

An Elevated Value on Drug-Induced Lymphocyte Stimulation Test for Immunoglobulin Is an Immunological Abnormality of Kawasaki Disease

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Key Words

Drug-induced lymphocyte stimulation · Immunoglobulin · Immunological abnormality · Kawasaki disease

Abstract

Background: Kawasaki disease (KD) is an acute febrile vasculitis in childhood. Currently, treatment with 2 g/kg of intravenous immunoglobulin (IVIG) is recommended. Previously we had encountered a patient with KD who showed persistent fever and a severe eruption after IVIG treatment. Using a drug-induced lymphocyte stimulation test (DLST), he was positive for an immunoglobulin product. The aim of this study was to clarify the importance of a positive value for the DLST for immunoglobulin products in KD patients. **Methods:** Subjects were 30 confirmed KD patients treated with IVIG at the Kagoshima Medical Association Hospital. DLST values were compared between patients with additional events and those without additional events using the stimulation index (SI = value of ³H-thymidine absorption with antigen/without antigen). Additional events were defined as symptoms observed after IVIG that were considered unexplainable by the symptoms of KD alone. **Results:** DLST results were evaluated in 13 patients with additional events and 17 patients without additional events. Elevated DLST values were observed not only in patients with additional

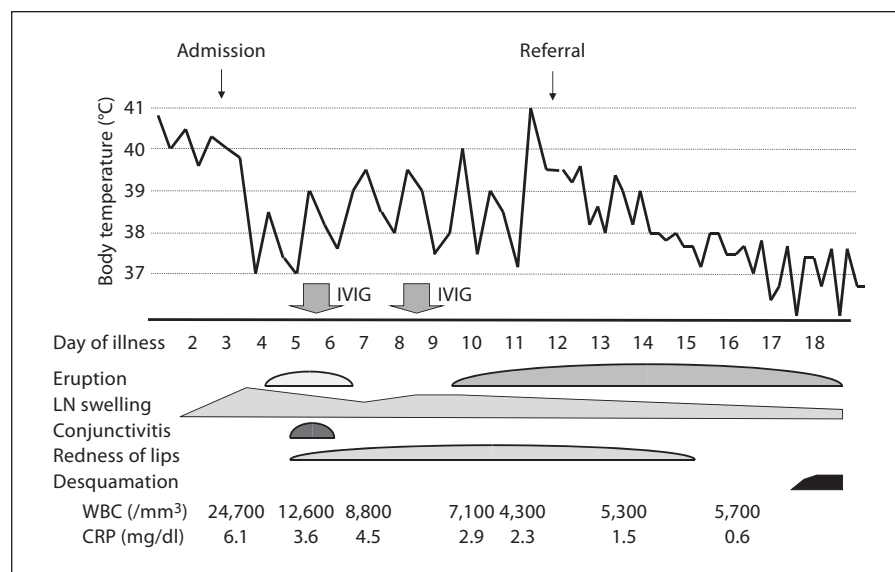
events but also in those without additional events. Elevated SI values were observed in the initial 14 days after IVIG and the SI values in this period were significantly higher than those after day 14 (initial 14 days, $n = 20$, $194 \pm 112\%$; after day 14, $n = 10$, $117 \pm 66\%$, $p = 0.010$). **Conclusions:** Elevated SI values of DLST for immunoglobulin products are not related with additional events. Our results show they may represent one of the immunological abnormalities of KD.

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Background

Kawasaki disease (KD) is an acute febrile vasculitis that occurs in childhood and is a leading cause of acquired heart disease in Japan and United States [1, 2]. Currently, 2 g/kg of intravenous immunoglobulin (IVIG) with oral administration of aspirin is the recommended treatment [1, 2]. The therapeutic efficacy of IVIG has been well established, not only in KD but also in many autoimmune/inflammatory diseases [3, 4]. IVIG has been used in many diseases, but occasionally adverse effects occur with its use. Common and mild adverse effects include headache, nausea, chills, and fever [3, 4]. In rare instances, more severe adverse effects, such as aseptic meningitis, hemolytic anemia, thrombosis, anaphylactic

Fig. 1. Clinical course of the patient. Despite initial IVIG treatment, fever persisted although white blood cell counts, CRP value, and most of the main manifestations of KD improved. After the second IVIG administration, an eruption appeared that was different from that observed during the first IVIG treatment. Admission = Admission to Kagoshima Medical Association Hospital; referral = referral to Kagoshima University Hospital; LN swelling = lymph node swelling.



shock and acute renal failure, have been reported [3, 4]. Dermatologic adverse effects are also rare and include pruritus, rash and erythema multiforme [5]. We encountered a patient with KD who showed persistent fever and severe eruption after IVIG, which could not be explained by KD alone. He was also positive for an immunoglobulin product using the drug-induced lymphocyte stimulation test (DLST). DLST is a method for detecting sensitized lymphocytes and can be applied to many causative drugs and allergic symptoms [6]. DLST is the most commonly used in vitro test to detect causative drugs of drug allergies.

A positive DLST value for the immunoglobulin product observed in our patient may indicate an allergic reaction to immunoglobulin products, but it may also represent an immunological abnormality of KD. To illuminate this issue, DLST for immunoglobulin products were examined in KD patients. The aim of this study was to clarify the importance of a positive DLST value for immunoglobulin products in KD patients.

Patients and Methods

Confirmed KD patients were enrolled after referral and admission to the Kagoshima Medical Association Hospital and were treated with IVIG between January 2006 and June 2008. Their diagnosis of KD was established using the Japanese criteria [7]. When informed consent was obtained from the patient's parents, a blood sample for DLST was taken during the convalescent phase of the disease. The same rod immunoglobulin product used for

each patient's treatment was prepared as the antigen for his/her DLST. DLST results for immunoglobulin products were evaluated using the conventional method [6] by a commercial laboratory testing service company (SRL, Tokyo, Japan). Briefly, patients' mononuclear cells and antigen solution were incubated for 48 h. They were then incubated for additional 24 h with a ³H-thymidine solution. After washing and lysing the cells, the quantity of ³H-thymidine absorbed by the cells was measured. The stimulation index (SI) was calculated by the following formula: SI = ³H-thymidine absorption of mononuclear cells with antigen/without antigen. An SI value above 180% was considered positive. SI values were compared between patients with additional events and those without additional events. Additional events were defined as symptoms observed after IVIG treatment and considered unexplainable by the symptoms of KD alone.

A correlation coefficient of SI values and number of days after IVIG initiation was calculated using Pearson's correlation coefficient. The Mann-Whitney U test was used to compare the SI values between early and late periods of the disease. The data were processed using the statistical program SPSS 14.0 (SPSS, Chicago, Ill., USA). $p < 0.05$ was considered statistically significant.

This study was approved by the Institutional Review Board of Kagoshima University Graduate School of Medical and Dental Sciences.

Case Report on the Patient Who Instigated This Study

A 22-month-old previously healthy boy presented with high fever and left cervical lymph node swelling. The patient's clinical course is shown in figure 1. He was referred and admitted to Kagoshima Medical Association Hospital on the 2nd day of illness. Because of an elevated white blood cell count (WBC) and C-reactive protein (CRP) value, he was given antibiotics (flomoxef) intravenously after a diagnosis of bacterial lymphadenitis. He developed a miliary rash on his lower abdomen, bilateral conjunctivitis, red lips, strawberry tongue, and bilateral red palms and soles. Once his diagnosis of KD was confirmed, IVIG treatment

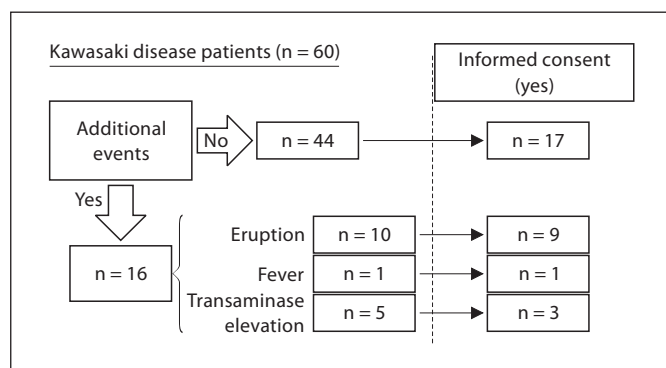


Fig. 2. Study outline. Informed consent was obtained from the parents of 30 patients, who were enrolled in the present study. Thirteen patients had additional events and 17 patients did not have any additional events.

(25 g, 2.3 g/kg of Venoglobulin IH®; Benesis, Tokyo, Japan) and oral administration of aspirin (30 mg/kg/day) were initiated on the 5th day of illness. Although the rash and conjunctivitis were alleviated, his fever persisted, and then a second IVIG by another company (25 g of Glovenin-I®; Takeda Pharmaceutical, Tokyo, Japan) was administered on the 8th day of illness. After the second dose of IVIG, he developed a rash on his abdomen and extremities, which was different from that observed on the 4–5th day of illness.

Because the rash progressed and the fever persisted, he was referred to Kagoshima University Hospital on the 12th day of illness. During the referral, he showed a high fever of 39.4°C, bilateral conjunctivitis, left lymph node swelling (2.5 cm). Miliary erythematous skin lesions were observed on the abdomen and dys-hidrotic eczematous skin lesions were mainly on the palms and soles. Since the laboratory tests revealed improved values of WBC and CRP compared with those before IVIG administration, no additional treatment was applied, and his body temperature gradually normalized. Echocardiography was performed every 2 or 3 days during admission, and no abnormal findings were observed during the disease course.

DLST for immunoglobulin revealed an elevated SI value for Venoglobulin IH (188%) and a slightly elevated value for Glovenin-I (153%) on the 31st day of illness. The SI value of DLST for Venoglobulin-IH decreased to 161% on the 89th day of illness and to 130% by the 130th day of illness. DLST for material contained in Venoglobulin-IH was performed on the 89th day of illness. SI values for polyethylene-glycol was 135% and 111% for D-sorbitol.

Results

During the study period, 60 patients were treated with a single infusion of 2 g/kg of IVIG and oral administration of 30 mg/kg aspirin. IVIG was initiated on the day 4.7 ± 1.2 (range 2–9) of illness. Of these, 16 patients

showed additional events after IVIG treatment (fig. 2). Among the additional events, eruption was observed in 10 patients (including 3 with fever), fever without eruption in 1 patient, and transient elevated transaminase values in 5 patients. Additional events were initially observed between the 9th and 17th day of illness. Eruptions were initially observed between the 9th and 17th day of illness (day 4–11 after IVIG initiation), fever on 11th day of illness (day 5 after IVIG initiation), and elevated transaminase values at 11th and 12th day of illness (day 6 after IVIG initiation).

Informed consents were obtained from 30 patients' parents. Of these, 13 patients showed additional events; 9 patients with eruption including 3 patients with fever, 1 patient with fever, and 3 patients with elevated transient transaminase values. Seventeen patients showed no additional events.

DLST for immunoglobulin products were examined on day 17 ± 8 (range 12–45) of illness and day 13 ± 8 (range 6–39) after IVIG initiation. Elevated DLST values were observed not only in patients with additional events, but also in patients without additional events (fig. 3). A 3-year-old boy with no additional events showed a 561% SI value of DLST at day 10 after IVIG initiation. He was admitted on the 3rd day of illness and showed elevated values of white blood cell counts (14,200/ μ l), CRP (6.2 mg/dl) and transaminases (aspartate aminotransferase 128 IU/l, alanine aminotransferase 73 IU/l). The next day, his 6 main manifestations of KD were confirmed, and he was given IVIG. His fever responded to IVIG administration, abnormal values of transaminases were gradually normalized, and he showed no recurrence. His SI value for DLST of the immunoglobulin product decreased to 129% at day 66 after IVIG initiation. A 4-year-old girl with no additional events showed an SI value of 288% on the 16th day of illness (day 9 after IVIG initiation). Her elevated SI value also decreased to 116% on day 84.

SI values of DLST for the immunoglobulin product showed a tendency for a negative correlation with number of days after IVIG initiation ($r = -0.346$, $p = 0.061$; fig. 4). Elevated SI values were mainly observed in the first 14 days after IVIG. SI values in this period were $194 \pm 112\%$ ($n = 20$), and they were significantly higher than those after day 14 ($n = 10$, $117 \pm 66\%$, $p = 0.010$).

Among the 10 patients with additional events of eruption, DLST for aspirin were examined in 7. Their SI values were $113 \pm 34\%$ (72–163%). The possibility of antibiotic allergies was suspected by clinical courses in 4 patients with additional events of eruption and 1 patient with fever. Their DLST for antibiotics used were examined, and

positive SI values were observed in 2 patients; 397% for panipenem in a patient with eruption and fever and 651% for flomoxef in a patient with fever. Their SI values for the immunoglobulin product were 99% and 93%, respectively.

Discussion

The presented case was positive for an immunoglobulin product as measured by DLST. His DLST values for material contained by immunoglobulins were not elevated. In the examination of DLST values of 30 KD patients, no relationship between additional events and DLST values for immunoglobulin products were observed. Additionally, significantly higher DLST values were observed in the first 14 days after IVIG than after day 14, suggesting a relationship between DLST values and activity of KD. Activation of monocytes/macrophages coincides with a variety of elevated cytokines and a variety of auto-antibodies have been observed in KD [8–11]. These immunological abnormalities are considered to be a special characteristic of KD. Positive DLST values observed in the present study might be one of these immunological abnormalities in KD and not an allergic reaction for immunoglobulin. A limitation of this study is that the DLST values prior to IVIG were not shown. There is a chance that positive DLST values for immunoglobulin might be observed before a KD episode. However, this possibility was unlikely because the reported case and the 2 patients with positive DLST values shown in the results later had normal DLST values. However, there remains a possibility that DLST values for immunoglobulin are elevated by the immunological abnormality of KD alone and so are not related to IVIG.

In the present study, eruptions were observed in 10 of 60 patients (17%). This incidence may be slightly higher than as usual. Since this study concerned allergic reactions for immunoglobulin products, an over-sensitive screening for adverse events might have caused this higher incidence. The present study showed that elevated SI values were present in some patients without additional events. It is important that the definition of no additional events was made with this over-sensitive screening condition.

Vecchietti et al. [5] reported 4 patients with a characteristic severe extensive eczematous reaction that occurred 10 days after IVIG for polyradiculoneuritis. They showed a pruriginous rash that started in the form of dysidrotic lesions of the palms. Their symptoms were identi-

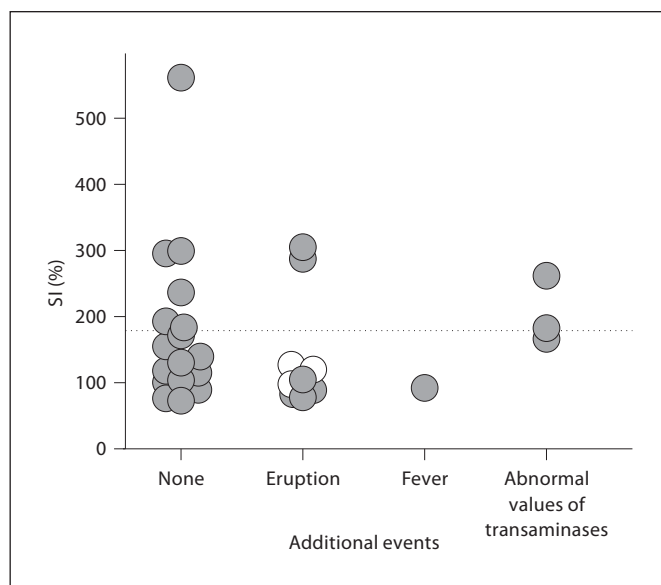


Fig. 3. Relationship between SI values of DLST for immunoglobulin products and additional events after intravenous immunoglobulin treatment. Elevated SI values were observed not only in patients with additional events but also in patients without additional events. Open circles indicate patients with additional events of fever in addition to eruption. Additional events were symptoms observed after IVIG and considered unexplainable by the symptoms of KD.

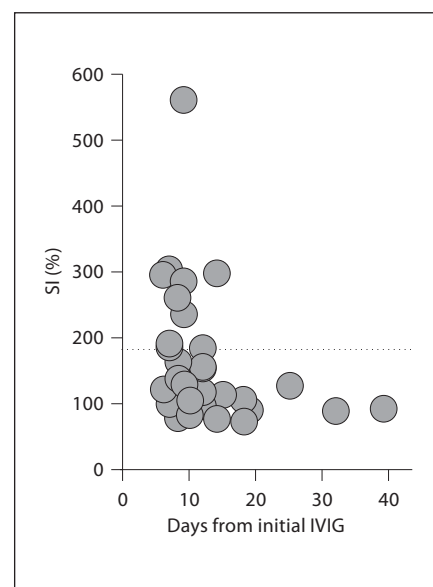


Fig. 4. Relationship between SI values of DLST for immunoglobulin products and number of days after IVIG treatment. Elevated SI values of DLST were observed in the initial 14 days after IVIG treatment. After that time point, elevated SI values were not observed.

cal to those of the first patient we presented. The eruption observed in our patient was, therefore, explained by an adverse reaction to IVIG.

There are problems performing DLST in children. As the test is expensive and needs a 5–10 ml blood sample, it is not easy to perform DLST in children. Still, DLST remains useful for determining a causative drug among many candidate ones. Although its sensitivity is not high, its specificity is sufficient. Saito et al. [6] reported the sensitivity of 25% and specificity 93%. From these findings, drug allergies can easily be determined in patients who have a positive DLST value, whereas it is difficult to deny a drug allergy in a patient with a negative DLST value. However, cross-reactivities among drugs or examinations in immunologically abnormal situations, such as KD, have not been reported. This study had a small number of subjects and there were no control patients, thus it was difficult to draw a firm conclusion. However, it is important that information about one of the immunological

abnormalities of KD is available. When a patient has a positive DLST value for immunoglobulin products with additional events, it possibly represents an immunologic abnormality of KD. However, the possibility of an allergic reaction remains.

Conclusions

Elevated SI values of DLST for immunoglobulin products are observed in some KD patients. Their values are not related to additional events. Elevated SI values may be one of the immunological abnormalities of KD.

Acknowledgment

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