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治療　ガンマグロブリン無効例への対応

ステロイドパルス療法

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Methylprednisolone pulse therapy in Kawasaki disease

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Abstract

A role of intravenous methylprednisolone pulse (IVMP) therapy has not been established for Kawasaki disease (KD) patients unresponsive to initial intravenous immunoglobulin (IVIG) therapy. We conducted a control study in 22 KD patients unresponsive to initial IVIG to compare IVMP with additional IVIG. IVMP induced faster but temporary resolution of fever and more adverse effects such as bradycardia. For the last four years, 62 KD patients unresponsive to initial IVIG have been treated with additional IVIG, and 17 unresponsive to additional IVIG with IVMP, followed by oral prednisolone. Among of them, coronary artery lesions were detected in two patients, but regressed in 6 months. This protocol may be useful for eradication of coronary artery lesions.

Key words: Kawasaki disease, non-responder to immunoglobulin therapy, steroid pulse therapy

はじめに

川崎病に対するステロイド療法は，一時の禁忌のイメージが払拭され，世界的に使用されるようになってきた．その方法は，

(1) 投与量：プレドニゾロンなどの通常量かメチルプレドニゾロンの超大量(ステロイドパルス療法：IVMP)か，

(2) 適応：免疫グロブリン療法(IVIG)と初期から併用するかIVIG不応例に使用するか，

に大別される．投与方法は確立していないので，施設により様々である．

本稿では，川崎病に対するIVMPについて，IVIG不応例に対する著者らの成績を中心に概説する．

1. IVMPの一般論と川崎病への応用

IVMPは腎移植後の急性拒絶反応を抑制するために開発され，以後，リウマチ性疾患，呼吸器疾患，腎疾患など様々な病態の重症例や難治例に用いられるようになった[1,2]．電解質作用が少ないメチルプレドニゾロンを，成人では1,000 mg/日，小児では30 mg/kg/日(1時間以上かけて点滴)，3日間投与する方法が標準的である．しかし，IVMPに関するエビデンスが乏しく，より少量(ミニパルス)でも十分な効果が

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あり副作用が少ないという報告もあり，適切な投与方法は完全に確立していない[1-3]．

IVMPによって炎症や免疫異常が強力に抑制されることが予想されるが，その詳細な作用機序はわかっていない．ステロイドは糖質コルチコイド受容体(GR)に結合し，炎症や免疫にかかわる遺伝子の転写を調節する．IVMPでは，GRの飽和量をはるかに上回る量を投与することから，GR以外の受容体の介在，直接的な細胞障害作用などの関与が推測される[1,3,4]．

IVMPの副作用としては，感染症をはじめ，顔面紅潮，味覚障害，頭痛，不眠，精神障害，血圧上昇，血栓症，高血糖，大腿骨頭壊死などがあげられる[1-3]．洞性徐脈，心房粗細動，心室頻拍，房室ブロックなどの不整脈，更には突然死も報告されていることから，心疾患の合併時には注意が必要とされている[1]．

1982年，IVIGが普及する前に，Kijimaら[5]はIVMPによる川崎病の初回治療の有用性を初めて報告した．1996年，Wrightら[6]の論文によって，IVIG不応例の治療法としてIVMPが注目されるようになった．我が国では，Hashinoら[7]が，IVIG追加不応例に対するIVMPは，IVIG再追加に比し医療経済的に安価で発熱期間も短縮するが，冠動脈の一過性拡張を来す恐れがあると述べている．Hungら[8]はIVIG不応例に対するIVMPに関する文献のレビューを行い，その有用性を示した．

2. IVIG初回不応例に対するIVMPとIVIG追加の比較

このような情勢を踏まえて，著者らは初回IVIG不応例に対するIVMPとIVIG追加の無作為化比較オープン試験を行った[9,10]．IVIG(2 g/kg/24時間)不応の22例を無作為に2群に分け，IVMP(メチルプレドニゾロン30 mg/kg/2時間，3日間；ヘパリン15-20単位/kg/時間併用)またはIVIG追加(2 g/kg/24時間)により加療し，臨床所見を比較した．

IVMPによって全例速やかな解熱が得られたが，一部の症例に発熱の再燃(リバウンド)が生じた(図1)．IVMP群における最高体温は，

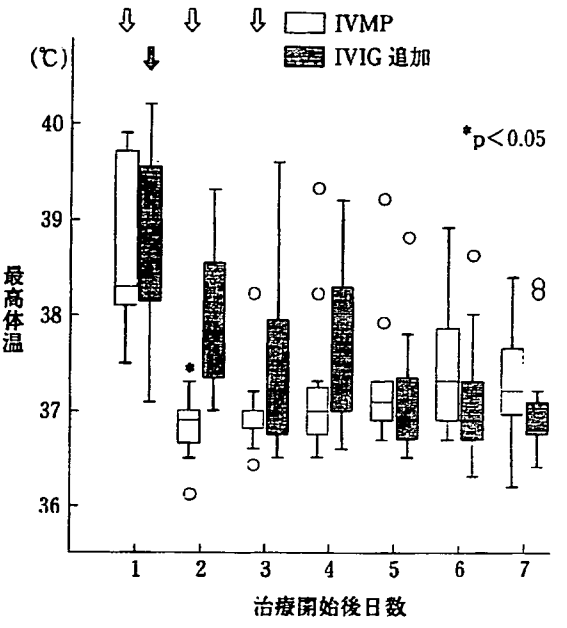

図1 免疫グロブリン療法(IVIG)不応例に対しステロイドパルス療法(IVMP)とIVIG追加を行った際の体温の変化(文献[9]より改変)

箱の中の線は中央値，下端は25%点，上端は75%点に相当する．ヒゲの下部境界線は下端から箱の長さの1.5倍以内の最小値を，上部境界線は上端から箱の長さの1.5倍以内の最大値を，○印ははずれ値を示す．両群の比較には反復測定分散分析を用いた．

IVIG追加群に比し，投与後2日目では有意に低値であったが(中央値36.9 vs 37.8℃，p=0.02)，3日目以降では有意差を認めなかった．IVMP群における発熱例は，投与後3日目までは有意に低率であったが(1/11 vs 8/11，p<0.001)，4日目以降は同数となり(6/11 vs 6/11)，治療を要した例の割合も有意差がなかった(IVMP群ではIVIG追加3例 vs IVIG追加群ではIVMP 2例)．30病日における両群の冠動脈病変の割合(2/11 vs 3/11)も同様であった．

血液検査では，ステロイドの効果を反映し，IVMP群における白血球数・好中球数，血糖値が，IVIG追加群に比し，投与後2日目で有意に高値を示した．CRP値は両群とも減少し，有意な相違はみられなかった．また，一部の症例(IVMP群7例，IVIG追加群8例)で測定したサイトカイン値では，TNF(腫瘍壊死因子)-αとMCP(単球走化性蛋白)-1が，IVMP群において投与後4日目で有意に抑制されたが，7日目

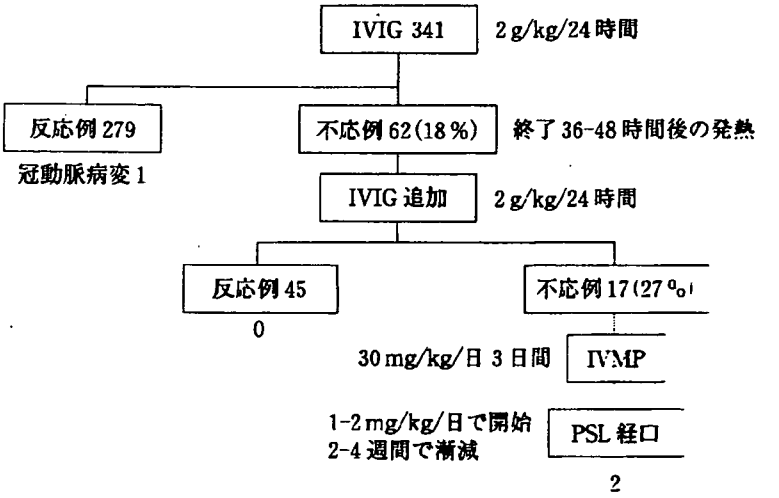

図2 現在の当院のプロトコール
IVIG: 免疫グロブリン療法, IVMP: ステロイドパルス療法, PSL: プレドニゾロン.

では有意差がなかった. すなわち, サイトカインに対するIVMPの抑制作用も, 早期に出現するがリバウンドを伴うことが示唆された.

高血糖以外のIVMPの副作用に関しては, 洞性徐脈が最も特徴的で, IVIG追加群に比し, 有意に高率に生じた(9/11 vs 1/11, p=0.01)[9]. その他の不整脈は認めなかったが, IVMPの際はモニター心電図を注意深く観察するべきである. IVMP群では体温が急速に降下し, 最低体温は有意に低値であった(平均値35.4 vs 36.1℃, p=0.002). 高血圧はIVMP群に多い傾向があったが, IVIG追加群と有意差はなかった(10/11 vs 6/11, p=0.15). これらのバイタルサインの変化は, いずれも一過性で自然に回復した. 塞栓症, 消化管出血, けいれん, 感染といった重篤な副作用は両群とも認めなかった.

以上の成績に基づくと, IVMPはIVIG追加に匹敵する効果が期待されるが, 症例数が少なく統計学的に非劣性(同等性)は主張できない. むしろ, 重篤ではなかったもののIVMPの副作用が目立ったことから, 米国のガイドライン[11]に準じ, 現状では, 初回IVIG不応例にはIVIG追加を早期に行い[12], 更に不応の際にIVMPを検討することが妥当と考えている.

3. IVIG追加不応例に対するIVMPおよびプレドニゾロン(PSL)後療法

上記成績に基づき, 現在は, IVIG追加(計4 g/kg)にも不応の際に, IVMPを行った後, リバウンドの発熱を防止するため後療法としてPSLを経口投与する(1-2 mg/kg/日から開始し2-4週間で漸減中止; IVMP・PSL後療法)方針とした. ステロイドによる副作用防止のため, ヘパリン, 抗生剤, 抗潰瘍剤も併用している.

最近4年間の成績では, 10病日未満にIVIGを開始した川崎病は341例であった. IVIG不応の62例(18.2%)にIVIGを追加し, 更に不応であった17例(27.4%, 全体の5.0%)にIVMP・PSL後療法を行った(図2). PSLの投与日数は13-40(中央値14)日で, 28日を超えたものが1例あった. PSL 14日投与後10日目に発熱が再燃した1例に3回目のIVIGを行った.

30病日の冠動脈病変は, IVIG反応例に1例(拡大), IVMP・PSL後療法を行った例に2例(拡大と瘤)の計3例(0.9%)で, 巨大瘤はなかった. 3例の冠動脈病変は, いずれも6カ月以内に正常化した. すなわち, 早期に診断すれば, IVIG追加不応例に対するIVMP・PSL後療法によって, 冠動脈瘤をほぼ抑制し得ると思う.

4. 初回IVIGとIVMPの併用療法

米国において，川崎病全例を対象に，初回IVIGとIVMP(メチルプレドニゾロン30mg/kg/2-3時間，IVIG前に1回のみ投与)の併用療法の効果を調べる目的で，二重盲検無作為化比較試験が行われた[13]．その結果，先行したパイロットスタディ[14]の予想に反し，IVMP併用の効果は認められなかった．IVMP+placebo群(98例)に比し，IVIG+IVMP群(101例)では，初回入院期間が短く炎症反応の改善も早い傾向を認めたが，全入院期間，発熱期間，IVIG追加の割合，冠動脈径のZスコア，冠動脈病変の割合は両群で有意差がなかった．したがって，IVMPは川崎病の初回治療としての適応はない，という結論が導かれた．

興味深いことに，IVIG追加を要した症例に対するpost hoc(後付け)解析では，IVMP併用群の冠動脈病変が有意に少なかった[13]．したがって，本研究はIVIG不応例あるいは不応予測例[15]に対するIVMPの治療効果を否定するものではなく，この点は新たな検討が必要である．

おわりに

IVIG不応の重症川崎病には，有効性が証明された治療法はなく，冠動脈病変抑制のためにIVMP・PSL後療法は有用な選択肢と考える．しかし，川崎病に対するステロイド療法には批判も根強く[16]，適応や妥当な使用法を確立する必要性が痛感される．近々，関東川崎病研究会を中心に，IVIG不応予測例[15]に対する初回IVIG+PSL併用療法の多施設共同研究が開始される．このような体制をもとに，ステロイド療法に関するエビデンスが，我が国から世界に発信されることを期待したい．

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port-wine stains through greater vessel heating and deeper vascular injury.[2] The improved technology targets the heterogeneity in blood-vessel sizes that is characteristic of port-wine stains.[3]

Any study evaluating the response of port-wine stains to treatment should include an analysis based on the location of the anatomical malformation and the patient's age. As compared with other areas of the face and neck, port-wine stains on the center of the face have been shown to respond less effectively to treatment and are more likely to recur.[4] An aggressive approach to treating infants and young children can also allow for more rapid and complete clearing.[5]

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THE AUTHORS REPLY: With ongoing research in medicine, investigating the 10-year follow-up results of any medical treatment inevitably leads to somewhat outdated results at the time of presentation. This is especially the case in a field that is subject to continuous development, such as pulsed-dye–laser treatment of port-wine stains. As Nelson and Geronemus point out, the results with newer pulsed-dye lasers have been reported to be promising and superior to the results with the laser used in our study. However, to date no controlled comparative studies have shown improved clinical efficacy. Whether the new lasers have improved long-term efficacy remains to be reported; in light of our observation of the recurrence of port-wine stains, we certainly hope they do.

No differences or trends in responses to treatment related to the anatomical locations of the port-wine stains were observed in either the original study[1,2] or the current follow-up study, possibly because of the relatively small number of patients. Furthermore, in the original study, age was shown to have no influence on the response to treatment. Therefore, we refrained from performing age-dependent analyses in the current long-term follow-up assessment.

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Treatment of Kawasaki Disease

TO THE EDITOR: In their trial of pulsed corticosteroid therapy for primary treatment of Kawasaki disease, Newburger et al. (Feb. 15 issue)[1] report that, as compared with placebo, a single pulsed dose of corticosteroid resulted in a shorter initial period of hospitalization but that the total numbers of days of fever and hospitalization, the rates of retreatment, and the coronary-artery outcomes did not differ significantly between the two groups. The use of a single-dose regimen without tapering most likely contributed to their results. A single application of a corticosteroid, even at a high dose, may have a strong but only short-lived effect, which could therefore be associated with a secondary increase in inflammation.

On the basis of nearly 10 years of clinical experience,[2] we designed a regimen involving a short intravenous course of prednisolone and subsequent oral administration of prednisolone followed by tapering.[3] In a randomized trial performed to test the effectiveness of the regimen as an adjunct to intravenous immune globulin, the incidences of retreatment and coronary-artery abnormalities within 1 month after the start of treatment were less frequent in the corticosteroid group than in the group receiving immune globu-

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lin alone. Our regimen therefore appears to be more efficacious than the control regimen. Nevertheless, the optimal corticosteroid regimen remains an issue in the primary therapy of Kawasaki disease.

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TO THE EDITOR: Newburger et al. studied the effects of adding intravenous methylprednisolone to conventional therapy for Kawasaki disease. The authors found a significantly lower frequency of coronary-artery abnormalities in the intravenous-methylprednisolone group than in the placebo group within the subgroup of patients who required retreatment with intravenous immune globulin.

The identification of predictors of coronary abnormalities in Kawasaki disease is still problematic. Failure of initial treatment with intravenous immune globulin remains the most consistent risk factor for cardiac abnormalities.[1] Administration of intravenous methylprednisolone after the failure of initial treatment with intravenous immune globulin does not seem to be effective in reducing the risk of coronary damage,[2] although the current data suggest that this might not be the case for patients who do not have a response to intravenous immune globulin and who have previously received intravenous methylprednisolone.

Since intravenous methylprednisolone administered as a single dose appears to be safe,[3] and given our inability to identify a priori the patients who will not have a response to intravenous immune globulin, it seems obvious that the concurrent use of a single dose of intravenous methylprednisolone and intravenous immune globulin may be our best choice at the moment. It is unrealistic to expect that trials powered to show the effectiveness of intravenous methylprednisolone could be accomplished anytime soon.

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TO THE EDITOR: Although the study by Newburger et al. involved assessment of coronary-artery outcomes with the use of transthoracic echocardiography, we were quite surprised by the inclusion of an example of a coronary aneurysm seen on multidetector computed tomography (CT) in the accompanying Perspective article by Burns.[1]

We and others[2,3] have shown the efficacy of noninvasive magnetic resonance imaging (MRI) of the heart for both the identification and characterization of coronary artery disease in patients with Kawasaki disease (Fig. 1). Patients with Kawasaki disease require frequent observation over many decades. Given the relatively high doses of ionizing radiation associated with multidetector

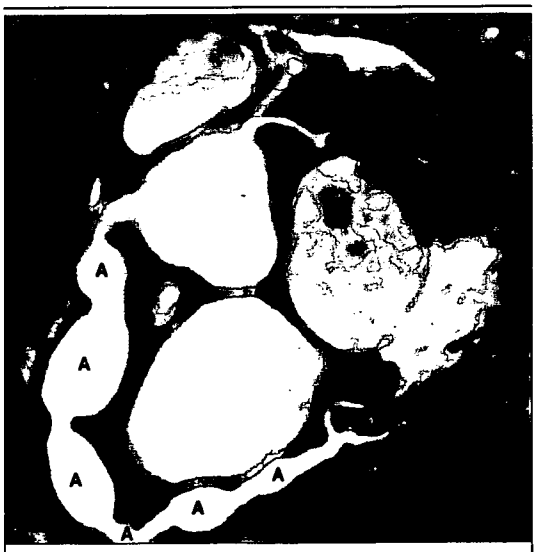

Figure 1. **Three-Dimensional Steady-State Free-Precession MRI of the Whole Heart in an 8-Year-Old Boy with Kawasaki Disease and Serial Aneurysms (A) in the Right Coronary Artery.**
No contrast material was administered.

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CT[4] and the heightened potential for radiation-induced fatal cancer in children,[5] we believe that, if transthoracic echocardiography is inadequate, these younger patients are best served by the use of coronary MRI.

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THE AUTHORS REPLY: Inoue and colleagues describe the results of their open trial using a prolonged course of corticosteroids, which we discuss in our article. We found that clinically significant coronary-artery abnormalities were infrequent in patients in both of our study groups. For this reason, although the optimal corticosteroid regimen may be unknown, we believe that corticosteroid regimens requiring a prolonged course of treatment would be difficult to rationalize for the primary treatment of all patients with Kawasaki disease.

Taddio and Rosé highlight an important question arising from our analyses. Our study was designed to test the hypothesis that the addition of intravenous methylprednisolone to conventional primary treatment of Kawasaki disease would improve coronary-artery outcomes; the study groups had similar overall coronary outcomes. A post hoc subgroup analysis suggested that primary corticosteroid therapy reduced the incidence of coronary-artery abnormalities in a high-risk subgroup of patients in our study who required retreatment with intravenous immune globulin because of persistent or recrudescent fever. However, such subgroup analyses must be interpreted with caution[1]; the literature is replete with subgroup analyses suggesting differential responses to therapy, findings that have been shown to be erroneous in subsequent prospective trials.[2] Children with Kawasaki disease can be characterized at the time of presentation with respect to their risk of resistance to intravenous immune globulin.[3] Until further studies are conducted in high-risk patients, we do not believe that corticosteroid therapy should be used in the primary treatment of Kawasaki disease.

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DR. BURNS REPLIES: Imaging of the coronary arteries is important in the long-term management of aneurysms in patients with Kawasaki disease. Transthoracic echocardiography can be used only to image the proximal arteries, is dependent on a high level of technical skill, and cannot reliably detect stenosis. Advantages of multidetector CT are the assessment of calcification and soft plaque, rapid collection of data, and straightforward interpretation of images. With proper gating to the cardiac cycle and lowering of the heart rate with beta-adrenergic blockade, exposures of approximately 0.67 mSv have been documented for coronary-artery studies of children involving multidetector CT (Larkin G, GE Healthcare: personal communication) (for comparison, one chest radiograph results in exposure to 0.02 mSv). MRI is safe, but many centers cannot image the coronary arteries with sufficient precision. All these approaches require general anesthesia for young patients, and MRI requires a longer time to capture images than does multidetector CT and thus increases the time under anesthesia and associated risks. Clearly, this is an area of medicine that is in flux. We look forward to the time when safe, noninvasive imaging methods are widely available at all centers for these children.

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Increased CD11b expression on polymorphonuclear leucocytes and cytokine profiles in patients with Kawasaki disease

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Summary

Clinical evidence implicates polymorphonuclear leucocytes in the pathogenesis of vasculitis in Kawasaki disease. We examined modulation of expression of adhesion molecules (CD11b and CD62L) on polymorphonuclear leucocytes and how this expression is related to serum cytokine concentrations. In 18 patients with Kawasaki disease and 15 control subjects, adhesion molecule expression was determined by two-colour immunofluorescence staining of blood leucocytes and flow cytometry. Eight cytokines and chemokines were also measured. In patients with Kawasaki disease, mean fluorescence intensity for CD11b before giving intravenous immunoglobulin was significantly higher than in normal subjects ($P < 0.005$). After intravenous immunoglobulin, mean fluorescence intensity for CD11b decreased significantly. With coronary artery lesions present, mean CD11b fluorescence intensity was significantly higher than without coronary artery lesions ($P = 0.005$ before intravenous immunoglobulin; $P = 0.024$ after intravenous immunoglobulin). No differences were seen in CD62L expression on polymorphonuclear leucocytes between patients with Kawasaki disease and normal subjects. CD11b expression on polymorphonuclear leucocytes correlated positively with serum interleukin (IL)-6, IL-10, granulocyte colony-stimulating factor, percentage of neutrophils among white cells and C-reactive protein. Polymorphonuclear leucocytes from patients with Kawasaki disease showed increased CD11b expression, which was associated with increased serum cytokines and appeared to be related to coronary artery lesions.

Keywords: CD11b, coronary artery lesions, cytokine, intravenous immunoglobulin, Kawasaki disease

Introduction

Kawasaki disease (KD), an acute multi-system vasculitis of unknown aetiology that affects primarily infants and children, is a major cause of acquired heart disease [1]. The most widely used therapy is intravenous immunoglobulin (IVIG) given with oral aspirin [2], although approximately 10–20% of patients have persistent fever after completion of IVIG treatment and 5% of patients still may develop coronary artery lesions (CAL) despite this therapy [3]. Clinical evidence suggests that polymorphonuclear leucocytes (PMN) may participate importantly in pathogenesis of vasculitis in KD. During the acute phase, PMN are seen in the peripheral blood, together with an overall leucocytosis including a left shift [4]. Children with KD have high serum granulocyte colony-stimulating factor (G-CSF) concentrations in the acute phase, which decrease rapidly to normal after resolution of the acute phase [5,6]. Furthermore, G-CSF has shown association with coronary artery dilation [7]. Histologically, vascular lesions in the acute phase of KD are associated with evidence of PMN activation and damage to endothelial cells caused by PMN extravasation [8,9]. PMN in the acute phase of KD are likely to damage the endothelium through secretion of oxygen radicals [10], matrix metalloproteinase and elastase [11]. Thus, excessively activated PMN and their inflammatory responses may be associated with coronary artery damage in KD.

One of the major influences on PMN migration from the blood to an inflammatory site involves modulation of adhesion molecules on both PMN and endothelial cells. In particular, proinflammatory mediators induce shedding of L-selectin (CD62L) [12] and increase expression of aMb2

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(CD11b/CD18) on the PMN surface as major events in transendothelial migration [13]. Such altered adhesion molecule expression can influence migration of PMN to inflammatory sites, where they release excessive reactive oxygen species that initiate tissue damage. Johnson *et al.* suggested that up-regulation of aMb2 by antibodies directed against neutrophil cytoplasmic antigens may represent part of the mechanism mediating neutrophil–endothelial cell interactions in systemic vasculitis [14]. However, the specific surface molecules on PMN that participate in the acute phase of KD remain unclear.

In this study, we evaluated modulation of expression of the adhesion molecules CD11b and CD62L on PMN in patients with KD. We also investigated how various circulating cytokines might be related to adhesion molecule expression on surfaces of PMN.

Materials and methods

Study populations

The study was reviewed and approved by the Gunma University Ethics Committee in May 2000. A total of 18 patients with KD were studied after written informed consent had been obtained from their parents. All patients were admitted to our hospital between September 2000 and August 2004 and fulfilled at least five of six criteria for diagnosis of KD. Patients were excluded when clinical or laboratory evidence of any other disease known to mimic KD was present or when KD was atypical. The patients received IVIG (1 g/kg/day for 2 consecutive days), aspirin (30 mg/kg/day) and dipyridamole (2 mg/kg/day). The IVIG used was S-sulphonated human immunoglobulin (Kenketu Venilon I; Teijin Pharma, Tokyo, Japan). CAL were diagnosed by two-dimensional echocardiography performed in all patients at the time of enrolment, at 2 weeks of illness and at 4 weeks of illness by the same paediatric cardiologist. CAL were defined as present if the internal luminal diameter was 3 mm or more in patients < 5 years old or 4 mm or more in patients 5+ years old; if the internal diameter of a segment was at least 1·5 times as large as that of an adjacent segment or if luminal contour was clearly irregular. Control values for cytokines and PMN expression of CD11b and CD62L were obtained from 15 healthy children who had no past history of KD.

Immunostaining and flow cytometric analysis

Blood samples were obtained before IVIG treatment, shortly after IVIG treatment (within 24 h) and in the convalescent phase (14–21 days after IVIG treatment). Samples were stored at room temperature and processed for flow cytometry within 1 h. Expression of adhesion molecules was determined by two-colour immunofluoroscent staining of blood leucocytes and flow cytometry. Saturating concentrations of fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD11b (Beckman Coulter, Tokyo, Japan) and phycoerythrin (PE)-conjugated mouse anti-human CD62L (Beckman Coulter) were added to the blood sample. After cells were exposed to antibodies for 30 min at room temperature in darkness, 0·5 ml of red cell lysing solution (Optilyse C; Beckman Coulter) was added for 10 min of incubation at room temperature in darkness. Cells were then suspended in phosphate-buffered saline and stored in darkness at room temperature for 15 min. After cells were centrifuged at 400 *g* for 5 min and the supernatant discarded, they were resuspended in 0·5 ml of phosphate-buffered saline and analysed with an EPICS XL cytometer (Beckman Coulter) within 5 min. Isotype-matched control antibodies, PE-conjugated IgG_2 and FITC-conjugated IgG_2 (Beckman Coulter) were used to define the cut-off for fluorescence positively as the 99th percentile of the distribution of the cells labelled with control antibody. PMN were gated based on forward- and side-scatter on the display. Ten thousand events were acquired from each sample. Antigen expression was determined as mean fluorescence intensity.

Cytokine and chemokine measurements

We measured eight cytokine and chemokine concentrations in a small volume of serum (50 µl) using a highly sensitive fluorescent microsphere system (Japan Bio-Rad Laboratories, Tokyo, Japan). Blood samples were centrifuged at 1500 *g* for 10 min, and the sera were stored at −80°C until use. Serum tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10 and G-CSF were measured in duplicate using the Bioplex Protein Array system (Japan Bio-Rad Laboratories). Capture antibodies and detection antibodies for microspheres coupled with TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10 and G-CSF were obtained from Japan Bio-Rad Laboratories. The appropriate standard and samples (50 µl) diluted in assay buffer (phosphate-buffered saline, 1% bovine serum albumin-fraction V and 0·02% azide, pH 7·4) were added to each well of a filter plate pre-equilibrated with assay buffer. Samples were incubated with 50 µl of the antibody-coupled microspheres at room temperature for 30 min on a plate shaker oscillating at 300 r.p.m. Freshly diluted detection antibody (25 µl) was added to wells for incubation at room temperature for 30 min on the shaker. Streptavidin–PE (50 µl) was added to wells, which were incubated further at room temperature for 10 min on the shaker. Samples were analysed by the Bioplex suspension array system according to the manufacturer's instructions. The lower limit of detection for each cytokine and chemokine measurement was 3–6 pg/ml.

Statistical analysis

All analyses were carried out by means of an SPSS statistical software package version 13·0 J (SPSS Japan Inc., Tokyo, Japan). Data are expressed as the mean ± standard deviation

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Table 1. Baseline characteristics and clinical outcomes of patients with Kawasaki disease.

	Kawasaki patients	Normal controls
Number	18	13
Male/female	11/7	9/4
Age (months)	24·2 ± 21·1 (range 3–12)	40·5 ± 33·7 (range 3–136)
Days of illness at initial IVIG	4·7 ± 1·2 (range 3–8)	
Clinical symptoms		
6/6	10/18	
5/6	8/18	
4/6	0/18	
Length of fever (days)	6·4 ± 2·9 (range 3–15)	
Coronary artery lesions (+)	3/18	

Data are presented as mean ± standard deviation. IVIG: intravenous immunoglobulin.

(SD) or median with range. Continuous variables were compared with the Mann–Whitney *U*-test or Wilcoxon's rank sum test. Using data from pre-IVIG, post-IVIG and convalescent phase, correlations between cell adhesion molecule expression on PMN and other laboratory values were assessed using Pearson's correlation coefficient. Differences with two-tailed *P*-values below 0·05 were considered significant.

Results

Baseline characteristics and clinical outcomes of patients

Table 1 shows baseline characteristics and clinical outcomes of patients with KD and characteristics of normal subjects. Patients with KD (11 boys and seven girls with an age range of 3–62 months; median 17 months) and normal subjects (11 boys and four girls with an age range of 3–136 months) were studied. CAL were found in three patients with KD (17%).

Serial changes in CD11b and CD62L expression on PMN

Figure 1 shows fluorescence activated cell sorting plots displaying the gates and gating sequences used. Figure 2 depicts CD11b and CD62L expression on PMN in pre-IVIG, post-IVIG and convalescent phases of KD, compared with expression in normal subjects. In patients with KD, mean fluorescence intensity of CD11b before IVIG treatment was significantly higher than that in normal subjects ($P < 0·005$). IVIG treatment caused a significant reduction in mean fluorescence intensity of CD11b from that before treatment ($P < 0·001$). More importantly, the mean fluorescence intensity of CD11b in KD patients with CAL was significantly higher than in KD patients without CAL ($P = 0·005$ pre-IVIG, $P = 0·024$ post-IVIG). In contrast, no significant differences in CD62L expression on PMN were seen between patients with KD and normal subjects or between KD patients with CAL and without CAL.

Serum cytokines and chemokines: correlation with expression of CD11b on PMN

To investigate how cytokines and chemokines are related to CD11b expression on PMN, we measured serum concentrations of eight cytokines and chemokines in KD patients. Serum IL-2, IL-4, IL-6, IL-8, IL-10 and G-CSF in patients with KD before IVIG treatment were significantly higher than in control subjects and IL-6, IL-10, G-CSF and CRP were significantly lower after IVIG treatment than before treatment (Fig. 3). Before IVIG treatment, serum IL-6, IL-10 and G-CSF concentrations tended to be higher in KD patients with CAL than in patients without CAL, while falling short of significance (IL-6, 2118·5 ± 1006·1 *versus* 1356·5 ± 1182·0 pg/ml, $P = 0·164$; IL-10, 87·2 ± 70·7 *versus* 30·9 ± 31·5 pg/ml, $P = 0·074$; G-CSF, 639·6 ± 321·7 *versus* 248·7 ± 363·6 pg/ml, $P = 0·076$). In the post-IVIG phase, serum IL-6 and G-CSF were significantly higher in KD patients with than without CAL (IL-6, 1556·0 ± 1131·3 *versus* 304·9 ± 291·9 pg/ml, $P = 0·027$; G-CSF, 265·6 ± 171·7 *versus* 32·3 ± 32·0 pg/ml, $P = 0·011$), while serum IL-10 tended to be higher in KD patients with CAL (16·1 ± 8·0 *versus* 8·5 ± 6·9 pg/ml, $P = 0·066$). Table 2 shows correlations between CD11b expression on PMN and serum cytokines and chemokines. CD11b expression on PMN showed a positive correlation with serum concentrations of IL-6, IL-10, G-CSF, CRP and percentage of neutrophils.

Table 2. Correlations with CD11b expression on polymorphonuclear leucocytes and other laboratory data.

	IL-2	IL-4	IL-6	IL-8	IL-10
R	0·065	0·067	0·410	0·060	0·574
P value	0·612	0·601	0·001	0·638	< 0·001
	TNF-α	IFN-γ	G-CSG	% PMN	CRP
R	0·014	−0·029	0·473	0·569	0·479
P value	10·910	0·819	< 0·001	< 0·001	< 0·001

IL: interleukin; TNF: tumour necrosis factor; IFN: interferon; G-CSF: granulocyte colony-stimulating factor; PMN: polymorphonuclear leucocytes; CRP: C-reactive protein.

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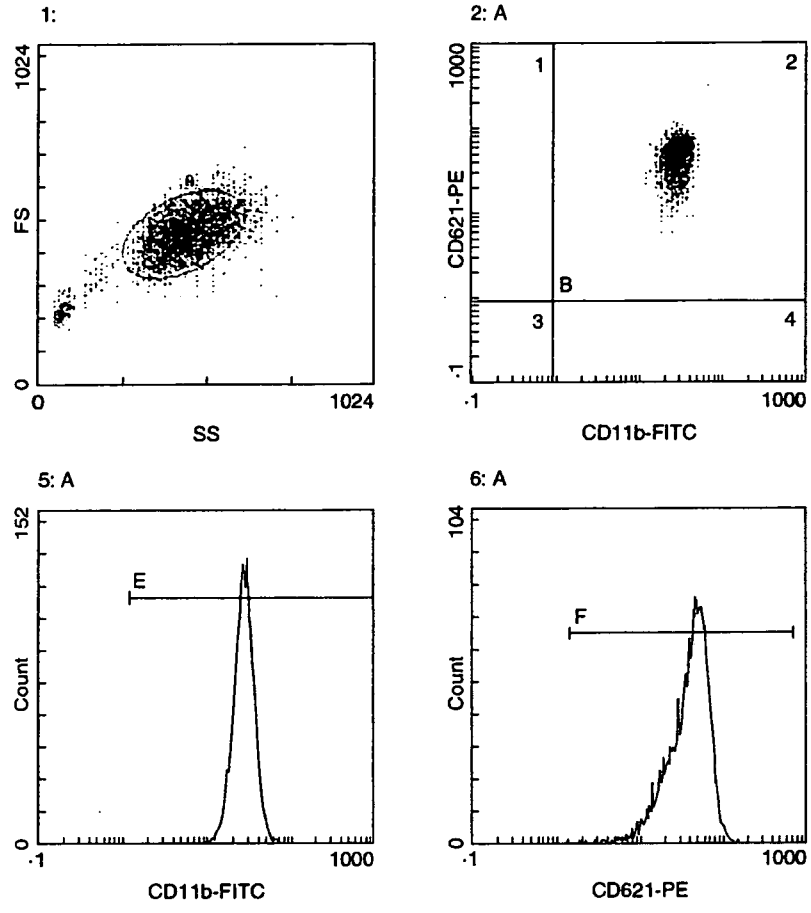

Fig. 1. Flow-cytometer measurements of CD11b and CD62L in peripheral blood of patients with Kawasaki disease.

Discussion

The main findings in this study were, first, that CD11b expression on PMN from patients with KD was increased significantly in the acute phase and decreased rapidly after therapy; secondly, CD11b expression on PMN was higher in KD patients with CAL; and thirdly, CD11b expression on PMN correlated with serum concentrations of several pro- and anti-inflammatory cytokines and chemokines, the percentage of PMN among white cells and CRP. To the best of our knowledge, this is the first report to demonstrate increased CD11b expression on PMN in the acute phase of KD.

Although we could not confirm the pathogenetic consequences of increased expression of CD11b on PMN and the high concentrations of various cytokines in acute KD

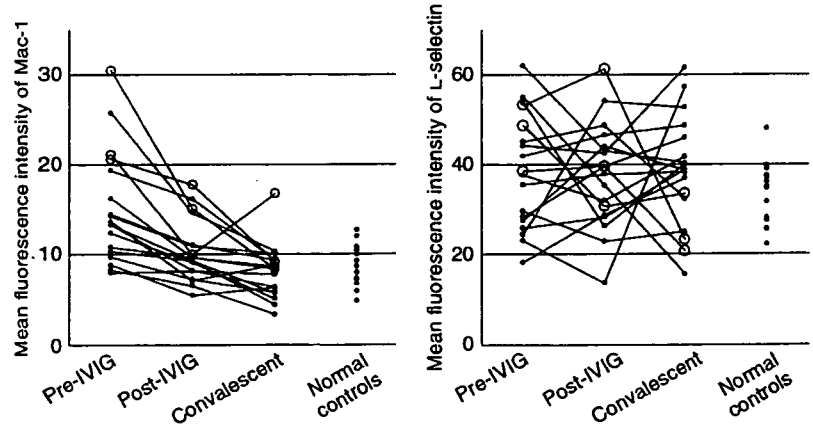

Fig. 2. Serial changes of mean fluorescence intensity of CD11b and CD62L on polymorphonuclear leucocytes. Open circles, Kawasaki disease patients with coronary artery lesions; closed circles, Kawasaki disease patients without coronary artery lesions. IVIG, intravenous immunoglobulin.

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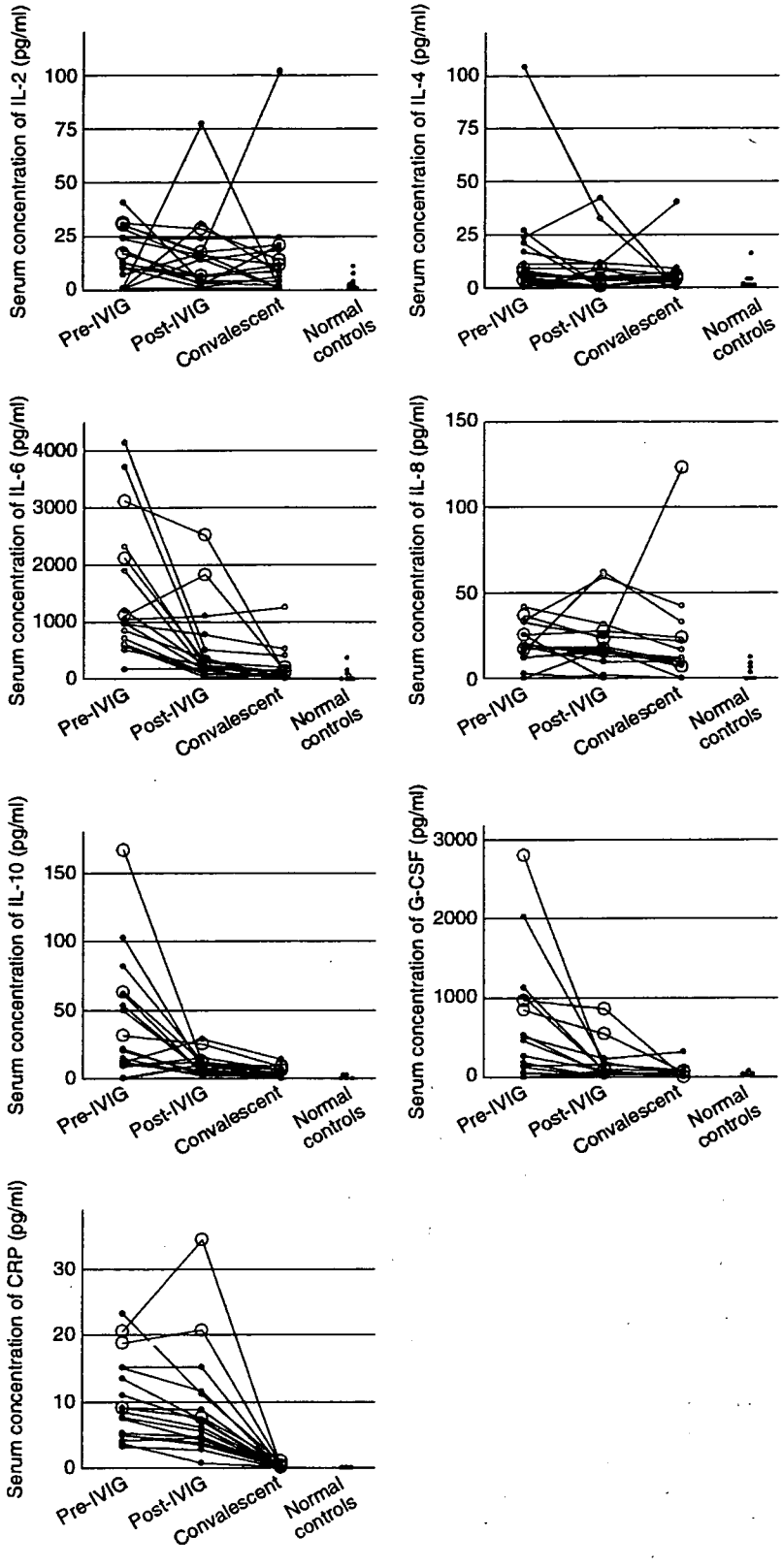

Fig. 3. Serial changes of serum cytokine levels in Kawasaki disease patients and normal controls. Open circles, Kawasaki disease patients with coronary artery lesions; closed circles, Kawasaki disease patients without coronary artery lesions. IVIG, intravenous immunoglobulin; IL, interleukin; G-CSF, granulocyte colony stimulating factor; CRP, C-reactive protein.

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in vitro, we postulated as follows. In general, CD11b promotes firm attachment of the PMN to the endothelium, which allows transendothelial migration into inflamed tissues. CD11b expression is up-regulated mainly by proinflammatory cytokines such as IL-6 and G-CSF. In addition, PMN generate large amounts of reactive oxygen species (superoxide radicals, hydrogen peroxide and hypochlolite) and release toxic granules containing myeloperoxidase. These toxic compounds may be associated with cardiovascular system injuries in KD patients. Therefore, enhanced expression of CD11b induced by circulating inflammatory cytokines is likely to promote adhesion and transendothelial migration of leucocytes in KD. In turn, these migrated leucocytes are likely to be an important source of inflammatory cytokines that further induced endothelial cell surface expression of intercellular adhesion molecules 1–3, resulting in progression of vasculitis in KD. Indeed, histopathological findings in KD show PMN adherent to endothelial cells and infiltration of neutrophils, monocytes and lymphocytes into the walls of small and medium-sized blood vessels [9,15–17]. However, our study lacked sufficient data to demonstrate that increased CD11b expression on polymorphonuclear cells actually promotes adhesion and transmigration of leucocytes in acute KD patients. Further examination using autopsy specimens or animal models of KD vasculitis is needed to prove our hypotheses.

Several clinical studies have reported that activation of PMN may contribute to the severity of KD. Suzuki *et al.* reported that increased peripheral blood white blood cell counts, particularly neutrophil counts with a morphological left shift, could be helpful in predicting CAL [18]. Niwa *et al.* suggested that oxygen radical generation and proteolytic enzymes produced by neutrophils induce endothelial cell damage in KD [10]. Senzaki *et al.* demonstrated a positive association between plasma concentrations of neutrophil proteinases such as elastase and matrix metalloproteinases with CAL formation in acute-phase KD patients [11]. Several studies have suggested that concentrations of proinflammatory cytokines known to stimulate neutrophils could predict CAL [18,19]. Taken together, these results suggest that PMN from the CAL group may have strong adhesive capacities as well as enhanced ability to release cytotoxic substances. However, further examinations will be needed to use CD11b expression on PMN as a predictor of CAL, because only three patients with CAL were included in this study.

We confirmed evaluation of serum concentrations of six of the eight cytokines and chemokines that we measured during the acute phase of KD. Evaluation of the anti-inflammatory cytokine IL-10 as well as proinflammatory cytokines correlated positively with CD11b expression on PMN. The latter findings would seem to be a matter of debate, because IL-10 has been reported to reduce the inflammatory response of monocytes and macrophages and to inhibit cytokine production by T helper 1 cells and inhibit CD11b expression on PMN *in vitro* [20]. However, in acute inflammation, IL-10 production is considered increasingly to be a modulatory response to proinflammatory cytokine production, tending to counteract excesses of proinflammatory mediators. In this study, serum concentrations of IL-10 showed strong correlations with serum concentrations of IL-6 ($R = 0{\cdot}614$, $P < 0{\cdot}0001$), G-CSF ($R = 0{\cdot}705$, $P < 0{\cdot}0001$), IL-8 ($R = 0{\cdot}214$, $P = 0{\cdot}036$) and CRP ($R = 0{\cdot}381$, $P = 0{\cdot}0001$). Thus, CD11b expression on PMN may stimulate synthesis indirectly, via proinflammatory cytokines.

No significant difference in CD62L expression on PMN was observed in the various phases of KD treatment in this study. The results are consistent with a report finding that during acute KD changes in soluble L-selectin in serum were not significant, despite dramatic changes in soluble E-selectin and P-selectin [21,22]. However, diminished L-selectin expression on cell surfaces as the result of a shedding process, associated with increased soluble L-selectin in blood, has been reported during PMN activation. Decreased CD62L expression, associated with increased CD11b surface expression, has been described in patients with inflammatory diseases other than KD [23]. Unfortunately, no such disease controls were included in the present study. Further studies will be necessary to determine whether the finding is specific to KD.

In conclusion, PMN from KD patients showed increased CD11b expression. This abnormality is associated with increased serum cytokines and chemokines, and appears to predict CAL. Understanding of the molecular mechanisms underlying ligand binding by CD11b, as well as the roles of CD62L in PMN functions, should guide us in regulating neutrophil function in KD patients.

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risk index increased with higher ponderal index (weight/height^3).[7]

If the clinical end point of treatment of pediatric obesity is prevention of adult cardiovascular disease, then creating a standardized MS definition may help identify the highest risk children who need immediate medical intervention. Conversely, if the clinical end point is prevention of type 2 diabetes mellitus, then following the American Heart Association and American Diabetes Association recommendations for lifestyle intervention may be sufficient.[11] Perhaps what is ultimately needed is a paradigm shift from MS to a composite "risk index" score that alerts patients and physicians of all potential health problems related to obesity. The "risk index" score would be composed of both physical and nonphysical attributes that are associated with obesity-related heath problems. Risk score cutoff points would not be absolute but "continuous" and dependent on the targeted health problem.

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External validation of a scoring system to predict resistance to intravenous immunoglobulin

To the Editor:

Egami et al[1] described a new scoring system that predicts resistance to intravenous immunoglobulin (IVIG) treatment in patients with Kawasaki disease (KD). Because no special equipment is needed, this score can be used in almost any children's hospitals and pediatric services and should help guide clinicians in decision making regarding primary therapy. Although the scoring has acceptable predictive values in the authors' database, they did not perform an external validation that involved completely new data to further assess the generalizability of their proposed scoring model. Therefore, we performed external validation using a database made up from clinical records of 750 consecutive KD patients treated with intravenous immunoglobulin (IVIG).[2]

Of the 750 KD patients, 137 were classified as having resistance to IVIG. We defined IVIG nonresponders as KD patients with persistent fever (≥37.5°C) that lasted more than 24 hours. In our database, the area under the receiver operating characteristics curve was 0.74 (95% confidence interval, 0.69 to 0.79) on Egami et al's scoring. Using a cutoff point of ≥3 with this prediction score, we could identify IVIG nonresponders with 66% sensitivity and 72% specificity and coronary artery lesions with 70% sensitivity and 68% specificity. We also compared the score of Harada[3] with the score of Egami et al. With the score of Harada, the area under the receiver operating characteristics curve was 0.62 (95% confidence interval, 0.56 to 0.67). IVIG nonresponders at a cutoff of ≥4 could be predicted at 69% sensitivity and 47% specificity; coronary artery lesions, at 46% sensitivity and 86% specificity. Although the score of Egami et al was superior to that of Harada, the prediction performance was not as high as the authors reported.

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治療　ガンマグロブリン無効例への対応

無効例の予測および層別化

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Risk stratification and prediction of resistance to intravenous immunoglobulin in Kawasaki disease

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Abstract

Intravenous immunoglobulin is effective to resolve inflammation of Kawasaki disease and reduce the incidence of coronary artery abnormalities. However approximately 20% of patients with Kawasaki disease had persistent or recurrent fevers after intravenous immunoglobulin and are considered to have a high risk for coronary artery abnormalities. Recently, we developed a new risk score that resistance to intravenous immunoglobulin could be identified with high sensitivity and specificity in advance using seven laboratory and demographic variables available before initiation of primary therapy. In this article, we will review prediction of intravenous immunoglobulin unresponsiveness. In addition, we will focus on the risk stratification of primary therapy using the risk score.

Key words: Kawasaki disease, risk score, intravenous immunoglobulin, prednisolone

はじめに

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，無治療では高率に冠動脈病変を合併することが知られている[1]．作用機序はいまだ不明であるものの免疫グロブリン超大量療法(IVIG)が臨床症状や炎症マーカーの改善，冠動脈病変合併の抑制に有効であると報告され，現在標準的な治療として川崎病患者に対し広く使用されている[2]．しかし10-20%はIVIGにより解熱しないIVIG無効例であり，冠動脈病変合併例の大部分がIVIG無効例に含まれる[3]．著者らはIVIGにて初期治療を行った750症例を解析することによってIVIG無効例か否かを予測する因子を見いだし，7つの変数からなるリスクスコアを作成した[4]．

本稿ではIVIG無効例を予測するリスクスコアとその意義を，またリスクスコアを用いた川崎病初期治療層別化の可能性について解説する．

1. IVIG無効例を予測する因子とリスクスコア

2000年9月-2006年1月の期間に群馬大学関連13病院において川崎病の診断でIVIGを施行

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0047-1852/08/¥40/ft/JCLS

表1 リスクスコアの項目と点数

	カットオフ値	点数
Na	133 mmol/*l* 以下	2点
AST	100 IU/*l* 以上	2点
治療開始(診断)病日	4病日以前	2点
好中球%	80%以上	2点
CRP	10 mg/dl 以上	1点
月齢	12カ月以下	1点
血小板数	30万/mm^{3}以下	1点

された連続750例を用いてIVIG無効例を予測するモデルを作成した．治療前に冠動脈拡大性病変が存在していた症例と初期治療としてステロイド投与を行った症例は除外した．なお，川崎病と診断した後速やかにIVIGを施行したため，診断病日と治療開始病日は同病日である．まず後方視的に患者背景，IVIG投与前の血液検査所見，初期治療不応，再燃，冠動脈病変の有無を調査した．IVIG無効例(初期治療不応例と再燃例)を従属変数に単変量解析を行って関連のある血液検査結果を抽出し，それらの変数に性別，月齢，治療開始病日の3変数を加え，ロジスティックモデルを用いて多変量解析を施行し転帰を予測する回帰式を作成した．そして実際の臨床現場で使いやすいように，作成された回帰式を簡易スコア化したリスクスコアを作成した．

対象患者のうち，154例(20.5%)がIVIG無効例，54例(7.2%)が冠動脈病変(経過中の一過性拡大を含む)合併例であった．多変量解析を行った結果，月齢，治療開始病日，好中球%，血小板数，AST，Na，CRPがIVIG無効例と関連する独立変数であることが明らかとなった．得られた偏回帰係数を整数のスコアに近似して重みづけを行い，11点満点のリスクスコアを作成した(**表1**)．リスクスコアとIVIG無効例，冠動脈病変合併例の割合を**図1**，2に示す．リスクスコアの点数が高いほどIVIG無効例と冠動脈病変合併例の割合が増していくことがわかる．リスクスコアのカットオフ値を5点以上と設定したところIVIG無効例を予測する確率は感度76%，特異度80%(ROC曲線下の面積0.85)，冠動脈病変を予測する確率は感度81%，特異度72%(ROC曲線下の面積0.84)と高い予測確率でIVIG無効例と冠動脈病変合併例を治療開始前に判定することが可能であった．

2. リスクスコアに抽出された変数の臨床意義と注意点

今回著者らが作成したリスクスコアのうち血液検査項目から抽出された5項目は，以前より冠動脈病変合併に関連する因子として報告されている[5-8]．IVIG無効例と冠動脈病変の合併は関連が深いことから，いずれの項目も血管炎の重症度を反映するためではないかと考えられる．乳児がIVIG無効例に関連する原因は不明であるが，過去の大規模疫学調査では冠動脈病変と関連が証明されている[9]．ではなぜ治療開始病日が早いとIVIG無効例が多くなるのであろうか．

診断の遅れが血管炎の増悪による冠動脈のリモデリングを促進し，冠動脈瘤発生に寄与することは周知の事実であるため，多くの小児科医は川崎病をより早期に診断してIVIGを行うことが予後改善につながるとして急性期治療を行ってきた[1]．早期治療例にIVIG無効例が多い結果は前述の治療方針に対する大いなる矛盾であるが，治療開始病日が早いとIVIG無効例が多いことは川崎病全国調査からも明らかとなっており間違いのない事実であろう[10]．早期治療例にIVIG無効例が多い理由として2つの可能性が考えられる．第1の仮説は早期に症状がそろう症例はより重症度が高いためIVIGが効きにくい可能性があるということである．この仮説を検証するため，前述の症例を対象とし治療開始病日以外の6変数を用いてスコア点数を算出したうえで，4病日以前にIVIG投与を行った早期治療群，5病日以降にIVIGを行った通常治療群とを比較検討した．結果，早期治療群は通常治療群に比べてIVIG無効例(31% vs 13%，$p<0.001$)と冠動脈病変合併例(12% vs 4%，$p<0.001$)は有意に多く，またリスクスコア点数も有意に高値であった(2.9±2.5 vs 2.1±2.0，$p<0.001$)．これらの結果からは，4病日以前に診

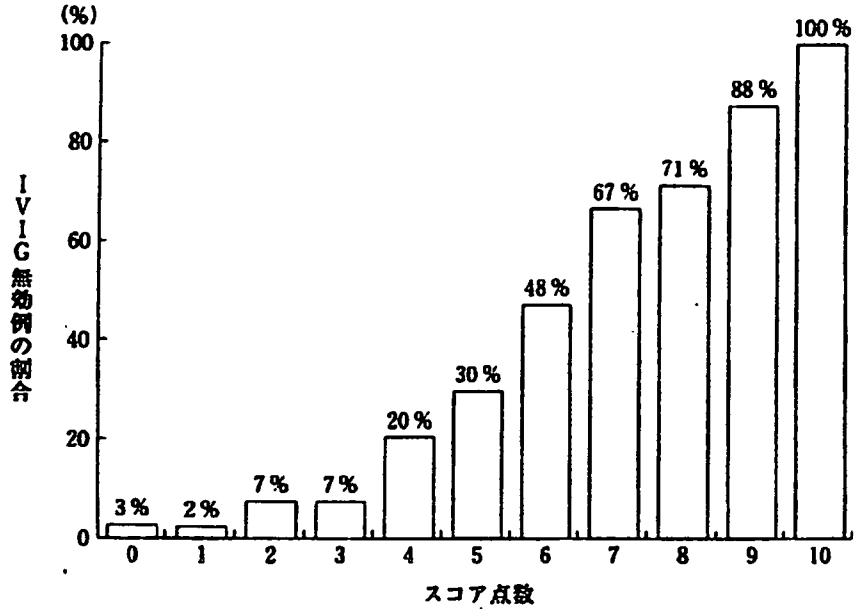

図1 リスクスコア点数とIVIG無効例の割合

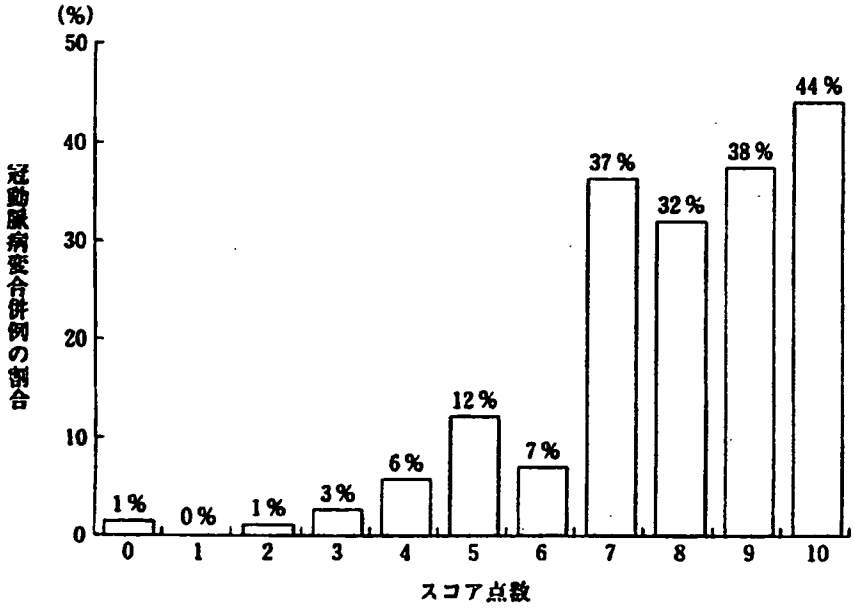

図2 リスクスコア点数と冠動脈病変合併例の割合

断された患者は5病日以降に診断された患者に比べて重症度が高いため，IVIGの効果が乏しく冠動脈予後も悪い可能性が考えられる．一方，理論上は早期にIVIGを投与すると血管炎を悪化させるといった第2の仮説も考えられる．この仮説を証明するためには早期診断例のみを対象にして，早期治療群と待機群とに無作為割付を行うcontrol studyが必要であるが，このような研究はまだ行われていない．確定的なことを述べることは困難ではあるが，前述したように早期治療群がよりリスクスコアが高値であることや，早期治療が血管炎を悪化させるといった証拠がない現時点においては，早期治療をためらう根拠は乏しく，冠動脈のリモデリングを抑制するためにより早期に血管炎を抑制する治療戦略を選択すべきであろう．

表2 リスク別・治療別の臨床経過と冠動脈予後

低リスク患者	IVIG (n=62)	IVIG+PSL (n=61)	p value
治療抵抗例, n(%)	3(4.8)	4(6.6)	0.717
初期治療不応例, n(%)	3(4.8)	2(3.3)	1.000
再燃例, n(%)	0(0)	2(3.3)	0.244
経過中の冠動脈病変合併例, n(%)	1(1.6)	0(0)	1.000
1カ月時の冠動脈病変合併例, n(%)	0(0)	0(0)	—
治療開始後解熱するまでの日数	1.0±1.2	0.4±1.4	<0.001
治療開始後CRP陰性化するまでの日数	10.0±6.0	8.3±4.1	0.114
高リスク患者	**IVIG (n=26)**	**IVIG+PSL (n=29)**	**p value**
治療抵抗例, n(%)	13(50)	5(17.2)	0.020
初期治療不応例, n(%)	13(50)	3(10.3)	0.002
再燃例, n(%)	2(7.7)	2(6.9)	1.000
経過中の冠動脈病変合併例, n(%)	9(34.6)	2(6.9)	0.017
1カ月時の冠動脈病変合併例, n(%)	3(11.5)	0(0)	0.099
治療開始後解熱するまでの日数	2.7±2.4	0.9±1.6	0.025
治療開始後CRP陰性化するまでの日数	14.0±7.4	8.5±3.3	<0.001

リスクスコアを実際の臨床で使用するために幾つかの注意点がある．まず血液検査の5項目だが，初診から診断時までに複数回にわたって血液検査を施行した場合は，好中球%・AST・CRPは最高値を，血小板数・Naは最低値をリスクスコア算出に用いる値とする．また，できるかぎりIVIG開始病日の採血を行うことが望ましい．リスクスコアを作成したコホートは川崎病と診断後速やかにIVIGを施行しているため，川崎病診断病日と治療開始病日(IVIG開始病日)は同一病日である．そのため，5病日以前に川崎病と診断してもアスピリン投与のみで5病日まで待機し，5病日以降にIVIGを投与するといった治療方針を採用している施設の患者に対して本リスクスコアを用いることはできない．リスクスコアを作成したコホートと重要な背景因子が異なってしまうため，同一のモデルでIVIG無効例を判定することができないからである．同様の理由で5病日以前に川崎病と診断した患者に対して5病日まで待ってIVIGを行い，治療開始病日の項目を0点にすることによってスコア点数を下げるといった人為的操作は全く意味をなさない．

3. リスクスコアを用いた初期治療層別化の可能性

前述したリスクスコアを用いることによっておよそ初期治療開始前にIVIG無効例を予測することが可能となった．今後の課題はリスクスコアを用いて川崎病患者の予後を改善させるためにどのような治療戦略を行うかであろう．血管炎の進行に伴い，冠動脈の血管構造が破壊され遠心性の拡大を来すという病理学的な機序を考えると，より早期に血管炎を鎮静化させ，冠動脈のリモデリングを抑制することが冠動脈病変発生を抑制するために重要であると考えられる．IVIGは約8割の患者で有効であるため，治療開始前に治療反応性を予測したうえで，IVIG無効例であることが予想される重症川崎病患者に対してより強力な初期治療を行うことによって，最大限の効果と最小限のリスクで予後を改善させることができるものと考えられる．より強力な初期治療の候補として，ステロイド，シクロスポリンといった免疫抑制剤[11][12]や抗TNF-α抗体(infliximab)[13]などがあげられる．著者らは初期治療としてのIVIG+プレドニゾロン療法(IVIG+PSL)の有用性を検討する多施設共