分担研究者:野村裕一

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Eguchi T, **Nomura Y**, Hashiguchi T, Masuda K, Arata M, Hazeki D, Ueno K, Nishi J, Kawano Y, Maruyama I.	An elevated value of high mobility group box 1 is a potential marker for poor response to high-dose of intravenous immunoglobulin treatment in patients with	Pedatr Infect Dis J.	28	339-341	2009
Suda K, Kudo Y, Higaki T, **Nomura Y**, Miura M, Matsumura M, Ayusawa M, Ogawa S, Matsuishi T.	Multicenter and retrospective case study of warfarin and aspirin combination therapy in patients with giant coronary aneurysms caused by Kawasaki disease.	Circ J	73	1319-1323	2009
Ueno K, **Nomura Y**, Masuda K, Morita Y, Hazeki D, Eguchi T, Hashiguchi T, Maruyama I, Kawano Y.	Platelet vascular endothelial growth factor is a useful predictor for prognosis in Kawasaki syndrome.	British J Haematol.	148	285-292	2010
Nomura Y, Arata M, Koriyama C, Masuda K, Morita Y, Hazeki D, Ueno K, Eguchi T, Kawano Y.	A severe form of Kawasaki disease presenting with only fever and cervical lymphadenopathy at admission.	J Pediatr	156	(in press)	2010
Nomura Y, Masuda K, Eguchi T, Morita Y, Ueno K, Kawano Y.	Elevated value of a drug-induced lymphocyte stimulation test for immunoglobulin is one of the immunological abnormalities of Kawasaki disease.	Int Arch Allergy Immunol	152	(in press)	2010

分担研究者:三浦　大

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
三浦　大	川崎病の新しい病因論－IgA免疫反応と細胞質封入体－	小児科	50	2049-2057	2009
Kobayashi T, Inoue Y, Otani T, Morikawa A, Kobayashi T, Takeuchi K, Saji T, Sonobe T, Ogawa S, **Miura M**, Arakawa H.	Risk stratification in the decision to include prednisolone with intravenous immunoglobulin in primary therapy of Kawasaki disease.	Pediatr Infect Dis J	28	498-502	2009
Suda K, Kudo Y, Higaki T, Nomura Y, **Miura M**, Matsumura M, Ayusawa M, Ogawa S, Matsuishi T.	Multicenter and retrospective case study of warfarin and aspirin combination therapy in patients with giant coronary aneurysms caused by Kawasaki disease.	Circ J	73	1319-1323	2009

書籍

研究代表者:佐地　勉

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
佐地勉	急性期治療　ウリナスタチン療法.	石井正浩	小児科 臨床ピクシス9　川崎病のすべて	中山書店	東京	2009	106-108

分担研究者:小林　徹

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
小林　徹、小林富男、荒川浩一	免疫グロブリン治療抵抗例の予測スコア	石井正浩	小児科臨床ピクシス9　川崎病のすべて	中山書店	東京都	2009	116-119

分担研究者:小川俊一

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
小川俊一	「巨大冠動脈瘤:トータルケア」	石井正浩	小児科臨床ピクシス９　川崎病のすべて	中山書店	東京	2009	139－141

分担研究者:竹内一夫

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
竹内一夫	第9章「学校保健」の一部	小山洋・辻一郎	シンプル衛生公衆衛生学2009	南江堂	東京	2009	p227-230 p239-241

分担研究者:市田蕗子

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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		東京	2009	167-175
廣野恵一、市田蕗子	インフリキシマブ療法	石井正浩	アクチュアル小児科診療 ⑦『川崎病のすべて』	中山書店		2009 in press	

分担研究者:阿部　淳

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
阿部淳	川崎病の病因．スーパー抗原．	石井正浩	小児科臨床ピクシス９　川崎病のすべて	中山書店	東京	2009	32-33
阿部淳	川崎病と遺伝．マイクロアレイからみた病因と病態．	石井正浩	小児科臨床ピクシス９　川崎病のすべて	中山書店	東京	2009	50-53
阿部淳	川崎病．	五十嵐隆	総合小児科診療のための小児科学レビュー	総合医学社	東京	2009 in press	

分担研究者:野村裕一

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
野村裕一	ブドウ球菌説	石井正浩	小児科臨床ピクシス９　川崎病のすべて	中山書店	東京	2009	36-37

分担研究者:三浦　大

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
三浦　大	ステロイドパルス療法：IVIG不応例	石井正浩	小児科臨床ピクシス９　川崎病のすべて	中山書店	東京	2009	102-105

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Case report

Mimicking Kawasaki disease in burned children: Report of four cases☆

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1. Introduction

Kawasaki's disease presents with fever, bulbar conjunctival injection, redness of the labial and oral mucosa, polymorphous rashes, changes at the distal ends of the extremities, and cervical lymphadenopathy [2,12]. Complications caused by coronary arterial lesions, which are adverse symptoms, markedly affect the long-term prognosis of patients. When such complications are predicted, high-dose intravenous gammaglobulin therapy is provided from an early stage according to 'Harada's score [8]' which is the most reliable assessment score for the medical treatment of Kawasaki disease in Japan (Table 1). It is a low-incidence disease encountered in the field of Plastic and Reconstructive Surgery, and is considered to occur in more than 100 out of 100,000 children from 0 to 4 years of age in Japan. Although the etiology is unknown, many studies have reported that pathogen-related factors and some additional host factors lead to the development of the disease [19,24,33]. We report four cases with Kawasaki disease, the onset of which was apparently triggered by burns.

2. Case report

Case 1: A 9-month-old boy was consulted to our department with chief complaints of scalds on the right forearm, palm, and lower limb. His past history was unremarkable. He suffered the scalds, developed fevers of 39.0–39.9 °C, and was therefore referred to our department 2 days later. He had superficial dermal burns (SDB) on the right forearm to fingers and on the right thigh to lower leg, and deep dermal burns (DDBs) on the dorsum of the right foot, with a total burn area of 8% (Fig. 1a and b). He was admitted in the hospital the same day, and received fluid and antibiotic (CFPN-PI) therapy and conservative treatment with topical application of amikacin-containing Eksalb® and silver sulfadiazine to the burn wound surface. The patient's general and local condition good, and he was scheduled for skin grafting. However, on the 9th hospital day, he developed a fever of over 40 °C, generalized diffuse erythema, redness at the Bacillus Calmette–Guerin vaccine (BCG) inoculation site, and bulbar conjunctival injection. Hematologic and biochemical tests showed an increased WBC count of 14,400 mm^{-3}, normal red cell and platelet

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Table 1 – The guidelines for gammaglobulin application established by the Ministry of Health and Welfare: 'Harada's Score'

Judgment is made based on data obtained by the 9th hospital day in the acute stage

1. WBC: more than 12,000 mm^{-3}
2. Platelet count: less than 350,000 mm^{-3}
3. CRP: more than 3+
4. Hct: less than 35%
5. Albumin: less than 3.5 g/dl
6. Age: 12 months or less
7. Male sex

Cases meeting four or more of these seven items are indicated for gammaglobulin treatment. When the test is performed several times, the lowest values during the course are adopted for assessment.

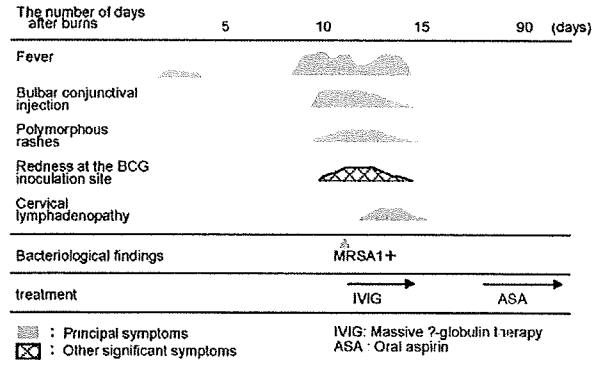

Fig. 2 – Case 1: clinical course.

counts, and evidence of inflammatory reaction and mild liver dysfunction such as a CRP of 5.5 mg/dl, GOT of 47 IU/l, GPT of 77 IU/l, but no abnormalities in BUN, Cr, or CPK. ASO and ASK antibody titers were negative. Urinalysis revealed proteinuria. On the 13th day, cervical lymphadenopathy was noted, and the patient was diagnosed with Kawasaki disease at the Department of Pediatrics of our hospital. He had a Harada score of 5/7 [8], and was immediately started on high-dose intravenous gammaglobulin therapy and oral aspirin, which achieved a complete response. During the course of the disease, no coronary artery aneurysms were observed. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in wound-surface cultures. The MRSA was positive for enterotoxin C and TSST-1 production, and was of coagulase II-type strain. Cultures of nasal and pharyngeal secretions and blood were negative. On the 58th day, the wounds epithelialized, and the patient was discharged (Fig. 2).

Case 2: A 1-year-and-1-month-old girl with chief complaints of scalds on the left face, auricle, neck, left anterior chest, back, and right thigh was referred to our department on the second day after suffering scalds. She had a history of infantile asthma. She suffered the scalds, was brought to the emergency room of our hospital, and was referred to our department on the second day after injury. She had superficial burns with deep areas on the thigh, with a total burn area of 20% (Fig. 3a and b), and was hospitalized the same day. She received fluid and topical antibiotic therapy and conservative treatment with topical application of Eksalb® to the burn wound surface. She showed good epithelialization of the wounds; however, she developed fevers of 39–39.9 °C, bulbar conjunctival injection, and lip redness from the 7th day after injury, and generalized erythema, redness at the BCG inoculation site, and strawberry tongue on the 9th day. Hematological and biochemical tests showed an increased WBC count of 17,600 mm^{-3}, increased platelet count of 473,000 mm^{-3}, and evidence of inflammatory reaction and liver dysfunction such as a CRP of 12.6 mg/dl, GOT of 66 IU/l, GPT of 32 IU/l, and LDH of 610 IU/l. ASO, ASK, and EBV-related antibody titers were negative. Urinalysis revealed no abnormalities. On the 10th day, she was diagnosed as having Kawasaki disease at the Department of Pediatrics of our hospital. She had a Harada score of 5/7, and was started on high-dose intravenous gammaglobulin therapy and oral aspirin, with a rapid decline of fever and improvement of other symptoms. From the 17th day, membranous desquamation of the fingers appeared. Though no coronary aneurysm nor other diseases that may cause coronary arterial lesions, such as viral infection and collagen disease-related disorders, developed, Moniliform dilation of the coronary artery was noted during the course. No apparent sign of local infection was noted, but *S. aureus* (MSSA) and MRSA were detected in the wound 8 and 16 days after injury, respectively. The bacteria were TSST-1-producing, enterotoxin C-positive, and coagulase type II. Cultures of nasal and pharyngeal secretions and blood were negative. The wounds epithelialized on the 58th day, and the patient was discharged (Fig. 4).

Case 3: A 10-month-old boy with a chief complaint of scalds on both lower limbs was referred to our department on the

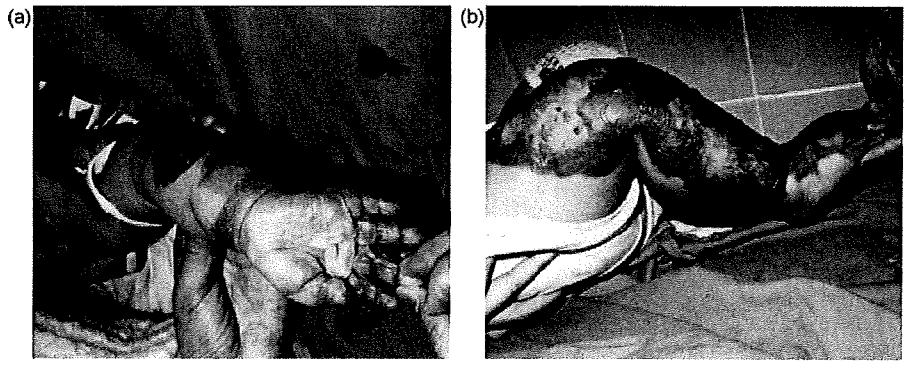

Fig. 1 – (a and b) Case 1: 9-month-old male. Scald burn, SDB-DDB, 8%TBSA.

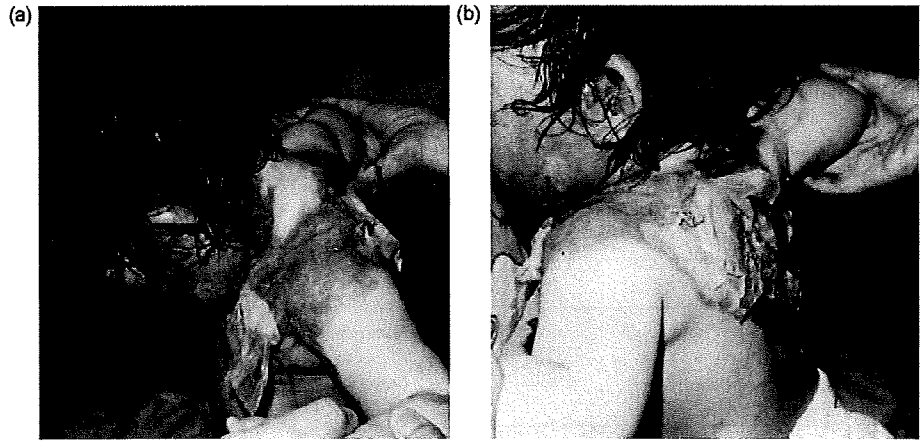

Fig. 3 – (a and b) Case 2: 1-year-and-1-month-old female. Scald burn, SDB-DDB, 20%TBSA.

second day after suffering scalds. His past history was noncontributory. He had superficial burns with deep areas on the dorsum of the feet, with a total burn area of 20%, and was hospitalized the same day. He received fluid and topical antibiotic therapy and conservative treatment with topical application of Eksalb®. He showed good epithelialization of the wounds with no signs of inflammation; however, he developed fevers of over 40 °C, facial and abdominal erythema, redness at the BCG inoculation site, and bulbar conjunctival injection from the 4th day after injury. Hematological and biochemical tests showed an increased WBC count of 24,500 mm^{-3} and evidence of inflammatory reaction and mild liver dysfunction such as a CRP of 10.1 mg/dl, GOT of 37 IU/l, GPT of 20 IU/l, and LDH of 699 IU/l. Urinalysis revealed no abnormalities. Based on the clinical course, atypical Kawasaki disease was diagnosed at the Department of Pediatrics of our hospital. The patient, placed on fluid therapy and topical ointment therapy, achieved symptomatic relief of Kawasaki disease. No coronary aneurysms were noted during the course of the disease. On the 22nd day after injury, membranous desquamation of the fingers appeared. In the absence of evidence of wound infection, MRSA was detected in wound cultures performed on the 9th day after injury, but blood cultures were negative. On the 26th day after injury, he was discharged, and the wounds epithelialized on the 40th day (Fig. 5).

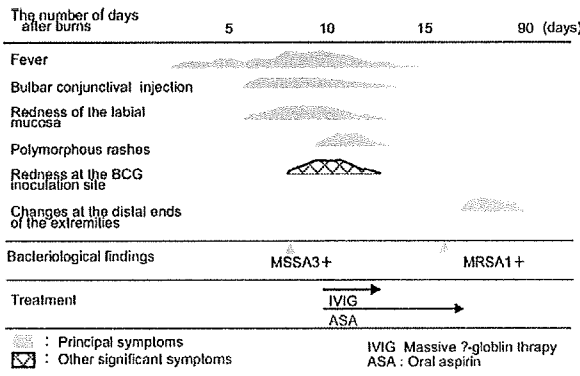

Fig. 4 – Case 2: clinical course.

Case 4: A 2-year-and-2-month-old girl was referred to our department with chief complaints of burns on the right palm and forearm. Her past history was noncontributory. She suffered hot iron contact burns, received conservative treatment at a local clinic, with no improvement, and was referred to our department on the 18th day after injury. She had superficial burns but deep in part, with a total burn area of 0.5%. After hospitalization, she received conservative treatment with topical application of Eksalb®. However, from the 19th day after injury, she had five symptoms of Kawasaki disease such as fevers of over 40 °C and generalized erythema, except for changes at the distal ends of the extremities, and was diagnosed with Kawasaki disease at the Department of Pediatrics of our hospital. She was immediately started on high-dose intravenous gammaglobulin therapy and oral aspirin, and rapidly achieved symptomatic improvement. No coronary aneurysms were noted during the course of the disease. On the 20th day after injury, MRSA was detected in the wounds, which epithelialized on the 48th day (Fig. 6).

3. Discussion

Kawasaki's disease was proposed as a vasculitis of unknown origin by Kawasaki et al. [12]. Kawasaki's disease is defined as a set of five or more of the following six symptoms, or a set of four symptoms in combination with proven coronary aneurysm in the absence of other possible diseases: (1) fever, (2) bulbar conjunctival injection, (3) redness of the labial and oral mucosa, (4) polymorphous rashes, (5) changes at the distal ends of the extremities, and (6) cervical lymphadenopathy. Other significant clinical and laboratory features include heart disease, including coronary artery aneurysm, respiratory and digestive symptoms, leukocytosis with left shift, hypoalbuminemia, anemia, redness at the BCG inoculation site, and neurological symptoms [2]. The incidence is high (68.0%) in infants younger than 3 years of age, 11.1% at 5 years of age or older, and very rare at 20 years of age or older in Japan [11]. The incidence of cardiac complications is about 20% in children and 13.6% in adults [28]. In some cases of Kawasaki's disease, the etiology of which is unknown by definition, potential

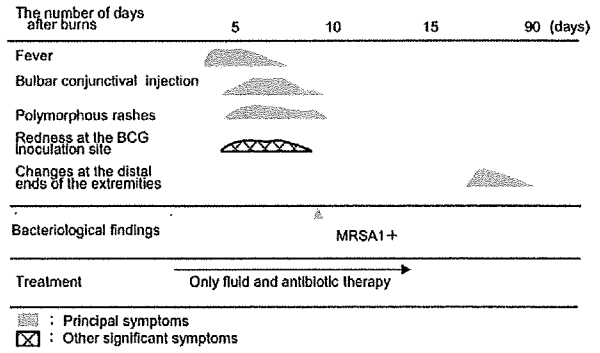

Fig. 5 – Case 3: clinical course.

causative pathogens, substances, or specific antibodies of other inflammatory diseases are detected, or other diseases precede the onset. The disease has many similarities to hemolytic streptococcal infection and measles, and epidemiologically exhibits onset seasonality and regional epidemicity, providing a basis for suspecting the involvement of pathogenic microorganisms as causative agents. Indeed, for the past 30 years, researchers have reported many cases of similar diseases [9,10,13,14,16,19,22–24,26,31–34] (Table 2). Therefore, the terms Kawasaki-like disease or mimicking Kawasaki disease are used to refer to a pathological condition which meets the diagnostic criteria for Kawasaki disease that is caused by an identified pathogen [23]. All the four patients reported here presented with characteristic symptoms meeting the diagnostic criteria for Kawasaki disease, and one of them showed moniliform dilatation of coronary arteries. In addition, antibiotics were ineffective, and high-dose intravenous gammmaglobulin therapy rapidly relieved the symptoms. These results indicate that three of them had typical Kawasaki's disease. However, since these patients developed symptoms after MRSA infection following burns, the condition was diagnosed as mimicking Kawasaki disease.

Recent studies suggested the involvement of infectious agents in the etiology of Kawasaki disease. Saji et al. indicated that Kawasaki disease itself should be understood as a syndrome presenting with similar symptoms [23]. Kawasaki's disease is also characterized by the development of coronary artery aneurysms due to coronary arteritis, an important factor influencing the long-term prognosis. Coronary arteritis

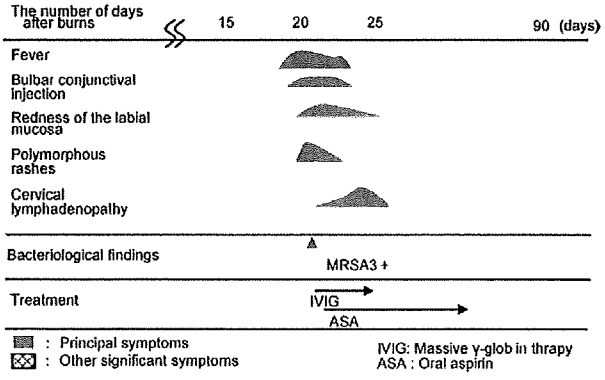

Fig. 6 – Case 4: clinical course.

Table 2 – Causes/diseases reported as mimicking Kawasaki disease

1. Infection (specific inflammatory diseases)
Yersinia
Streptcoccus pyogenes
Staphylococcus aureus (TSS, NTED, etc.)
Virus
Measles
Influenza
HIV
EBV
Candida albicans
Mycoplasma
Rickettsiosis
Others
2. Nonspecific inflammatory diseases
After vaccine inoculation (Measles, smallpox and DPT)
After burns
After cellulitis
Insect bite
Systemic rheumatoid arthritis
Infantile periarteritis nodosa
Stevens-Johnson syndrome
Hemophagocytosis syndrome
Henoch–Schonlein purpura
Drug sensitivity
Carbamazepine, mesalazine
Acetoaminophen

or the resulting aneurysms have been reported in 72.5% of childhood vasculitides seen in diseases except Kawasaki disease, such as EB virus-related diseases [20]. The causes that induce childhood coronary arterial complications include EB virus infection described above, nodular periarteritis, and other viral and collagen disease-related disorders. In adults, only arteriosclerotic lesions and systemic lupus erythematosus (SLE) are known as the causes. However, the current International understanding of Kawasaki disease is that the classification of vasculitides includes Kawasaki disease in "primary vasculitides", and distinguishes it from secondary vasculitides related to infections, collagen disease, drug hypersensitivities, cryoglobulinemia, malignant neoplasms, hypocomplementemic urticaria, or transplantation, and post-transplant vasculitis and pseudovasculitis, of which the causes or predisposing causes are present [15].

To our knowledge, there have been only two reported cases of Kawasaki disease that developed after burns [33], excluding our patient, and no adult case has been reported. These cases and ours had the same clinical features: (1) age at onset less than 3 years, (2) burns on relatively small areas of the body, and (3) early onset after burn injury. In general, milder burns epithelialize without becoming infected, whereas deep burns take 3–4 weeks to epithelialize, increasing the chance of infection. All our patients had DDB in all or part of the burn surface, and developed Kawasaki disease during burn wound infection. Kawasaki disease tends to develop at about 1 year of age when passive immunity from the mother declines, a fact also consistent with the observations in our patients. The development of Kawasaki disease in association with burns on relatively small areas of the body suggests the involvement of various factors. Extensive burns are often treated early by massive fluid therapy, antibiotic therapy, and anti-shock

therapy (high-dose intravenous gammaglobulin therapy), resulting in the prevention of the development of Kawasaki disease. Moreover, severe cases are associated with decreased immune function, particularly T cell-mediated immunity, a factor related to vulnerability to infection. In any event, the reported patients and ours had common clinical features that favored the establishment of infection.

Wong et al. explained that the breakdown of the microbiological barrier function of epithelium due to burns was associated with the entry of infectious antigens, and exotoxins or superantigens caused Kawasaki disease [33]. Unlike other antigens, superantigens refer to antigens that are bound to self-MHC class II molecules via TcR, and are recognized, not by the CDR formed in the V region of a particular TcR, but via particular regions other than the CDR of a particular Vbeta element. Thus, superantigens activate large numbers of T cells, inducing overproduction of cytokines. Bacterial superantigens collectively refer to exotoxins produced by *S. aureus* and streptococci, and cause toxic shock syndrome (TSS) and scarlet fever [29]. Although more than 50% of individuals over 5 years of age carry anti-TSST-1 antibodies, TSS patients have been reported to have low titers of anti-TSST-1 antibodies [30]. Some studies have reported little or no involvement of superantigen-producing bacteria in Kawasaki disease [14,18], or the development of Kawasaki disease after cellulitis due to TSST-1-positive staphylococci [19]. The present patients also showed an increase in anti-TSST-1 antibody titer in the convalescent stage; moreover, the patients suddenly developed symptoms of Kawasaki disease in the absence of signs of infection and with a normal general condition. The superantigen activity of exotoxins produced by *S. aureus* has been considered to induce abnormal activation of cell-mediated immunity even in the stage of colonization [4,6]; therefore, it is very likely that the infection secondary to burns directly or indirectly caused Kawasaki disease.

TSS was first described in 1978 by Todd et al. [27] It is an acute toxic disease that manifests itself with high fever, muscle pain, vomiting, and diarrhea, followed by hypotension, diffuse rashes and peeling of the skin, and multi-organ failure, presenting clinical features similar to those of Kawasaki disease. Since Frame et al. [7] first described seven patients with TSS following burns in 1985, many studies have reported its development in patients under 6 years of age after burns [6,7,25], and this is well known in the field of Plastic and Reconstructive Surgery. However, in some patients with incomplete forms of TSS, Kawasaki disease should be suspected [21,25]. Reported patients with TSS or neonatal TSS-like exanthematous disease, which develops by a mechanism similar to that of TSS, included some who developed coronary artery abnormalities [17]. The possibility cannot be excluded that instead of Kawasaki disease, TSS was diagnosed because of the detection of *S. aureus* or MRSA in wound areas.

Regarding differences between Kawasaki disease and TSS, blood pressure reduction, central nervous symptoms, such as convulsion, digestive symptoms, elevations of CK, BUN, and Cr, and thrombocytopenia in peripheral blood occur more frequently in TSS [1,14]. However, all these findings are nonspecific, and precise distinction of the two diseases is difficult. Edwards-Jones et al. reported that the incidence of TSS after burns was about 2.5% [5]. Thus, TSS should be initially suspected when distinction is difficult. However, in the field of Pediatrics, the early treatment of Kawasaki or analogous diseases has been increasingly applied when the condition meets the diagnostic criteria of Kawasaki disease, and no other disease is apparent, which is also based on a report [3] that high-dose intravenous gammaglobulin therapy is effective for TSS because they contain anti-TSS-1 antibodies.

4. Conclusions

We encountered four patients who developed mimicking Kawasaki disease after sustaining burns. Kawasaki disease is not sufficiently understood because of the few cases reported in the field of Plastic and Reconstructive Surgery. In the future, the differential diagnosis of fever and polymorphous exanthem following infection of burns and other wounds should include Kawasaki disease. We need further understanding of the importance of local management, and consider the period of susceptibility to Kawasaki disease, the depth of burns, and their treatment after the development of Kawasaki disease.

REFERENCES

[1] Abe J, Kotzin BL, Jujo K, Melish ME, Glode MP, Kohsaka T, et al. Selective expansion of T cells expressing T-cell receptor variable regions Vβ2 and Vβ8 in Kawasaki disease. Proc Natl Acad Sci USA 1992;89:4066–70.
[2] Ayusawa M, Sonobe T, Uemura S, Ogawa S, Nakamura Y, Kiyosawa N, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). Pediatr Int 2005;47:232–4.
[3] Curtis N, Chan B, Levin M. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* from Kawasaki syndrome. Lancet 1994;343:299.
[4] Edward-Jones V, Shawcross SG. Toxic shock syndrome in the burned patient. Br J Biomed Sci 1997;54:110–7.
[5] Edward-Jones V, Dawson M, Childs C. A survey into toxic shock syndrome (TSS) in UK burns units. Burns 2000;26:323–33.
[6] Egan WG, Clark WR. The toxic shock syndrome in a burn victim. Burns 1988;14(2):135–8.
[7] Frame JD, Eve MD, Hackett MEJ, Dowsett EG, Brain AN, Gault DT, et al. The toxic shock syndrome in burned children. Burns 1985;11:234–41.
[8] Harada K. Intravenous gamma-globulin treatment in Kawasaki disease. Acta Paediatr Jpn 1991;33:805–10.
[9] Hurvitz H, Branski D, Gross-Kieselstein E, Klar A, Abrahamov A. Acetaminophen hypersensitivity resembling Kawasaki disease. Isr J Med Sci 1984;20(2): 145–7.
[10] Jackson MA, Burry VF. Influenza mimicking Kawasaki disease. Pediatr Infect Dis J 1993;12(3):787–8.
[11] Japan Kawasaki Disease Research Committee. Results of the 18th nationwide survey on Kawasaki disease. J Pediatr Pract 2006;69:281–91 (in Japanese).
[12] Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics 1974;54:271–6.

[13] Kikuta H, Taguchi Y, Tomizawa K, Kojima K, Kawamura N, Ishizaka A, et al. Epstein-Barr virus genome-positive T lymphocytes in a boy with chronic active EBV infection associated with Kawasaki-like disease. Nature 1988;333:455–7.
[14] Leung DYM, Meissner HC, Fulton DR, Muray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. Lancet 1993;342:1385–8.
[15] Lie JT. Nomenclature and classification of vasculitis: plus ça change, plus c'est lamême chose. Arthritis Rheum 1994;37(2):181–6.
[16] Martin L, Goudeau A, Lanotte P, Besnier JM, Lorette G, Vaillant L. Kawasaki syndrome or atypical measles mimicking Kawasaki syndrome? Acta Derm Venereol 1997;77(4):329.
[17] Miki M, Haneda N, Kato H, Uchiyama T. (Personal communication) 2002.
[18] Morita A, Imada Y, Igarashi H, Yutsudo T. Serologic evidence that streptococcal superantigens are not involved in the pathogenesis of Kawasaki disease. Microbiol Immunol 1997;41:895–900.
[19] Nakano M. A case of typical kawasaki disease (MCLS) secondary to cellulitis caused by toxic shock syndrome toxin-1(TSST-1)-producing staphylococci A hypothesis on the etiology of MCLS. In: Proceedings of the Fourth International Symposium on Kawasaki Disease; 1991. p. 132–6.
[20] Naoe S, Takahashi K, Oharaseki T. (Personal communication) 1997.
[21] Ogawa M, Tanaka H, Kagawa K. Toxic shock syndrome in minor burned patients: reports of two cases. Shizuoka J Med 2000;15(1):35–9 (in Japanese).
[22] Parha S, Garoufi A, Yiallouros P, Theodoridis C, Karpathios T. Carbamazepine hypersensitivity and rickettsiosis mimicking Kawasaki disease. Eur J Pediatr 1993;152(12):1040–1.
[23] Saji T, Ishikita T, Kojima Y, Nakayama T, Ozawa Y, Hoshida H, et al. Mimicking Kawasaki disease. Disease or Syndrome? Pediatr Jpn 2000;41(4):554–64 (in Japanese).
[24] Sato K. Clinical findings and epidemiology of Yersinia pseudotuberculosis Infection, especially concerned with Izumi fever. Kansenshogaku Zassi 1987;61(7):746–62.
[25] Sugamata A, Sato M, Narumi A, Suzuki Y, Okizu H, Tanihira S, et al. Toxic shock syndrome in patients with a burn wound infected with MRSA. Jpn J Plast Reconstr Surg 1989;32(6):567–73 (in Japanese).
[26] 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(2):72–7.
[27] Todd JK, Fishaut M, Welch T. Toxic shock syndrome associated with phage-group-1 staphylococci. Lancet 1978;2:1116–8.
[28] Tomiyama J, Hasegawa Y, Kumagai Y, Adachi Y, Karasawa K. Acute febrile mucocutaneous lymph node syndrome (Kawasaki disease) in adults: case report and review of the literature. Jpn J Med 1991;30(3):285–9.
[29] Uchiyama T, Yan X, Imanishi K, Yagi J. Bacterial superantigens-mechanism of T cell activation by the superantigens and their role in the pathogenesis of infectious diseases. Microbiol Immunol 1994;38(4): 245–56.
[30] Vergeront JM, Stolz SJ, Crass BA, Nelson DB, Davis JP, Bergdoll MS. Prevalance of serum antibody to staphylococcal enterotoxin F among Wisconsin residents:implications for toxic shock syndrome. J Infect Dis 1983;148(4):692–8.
[31] Waanders H, Thompson J. Kawasaki-like syndrome after treatment with mesalazine. Am J Gastroenterol 1991;86(2):219–21.
[32] Whitby D, Hoad JG, Tizard EJ, Dillon MJ, Weber JN, Weiss RA, et al. Isolation of measles virus from child with Kawasaki disease. Lancet 1991;338:1215.
[33] Wong D, Nutting A, Yeung RSM, McCrindle BW. Kawasaki disease and scald injuries: a possible association. Can J Cardiol 2004;20(11):1147–9.
[34] Yoganathan K, Goodman F, Pozniak A. Kawasaki-like syndrome in an HIV positive adult. J Infect 1995;30(2):165–6.

Risk Stratification in the Decision to Include Prednisolone With Intravenous Immunoglobulin in Primary Therapy of Kawasaki Disease

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Background: We reported previously that intravenous immunoglobulin (IVIG) plus prednisolone for initial therapy for Kawasaki disease (KD) prevented coronary artery abnormalities (CAA) more effectively than IVIG alone. However, questions remain as to whether PSL has potential benefit in all KD patients. The present study was designed to explore the possibility of stratified initial therapy including PSL in patients with and without a high predicted risk of being an IVIG nonresponder.

Methods: We retrospectively analyzed data from KD patients who received IVIG (n = 896) or IVIG + PSL (n = 110) by scoring the likely risk of being an IVIG nonresponder. We compared clinical and coronary outcomes between treatment-defined groups separately for high- and low-risk patients.

Results: Among low-risk patients (score 0–4), clinical and coronary outcomes were similar. Among high-risk patients (score 5 or more), incidences of treatment failure and coronary artery abnormalities until 1-month follow-up were more frequent in the IVIG than in the IVIG + PSL group. Sex- and score point-adjusted odds ratios for IVIG + PSL were 0.17 (95% confidence interval, 0.08–0.39) for treatment failure and 0.27 (95% confidence interval, 0.07–0.85) for coronary artery abnormalities A among high-risk patients.

Conclusions: IVIG + PSL treatment was associated with improving clinical and coronary outcomes in patients at high risk of being IVIG nonresponders.

Key Words: risk stratification, treatment failure, prednisolone, intravenous immunoglobulin, coronary artery abnormalities

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Kawasaki disease (KD) is an acute febrile illness of childhood characterized by clinical, biochemical, and histopathologic manifestations of systemic vasculitis.[1] Echocardiographic and cardiac angiographic data indicate that 20% to 25% of untreated KD patients develop coronary artery abnormalities (CAA).[2] Use of high-dose intravenous immunoglobulin (IVIG) together with aspirin is clearly effective in resolving inflammation in KD and reducing the occurrence of CAA.[3] However, about 10% to 20% of patients still have persistent or recurrent fever after completion of IVIG treatment, while CAA occur in about 10% of KD patients despite this therapy.[1] KD is presently considered to be the most common pediatric cause of acquired heart disease in developed countries.

Although corticosteroids are considered as a treatment option for various types of vasculitides, many physicians were uncomfortable with the use of corticosteroids in KD because of an early report,[4] showing a high incidence of CAA in a group that received a prolonged course of oral prednisolone (PSL) alone. Subsequent retrospective studies on effects of corticosteroids in KD, however, have shown either no ill effects or possible benefits.[5,6] Wooditch and Aronoff[7] concluded from a metaanalysis that inclusion of corticosteroids in aspirin-containing regimens for primary therapy for KD reduced the incidence of CAA. Recently, we reported a multicenter, prospective, randomized controlled trial demonstrating that primary therapy with IVIG plus PSL had a significant advantage over IVIG alone with respect to prevention of CAA and rapid resolution of inflammation.[8] Nonetheless, corticosteroid therapy may be associated with potential adverse reactions.[9–11] Additionally, a large fraction of IVIG responders, who account for 80% of KD patients, ultimately remain free of CAA.[12] Whether or not corticosteroids should be administered to all KD patients, therefore, is uncertain.

Recently, we developed a risk score that identified IVIG nonresponders in advance, with high sensitivity and specificity, based on 7 laboratory and demographic variables available before initiation of primary therapy.[13] This risk score could define 2 risk strata in patients with KD, indicating high- or low-risk for IVIG unresponsiveness. The risk score thus might enable us to identify KD patients who require more intensive primary therapy. We hypothesized that the addition of PSL to IVIG as primary therapy for KD patients would offer important therapeutic benefits to patients in this high-risk stratum. To explore the possibility of stratified primary therapy in KD patients, we retrospectively introduced risk stratification to identify the benefits of primary therapy, including PSL, in patients with and without a predicted high risk of IVIG unresponsiveness.

MATERIALS AND METHODS

Study Patients and Outline of Therapies

Data used for the present study were obtained from consecutive KD patients from August 2000 to August 2007 at 13 medical institutions in Gunma and Saitama prefectures in Japan. KD was diagnosed using the Japanese Diagnostic Guidelines for KD (fifth revised edition).[14] The first day of illness was defined as the first day of fever. Patients in the IVIG group received IVIG (1 g/kg/d

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for 2 consecutive days or 2 g/kg/d for 1 day), whereas patients in the IVIG + PSL group received IVIG plus PSL (2 mg/kg/d, in 3 divided doses) given by intravenous injection until the fever resolved, and then orally until C-reactive protein (CRP) levels normalized (<0.5 mg/dL). After CRP normalized, doses of PSL were tapered over 15 days in 5-day steps (2 mg/kg/d for 5 days, 1 mg/kg/d for 5 days, and 0.5 mg/kg/d for 5 days). These methods of administration were based on our previous report.[5] Patients also received aspirin (30 mg/kg/d). The dose of aspirin was reduced to 5 mg/kg/d after normalization of CRP.

Clinical Outcomes and Stratification Using a Risk Score

The patient was considered afebrile when body temperature remained below 37.5°C for more than 24 hours. Nonresponse to initial treatment was defined as the patient having fever that persisted for more than 24 hours after completion of initial IVIG. Recurrence was defined as recrudescent fever associated with KD symptoms 24 hours after an afebrile period. These patients were defined as having treatment failure. CAA, detected by 2-dimensional echocardiography, were defined as present if the internal lumen diameter reached 3 mm in a child less than 5 years old, or at least 4 mm in a child aged 5 years or more; if the internal diameter of a segment was at least 1.5 times as large as that of an adjacent segment; or if the lumen was irregular. We stratified KD patients according to a risk scoring system developed to predict IVIG unresponsiveness. This risk score was based on a multiple logistic regression analysis, including 750 consecutive KD patients given IVIG. Seven variables were included in the risk score (Table 1). Point scores and cut-off values for each variable were as follows: 2 points each for sodium 133 mmol/L or less, 4 or less days of illness before initial treatment, aspartate aminotransferase (AST) 100 IU/L or more, percentage of white cells representing neutrophils at least 80%; and 1 point for platelet count 30.0 × $10^4/mm^3$ or less, CRP at least 10 mg/dL, and age 12 months or less. If a laboratory test was performed twice or more before primary therapy, the highest value was chosen for % neutrophils, AST, and CRP, while the lowest value was chosen for platelet count and sodium. We considered KD patients as high risk when they had risk scores of 5 points or more.

Statistical Analysis

All analyses were carried out by means of the SPSS statistical package program (version 16.0J; SPSS Japan, Tokyo). Data were presented as mean ± SD for continuous variables or as a percentage for categorical variables. Patients who had missing values were excluded from this study. We compared clinical and coronary outcomes by dividing both treatment groups into high- and low-risk patients. Categorical data were compared between the IVIG group and the IVIG + PSL group using Fisher exact test. Two-sample *t* tests were used for analysis of normally distributed continuous variables, and the Mann-Whitney *U* test was used for continuous variables with a non-normal distribution. Normality was determined with the Kolmogorov-Smirnov algorithm. In high-risk patients, multiple logistic regression analysis was performed and odds ratios were adjusted for sex and score points. For all analyses, a 2-sided *P* value less than 0.05 was considered to indicate statistical significance.

TABLE 1. Risk Score for Prediction IVIG Unresponsiveness

Variable	Cut-Off Point	Score Point
AST	100 IU/L or more	2
Sodium	133 mmol/L or less	2
Illness days of initial treatment	4 or previous	2
Neutrophils	80% or more	2
CRP, (mg/dL)	10 mg/dL or more	1
Age in months	12 or less	1
Platelet counts	300,000 /mm^3 or less	1

RESULTS

Characteristics and Laboratory Findings of Patients

During the study period, 1123 KD patients were admitted and treated at our hospitals. Eight KD patients who presented with CAA at admission and 109 KD patients who had missing values were excluded from this study. Thus 1006 KD patients (IVIG group n = 896, IVIG + PSL group n = 110) were analyzed in this study. Of the 110 subjects in the IVIG + PSL group, 90 patients were participants in a previously reported randomized trial.[9] The other 20 patients were administered IVIG + PSL according to their pediatrician's selection except for one patient whose parents requested treatment with IVIG + PSL. Table 2 shows the baseline characteristics and laboratory findings of IVIG and IVIG + PSL groups. The IVIG + PSL group had significantly lower sodium, earlier illness days of initial treatment, and higher CRP and score points.

Univariate Analyses of Clinical Outcomes

Using the risk score, 298 patients (33.3%) in the IVIG group and 48 patients (43.6%) in the IVIG + PSL group were classified as high-risk patients ($P = 0.034$). In low-risk patients, clinical course and coronary outcomes did not show significant differences between IVIG and IVIG + PSL groups (Table 3). Only 5 patients in the IVIG group showed coronary dilation at 1 month, without giant coronary aneurysms exceeding 8-mm diameter. Table 4 summarizes clinical and coronary outcomes in high-risk patients. Treatment failure and nonresponse to initial treatment were less frequent in the IVIG + PSL group (20.8% and 8.3%, respectively) than in the IVIG group (51.7% and 47.3%, respectively; $P < 0.001$ both for comparison of treatment failure and for comparison of nonresponse to initial treatment). Similarly, the incidence of CAA before 1 month of treatment was significantly less in the IVIG + PSL group (3 patients, 6.3%; $P = 0.037$) than in the IVIG group (54 patients, 18.1%).

TABLE 2. Baseline Characteristics Both IVIG and IVIG + PSL Group

Variable	IVIG Group (n = 896)	IVIG + PSL Group (n = 110)	*P*
Male, n (%)	512 (57.1)	70 (63.6)	0.22
Age in months	30.3 ± 22.3	30.6 ± 23.9	0.87
Illness days of initial treatment	4.8 ± 1.4	4.5 ± 1.4	0.02
Past history of Kawasaki disease, n (%)	30 (3.3)	4 (3.6)	0.78
White blood cell counts, ($\times 10^3/mm^3$)	14.8 ± 5.0	14.9 ± 4.4	0.91
Neutrophils, (%)	68.8 ± 14.9	70.7 ± 15.0	0.20
Platelet counts, ($\times 10^4/mm^3$)	34.4 ± 10.7	36.1 ± 10.3	0.10
AST, (IU/L)	116 ± 222	127 ± 238	0.65
Sodium, (mmol/L)	134.6 ± 2.8	133.9 ± 3.0	0.01
CRP, (mg/dL)	8.6 ± 5.2	9.7 ± 5.2	0.05
Score points	3.5 ± 2.4	4.3 ± 2.5	0.002

TABLE 3. Clinical and Coronary Outcomes Among Low-Risk Patients

Variable	IVIG Group (n = 598)	IVIG + PSL Group (n = 62)	P
Treatment failure, n (%)	57 (9.5)	5 (8.1)	1.00
Nonresponse to initial treatment, n (%)	40 (6.7)	2 (3.2)	0.42
Relapse, n (%)	19 (3.2)	3 (4.8)	0.45
CAA until 1 mo, n (%)	14 (2.3)	0 (0.0)	0.38
CAA at 1 mo, n (%)	5 (0.8)	0 (0.0)	1.00

TABLE 4. Clinical and Coronary Outcomes Among High-Risk Patients

Variable	IVIG Group (n = 298)	IVIG + PSL Group (n = 48)	P
Treatment failure, n (%)	154 (51.7)	10 (20.8)	<0.001
Non-response to initial treatment, n (%)	141 (47.3)	4 (8.3)	<0.001
Relapse, n (%)	17 (5.7)	7 (14.6)	0.06
CAA until 1 mo, n (%)	54 (18.1)	3 (6.3)	0.04
CAA at 1 mo, n (%)	25 (8.4)	2 (4.2)	0.40

TABLE 5. Sex- and Risk Score Point-Adjusted Odds Ratios and 95% Confidence Intervals of Clinical and Coronary Outcomes Among High-Risk Patients

Variable	IVIG Group (n = 298)	IVIG + PSL Group (n = 48)	
		Adjusted OR	95% CI
Treatment failure	1.00 (reference)	0.18	(0.08–0.39)
Nonresponse to initial treatment	1.00 (reference)	0.07	(0.02–0.20)
Relapse	1.00 (reference)	2.75	(1.07–7.08)
CAA until 1 mo	1.00 (reference)	0.25	(0.07–0.85)
CAA at 1 mo	1.00 (reference)	0.41	(0.09–1.81)

OR indicate odds ratio.

Multivariate Analyses of Clinical Outcomes in High-Risk Group

Table 5 shows the sex- and risk score point-adjusted odds ratio of each of the end points. The IVIG + PSL group was at significantly lower risk of treatment failure (adjusted odds ratio 0.17; 95% confidence interval [CI], 0.08–0.39), nonresponse to initial treatment (adjusted odds ratio 0.07; 95% CI, 0.02–0.20), and CAA until 1 month of treatment (adjusted odds ratio 0.25; 95% CI, 0.07–0.85), despite having a significantly higher risk of relapse (adjusted odds ratio 2.75; 95% CI, 1.07–7.08).

DISCUSSION

The current study shows that risk-based stratification might guide decision making concerning initial treatment for KD. Among patients with high scores for risk of treatment failure with IVIG monotherapy, KD patients assigned to the IVIG + PSL treatment group were more likely to respond to therapy and avoid CAA. On the other hand, among patients with a low-risk of IVIG treatment failure, coronary and clinical outcomes were similar between the treatment-defined groups. These data suggest that primary therapy with a combination of IVIG and PSL might be a better therapeutic option than IVIG alone for this more severe form of KD. Patients with low risk of treatment failure might fare equally well with IVIG alone, thus limiting potential toxicities or adverse events due to steroid complications.

Stratification of primary therapy in KD patients was attempted in Japan in the 1990s. Harada[15] developed a risk score for prediction of coronary aneurysms for use when a child first presented with KD. Accordingly, at centers in Japan adopting this scoring system, IVIG was given to children fulfilling 4 of the following criteria: white blood cell count over 12000/mm^3; platelet count below 350000/mm^3; CRP exceeding 3+ by a semiquantitative analysis; hematocrit below 35%; albumin below 3.5 g/dL; age not greater than 12 months; and male gender. However, the influence of the Harada score in selecting KD patients who require IVIG has waned in Japan because IVIG is now given to almost all KD patients, as first-choice primary therapy. In addition, the American Heart Association and the American Academy of Pediatrics have recommended that all patients diagnosed with KD should be treated with IVIG, given the limited ability of scoring systems to predict CAA before initiation of IVIG.[16]

Pathologically, an affected coronary artery shows an influx of neutrophils in the early stage of lesion development (7–9 days after onset), followed by rapid transition to large mononuclear cells as well as lymphocytes (predominantly CD8$^+$ T cells) and IgA plasma cells.[17,18] Destruction of the internal elastic lamina followed by fibroblast proliferation occurs at this stage to form a coronary aneurysm. These findings underscore the importance of treating inflammation and vasculitis as soon as possible, before pathologic changes become irreversible. Recent clinical trials have focused on additional rescue therapy for KD patients who fail to respond to initial IVIG rather than primary therapy in KD. These rescue therapies have included corticosteroids,[19–21] tumor necrosis factor α blockade,[22] cyclosporine,[23] or plasma exchange.[24] Although these therapies were reported to bring about improvement in symptoms without significant worsening of adverse effects, no report concluded that these additional rescue therapies ultimately reduced the occurrence of CAA. Because IVIG nonresponders were mostly identified 24 to 48 hours after completion of the initial course of IVIG, rescue therapies generally were initiated 2 to 3 days after diagnosis of KD. Such a delay in administration of rescue therapies would permit initiation of CAA development. Because many studies have indicated that most KD patients with CAA are IVIG nonresponders,[12,13,25–26] the predictive identification of IVIG nonresponders permitting use of the additional intensive primary therapy, for IVIG plus corticosteroids should improve overall clinical and coronary outcomes.

Although we found addition of corticosteroids to IVIG primary therapy to benefit high-risk patients, the mechanism by which combining PSL with IVIG reduces the incidence of CAA in high-risk KD patients remains unclear. At this point, we suspect that rapid down-regulation of cytokine secretion by corticosteroid treatment might benefit high-risk patients because many investigators have concluded that various proinflammatory cytokines might take part in the pathogenesis of KD.[27,28] We previously reported that adding corticosteroids to IVIG when treating KD children rapidly ameliorated symptoms while reducing circulating cytokines, including interleukin IL-2, IL-6, IL-8, and IL-10.[29] Lin et al[30] reported that KD patients with CAA were found to have more abundant circulating pro-inflammatory cytokines, including IL-6, IL-8, and tumor necrosis factor α than patients with normal coronary arteries. Production of CRP occurs almost exclusively in hepatocytes as part of the acute-phase response upon stimulation by IL-6, tumor necrosis factor α, and IL-1β, produced at sites of inflammation. Although further studies are needed, these findings strongly support our inference that cytokine down-regulation con-

tributed to the therapeutic benefit, gained from adding PSL in high-risk patients.

On the other hand, the IVIG + PSL group was at significantly higher risk of relapse despite having a lower risk of overall treatment failure in high-risk patients. In the IVIG + PSL group, 6 of 10 patients relapsed at the steroid tapering phase. Although further examinations are needed to confirm this, we speculate that patients who relapse in the IVIG + PSL group have a severe form of KD, and the relapse is coupled with steroid tapering.

As our simple risk score predicts IVIG unresponsiveness with high sensitivity and specificity, patients in the low-risk group are unlikely to benefit from the addition of corticosteroids. This is important, given that corticosteroids may be associated with many potential adverse reactions.[9–11] Furthermore, epidemiologic and clinical features of KD suggest an infectious etiology,[31] and in general corticosteroid therapy can aggravate infectious processes. This study did not have sufficient statistical power to assess either the likelihood of adverse effects or the effectiveness of therapies in the low-risk group. Therefore, considering the balance between benefit and risk, only KD patients at high risk for nonresponse to IVIG should be given both corticosteroids and IVIG, as part of primary therapy. In addition, a randomized controlled trial suggested the usefulness of corticosteroid therapies in children at the highest risk for resistance to IVIG.[32]

There were several limitations to the study. First, our initial dose of aspirin, which is commonly used in Japan, is lower than that used in the United States. Second, Japanese Ministry of Health criteria used to diagnose CAA might underestimate the true incidence of CAA in patients with KD,[33] though it is a simple set of criteria that are easy to use in clinical settings. Third, this was a retrospective study not a randomized controlled study. A residual confounding effect was not completely ruled out, though we adjusted odds ratios of treatment for sex and for the risk score associated with clinical and coronary outcomes.

In conclusion, IVIG plus PSL might improve coronary and clinical outcomes in patients at high risk of IVIG failure as defined by our simple risk score. Future controlled, randomized clinical trials will define the benefit of this stratification and the role of IVIG + PSL therapy in high-risk KD patients with IVIG failure.

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REFERENCES

1. Burns JC, Glode MP. Kawasaki syndrome. *Lancet.* 2004;364:533–544.
2. Suzuki A, Kamiya T, Kuwahara N, et al. Coronary arterial lesions of Kawasaki disease: cardiac catheterization findings of 1100 cases. *Pediatr Cardiol.* 1986;7:3–9.
3. Newburger JW, Takahashi M, Beiser AS, et al. 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.
4. Kato H, Koike S, Yokoyama T. Kawasaki disease: effect of treatment on coronary artery involvement. *Pediatrics.* 1979;63:175–179.
5. Shinohara M, Sone K, Tomomasa T, et al. Corticosteroids in the treatment of the acute phase of Kawasaki disease. *J Pediatr.* 1999;135:411–413.
6. Jibiki T, Terai M, Kurosaki T, et al. Efficacy of intravenous immune globulin therapy combined with dexamethasone for the initial treatment of acute Kawasaki disease. *Eur J Pediatr.* 2004;163:229–233.
7. Wooditch AC, Aronoff SC. Effect of initial corticosteroid therapy on coronary artery aneurysm formation in Kawasaki disease: a meta-analysis of 862 children. *Pediatrics.* 2005;116:989–995.
8. Inoue Y, Okada Y, Shinohara M, et al. A multicenter prospective randomized trial of corticosteroids in primary therapy for Kawasaki disease: clinical course and coronary artery outcome. *J Pediatr.* 2006;149:336–341.
9. Miura M, Ohki H, Yoshiba S, et al. Adverse effects of methylprednisolone pulse therapy in refractory Kawasaki disease. *Arch Dis Child.* 2005;90:1096–1097.
10. Takahashi K, Oharaseki T, Wakayama M, et al. Pathological study of ruptured coronary artery aneurysms of Kawasaki disease (KD). In: Abstracts from the 2nd World Congress of Pediatric Cardiology and Cardiac Surgery; May 11–15, 1997; Honolulu, HI. Abstract 202.
11. Shulman ST. Is there a role for corticosteroids in Kawasaki disease? *J Pediatr.* 2003;142:601–603.
12. Burns JC, Capparelli EV, Brown JA, et al. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. *Pediatr Infect Dis J.* 1998;17:1144–1148.
13. Kobayashi T, Inoue Y, Takeuchi K, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. *Circulation.* 2006;113:2606–2612.
14. Ayusawa M, Sonobe T, Uemura S, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). *Pediatr Int.* 2005;47:232–234.
15. Harada K. Intravenous gamma-globulin treatment in Kawasaki disease. *Acta Paediatr Jpn.* 1991;33:805–810.
16. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation.* 2004;110:2747–2771.
17. Rowley AH, Eckerley CA, Jack HM, et al. IgA plasma cells in vascular tissue of patients with Kawasaki disease. *J Immunol.* 1997;159:5946–5955.
18. Brown TJ, Crawford SE, Cornwall MI, et al. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *J Infect Dis.* 2001;184:940–943.
19. Wright DA, Newburger JW, Baker A, et al. Treatment of immune globulin-resistant Kawasaki disease with pulsed doses of corticosteroids. *J Pediatr.* 1996;128:146–149.
20. Dale RC, Saleem MA, Daw S, et al. Treatment of severe complicated Kawasaki disease with oral prednisolone and aspirin. *J Pediatr.* 2000;137:723–726.
21. Hashino K, Ishii M, Iemura M, et al. Re-treatment for immune globulin-resistant Kawasaki disease: a comparative study of additional immune globulin and steroid pulse therapy. *Pediatr Int.* 2001;43:211–217.
22. Burns JC, Mason WH, Hauger SB, et al. Infliximab treatment for refractory Kawasaki syndrome. *J Pediatr.* 2005;146:662–667.
23. Raman V, Kim J, Sharkey A, et al. Response of refractory Kawasaki disease to pulse steroid and cyclosporin A therapy. *Pediatr Infect Dis J.* 2001;20:635–637.
24. Imagawa T, Mori M, Miyamae T, et al. Plasma exchange for refractory Kawasaki disease. *Eur J Pediatr.* 2004;163:263–264.
25. Wallace CA, French JW, Kahn SJ, et al. Initial intravenous gamma globulin treatment failure in Kawasaki disease. *Pediatrics.* 2000;105:e78.
26. Durongpisitkul K, Soongswang J, Laohaprasitiporn D, et al. Immunoglobulin failure and retreatment in Kawasaki disease. *Pediatr Cardiol.* 2003;24:145–148.
27. Furukawa S, Matsubara T, Yone K, et al. Kawasaki disease differs from anaphylactoid purpura and measles with regard to tumor necrosis factor-alpha and interleukin 6 in serum. *Eur J Pediatr.* 1992;151:44–47.

28. Inoue Y, Kato M, Kobayashi T, et al. Increased circulating granulocyte colony-stimulating factor in acute Kawasaki disease. *Pediatr Int.* 1999;41: 330–333.
29. Okada Y, Shinohara M, Kobayashi T, et al. Effect of corticosteroids in addition to intravenous gamma globulin therapy on serum cytokine levels in the acute phase of Kawasaki disease in children. *J Pediatr.* 2003;143:363–367.
30. Lin CY, Lin CC, Hwang B, et al. Cytokines predict coronary aneurysm formation in Kawasaki disease patients. *Eur J Pediatr.* 1993;152:309–312.
31. Burns JC, Cayan DR, Tong G, et al. Seasonality and temporal clustering of Kawasaki syndrome. *Epidemiology.* 2005;16:220–225.
32. Newburger JW, Sleeper LA, McCrindle BW, et al. Randomized trial of pulsed corticosteroid therapy for primary treatment of Kawasaki disease. *N Engl J Med.* 2007;356:663–675.
33. de Zorzi A, Colan SD, Gauvreau K, et al. Coronary artery dimensions may be misclassified as normal in Kawasaki disease. *J Pediatr.* 1998; 133:254–258.

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Infliximab Reduces the Cytokine-Mediated Inflammation but Does Not Suppress Cellular Infiltration of the Vessel Wall in Refractory Kawasaki Disease

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ABSTRACT: The aim of our study was to evaluate the efficacy of infliximab for the treatment of patients with refractory Kawasaki disease (KD) and investigate the dynamic changes of cytokines during infliximab treatment. We have performed a study of cytokine and proinflammatory molecule levels in 43 KD patients including 18 responders to IVIG, 14 nonresponders, and 11 patients treated with infliximab. We determined serum levels of soluble TNF receptor I (sTNFR I) and IL-6, as well as VEGF, damage associated molecular pattern (DAMP) molecules; myeloid-related protein (MRP)8/MRP14 and S100A12 sequentially. In eight patients, fever subsided immediately upon infliximab treatment. Four patients, who started infliximab after 12 d of illness, developed coronary artery lesions. Each of the cytokines was elevated before infliximab treatment in all patients. Although serum levels of proinflammatory cytokines decreased dramatically after infliximab treatment, DAMP molecules and VEGF and markers of local tissue damage were not suppressed. In contrast, in IVIG responders all cytokines decreased markedly after IVIG treatment. We show that infliximab is one of the adoptive therapies in refractory KD patients. Different behaviors of proinflammatory cytokines and DAMP molecules and VEGF after infliximab treatment suggest that infliximab is effective for suppression of cytokine-mediated inflammation, but could not completely block local vasculitis. (***Pediatr Res* 65: 696–701, 2009**)

Kawasaki disease (KD) is the most common systemic vasculitis syndrome primarily affecting small and medium-sized arteries, particularly the coronary artery. Although timely treatment with high-dose i.v. immune globulin (IVIG) is now accepted as reducing the incidence of coronary artery lesions (CAL), approximately 15% of the patients do not respond to IVIG treatment and have persistent fever as a manifestation of ongoing inflammation. These patients are at highest risk for development of CAL (1). The current practice for patients with KD and persistent or recrudescent fever after IVIG is to institute additional therapies, which may include one or more repeat doses of IVIG, high-dose pulse methylprednisoline, cyclophosphamide, methotrexate, ulinastatin, cyclosporine A (CyA), or plasmapheresis (2,3). Recently, potential new therapeutic approaches with infliximab (Remicade), a chimeric mouse-human MAb against tumor necrosis factor (TNF)-α, have been reported in refractory KD patients (4).

During the acute phase of KD, serum levels of proinflammatory cytokines such as TNF-α are elevated (5). In experimental studies of this syndrome, characterized by vasculitis resulting in coronary, as well as extracoronary, aneurysms, and stenosis, the attenuation of cytokine responses, especially IL-6, after infusions of IVIG may play an integral role in the rapid resolution of most of the symptoms in children with KD (6). In addition to these proinflammatory cytokines, VEGF, and markers of local inflammation of the family of damage-associated molecular pattern molecules (DAMPs) such as myeloid-related protein (MRP) 8/MRP14 and S100A12 have been reported to increase in acute KD and to play a crucial role in inflammation and are probably involved in the pathophysiology of acute vasculitis (7–13).

MRP8/MRP14, two calcium-binding proteins in the S-100 family, binds to microvascular endothelial cells and may participate in the genesis of a proinflammatory and prothrombotic state during systemic vasculitis (9,13). 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) (12,14). We reported that MRP8/MRP14 levels closely correlate with disease activity in acute KD and potential biomarker to predict both responses to IVIG therapy and coronary artery sequelae in the acute stage of KD (8,9). Another member of the S100 family, S100A12, also binds to endothelial cells *via* the receptor for advanced glycation end

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Abbreviations: **CAL,** coronary artery lesions; **CRP,** C-reactive protein; **IVIG,** intravenous immune globulin; **KD,** Kawasaki disease; **MRP,** myeloid-related protein; **sRAGE,** soluble receptor for advanced glycation end product; **sTNFRI,** soluble tumor necrosis factor-alpha receptor I; **DAMP,** damage associated molecular pattern molecules

products (RAGE) and induces cell activation and cytokine production through the nuclear factor-kappa-B signaling pathway (10,11). Transcript and protein levels of these three S100 proteins are reported to be strongly up-regulated during the acute stage of KD and decrease significantly in response to IVIG treatment and are possibly involved in the pathophysiology of acute vasculitis (8–11,15).

Here, we report the distinct effects of infliximab on systemic inflammation and local vasculitis, evaluating both proinflammatory and DAMP molecules and VEGF expression during infliximab treatment.

METHODS

Study population and blood samples. Patients with acute KD seen at the University Hospitals of Toyama University and Toho University School of Medicine between January 2005 and December 2007 were enrolled. All patients fulfilled the diagnostic criteria for KD and were initially treated with IVIG (2 g/kg body weight for 1 d) and oral aspirin (30 mg/kg/d) (16). Patients whose fever subsided within 48 h of IVIG treatment (2 g/kg body weight for 1 d) were considered responders. Patients whose fever did not subside within 48 h of the first IVIG treatment (2 g/kg body weight for 1 d) but responded to the second IVIG were designated nonresponders. Refractory KD was defined as the persistence or recrudescence of fever at least 48 h after the end of the multiple administrations of IVIG or IVMP infusion. Infliximab was used to the patient who was more than 1-y-old and received BCG vaccine. Thirty-one age-matched healthy control patients (16 males and 15 females), aged 3 mo to 7 y 5 mo (median 3.8 y), were enrolled during the same period.

Two-dimensional echocardiography was performed before and after treatment with IVIG as well as at 2 and 4 wk after the onset of KD, which was defined as the day on which fever developed. A coronary artery with a diameter of 3 mm or more (4 mm if the subject was over the age of 5 y) by echocardiogram was considered abnormal (16). The presence of CAL was assessed 1 mo after the onset of KD.

Patient demographic characteristics, therapies administered for KD before and after infliximab treatment, C-reactive protein (CRP) levels, dose, patient response to infliximab, and coronary artery outcome were recorded for all patients. Blood samples were collected from patients before and within 48 h after infliximab treatment in refractory KD and before and within 48 h after the first IVIG treatment in responders and nonresponders to IVIG. Simultaneously, blood samples were collected from healthy control patients on a single occasion. Parental informed consent was obtained for each child enrolled in this study, which was approved by the Research Ethics Committee of Toyama University Hospital.

Determination of serum cytokine levels. Serum concentrations of soluble tumor necrosis factor-alpha receptor 1 (sTNFRI), IL-6, VEGF, MRP 8/MRP 14, S100A12, and soluble receptor for advanced glycation end product (sRAGE) were determined by a sandwich enzyme-linked immunosorbent assay (ELISA). Commercial kits to quantitative human sTNFRI (Bender MedSystem, Vienna, Austria), human IL-6 (BioSource International, CA), human VEGF (Immuno-Biologic Laboratories, Fujioka, Japan), and sRAGE (Quantikine; R&D Systems, Minneapolis, MN) were used according to the manufacturer-recommended procedures (17,18). Serum concentrations of S100A12 and MRP8/MRP14 were determined by a double-sandwich ELISA using a specific antibody without cross reactivity to other S100 proteins, as described previously (19).

Statistical analysis. All results were expressed as mean ± SD. Paired t test was used to test for significance of the same parameter, within the same group, respectively. Analysis of variance or χ^2 test was used to test for significance of variables among three different groups. If data did not follow a normal distribution, the paired t test was replaced by a Wilcoxon signed rank test. When the data followed a normal distribution determined by Shapiro-Wilks test, comparisons between the two groups were performed using an unpaired t test or a Welch's test depending on equal or unequal variance. If the data did not follow a normal distribution, then a Mann Whitney U test was used.

RESULTS

Patient clinical characteristics and laboratory data. Table 1 shows the clinical characteristics of the 43 KD patients enrolled, comprising 18 responders, 14 nonresponders to the first IVIG, and 11 refractory KD patients treated with infliximab. The refractory KD patients and nonresponders had a significantly higher maximum concentration of CRP, a higher incidence of CALs, and a longer duration of fever compared with the responders. There were no significant difference between the groups with respect to age and the maximum number of white blood cells (WBCs).

In the refractory KD group, 10 patients received three or more IVIG infusions and eight patients received one to three doses of pulse methylprednisolone (30 mg/kg/dose i.v.) (Table 2). One patient did not use IVIG because she had a history of allergic reaction to immunglobulin. In addition, CyA was used in one patient (Table 2). All failed to become persistently afebrile after these treatments.

Patient outcome. Of the 11 refractory patients, 10 received a single infusion of 5 mg/kg of infliximab: one patient received a second infusion of 5 mg/kg. In eight of the 11 patients, fever subsided dramatically in response to the infliximab treatment and other symptoms disappeared (Table 2). In three patients, fever persisted and needed additional therapy after infliximab treatment. Four patients had coronary artery abnormalities documented by echocardiography before infliximab therapy and had transient dilatation that resolved postinfliximab infusion (Table 2); these patients continued to have CAL after treatment. Seven patients had normal coronary arteries preinfliximab and postinfliximab treatment. All 11 patients (excluding Patient 1, who died) were followed for 6–26 mo (median follow-up 17 mo) with no apparent complications of their infliximab therapy. Tuberculin reactions before and 6 mo after infliximab treatment disclosed no evidence of tuberculosis in all patients. Chest CT after 6 mo of infliximab treatment revealed normal findings in all patients.

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	Nonresponders to IVIG	p
No. 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 y (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 (d)		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 no.	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 No. 3 did not use IVIG because she had a history of allergic reaction to immunoglobulin.

† Patient No. 4 received 5 mg/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 controls	Refractory patients treated with infliximab	Responders to IVIG	Nonresponders to IVIG	*p*
No. patients	33	11	18	14	
Proinflammatory cytokines cytokines					
CRP (mg/dL)					
Before treatment		13.9 ± 9.5	7.1 ± 3.8	12.3 ± 5.6	0.00590
After treatment		10.6 ± 10.3	4.5 ± 4.2	14.6 ± 8.8	0.01595
STNFR (ng/mL)					
Before treatment	0.217 ± 0.080	0.714 ± 0.161	0.580 ± 0.180	0.600 ± 0.241	0.2155
After treatment		0.391 ± 0.161	0.326 ± 0.147	0.474 ± 0.220	0.0402
IL-6 (pg/mL)					
Before treatment	20 ± 10	1013 ± 1386	245 ± 321	276 ± 167	0.01786
After treatment		233 ± 561	69 ± 217	182 ± 387	0.14883
Endothelial cell specific cytokines specific cytokines					
MRP8/MRP14 (ng/mL)					
Before treatment	220 ± 40	4859 ± 2997	3261 ± 1724	4818 ± 3983	0.03907
After treatment		5860 ± 5468	2063 ± 1499	4588 ± 4397	0.00459
S100A12 (ng/mL)					
Before treatment	52 ± 32	1027 ± 615	412 ± 315	1148 ± 1837	0.00165
After treatment		1180 ± 1201	244 ± 286	651 ± 574	0.00201
sRAGE (pg/mL)					
Before treatment	1794 ± 368	868 ± 613	1495 ± 834	637 ± 404	0.00210
After treatment		1224 ± 497	3212 ± 1597	864 ± 553	0.00023
VEGF (pg/mL)					
Before treatment	92 ± 12	970 ± 1030	525 ± 607	790 ± 674	0.00809
After treatment		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.

Serum cytokine levels. The changes in serum levels of cytokines in refractory KD, and responders and nonresponders are shown in Table 3 and Figures 1 and 2.

Serum CRP levels were higher in the refractory KD and nonresponders groups than the responders before treatment (Fig. 1). Levels decreased in both the refractory and responders after treatment but increased further in the nonresponders. Before treatment, SNTFR was increased in all three groups by comparison with controls before treatment and declined to the normal level after treatment in all three groups. Serum IL6 was increased in all three patient groups by comparison with controls before treatment and was significantly higher in the refractory group than the other two groups. After treatment, all three groups decreased but remained higher than controls and were indistinguishable from each other.

Serum levels of MRP8/MRP14 and S100A12 were higher than controls in all three patient groups before treatment with the levels in the refractory patients and nonresponders higher than the responders (Fig. 2). After treatment, levels decreased in the responders, but increased further in the refractory patients and nonresponders. Serum levels of sRAGE were lower than controls in the refractory and nonresponder groups before treatment, whereas they were similar to controls in the responder group. Levels of sRAGE increased in all three

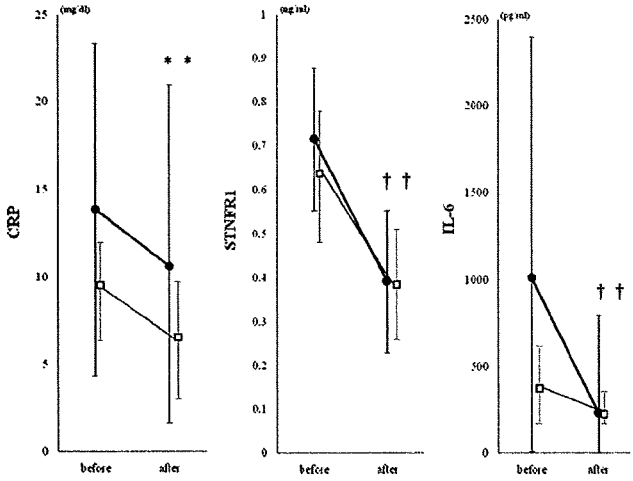

Figure 1. Sequential changes of proinflammatory cytokines, CRP (*left*), sTNFR 1 (*middle*), and IL-6 (*right*), in refractory KD patients who were treated with infliximab (●), compared with responders (□). Blood samples were collected from patients before and within 48 h after infliximab treatment in refractory KD and before and within 48 h after the first IVIG treatment in responders to IVIG. The *p* value shows changes between before and after treatment in each group. $*p < 0.05$, $\dagger p < 0.01$.

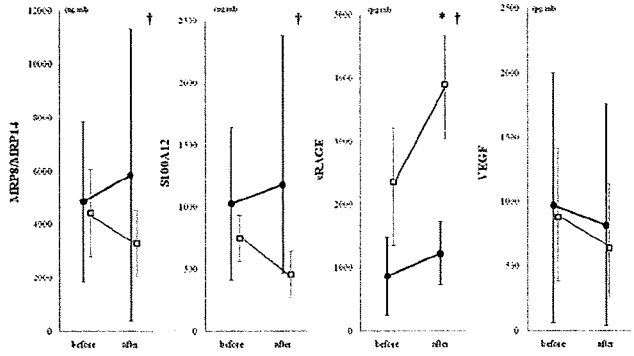

Figure 2. Sequential changes of endothelial cell specific cytokines and markers of local tissue damage, myeloid-related protein (MRP8/MRP14) (*left*), S100A12 (*left middle*), sRAGE (*right middle*), and VEGF (*right*), in refractory KD patients who were treated with infliximab (●), compared with responders (□). *p* value shows changes between before and after treatment in each group. $*p < 0.05$, $\dagger p < 0.01$.

groups after treatment but remained below normal in the refractory and nonresponder groups. Serum levels of VEGF were higher than controls in all three patient groups before and after treatment with only moderate changes seen after treatment.

As a result, each of the cytokines was elevated compared with healthy controls before treatment in all patients. Although serum IL-6 and sTNFR1 levels dramatically decreased after infliximab treatment and correlated with serum CRP levels and fevers, the serum levels of VEGF and DAMP-molecules such as MRP8/MRP14 and S100A12 remained high after infliximab treatment in refractory KD. This pattern of cytokines in refractory KD is different from responders or nonresponders after IVIG treatment; all cytokines decreased markedly in responders and did not decrease in nonresponders.

DISCUSSION

In this study, we show for the first time that different cytokines and proinflammatory DAMP-molecules are up-regulated and changed dynamically during IVIG and infliximab treatment in refractory KD patients (Table 3, Fig. 1 and 2). In refractory KD, the serum sTNFR1 and IL-6 levels reacted to infliximab treatment, but VEGF and DAMP-molecules like MRP8/MRP14 and S100A12 as well as sRAGE were not significantly affected.

Previous studies have shown that sTNFR1 is the natural homeostatic regulator of TNF-α activity and may reflect the true biologic activity of TNF-α more closely than serum TNF-α level (20). IL-6 causes many of the clinical and laboratory features of KD and is a reflection of a vigorous acute phase response (21). A recent case study reported that serum IL-6 was elevated and then decreased after infliximab treatment in a refractory KD patient (22). In this study, the fact that the serum levels of sTNFR1 and IL-6 are elevated and decrease after infliximab treatment suggest infliximab blocks systemic inflammation and inhibits the process of signaling of cytokines *via* TNF-α in KD.

In contrast, the serum levels of MRP8/MRP14, S100A12, and VEGF remained high after infliximab treatment. MRP8/MRP14, a complex of two calcium-binding proteins of the S-100 family, form heterodimers and are secreted by neutrophils and monocytes in response to inflammatory signaling cascades (Fig. 3) (8,9,23). The MRP8/MRP14 heterodimer binds to microvascular endothelial cells and phagocytes and may participate in the genesis of a proinflammatory and prothrombotic state during systemic vasculitis (24). Specifi-

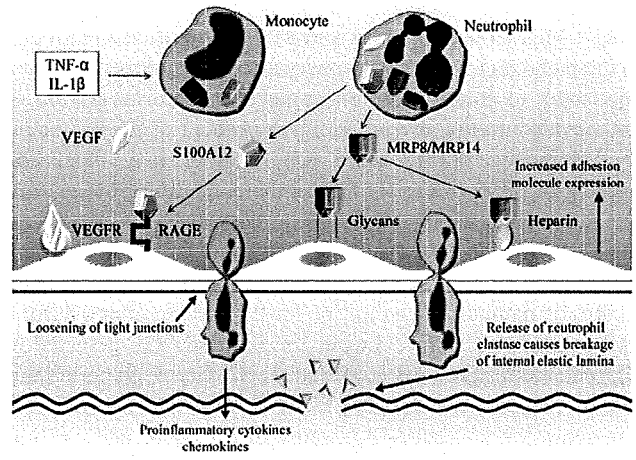

Figure 3. TNF-α and other proinflammatory cytokines activate endothelium and lead to the expression of carboxylated *N*-glycans. MRP8/MRP14 are released in high amounts at local sites of inflammation and have been recently described as novel members of the DAMP-family acting as endogenous ligands of 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 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 J, J Am Coll Cardiol 48:1265–1267, copyright © 2006 American College of Cardiology Foundation Published by Elsevier Inc., with permission.

cally, these proteins regulate adhesion of neutrophils and monocytes to endothelial cells and are implicated in their transmigration into the vessel wall (25). MRP8/MRP14 is believed to have an important functional role in vasculitis syndromes and serves as a marker identifying patients at risk for developing CAL during acute phase of KD (8). Interestingly, MRP8/MRP14 has recently been identified as novel endogenous ligands of TLR4, which directly promote inflammation. MRP14 −/− mice are protected from LPS-induced shock due to a lack of TNF-α production compared with wild-type mice indicating that MRP8/MRP14 act up-stream of TNF-α in the inflammatory cascade (12). This hypothesis is supported by our data showing that blockade of TNF-α efficiently inhibits down-stream pathways of systemic inflammation, such as the expression of IL-6, but does not completely block expression of local proinflammatory mechanisms. Whether this lack of action has negative consequences with respect to the long-term prognosis of KD-patients after infliximab treatment cannot be answered by this relatively small cohort but requires a prospective follow-up study including a larger number of patients.

Another member of the S100 family, S100A12, is also released by neutrophils, binds to the RAGE on endothelial cells and leukocytes, and induces cell activation and cytokine production through the nuclear factor-kappa-B signaling pathway (26) (Fig. 3). Transcript and protein levels of all three of these S100 proteins are increased in circulating leukocytes and in serum during the acute phase of KD (8–11,15,23). Taken together, these observations support the hypothesis that MRP8/MRP14 heterodimers and S100A12 participate directly in the pathogenesis of the coronary artery vasculitis in KD and contribute to endothelial cell damage and transmigration of leukocytes into the arterial wall independent of TNF-α activity. Moreover, during acute KD, sRAGE may block the binding of S100A12 to RAGE on the cell surface by neutralizing this proinflammatory protein. The fact that concentrations of MRP8/MRP14 and S100A12 did not change during infliximab treatment indicates that MRP8/MRP14 and S100A12 maintain signaling of local vascular injury despite the blockade of TNF-α. Lower levels of sRAGE detected in KD patients might thus confer susceptibility to hyperinflammation (27). Lower levels of sRAGE may reflect insufficient compensation of S100A12 because of the occurrence of dramatic and strong inflammation in refractory KD patients during infliximab treatment.

VEGF enhances proliferation and migration of endothelial cells in collaboration with nitric oxide and may contribute to later vascular remodeling after the acute phase of KD (7). Because systemic overproduction of VEGF has been demonstrated in acute KD, VEGF is considered to be involved in the pathophysiology of KD, especially in the development of CAL (28,29).

This study shows that serum IL-6 and sTNFR1 levels dramatically decreased after treatment and correlated with serum CRP levels and fevers; however, the serum levels of VEGF, MRP8/MRP14, and S100A12 remained high after infliximab treatment, especially in patients with CAL. In contrast, in IVIG responders all cytokines decreased markedly after IVIG treatment. These data indicate that proinflammatory cytokines decrease in response to infliximab treatment, but VEGF and local inflammatory proteins of the DAMP-family such as MRP8/MRP14 and S100A12, which were reported to be important factors in development of CAL during acute stage of KD, do not. Thus, it seems that infliximab is effective for the suppression of systemic inflammation, but could not completely block the local vasculitis in KD.

We have now successfully treated eight of the 11 refractory KD patients. Three patients who did not respond to infliximab treatment needed additional therapy. Four patients developed CAL despite infliximab therapy: of note, these patients were treated later than the patients without CAL and had signs of CAL before treatment. Histopathological studies have shown that transient infiltration by granulocytes occurs in the very early stage of acute KD, before infiltration of mononuclear cells, suggesting granulocytes act as a trigger in the pathogenesis of CAL (30). In light of our data, to prevent the progression of CAL and to prevent the secretions of MRP8/MRP14 and S100A12 from inflammatory cells such as neutrophils and monocytes, early administration of infliximab, or combination therapy with IVIG may lead to the successful cessation of the inflammatory response and vasculitis.

Study limitations. Limitations of this study include its retrospective nature, small number of patients, and the administration of multiple different therapies after the first IVIG infusion failure. Different IVIG preparations were used at different centers and concomitant or sequential antiinflammatory therapies administered to several of these patients precluded a final assessment of the effect of infliximab infusion. In conclusion, in this study, we show for the first time that inflammatory cytokines are up-regulated and changed dynamically during Infliximab treatment in refractory KD patients. Infliximab was effective for suppression of systemic inflammation, but could not completely block the local vasculitis in refractory KD patients. The early administration of infliximab or combination therapy with IVIG might be recommended for refractory KD patients.

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REFERENCES

1. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, Martin DD, Newburger JW, Burns JC 2008 Resistance to intravenous immunoglobulin in children with Kawasaki disease. J Pediatr 153:117–121
2. Pinna GS, Kafetzis DA, Tselkas OI, Skevaki CL 2008 Kawasaki disease: an overview. Curr Opin Infect Dis 21:263–270
3. Hashino K, Ishii M, Iemura M, Akagi T, Kato H 2001 Re-treatment for immune globulin-resistant Kawasaki disease: a comparative study of additional immune globulin and steroid pulse therapy. Pediatr Int 43:211–217
4. 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 2008 Infliximab treatment of intravenous immunoglobulin-resistant kawasaki disease. J Pediatr 153:833–838
5. Gupta M, Noel GJ, Schaefer M, Friedman D, Bussel J, Johann-Liang R 2001 Cytokine modulation with immune gamma-globulin in peripheral blood of normal children and its implications in Kawasaki disease treatment. J Clin Immunol 21:193–199

6. Nishimura S, Zaitsu M, Hara M, Yokota G, Watanabe M, Ueda Y, Imayoshi M, Ishii E, Tasaki H, Hamasaki Y 2003 A polymorphism in the promoter of the CD14 gene (CD14/-159) is associated with the development of coronary artery lesions in patients with Kawasaki disease. J Pediatr 143:357–362
7. Hamamichi Y, Ichida F, Yu X, Hirono KI, Uese KI, Hashimoto I, Tsubata S, Yoshida T, Futatani T, Kanegane H, Miyawaki T 2001 Neutrophils and mononuclear cells express vascular endothelial growth factor in acute Kawasaki disease: its possible role in progression of coronary artery lesions. Pediatr Res 49:74–80
8. Hirono K, Foell D, Xing Y, Miyagawa-Tomita S, Ye F, Ahlmann M, Vogl T, Futatani T, Rui C, Yu X, Watanabe K, Wanatabe S, Tsubata S, Uese K, Hashimoto I, Ichida F, Nakazawa M, Roth J, Miyawaki T 2006 Expression of myeloid-related protein-8 and -14 in patients with acute Kawasaki disease. J Am Coll Cardiol 48:1257–1264
9. Viemann D, Strey A, Janning A, Jurk K, Klimmek K, Vogl T, Hirono K, Ichida F, Foell D, Kehrel B, Gerke V, Sorg C, Roth J 2005 Myeloid-related proteins 8 and 14 induce a specific inflammatory response in human microvascular endothelial cells. Blood 105:2955–2962
10. Foell D, Ichida F, Vogl T, Yu X, Chen R, Miyawaki T, Sorg C, Roth J 2003 S100A12 (EN-RAGE) in monitoring Kawasaki disease. Lancet 361:1270–1272
11. Ye F, Foell D, Hirono KI, Vogl T, Rui C, Yu X, Watanabe S, Watanabe K, Uese K, Hashimoto I, Roth J, Ichida F, Miyawaki T 2004 Neutrophil-derived S100A12 is profoundly upregulated in the early stage of acute Kawasaki disease. Am J Cardiol 94:840–844
12. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, Nacken W, Foell D, van der Poll T, Sorg C, Roth J 2007 MRP8 and MRP14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat Med 13:1042–1049
13. Viemann D, Barczyk K, Vogl T, Fischer U, Sunderkötter C, Schulze-Osthoff K, Roth J 2007 MRP8/MRP14 impairs endothelial integrity and induces a caspase-dependent and -independent cell death program. Blood 109:2453–2460
14. Foell D, Wittkowski H, Vogl T, Roth J 2007 S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol 81:28–37
15. Abe J, Jibiki T, Noma S, Nakajima T, Saito H, Terai M 2005 Gene expression profiling of the effect of high-dose intravenous Ig in patients with Kawasaki disease. J Immunol 174:5837–5845
16. Shulman ST, Inocencio J, Hirsch R 1995 Kawasaki disease. Pediatr Clin North Am 42:1205–1222
17. Adolf GR, Apfler I 1991 A monoclonal antibody-based enzyme immunoassay for quantitation of human tumor necrosis factor binding protein I, a soluble fragment of the 60 kDa TNF receptor in biological fluids. J Immunol Methods 143:127–136
18. Hirata C, Nakano K, Nakamura N, Kitagawa Y, Shigeta H, Hasegawa G, Ogata M, Ikeda T, Sawa H, Nakamura K, Ienaga K, Obayashi H, Kondo M 1997 Advanced glycation end products induce expression of vascular endothelial growth factor by retinal Muller cells. Biochem Biophys Res Commun 236:712–715
19. Vogl T, Propper C, Hartmann M, Strey A, Strupat K, van den Bos C, Sorg C, Roth J 1999 S100A12 is expressed exclusively by granulocytes and acts independently from MRP8 and MRP14. J Biol Chem 274:25291–25296
20. Furukawa S, Matsubara T, Umezawa Y, Okumura K, Yabuta K 1994 Serum levels of p60 soluble tumor necrosis factor receptor during acute Kawasaki disease. J Pediatr 124:721–725
21. Ueno Y, Takano N, Kanegane H, Yokoi T, Yachie A, Miyawaki T, Taniguchi N 1989 The acute phase nature of interleukin 6: studies in Kawasaki disease and other febrile illnesses. Clin Exp Immunol 76:337–342
22. Saji T, Kemmotsu Y 2006 Infliximab for Kawasaki syndrome. J Pediatr 149:426
23. Burns JC 2006 S100 proteins in the pathogenesis of Kawasaki disease. J Am Coll Cardiol 48:1265–1267
24. Eue I, Konig S, Pior J, Sorg C 2002 S100A8, S100A9 and the S100A8/A9 heterodimer complex specifically bind to human endothelial cells: identification and characterization of ligands for the myeloid-related proteins S100A9 and S100A8/A9 on human dermal microvascular endothelial cell line-1 cells. Int Immunol 14:287–297
25. Srikrishna G, Panneerselvam K, Westphal V, Abraham V, Varki A, Freeze HH 2001 Two proteins modulating transendothelial migration of leukocytes recognize novel carboxylated glycans on endothelial cells. J Immunol 166:4678–4688
26. Hoffmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM 1999 RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/ calgranulin polypeptides. Cell 97:889–901
27. Wittkowski H, Hirono K, Ichida F, Vogl T, Ye F, Yanlin X, Saito K, Uese K, Miyawaki T, Viemann D, Roth J, Foell D 2007 Acute Kawasaki disease is associated with reverse regulation of soluble receptor for advance glycation end products and its proinflammatory ligand S100A12. Arthritis Rheum 56:4174–4181
28. Terai M, Yasukawa K, Narumoto S, Tateno S, Oana S, Kohno Y 1999 Vascular endothelial growth factor in acute Kawasaki disease. Am J Cardiol 83:337–339
29. Yasukawa K, Terai M, Shulman ST, Toyozaki T, Yajima S, Kohno Y, Rowley AH 2002 Systemic production of vascular endothelial growth factor and fms-like tyrosine kinase-1 receptor in acute Kawasaki disease. Circulation 105:766–769
30. Naoe S, Shibuya K, Takahashi K, Wakayama M, Masuda H, Tanaka M 1991 Pathological observations concerning the cardiovascular lesions in Kawasaki disease. Cardiol Young 1:212–220