IgG group. On the other hand, increased cytokines and chemokines, such as IL-1α TNF-α, KC, MIP-1α, GM-CSF, and IL-13, in the nontreatment group were significantly suppressed by treatment with MZR, but the MCP-1 level increased. In addition, IL-1α, TNF-α, IL-10, IL-13, and MIP-1α were suppressed by treatment in the IgG group.

Conclusion: MZR treatment suppressed not only the incidence, range, and degree of vasculitis, but also inflammatory cytokines and chemokines in the plasma of the KD vasculitis model mice, suggesting that MZR may be useful for treatment of KD.

Keywords: Kawasaki disease, an animal model, IVIg, coronary arteritis, inflammatory cytokines and chemokines, mizoribine

* Correspondence: ksuzuki@faculty.chiba-u.jp
[2]Inflammation Program, Dept. of Immunology, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, 260-8670, Japan
Full list of author information is available at the end of the article

Background

Kawasaki disease (KD) is an acute febrile illness that manifests mainly in infancy and early childhood [1]. The most important complication of KD is coronary arteritis, which leads to formation of aneurysms. KD has attracted special interest because it may cause ischemic heart disease in children due to thrombosed coronary aneurysms [2]. Since the etiology and development of KD are thought to be due to the dysfunction of the immune system, intravenous immunoglobulin (IVIg) during the early acute phase has been used with an excellent response in most patients [3]. However, 16.5% of patients did not respond to the first IVIg treatment [4], and some nonresponders to the first IVIg treatment manifested severe coronary arteritis with large aneurysm [5]. Therefore, additional treatments have been tried on the nonresponders to the first treatment with IVIg. To date, a second IVIg treatment [6], plasmapheresis [7-10], pulse steroids [11], cyclophosphamide plus steroids [12], ulinastatin as an elastase inhibitor [13-16], cyclosporin A plus steroids and methotrexate plus steroids [17,18], and anti-tumor necrosis factor-α (infliximab) therapy [19-23] have been tried. Thus, for treatment of patients with KD who do not respond to IVIg, other medicines for immune response and suppression of lymphocyte proliferation have been applied due to immune dysfunction in the patients. One immune modulating medicine, mizoribine (MZR), a drug that inhibits synthesis of purine compounds (GMP), blocks proliferation of lymphocytes and will be useful for application to nonresponders to IVIg treatment. MZR has long been used as therapy for kidney transplantation, lupus nephritis, nephrotic syndrome, and rheumatoid disease with few side-effects [24]. Moreover, it has been reported to have been used for lupus nephritis, nephrotic syndrome, and IgA nephritis in children [25-28], and as a maintenance therapy in anti-neutrophil cytoplasmic autoantibody (ANCA)-associated renal failure, frequently relapsing nephrotic syndrome, and purpura nephritis [29,30]. Therefore, MZR will be a valuable therapeutic strategy for patients with KD who are nonresponsive to IVIg.

Prior to a clinical trial in children with KD, it was necessary to test MZR in a mouse model of KD, which has been established. The model we chose was the mouse model in which coronary arteritis can be induced by administration of *Candida albicans* water-soluble fractions (CAWS) [31]. This model mouse has previously been useful for evaluation of other drug treatments.

Therefore, in the present study, we tested MZR as a immunomodular for treatment of this CAWS-induced coronary arteritis. The evaluation of MZR was performed by histopathological findings and profiles of chemokines and cytokines. Also, this treatment effect was compared with that of IgG.

Methods

Animals

Four-week-old male C57BL/6N mice were purchased from Charles River Japan (Yokohama, Japan). All mice were kept under specific pathogen-free (SPF) conditions, according to the guidelines for animal care of the National Institute of Infectious Diseases in Tokyo (NIID).

Preparation of CAWS

CAWS was prepared from *C. albicans* strain IFO1385 in accordance with the reported method [31]. Briefly, 5 liters of medium (C-limiting medium) was added to a glass incubator, and the culture was maintained for 2 days at 27°C while air was supplied at a rate of 5 liters/min and the mixture was swirled at 400 rpm. Following culture, an equal volume of ethanol was added. After allowing this to stand overnight, the precipitate was collected. After dissolving the precipitate in 250 ml of distilled water, ethanol was added and the mixture was allowed to stand overnight. The precipitate was collected and dried with acetone to obtain CAWS.

Administration of MZR and IgG to the mice

CAWS (4 mg/mouse/day) in a volume of 0.2 ml was intraperitoneally injected into a C57BL/6N mouse (4-week old male) on each of 5 consecutive days. Subsequently, MZR (a kind gift of Asahikasei Pharma Corporation (Tokyo, Japan)) was administered at a dose of 30 mg/kg/day intraperitoneally for 5 days from the third day of CAWS injection (MZB group), according to the schecule for treatments such as IgG for patients with KD, and the dosage as described elsewhere [32]. Mice for the control group were intraperitoneally treated with 0.2 ml of Dulbecco's phosphate-buffered saline (PBS). After 35 days, the mice were sacrificed by carbon dioxide asphyxiation; autopsy was performed to obtain plasma, and hearts were fixed with 10% neutralized formalin. For a positive control, treatment with intraperitoneal human IgG (Kenketsu Glovenin I, a kind gift of Nihon Pharmaceutical Co. Ltd., Tokyo, Japan) was performed at a dose of 400 mg/mouse/day, or for a negative control saline containing 0.1% glucose (SG) was injected for 5 days according to the same procedures as described elsewhere (IgG group) [33]. The start date of the drug administration was based on the results that the administration from the third experimental day had been the most effective to suppress the development of vasculitis.

Histological evaluation

The fixed hearts were embedded in paraffin and sectioned. To observe the histological changes in the coronary arteries and the aorta in detail, 20 to 30 horizontal step sections per mouse were made every 20 μm. Hematoxylin and eosin (H&E)-stained sections were prepared by using routine techniques for examination by light microscopy [31]. First, we investigated the incidence of mice with panvasculitis in each group. Panvasculitis was defined as inflammation of all layers of the walls of the coronary arteries and/or the aorta. Then, for quantitative evaluation of vascular inflammation, we divided the area of the aortic root and coronary arteries into five segments and graded the intensity of inflammation in each segment as follows: score 3, panvasculitis; score 2, inflammation involving the tunica intima and adventitia; score 1, inflammation localized to the tunica intima; and score 0, no inflammatory cell infiltration in the vascular wall. A section with the severe inflammation was observed in each segment. The scope of inflammation was defined as the number of segments evaluated as score 1 or more in each mouse, and the severity of the arteritis was defined as the average score of the five segments in each mouse.

Measurement of cytokines and chemokines with Bio-Plex

Cytokines and chemokines in the plasma of mice autopsied were measured by a Bio-Plex system. An aliquot of serum (12 μl) collected from peripheral blood and diluted 4-fold with the dilution solution was measured for concentration of cytokines by the 23-Plex kit using Bio-Plex 200 according to the manufacturer's protocol and analyzed by the Bio-Plex Luminex 100 XYP instrument (Bio-Rad, Hercules, California, USA). We assayed the following 23 cytokines and chemokines: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, eotaxin, G-CSF, GM-CSF, INF-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF-α as estimated with a single assay to a single standard curve described in the kit instructions. Concentrations of cytokines and chemokines were calculated using Bio-Plex Manager 3.0 software (Bio-Rad, Tokyo) with a five-parameter curve-fitting algorithm applied for standard curve calculations [34].

Statistical analysis

Fisher's exact probability test was used to analyze the differences in the incidence of arteritis among the groups. The data on the scope and severity of the arteritis and cytokine/chemokine levels were analyzed using the two-sample *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

Histological evaluation of panvasculitis in treatment with MZR

Panvasculitis developed in the coronary arteries and the aortic root, and histology was similar to that previously described [33]. Specifically, vascular changes were classified as proliferative inflammation that consisted mainly of large mononuclear cells such as histiocytes and fibroblasts and of a small number of neutrophils. The normal structure of the arteries was completely destroyed, and the internal elastic lamina, external elastic lamina, and smooth muscle layer of the tunica media were severely damaged. However, fibrinoid necrosis was not observed in any of the mice. In addition, the histology of panvasculitis was similar in the three groups (Figure 1).

Decrease of coronary arteritis by treatment with MZR

Panvasculitis of the coronary arteries and the aortic root was observed in 5 of 5 mice (100%) in the nontreated control group. On the other hand, the incidence of panvasculitis in the MZR group was 3 of 6 mice (50%), and the IgG group as an effective control showed 3 of 7 (43%) (Figure 2A). In addition, the number of segments evaluated as score 1 or more in each MZR group was decreased compared with the nontreated control group ($P = 0.06$), and the scope of inflammation in IgG groups was significantly lower than in the control group ($P < 0.05$) (Figure 2B). Furthermore, the severity of the arteritis, i.e., the scores of each of five segments in the mice in the MZR and IgG groups, was significantly lower than in the nontreated control group ($P < 0.01$) (Figure 2C).

Reduction of inflammatory cytokines and chemokines by treatment with MZR and IgG

Inflammatory cytokines IL-1α, TNF-α, chemokines KC, MIP-1α, GM-CSF, and Th2, and cytokine IL-13 in plasma of mice, which were inoculated with CAWS in the control group, were elevated (Figure 3). However, in the MZR group, plasma levels of inflammatory cytokines IL-1α ($P < 0.01$) and TNF-α ($P < 0.05$), and chemokines KC ($P < 0.01$), MIP-1α ($P < 0.01$), and GM-CSF ($P < 0.05$) were significantly suppressed (Figure 3A). Inversely, the MCP-1 level increased with MZR treatment (Figure 3A). On the other hand, IL-1α ($P < 0.05$), TNF-α ($P < 0.05$), IL-10 ($P < 0.05$), and IL-13 ($P < 0.01$) were suppressed by administration of IgG (Figure 3B).

Furthermore, we analyzed levels of cytokines/chemokines in plasma, which were related with suppression of the development of coronary arteritis by treatment with MZR. As shown in Figure 4A, the suppression levels were almost the same in all plasmas of MZR-treated mice. These results are not the same as those in the IgG

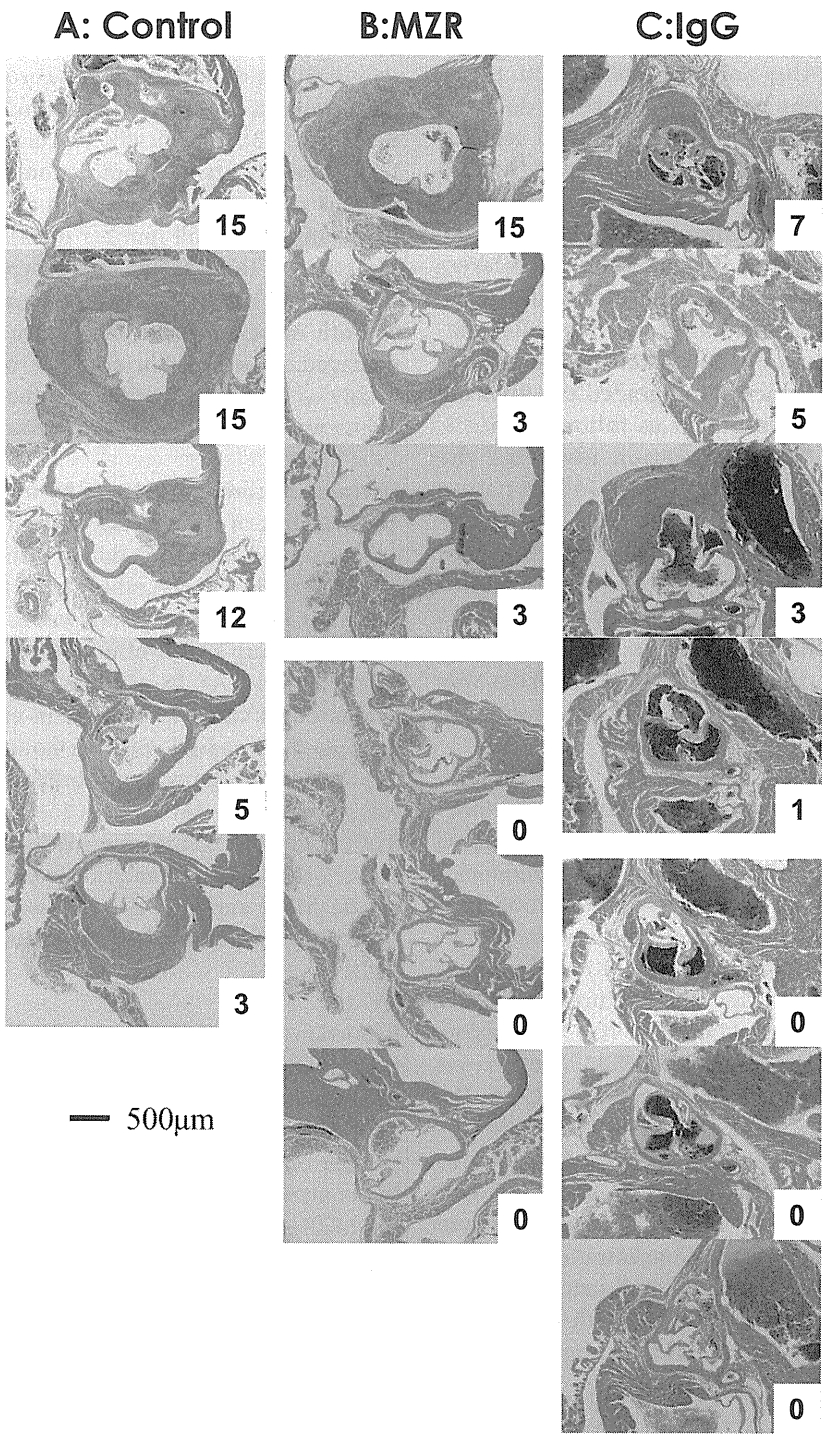


Figure 1 Histological observations of coronary arteritis induced by treatment with MZR and IgG. A, Control (PBS); B, MZR group; C, IgG group. Each micrograph represents an individual mouse. H&E stain, Bar: 500 μm. Numbers (white) are coronary arteritis score.

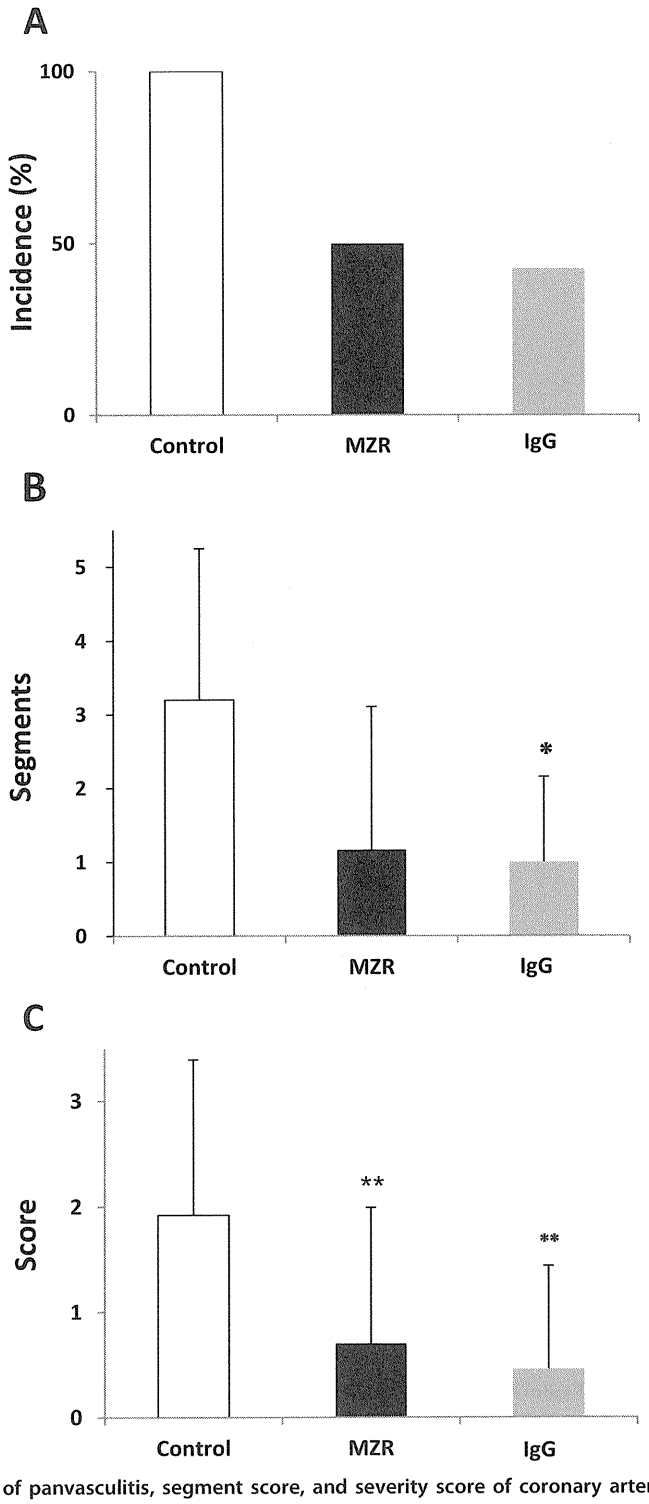


Figure 2 Decrease of incidence of panvasculitis, segment score, and severity score of coronary arteritis by treatment with MZR and IgG. A, incidence of development of panvasculitis; B, scope as number of segments with inflammation evaluated as score 1 or more at aortic root and coronary arteries; C, severity score of each segment. Data are expressed as mean ± SD of results from three individuals. *$P < 0.05$ and **$P < 0.01$ (Student's *t*-test).

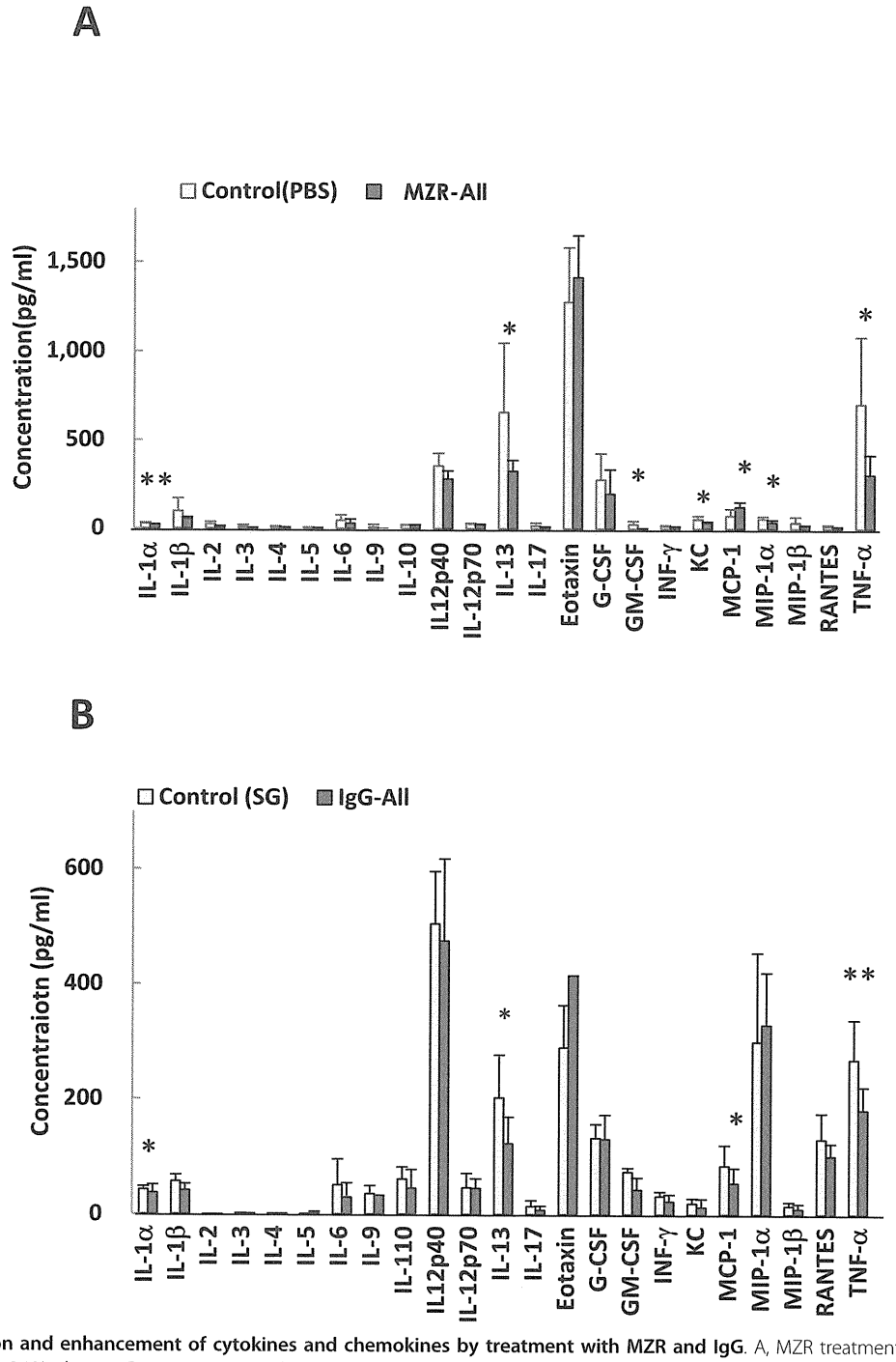


Figure 3 Reduction and enhancement of cytokines and chemokines by treatment with MZR and IgG. A, MZR treatment; B, IgG treatment. SG: saline including 0.1% glucose. Data are expressed as mean ± SD of results from three individuals. *$P < 0.05$ and **$P < 0.01$.

group, showing good response for suppression of the development of coronary arteritis (Figure 4B).

Discussion

Decrease of coronary arteritis by treatment with MZR

We here have shown the efficacy of MZR on vascular inflammation by using a KD vasculitis mouse model to develop alternative treatments for KD patients who are nonresponsive to IVIg treatment. The results here show that the incidence, scope, and degree of inflammation of the coronary arteries and the aortic root were suppressed by MZR administration. Coronary arteritis in this CAWS-induced vasculitis mouse model is also suppressed after administration of IVIg [33]. Furthermore,

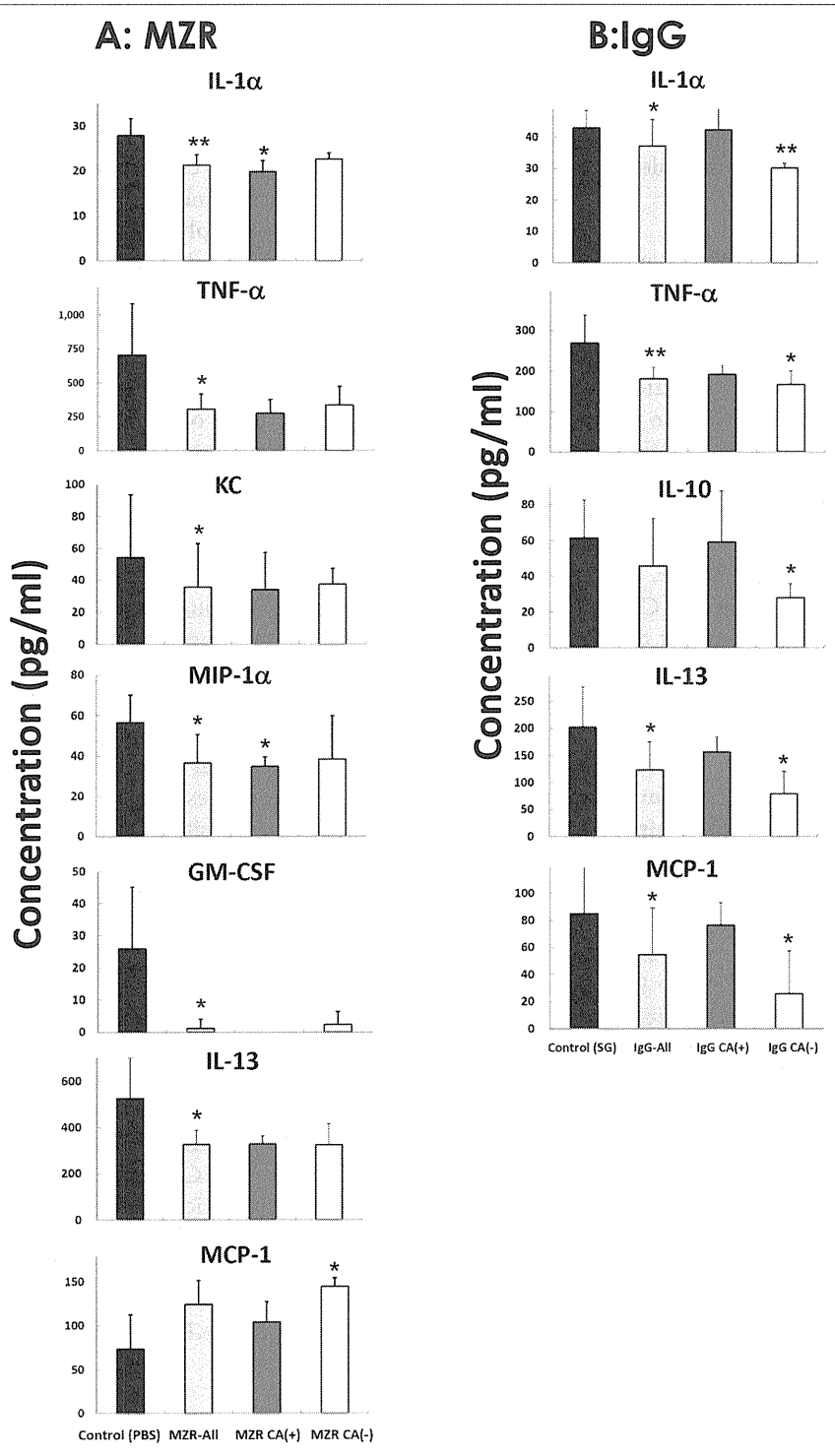


Figure 4 Reduction and enhancement of cytokines and chemokines in coronary arteritis treated with MZR and IgG. A, MZR treatment; B, IgG treatment. CA(+): coronary arteritis score > 0, CA(-): coronary arteritis score = 0, SG: saline including 0.1% glucose. Data are expressed as mean ± SD of results from three individuals. *$P < 0.05$ and **$P < 0.01$.

we have also demonstrated that the anti-TNF-α therapy that has been shown to be effective in treating some children unresponsive to IVIg therapy also dramatically suppresses the development of vasculitis in this mouse model of KD (manuscript in preparation). Thus this mouse model appears to be valuable for evaluation of alternative therapies for KD arteritis.

Reduction of inflammatory cytokines and chemokines by treatment with MZR

Some cytokines and chemokines such as IL-1β, IL-2, sIL-2R, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, RANTES, MCP-1, M-CSF, G-CSF, and MIPs are elevated in the blood of patients with acute-phase KD. Some elevated cytokines and chemokines are decreased by IVIg treatment in the acute phase, when it is effective [35]. On the other hand, IL-6, TNF-α, IL-4, and IL-12 were increased in the plasma of the KD mouse model induced with *C. albicans*-derived substances (CADS) [36]. Moreover, IL-6 and IFN-γ in splenocytes administered CAWS in C57BL/6 mice were elevated [37]. With IVIg treatment of KD model mice induced with CAWS, elevated proinflammatory cytokines IL-1α, TNF-α, IL-10, IL-13, and MCP-1 were decreased in our data. Furthermore, chemokines IL-1α, TNF-α, KC, GM-CSF, IL-13, and MIP-1α in plasma of autopsied mice were decreased in the MZR treatment group in the present study. The results with MZR treatement show similar effects as well as IgG treatment for KD model mice. However, supression levels of IL-1α, TNF-α, IL-10, IL-13, and MIP-1α in the recovery group from the coronary arteritis (CA(-)) in the MZR group differed from those in the IgG group. Levels in the recovery group (CA(-)) after MZR treatment were not suppressed, whereas those in the CA (-) group after IgG treatment were suppressed in the present study, which suggests that MZR may have a stronger effect than a high dose of IgG (400 mg/kg/day for 5 days). Because these cytokines/chemokines decrease slightly after MZR treatment, they may have a role in the development of coronary arteritis in the KD model.

Effective treatment with MZR of model mice for KD induced by CAWS

In the present study, MZR treatment of the KD model mice significantly suppressed the development of coronary arteritis associated with significant suppression of levels of proinflamatory cytokines and chemokines in plasma. These results suggest association of the suppression of lymphocyte proliferation with MZR [38]. The mode of action of MZR is that it mainly blocks immunosuppression related to lymphocyte proliferation through inhibition of purine synthesis [32,39,40]. In the present study, the incidence of panarteritis decreased to half, and both the scope and severity of inflammation were limited after administration of MZR. In addition to the lymphocyte action, these observations suggest that MZR may act on functions of monocytes/macrophages and neutrophils, which are mainly involved in the development of inflammation, resulting in the possible suppression of coronary arteritis through suppression of proinflammatory cytokines and chemokines released from these cells. Indeed, recently, MZR acted to inhibit functions of lymphocytes as well as those of macrophages, such as migration and production of Nitrous Oxide Systems (NOS), IL-1β, and TNF-α in a dose-dependent manner [41,42]. Furthermore, in the mixed lymphocyte reaction method (MLR) of human peripheral blood mononuclear cells, the IC_{50} is 1 μg/ml [43]. In addition, MLR of T-cells in human peripheral blood, which are stimulated with anti-CD3 monoclonal antibody, shows an IC_{50} of less than 1 μg/ml for MZR and also phorbol myristate stimulation less than 5 μg/ml [44]. In addition, MZR also inhibits activation of M1 macrophages [42], which are classified as inflammatory, showing tissue injury and activation with IFN-γ. In the present study, suppression profiles of proinflammatory cytokines and chemokines by MZR treatment of KD model mice seem to be associated in the literature with those in the M1 macrophage. Therefore, the effect of MZR on KD model mice may be to inhibit the proliferation of lymphocytes and activation of macrophages and neutrophils associated with elevation of proinflamatory cytokines and chemokines.

Based on these observations, suppression of development of coronary arteritis associated with suppression of proinflammatory cytokines and chemokines by MZR treatment for the KD model mice suggests that MZR may be useful for patients with KD in the acute phase. MZR has been used as therapy for kidney transplantation, lupus nephritis, nephrotic syndrome, and rheumatoid disease with few side effects [24]. Furthermore, MZR has been used as maintenance treatment for ANCA-associated vasculitis, frequently relapsing nephrotic syndrome, and purpura nephritis [29,30]. Clinical use will be recommended for immune dysfunctions when the safety of long-time use becomes known. Therefore, MZR is a possible therapy for patients with KD who are nonresponsive to IVIg.

Conclusions

MZR treatment suppressed not only the incidence, range, and degree of vasculitis, but also inflammatory cytokines and chemokines in the plasma of the KD vasculitis model mice. It appears likely that MZR may prove to be a useful for alternative treatment for KD.

Abbreviations used

ANCA: anti-neutrophil cytoplasmic autoantibody; CAWS: *Candida albicans* water-soluble fractions; H&E:

hematoxylin and eosin; IVIg: intravenous immunoglobulin; KD: Kawasaki disease; MLR: mixed lymphocytes reaction method; MZR: mizoribine; NOS: Nitrous Oxide Systems: PBS; Dulbecco's phosphate- buffered saline; SG: saline containing 0.1% glucose.

Contribution of authors

KT: Histological evaluations of coronary arteritis. TO: Histological evaluations of coronary arteritis. TN: Measurement and analysis of cytokines and chemokines. YY: Measurement and analysis of cytokines and chemokines. HY: Histological evaluations of coronary arteritis. NNM: Preparation of CAWS. NO: Preparation of CAWS. TS: Planning treatments with MZR and IgG, and clinical evaluation. TO: Planning treatments with MZR and IgG, and clinical evaluation. KS: Measurement and analysis of cytokines and chemokines, correspondence to all evaluation of this study. All authors read and approved the final manuscript.

Acknowledgements

We thank Dr. Shiro Naoe (Department of Biomedical Engineering, Toin University of Yokohama, Yokohama). We are also grateful to Mr. Kazuo Tomizawa of NIID for excellent assistance with animal experiments.

Author details

[1]Department of Pathology, Toho University Ohashi Medical Center, Meguro-ku, Tokyo, 153-8515, Japan. [2]Inflammation Program, Dept. of Immunology, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, 260-8670, Japan. [3]Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 192-0392, Japan. [4]Department of Pediatrics, Toho University Omori Medical Center, Ota-ku, Tokyo, 143-8541, Japan. [5]Kure Kyosai Hospital, Kure, Hiroshima, 737-8505, Japan.

Competing interests

The authors declare that they have no competing interests.

Received: 3 April 2011 Accepted: 29 September 2011
Published: 29 September 2011

References

1. Kawasaki T: **Acute febrile muco-cutaneous lymph node syndrome in young children with unique digital desquamation.** *Jpn J Allergol* 1967, **16**:178-222.
2. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H: **New infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan.** *Pediatrics* 1974, **54**:271-276.
3. Furusho K, Sato K, Soeda T, Matsumoto H, Okabe T, Hirota T, Kawada S: **High-dose intravenous gammaglobulin for Kawasaki disease.** *Lancet* 1983, **10(8363)**:1359.
4. Nakamura Y, Yashiro M, Uehara R, Sadakane A, Chihara I, Aoyama Y, Kotani K, Yanagawa H: **Epidemiologic features of Kawasaki disease in Japan: results of the 2007-2008 nationwide survey.** *J Epidemiol* 2010, **20**:302-307.
5. Sundel RP, Burns JC, Baker A, Beiser AS, Newburger JW: **Gamma globulin re-treatment in Kawasaki disease.** *J Pediatr* 1993, **123**:657-659.
6. Shinohara M, Sone K, Tomomasa T, Morikawa A: **Corticosteroids in the treatment of the acute phase of Kawasaki disease.** *J Pediatr* 1999, **135**:465-469.
7. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, Kobayashi T, Morikawa A: **Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease.** *Circulation* 2006, **113**:2606-2612.
8. Villain E, Kachaner J, Sidi D, Blaysat G, Piéchaud JF, Pedroni E: **Trial of prevention of coronary aneurysm in Kawasaki's disease using plasma exchange or infusion of immunoglobulins.** *Arch Fr Pediatr* 1987, **44**:79-83.
9. Imagawa T, Mori M, Miyamae T, Ito S, Nakamura T, Yasui K, Kimura H, Yokota S: **Plasma exchange for refractory Kawasaki disease.** *Eur J Pediatr* 2004, **163**:263-264.
10. Mori M, Imagawa T, Katakura S, Miyamae T, Okuyama K, Ito S, Nakamura T, Kimura H, Yokota S: **Efficacy of plasma exchange of plasma exchange therapy for Kawasaki disease intractable to intravenous gamma-globulin.** *Mod Rheumatol* 2004, **14**:43-47.
11. Wright DA, Newburger JW, Baker A, Sundel RP: **Treatment of immune globulin-resistant Kawasaki disease with pulsed doses of corticosteroids.** *J Pediatr* 1996, **128**:146-149.
12. Wallace CA, French JW, Kahn SJ, Sherry DD: **Initial intravenous gammaglobulin treatment failure in Kawasaki disease.** *Pediatrics* 2000, **105**:E78.
13. Zaitsu M, Hamasaki Y, Tashiro K, Matsuo M, Ichimaru T, Fujita I, Tasaki H, Miyazaki S: **Ulinastatin, an elastase inhibitor, inhibits the increased mRNA expression of prostaglandin H2 synthase-type 2 in Kawasaki disease.** *J Infect Dis* 2000, **181**:1101-1109.
14. Miura M, Ohki H, Tsuchihashi T, Yamagishi H, Katada Y, Yamada K, Yamashita Y, Sugaya A, Komiyama O, Shiro H: **Coronary risk factors in Kawasaki disease treated with additional gammaglobulin.** *Arch Dis Child* 2004, **89**:776-780.
15. Uehara R, Yashiro M, Oki I, Nakamura Y, Yanagawa H: **Re-treatment regimens for acute stage of Kawasaki disease patients who failed to respond to initial intravenous immunoglobulin therapy: analysis from the 17th nationwide survey.** *Pediatr Int* 2007, **49**:427-430.
16. Iwashima S, Seguchi M, Matubayashi T, Ohzeki T: **Ulinastatin therapy in Kawasaki disease.** *Clin Drug Investig* 2007, **27**:691-696.
17. Raman V, Kim J, Sharkey A, Chatila T: **Response of refractory Kawasaki disease to pulse steroid and cyclosporine A therapy.** *Pediatr Infect Dis* 2001, **20**:635-637.
18. Adachi S, Sakaguchi H, Kuwahara T, Uchida Y, Fukao T, Kondo N: **High regression rate of coronary aneurysms developed in patients with immune globulin.** *Tohoku J Exp Med* 2010, **220**:285-290.
19. Weiss JE, Eberhard BA, Chowdhury D, Gottlieb BS: **Infliximab as a novel therapy for refractory Kawasaki disease.** *J Rheumatol* 2004, **31**:808-810.
20. Saji T, Kemmotsu Y: **Infliximab for Kawasaki syndrome [reply].** *J Pediatr* 2006, **149**:426.
21. Burns JC, Best BM, Mejias A, Mahony L, Fixler DE, Jafri HS, Melish ME, Jackson MA, Asmar BI, Lang DJ, Connor JD, Capparelli EV, Keen ML, Mamun K, Keenan GF, Ramilo O: **Infliximab treatment of intravenous immunoglobulin-resistant Kawasaki disease.** *J Pediatr* 2008, **153**:833-838.
22. Shirley DA, Stephens I: **Primary treatment of incomplete Kawasaki disease with infliximab and methylprednisolone in a patient with a contraindication to intravenous immune globulin.** *Pediatr Infect Dis J* 2010, **29**:978-979.
23. Son MB, Gauvreau K, Burns JC, Corinaldesi E, Tremoulet AH, Watson VE, Baker A, Fulton DR, Sundel RP, Newburger JW: **Infliximab for intravenous immunoglobulin resistance in Kawasaki disease: a retrospective study.** *J Pediatr* 2011, **158**:644-649.
24. Ishikawa H: **Mizoribine and mycophenolate mofetil.** *Curr Medicinal Chem* 1999, **6**:575-597.
25. Yokota S: **Mizoribine: Mode of action and effects in clinical use.** *Pediatr Int* 2002, **44**:196-198.
26. Kawasaki Y, Hosoya M, Suzuki J, Onishi N, Takahashi A, Isome M, Nozawa R, Suzuki H: **Efficacy of multidrug therapy combined with mizoribine in children with diffuse IgA nephropathy in comparison with multidrug therapy without mizoribine and with methylprednisolone pulse therapy.** *Am J Nephrol* 2004, **24**:576-581.
27. Yoshikawa N, Nakanishi K, Ishikura K, Hataya H, Iijima K, Honda M: **Japanese Pediatric IgA Nephropathy Treatment Study Group: combination therapy with mizoribine for severe childhood IgA nephropathy: a pilot study.** *Pediatr Nephrol* 2008, **23**:757-763.
28. Honda M: **Nephrotic syndrome and mizoribine in children.** *Pediatr Int* 2002, **44**:210-216.
29. Hirayama K, Kobayashi M, Hashimoto Y, Usui J, Shimizu Y, Hirayama A, Yoh K, Yamagata K, Nagase S, Nagata M, Koyama A: **Treatment with the purine synthesis inhibitor mizoribine for ANCA-associated renal vasculitis.** *Am J Kidney Dis* 2004, **44**:57-63.

30. Ohtomo Y, Fujinaga S, Takada M, Murakami H, Akashi S, Shimizu T, Kaneko K, Yamashiro Y: **High-dose mizoribine therapy for childhood-onset frequently relapsing steroid-dependent nephritic syndrome with cyclosporine nephrotoxicity.** *Pediatr Nephrol* 2005, **20**:1744-1749.
31. Takahashi K, Oharaseki T, Wakayama M, Yokouchi Y, Naoe S, Murata H: **Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract - an animal model of Kawasaki disease.** *Inflamm Res* 2004, **53**:72-77.
32. Okubo M, Chen XM, Kamata K, Masaki Y, Uchiyama T: **Suppressive effect of mizoribine on humoral antibody production in DBA/2 mice.** *Transplantation* 1986, **41**:495-498.
33. Takahashi K, Oharaseki T, Nagai-Miura N, Ohno N, Ishida-Okawara A, Yamada H, Kaneshiro Y, Naoe S, Suzuki K: **Administration of human immunoglobulin inhibited development of vasculitis in a murine model of vasculitis induced with CAWS, *Candida albicans* water soluble fraction.** *Modern Reumatol* 2010, **20**:160-167.
34. Tomizawa K, Nagao T, Kusunoki R, Saiga K, Oshima M, Kobayashi K, Nakayama T, Tanokura M, Suzuki K: **Reduction of MPO-ANCA epitopes in SCG/Kj mice by 15-deoxyspergualin treatment restricted by IgG2b associated with crescentic glomerulonephritis.** *Rheumatology (Oxford)* 2010, **49**:1245-1256.
35. Jibiki T, Terai M, Kohno Y: **High concentrations of interleukin-8 and monocyte chemoattractant protein-1 in urine of patients with acute Kawasaki disease.** *Eur J Pediatr* 2004, **163**:749-750.
36. Oharaseki T, Kameoka Y, Kura F, Persad AS, Suzuki K, Naoe S: **Susceptibility loci to coronary arteritis in animal model of Kawasaki disease induced with *Candida albicans*-derived substances.** *Microbiol Immunol* 2005, **49**:181-189.
37. Nagi-Miura N, Shingo Y, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, Naoe S, Suzuki K, Ohno N: **Induction of coronary arteritis with administration of CAWS (Candida albicans water-soluble fraction) depending on mouse strains.** *Immunopharmacol Immunotoxicol* 2004, **26**:527-543.
38. Koyama H, Tsuji M: **Genetic and biochemical studies on the activation and cytotoxic mechanism of bredinine, a potent inhibitor of purine biosynthesis in mammalian cells.** *Biochem Pharmacol* 1983, **32**:3547-3553.
39. Gan L, Mohammad R, Seyedsayamdost , Shuto S, Matsuda A, Gregory A, Hedstrom PL: **The immunosuppressive agent mizoribine monophosphate forms a transition state analogue complex with inosine monophosphete dehydrogenase.** *Biochemistry* 2003, **42**:857-863.
40. Kusumi T, Tsuda M, Katsunuma T, Yamamura M: **Dual inhibitory effect of bredinin.** *Cell Biochem Func* 1988, **7**:201-204.
41. Kikuchi Y, Imakiire T, Yamada M, Saigusa T, Hyodo T, Hyodo N, Suzuki S, Miura S: **Mizoribine reduces renal injury and macrophage infiltration in non-insulin-dependent diabetic rats.** *Nephrol Dial Transplant* 2005, **20**:1573-1581.
42. Ikezumi Y, Suzuki T, Karasawa T, Hasegawa H, Kawachi H, Nikolic-Paterson DJ, Uchiyama M: **Contrasting effects of steroids and mizoribine on macrophage activation and glomerular lesions in rat Thy-1 mesangial proliferative glomerulonephritis.** *Am J Nephrol* 2010, **31**:273-282.
43. Sonda K, Takahashi K, Tanabe K, Funchinoue S, Hayasaka Y, Kawaguchi H, Teraoka S, Toma H: **Clinical pharmacokinetic study of mizoribine in renal transplantation patients.** *Transplant Proc* 1996, **28**:3643-3648.
44. Turka LA, Dayton J, Sinclair G, Thompson CB, Mitchell BS: **Guanine ribonucleotide depletion inhibits T cell activation.** *J Clin Invest* 1991, **87**:940-948.

doi:10.1186/1546-0096-9-30
Cite this article as: Takahashi *et al.*: Mizoribine provides effective treatment of sequential histological change of arteritis and reduction of inflammatory cytokines and chemokines in an animal model of Kawasaki disease. *Pediatric Rheumatology* 2011 **9**:30.

⑩ 川崎病

群馬大学大学院 小児科学分野
助教 小林 徹

1997年群馬大学卒業後，主に群馬県立小児医療センターで小児循環器疾患の診療に携わり，2008年より現職。先天性心疾患診療と共に川崎病に関連した臨床研究を行い，現在，重症川崎病患者に対する大規模臨床試験（RAISE Study）の研究事務局代表として新たな治療法の開発に携わっている。

東邦大学医療センター大森病院
小児科 教授 佐地 勉

川崎病は乳児・幼児に発症する原因不明の血管炎症候群である。川崎病全国調査結果によると，現在年間１万人以上の新規川崎病患者が発生しており（図），累積患者数は20万人を超える。罹患率は性差があり（男女比1.4：１），人種差も認める（日本人＞アジア系＞黒人＞白人）。後遺症として冠動脈病変（心臓を栄養する血管のこぶ）を合併することが知られており，先進国における後天性心疾患最大の原因である。

本稿では，川崎病の病態，臨床症状，治療，後遺症等を概説し，看護のポイントについて解説する。次に挙げるホームページより川崎病関連の情報を得ることができるため，一度サイトを訪れていただくことをお勧めする。

●日本川崎病学会ホームページ
http://www.jskd.jp/
●日本川崎病研究センターホームページ
http://www.kawasaki-disease.org/index2.html
●自治医科大学公衆衛生学ホームページ（川崎病全国調査結果）
http://www.jichi.ac.jp/dph/kawasaki.html
●RAISE Studyホームページ　http://raise.umin.jp/
●急性期川崎病治療のガイドライン
http://www.jskd.jp/info/pdf/guide.pdf
●川崎病心臓血管後遺症の診断と治療に関するガイドライン（2008年改訂版）
http://www.j-circ.or.jp/guideline/pdf/JCS2008_ogawasy_h.pdf
●川崎病の管理基準（日本川崎病研究会運営委員会編〈2002年改訂〉）
http://www.jskd.jp/info/pdf/kawakijun.pdf

川崎病の基礎知識

■病態

川崎病は，小児期に好発する原因不明の血管炎症候群であり，組織学的には全身の小～中型動脈における血管炎である。その疫学像からは，何らかの感染症がきっかけになって体内で免疫システムの異常活性化が生じ，さまざまな臨床症状・合併症が出現すると考えられている。流行はするが伝染はしないため，病棟内で集団発生することはない。

急性期はさまざまな免疫反応を誘導する炎症性サイトカインの上昇が報告されており，高サイトカイン血症が川崎病の主病態であることが分かってきた。歴史的にも多くの病原体の関与が研究されてきたが，いずれも確証とはなっていない。この宿主側の因子としては，リンパ球の異常な活性を示す一部の子どもたちがかかりやすく，後遺症を残しやすいことが判明している。

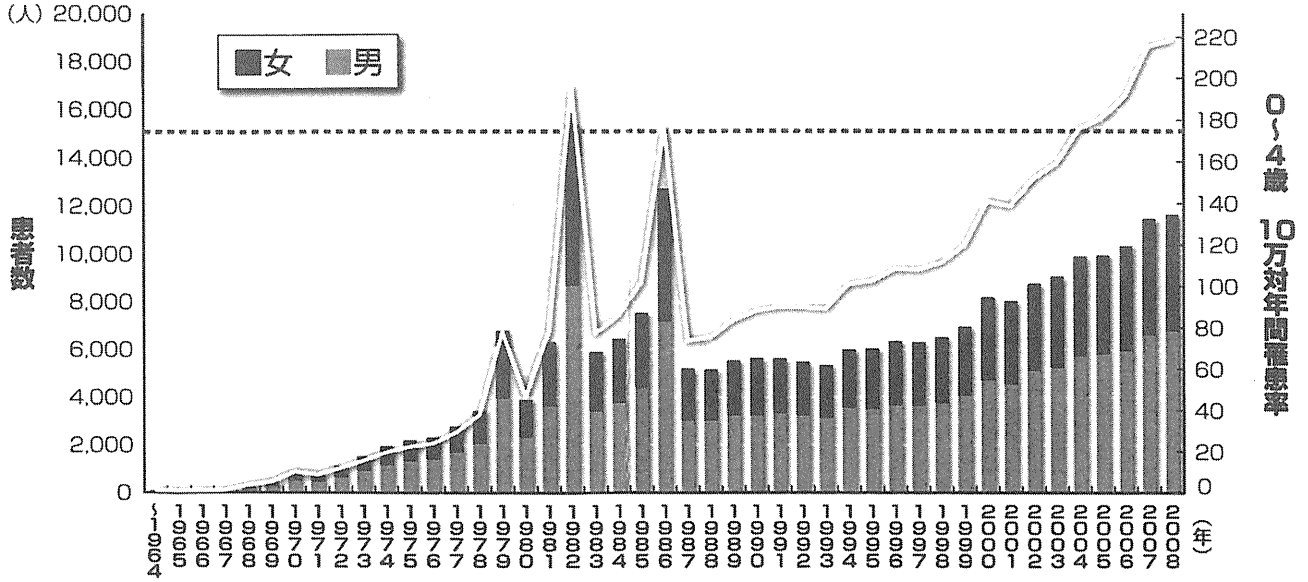
■臨床症状と臨床経過

川崎病は，疾患概念の提唱後40年が経過したが，いまだ原因は特定されていない。そのため，川崎病に特徴的な症状に基づき，いわゆる症候群として確定診断されている。川崎病は，川崎病診断の手引き改訂５版（付表）に基づき，診断する。主要症状の特徴を次に示す[1]。

発熱

通常初発症状が発熱で，39℃以上の高熱であ

図■新規川崎病患者数と罹患率（第20回川崎病全国調査成績）



日本川崎病学会ホームページ

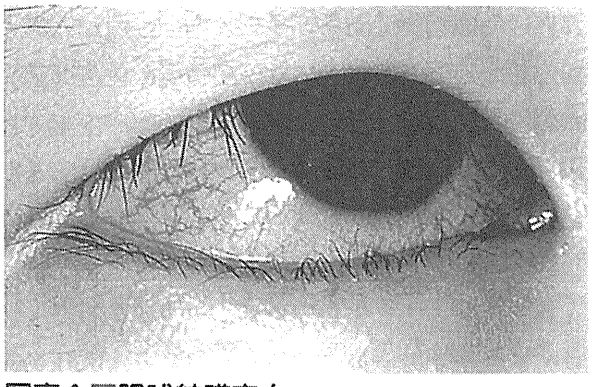


写真１■眼球結膜充血

日本川崎病学会ホームページ

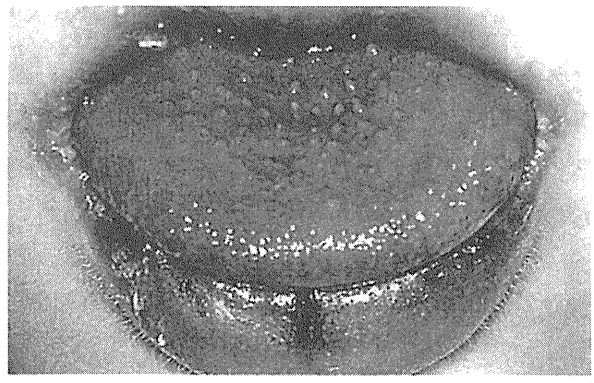


写真２■口唇の紅潮といちご舌

ることが多い。乳児ではぐったりとして非常に機嫌が悪く，年長児では全身倦怠感を訴えることが多いため，多くの症例で一般感冒とは異なる重症感を感じる。

眼球結膜充血

片側ではなく両側の眼球結膜（いわゆる白眼）の血管が拡張し，結膜全体がピンク～赤色に充血する（**写真１**）。眼脂はないかごくわずかであるところがアデノウイルス感染症（プール熱）と異なる。

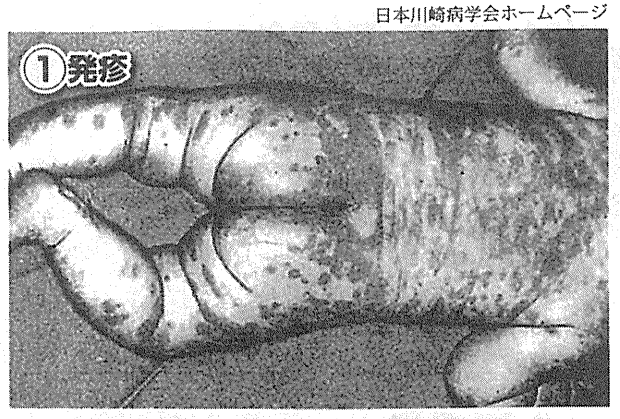
口唇・口腔所見

口唇は口紅を塗ったように赤くなり，所見の強い症例では口唇全体が腫脹して亀裂や出血を伴うことがある。舌は全体的に発赤腫脹し，舌乳頭の肥大が起こるため，溶連菌感染症類似の「いちご舌」の所見を認める（**写真２**）。口腔粘膜は全体に発赤するが，扁桃に白苔が付着することがほとんどないところがEBウイルス感染症や溶連菌感染症と異なる点である。

不定形発疹

どのような発疹でも川崎病の主要症状の一つとなり得るとされているが，典型的な発疹の形態は多形滲出性紅斑のような大小不同で部分的に癒合する平坦，ないしはやや膨隆する斑状疹である（**写真３－①**）。左上腕のBCG接種部位が発赤・腫脹することは年少児における特徴的な所見である

日本川崎病学会ホームページ



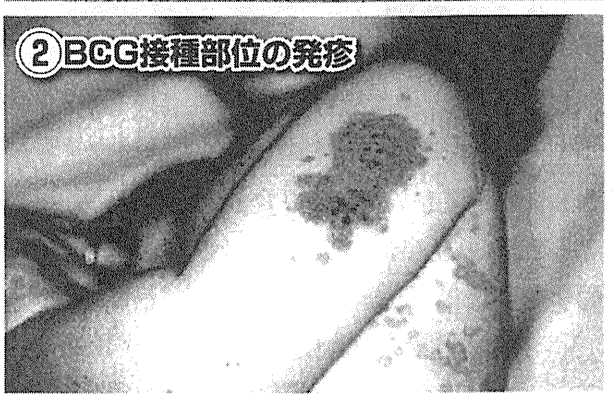
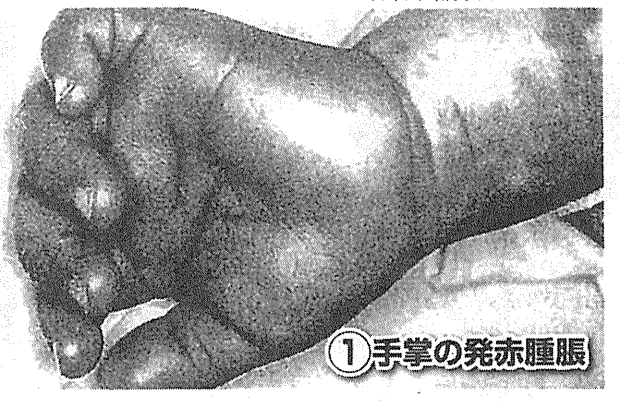


写真３■不定形発疹

日本川崎病学会ホームページ



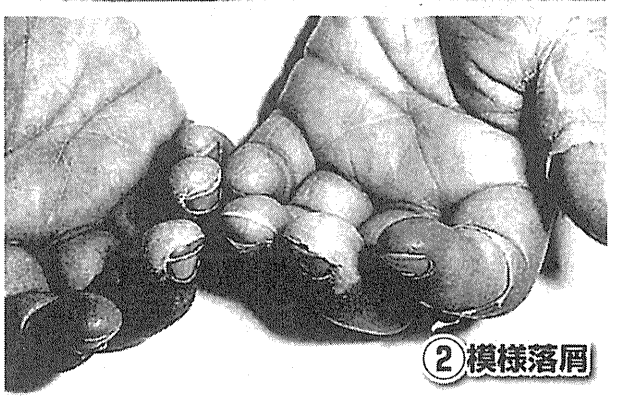


写真４■四肢末端の変化

（写真３－②）。BCG発赤は，接種後１年以内に生じるものが大部分のため，年長児における診断価値は乏しい。川崎病診断基準としては参考条項として位置づけられている。

四肢末端の変化

手掌と足底や指関節部分が発赤腫脹し，指で圧迫しても圧痕が残らない硬性浮腫を呈する。典型的な症例では，指全体がソーセージのように腫脹して光沢を持つ（写真４－①）。病状が回復期に入ると（発症後１～２週間程度）指先部と爪床の境界（指の最先端）からべろっと皮が一塊になって剝け落ちる膜様落屑を認める（写真４－②）。四肢末端の発赤腫脹の程度が強かった症例ほど膜様落屑の範囲・程度共に増強し，手袋を取ったように手掌全体が膜様落屑する症例もある。

非化膿性頸部リンパ節腫脹

観察できる頸部リンパ節腫脹の多くは胸鎖乳突筋下リンパ節腫脹である。典型例では，大人の拇指頭大以上（直径1.5cm以上）の大きさとなり，１個以上のリンパ節が集塊として触知される。リンパ節は，しばしば片側性で，比較的硬く，波動を生じず，穿刺しても膿は吸引されない。鶏卵大以上の大きな頸部リンパ腫大を呈する症例では，周辺皮膚は発赤し，強い圧痛がある（写真５）。痛みのため患側に首を曲げ続ける症例も時に認める。

他の主要症状と比較してその出現頻度は70%前後と低く，特に１歳以下の乳児ではさらに低頻度である。しかし４歳以上の年長例においては，発熱と共に病初期から出現することが多く，川崎病早期診断の大きな補助所見となる。抗菌剤に反応不良な化膿性頸部リンパ節炎や流行性耳下腺炎として治療をされる場合もあるため，注意が必要である。

各症状の出現する順序は症例によって異なり，同時にいくつかの主要症状が出現することもあれば，１つずつ出現して気づかれる場合もある。また，いくつかの症状が同時に出現していることも

日本川崎病学会ホームページ

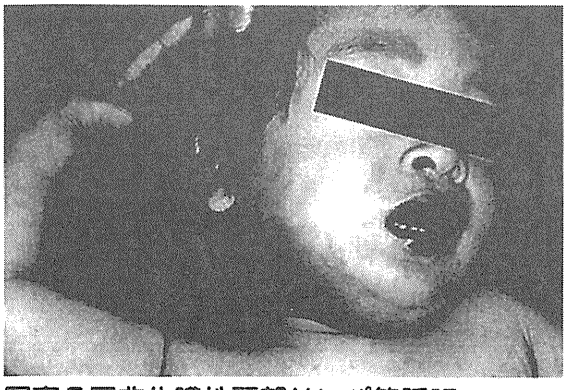


写真５■非化膿性頸部リンパ節腫脹

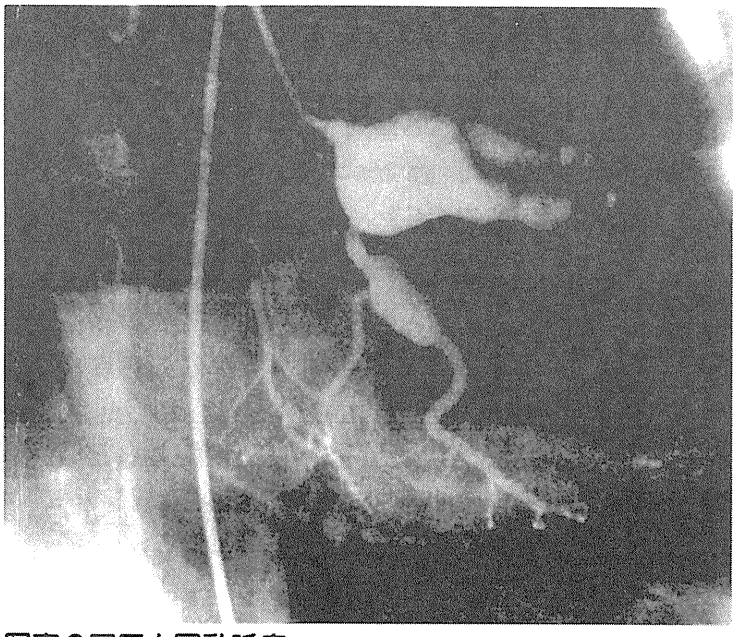


写真６■巨大冠動脈瘤

あれば，出現している日が別のこともあるため，注意深く経過を観察し，記録しておくことが，正しく川崎病を診断する上で重要な点である。

川崎病は，無治療でも数週間の経過で解熱し，すべての急性期症状は消失する。しかし，発熱が長期間続いた症例ほど，後遺障害である冠動脈障害の出現頻度が増すため，早期診断と早期治療による冠動脈障害出現の予防が重要となる。

■合併症・後遺症

川崎病に起因する急性期合併症は非常に多彩である（『川崎病診断の手引き改訂５版』参考条項参照）。中でも，肝逸脱酵素上昇，無菌性膿尿，胆嚢腫大，麻痺性イレウスは比較的頻度が高く，川崎病の早期診断において有用な所見である。

川崎病の後遺障害で最も重要なものは，心臓障害，特に冠動脈病変である。冠動脈病変は狭心症や心筋梗塞，突然死といった心事故を起こす原因となるため，川崎病急性期治療最大の目標は，いかに冠動脈病変を抑制するかの１点に尽きる。冠動脈病変は，無治療ではその25％に形成するが，近年の治療法の進歩によって，冠動脈瘤の頻度は急性期では10％，１カ月時では３％程度にまで減少した。しかし，高率に心事故を起こす冠動脈内径８mm以上の巨大冠動脈瘤患者（**写真６**）は年間30例程度新たに発生しており，しかも巨大冠動脈瘤患者は近年減少傾向が認められないことが大きな問題である。

治療

■急性期治療

基本的には，2003年に日本小児循環器学会より提唱された『川崎病急性期治療のガイドライン』に沿って治療する。急性期治療の核となる治療法は，２g/kg１回投与の免疫グロブリン超大量（IVIG）療法と，アスピリン30mg/kg/dayの併用である。診断後できるだけ早期にIVIGを行うことが，冠動脈病変抑制のためには極めて重要である。遅くとも，発症後10病日以内にIVIGを実施することを目標とする。

一方で，IVIG後も臨床症状や血液検査所見が改善しないIVIG不応例も約15～20％存在する。このような重症川崎病患者に対する治療法は，残念ながらいまだ確立されていない。現在は，IVIGの再投与，ステロイドやシクロスポリンなどの免疫抑制薬，生物学的製剤であるインフリキシマブ，エラスターゼ阻害薬であるウリナスタチン，血漿交換などの追加治療が各施設の方針に従って実施されている。

また，一般的な管理方法として，過剰な輸液を避けることは極めて重要である。川崎病急性期は，血管炎によって血漿成分が血管外に漏出しており，

抗利尿ホルモンの過剰分泌によって体内は水分過多となっている。過剰輸液は冠動脈に対する圧力を増加する方向に働くため，冠動脈障害を助長する。そのため筆者は，IVIG投与中はIVIG以外の輸液を行わず，体重が増加していく児やIVIG不応例といった重症患者に対しては利尿剤やβ遮断薬を積極的に使用し，できる限り血管に対する圧力を下げるよう心がけている。

■慢性期治療

解熱し，急性期症状が消失した児に対しては，冠動脈病変の程度によって治療法を選択する。

冠動脈病変を生じなかった患者には，２カ月間抗血小板薬を継続する。アスピリンは３～５mg/kg/day分１に減量して投与し，２カ月後の検査で異常所見がないことを確認したら内服終了とする。軽度～中程度の冠動脈病変を残存した患者には，アスピリンの投与を継続し，冠動脈病変が正常化するようならアスピリンの投与を中止する。８mmを超える重度の冠動脈病変を合併した患者は，心事故の高リスク群であるため，抗血小板薬に抗凝固療法（ワーファリン0.05～0.1mg/kg分１）を併用する。ワーファリンの効果は個人差が大きいため，PTINRを2.0前後に保つよう用量を調節する。

看護のポイント

■患児へのケアポイント

診断前

川崎病患者の中には，急性期症状がそろう前に入院となり，入院中に川崎病と診断される症例も散見される。特に６カ月未満の乳児は不明熱として入院となり，４歳以上の幼児は化膿性頸部リンパ節炎の初期診断で入院することもある。そのような患者の場合は，発疹や眼球結膜の充血，手足の発赤やむくみ，口唇の所見が出現しないか，注意深く観察することが重要である。

急性期

川崎病と診断された症例の大部分はIVIGが投与されるが，IVIG投与時にショック症状（血圧低下，頻脈，顔色不良，多呼吸）を呈する患者が２%程度存在するため，注意が必要である。IVIG製剤の投与速度が速いほどショック症状を起こしやすいため，投与開始直後は投与速度を遅くし，心電図やSpO_2モニターを装着して頻繁に血圧や心拍数，呼吸数等をモニタリングすることが重要である。ショック症状がないことを確認できたら，投与速度を上げる。

また，冷蔵保存されている液状製剤を室温に戻さずに投与するとショック症状を起こしやすいため，液状製剤は必ず室温に戻してから投与する。自施設では，投与前，５分，10分，15分，30分とバイタルチェックを行い，ショック症状がないことを確認した後に投与速度を上げ，バイアル交換時にバイタルサインのチェックを行っている。

川崎病患者のIVIG投与時のクリニカルパスを作成することが望ましい。

IVIG投与によって主要症状がどのように変化するかを詳細に観察することも重要である。一般的