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G. 知的財産の出願・登録状況

１、特許取得

なし

２、実用新案登録

なし

３、その他

なし

資料

川崎病に対するインフリキシマブ使用における目安

インフリキシマブを川崎病に使用する場合、下記の要領で使用することが望ましい。この基準は、班研究初年度の指針であり、今後、適宜変更がありうる。

6、適応

5） 充分量の超大量γグロブリン静注療法不応例（いわゆる難治性川崎病）に使用する。

6）生ワクチン種後1ヶ月以上経過している。

7）下記感染症スクリーニングで感染症の可能性が否定されている。

8）冠動脈瘤発症予防に対しては第10病日以内の使用が望ましい。

7、投与前の感染症スクリーニングとして、下記項目を行っておく。

7）結核患者との接触歴の有無

8）胸部X線

9）造影なしの胸部CT（施行が望ましい）

10） 血液、尿細菌培養、細菌培養など

11） B型肝炎ウイルス(HBs抗原、HBe抗原)

12） クオンティフェロン検査（望ましい）

8、投与方法

5mg/kg（最大100mg）を生理食塩水100mlで希釈して、2時間以上かけて静注する。

9、短期及び長期の副作用出現の可能性がある。投与後1時間は、インフュージョンリアクションのチェックを含めて注意深い観察を行う。急性期以後も長期の視察を要す。

10、使用する施設の倫理委員会の承認、及び両親からインフォームドコンセントが得られている。

研究成果の刊行に関する一覧表

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Ⅲ．研究成果の刊行物・別冊（主なもの）

Kawasaki Disease Patients With Redness or Crust Formation at the Bacille Calmette-Guérin Inoculation Site

Ritei Uehara, MD, Hiroshi Igarashi, MD,†‡ Mayumi Yashiro, BA,* Yosikazu Nakamura, MD, MPH,* and Hiroshi Yanagawa, MD**

Background: A specific diagnostic test for Kawasaki disease (KD) is currently unavailable. Redness or crust formation at the Bacille Calmette-Guérin (BCG) inoculation site is listed as a positive sign in the diagnostic guidelines of KD. The purpose of this study was to investigate the epidemiologic features of KD patients with such changes at the BCG inoculation site and to evaluate the specificity of this sign in KD diagnosis.

Methods: Data on KD patients who received BCG vaccination were analyzed from a Japanese nationwide epidemiologic survey on KD conducted in 2007. Patients who had 5 or 6 principal signs (complete cases) with redness or crust formation at the BCG inoculation site were compared by sex, year of hospital visit, day of first hospital visit, recurrent status, and presence of KD in siblings. To evaluate the specificity of the sign for KD diagnosis, patients aged 2 years or younger who were diagnosed as having respiratory syncytial virus or rotavirus infection using a commercial rapid test and who required hospitalization were observed.

Results: Of the 15,524 KD patients with a history of BCG vaccination, 7745 (49.9%) had redness or crust formation at the BCG inoculation site. This was observed in more than 70% of complete KD patients aged 3 to 20 months. Of these patients, the proportion with this sign in the group whose first day of hospital visit was within 1 to 4 days from the onset was significantly larger than that of the other patients groups (5–9 or 10+ days) (52.1%, $P < 0.001$). Among the patients with respiratory syncytial virus or rotavirus infection, none showed these changes at BCG inoculation site.

Conclusions: Redness or crust formation at the BCG inoculation site is a useful diagnostic sign for KD among children aged 3 to 20 months in countries with a BCG vaccination program. Even if patients have 4 or fewer signs of the clinical criteria for KD, physicians should consider that patients with redness or crust formation at the BCG inoculation site could suffer from KD.

Key Words: Kawasaki disease, BCG vaccine, diagnosis, epidemiology

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Kawasaki disease (KD) is a systemic vasculitis with unknown etiology mostly affecting children aged 5 years or younger. As no specific diagnostic test is currently available, diagnosis is based on clinical signs and exclusion of other diseases. KD is defined as an illness in patients with at least 5 of the following 6 principal clinical signs: (1) fever persisting for 5 days or more (inclusive of patients whose fever subsided before the fifth day in response to therapy), (2) bilateral conjunctival injection, (3) changes to the lips and oral cavity (eg, reddening of the lips, strawberry tongue), (4) polymorphous exanthema, (5) changes to peripheral extremities (eg, reddening of the palms and soles, edema, desquamation), and (6) cervical lymphadenopathy.[1]

Bacille Calmette-Guérin (BCG) vaccine is used to prevent meningitis and disseminated tuberculosis in children,[2] and about 100 million children receive this vaccine each year. Japan has been conducting universal BCG vaccination of infants using a multiple puncture technique since 1951[3] and the vaccination policy regarding BCG for infants was changed in 2005. Since then, it has been recommended that all children should receive the BCG vaccine by 6 months of age. According to a BCG vaccination survey in Japan in 2006, 97% of children received the BCG vaccine by that time.[4]

Redness or crust formation at the BCG inoculation site is listed as a symptom or finding both on the fifth revised edition of the diagnostic guidelines of KD in Japan[1] and on the American Heart Association scientific statement.[5] Japanese pediatricians previously reported findings of erythema at the BCG inoculation site in 281 KD patients who visited their hospital between 1976 and 1980.[6] In this report, erythema was observed at the BCG site in more than 50% of KD patients 1 to 12 months after inoculation. Several KD cases with BCG reactivation, inflammation, or induration were also reported from other countries.[7–10] According to an investigation of skin biopsy specimens from the BCG inoculation site in KD patients, extensive edema in the papillary dermis with marked dilation of the capillaries was found.[11] In addition, raised levels of cytokines such as interleukin-1 alpha and tumor necrosis factor alpha were detected at the site. Redness or crust formation at the BCG inoculation site in KD patients was hypothetically ascribed to cross-reactivity between mycobacterial heat shock protein 65 and human homolog HSP63.[12,13]

The epidemiology of KD patients with redness or crust formation at the BCG inoculation site is poorly understood. In this study, we investigated the epidemiologic characteristics of KD patients with these changes using data from a large-scale nationwide survey of the disease in Japan, and evaluated the specificity of this sign for KD diagnosis.

METHODS

Epidemiologic Characteristics of KD Patients With Redness or Crust Formation at the BCG Inoculation Site

Nationwide epidemiologic surveys on KD have been conducted approximately every 2 years in Japan since 1970. The 19th survey on KD was conducted in January 2007 and included patients who visited hospitals from January 1, 2005 to December 31, 2006. All pediatric hospitals and other general hospitals with a pediatric department and 100 or more beds were included in the nationwide survey. Pediatricians were asked to complete a questionnaire for all KD cases they had diagnosed over the 2-year

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period.[14] This survey protocol and the questionnaire were reviewed and approved by the institutional review board at Jichi Medical University, Japan.

Two items related to BCG vaccination were included in the questionnaire of the 19th survey. One item asked whether patients had received the BCG vaccination and the other item asked whether redness or crust formation was observed at the BCG inoculation site. Patients who did not receive the BCG vaccination or whose history of BCG vaccination was unknown were excluded from the analysis.

The age-specific proportion of KD patients with redness or crust formation at the BCG inoculation site was observed. Clinical criteria were divided into 2 groups: 5 or more signs (complete KD) and 4 or fewer (incomplete KD). These patients were compared by sex, year of hospital visit, day of first hospital visit, recurrent status, and presence of KD in siblings. Day of first hospital visit was divided into 3 categories: 1 to 4 days, 5 to 9 days, and 10 days or more from the onset. The proportion of coronary artery abnormalities (CAA) among complete KD patients with redness or crust formation at the BCG site was compared with that of patients without such changes. The presence of CAA was also examined according to time period: <30 days or ≥30 days after KD onset. CAA was defined as a giant coronary aneurysm, coronary aneurysm, or coronary dilatation.[15]

Comparisons between redness or crust formation at the BCG inoculation site and categorical variables were made using χ^2 analysis or Fisher exact test; 95% confidence intervals were also calculated for these proportions. The significance level was $P < 0.05$. Statistical analyses were performed using the SAS 9.1 software program (SAS Institute Inc., Cary, NC).

Specificity of Redness or Crust Formation at the BCG Inoculation Site for Diagnosis of KD

To identify the prevalence of redness or crust formation at the BCG inoculation site in patients with febrile illness except for KD, we observed serial patients diagnosed with respiratory syncytial virus (RSV) or rotavirus infection and who required hospitalization at a general hospital (Oyama Municipal Hospital, Tochigi, Japan) between October 2008 and May 2009. The rapid diagnostic test for RSV infection (Check RSV, Alfresa Pharma Corp., Osaka, Japan) detects RSV antigen in respiratory tract specimen by using immunochromatography testing. Rapid diagnostic test for rotavirus infection (Rapid Testa Rota Adeno, Sekisui Medical Co. Ltd, Tokyo, Japan) detects rotavirus antigen in feces by using immunochromatography testing. Patients who were 2 years of age or younger and who had received BCG vaccination were included in this observation to compare with the prevalence of changes at the BCG inoculation site in KD patients.

RESULTS

Epidemiologic Characteristics of KD Patients With Redness or Crust Formation at the BCG Inoculation Site

Completed questionnaires were returned from 1543 (70.7%) of the 2183 hospitals contacted. A total of 20,475 patients diagnosed with KD by a physician were reported: 10,041 in 2005 and 10,434 in 2006. A total of 15,524 patients had a history of BCG vaccination. Of these, 7745 (49.9%) had redness or crust formation at the BCG inoculation site. The age-specific proportion of these patients is shown in Figure 1. Of those patients aged 3 to 20 months, more than 70% had redness or crust formation at the BCG inoculation site. The same finding was obtained in complete KD patients (n = 12,783).

Among all complete KD patients receiving the BCG vaccination, the proportion of male patients with redness or crust formation at the BCG site significantly larger than that of female patients with the same sign (52.2% vs. 46.4%, $P < 0.001$) (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A353).

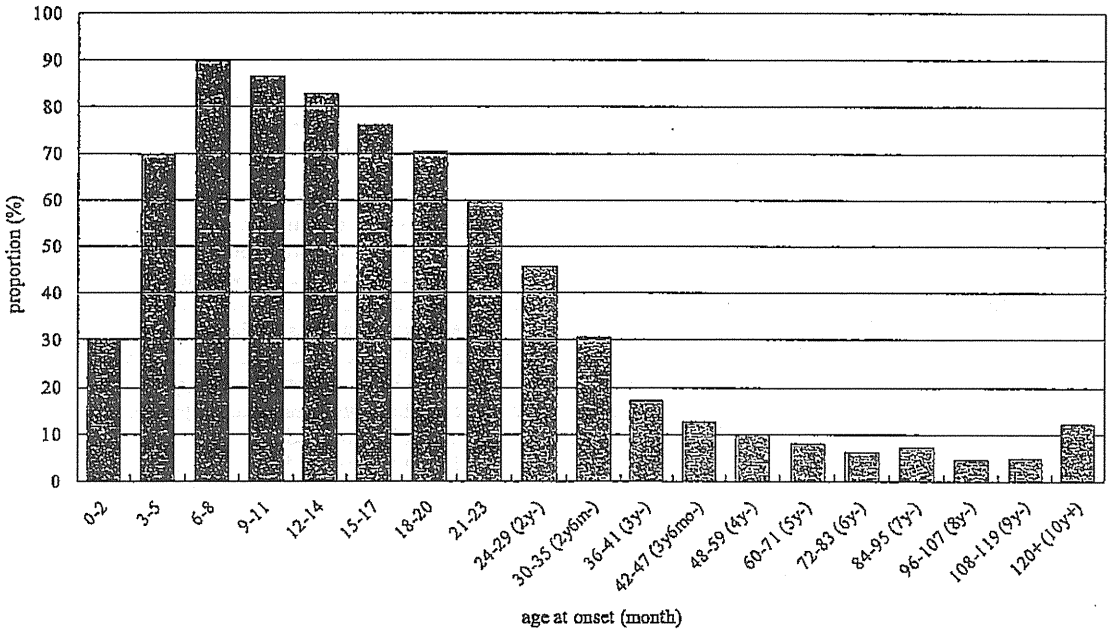

FIGURE 1. Age-specific proportion of Kawasaki disease patients who had redness or crust formation at the Bacille Calmette-Guérin inoculation site. Data of the nationwide survey of Kawasaki disease in Japan which was conducted in 2007 was used. This figure showed the proportion of 7745 patients with Kawasaki disease who had redness or crust formation at the Bacille Calmette-Guérin inoculation site by age at onset.

TABLE 1. Association Between Redness or Crust Formation at the Bacille Calmette-Guérin Inoculation Site and Coronary Artery Abnormality in Complete Kawasaki Disease

Redness or Crust Formation	CAA (<30 d of Illness)			CAA (≥30 d of Illness)		
	No. Patients	Proportion (95% CI)	P	No. Patients	Proportion (95% CI)	P
All patients (n = 12783)*						
(+)	623/6302	9.9 (9.2–10.7)	<0.001	196/6221	3.2 (2.7–3.6)	0.87
(−)	756/6400	11.8 (11.0–12.6)		196/6323	3.1 (2.7–3.6)	
3–20 mo of age (n = 5448)†						
(+)	451/4404	10.2 (9.4–11.2)	0.46	143/4358	3.3 (2.8–3.9)	0.91
(−)	95/1004	9.5 (7.7–11.4)		32/996	3.2 (2.2–4.5)	

* Number of missing denominator were 81 before 30 d of illness and 239 in 30 d of illness or after.
† Number of missing denominator were 40 before 30 d of illness and 94 in 30 d of illness or after.
CAA indicates coronary artery abnormality; CI, confidence interval.

The proportion of patients with the changes at the BCG inoculation site in the group whose first day of hospital visit was within 1 to 4 days from the onset was also significantly larger than that of the other patient groups (52.1%, $P < 0.001$). Patients with a recurrent KD status were significantly less likely to have redness or crust formation at the BCG inoculation site (24.0%, $P < 0.001$). When data from patients aged 3 to 20 months were analyzed, similar findings were obtained (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A353). These findings except for sex were also observed among incomplete KD patients.

Comparing complete KD patients without changes at the BCG inoculation site, the proportion of CAA <30 days after disease onset was smaller in patients with changes at the BCG inoculation site (9.9% vs. 11.8%, $P < 0.001$) (Table 1). However, a similar association was not observed in patients aged 3 to 20 months. There was no association between redness or crust formation at the BCG inoculation site and CAA ≥30 days after the onset.

Specificity of Redness or Crust Formation at the BCG Inoculation Site for Diagnosis of KD

A total of 53 patients met the inclusion criteria for the identification of redness or crust formation at the BCG inoculation site among those with other febrile illnesses during the observation period. The mean age was 11.6 months (standard deviation: 5.5) and the range was 20 months (3–23 months). Forty-nine patients were diagnosed with RSV infection, and 4 with rotavirus infection. None of these patients had redness or crust formation at the BCG site.

DISCUSSION

Redness or crust formation at the BCG inoculation site is a common finding among Japanese KD patients. More than 70% of complete KD patients aged 3 to 20 months had this finding. Cervical lymphadenopathy, which is one of the 6 principal signs of KD, was found in less than 60% of patients aged 2 years or younger.[16,17] Among the complete KD patients who were 2 years of age or younger, redness or crust formation at the BCG inoculation site was more prevalent than cervical lymphadenopathy. Although there was a high prevalence of redness or crust formation at the BCG inoculation site in complete KD patients, especially those aged 3 to 20 months, no patient with RSV or rotavirus infection showed the same changes at the BCG inoculation site. Even if patients have 4 or fewer signs of the clinical criteria for KD, physicians should consider that patients with redness or crust formation at the BCG inoculation site could have KD. Regarding observation of the prevalence of changes at the BCG inoculation site among patients with other febrile illness, most patients were diagnosed as having RSV or rotavirus infection during the observation period. Only one patient was diagnosed with influenza virus infection and required hospitalization. No patients with adenovirus infection or group A streptococcus infection met the inclusion criteria. One patient with human herpes virus 6 infection having erythema at the BCG inoculation site has been reported.[18] Further investigation of the prevalence of redness or crust formation at the BCG inoculation site in patients with other infectious diseases or febrile illnesses may be needed.

A higher prevalence of redness or crust formation at the BCG inoculation site was observed in complete KD patients who visited a hospital between 1 and 4 days after the onset of illness, suggesting that this sign appears during the early stages of the disease.[19] In addition, parents or guardians of children with changes at the BCG inoculation site are likely to take the child to hospital quickly if the symptoms are accompanied by fever or other principal signs of KD. Patients with recurrent KD status were less likely to have redness or crust formation at the BCG inoculation site; this could be because the age distribution of KD patients with changes at the BCG inoculation site was skewed toward old children. The proportion of KD patients aged 2 years or younger was only 17.0% among complete KD patients with recurrent KD status in the present survey. No association between redness or crust formation at the inoculation site and the development of CAA was found among patients aged 3 to 20 months, suggesting that these changes are not useful for predicting the presence of CAA.

Although the association between redness or crust formation at the BCG inoculation site and each principal sign is an important issue, we were unable to investigate this because information about principal signs was not collected in the 19th survey. Similarly, we were not able to assess the severity of inflammation in patients with changes at the inoculation site as laboratory data were not obtained in the survey. Regarding a BCG inoculation method, multiple puncture technique may be unique. Also in countries where an intradermal injection is used, similar investigation should be needed.

In conclusion, redness or crust formation at the BCG inoculation site is useful for the diagnosis of KD among children aged 3 to 20 months in countries with a BCG vaccination program. The prevalence of this sign among complete KD patients aged 3 to 20 months was higher than that of cervical lymphadenopathy. Even if such patients have 4 or fewer signs of the clinical criteria for KD, physicians should assess them for KD development.

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Common variants in *CASP3* confer susceptibility to Kawasaki disease

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Kawasaki disease (KD; OMIM 611775) is an acute vasculitis syndrome which predominantly affects small- and medium-sized arteries of infants and children. Epidemiological data suggest that host genetics underlie the disease pathogenesis. Here we report that multiple variants in the caspase-3 gene (*CASP3*) that are in linkage disequilibrium confer susceptibility to KD in both Japanese and US subjects of European ancestry. We found that a G to A substitution of one commonly associated SNP located in the 5′ untranslated region of *CASP3* (rs72689236; $P = 4.2 \times 10^{-8}$ in the Japanese and $P = 3.7 \times 10^{-3}$ in the European Americans) abolished

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binding of nuclear factor of activated T cells to the DNA sequence surrounding the SNP. Our findings suggest that altered *CASP3* expression in immune effecter cells influences susceptibility to KD.

INTRODUCTION

Kawasaki disease (KD) is characterized by high fever, polymorphous skin rash, injection of the conjunctiva, erythema of the palms and soles followed by desquamation, redness of oral mucosa and lips and non-supprative cervical lymphadenopathy (1,2). Despite clinical and epidemiological features suggesting an infectious trigger in the pathogenesis of KD, the etiology remains unknown. Marked activation of the immune system accompanied by infiltration of lymphocytes, macrophages and neutrophils into the vascular wall occurs during the acute phase of KD. The coronary arteries are selectively targeted and coronary artery lesions (CALs) develop in 20–25% of the patients without treatment (3). KD is now a leading cause of acquired cardiac disease in children in developed countries.

Previously, we performed an affected sibpair linkage study and identified several candidate regions (4q35, 5q31.4, 6q27, 7p15, 8q24, 12q24, 18q23, 19q13.2, Xp12 and Xq26) for KD susceptibility (4). Recently, we identified a functional SNP in *ITPKC*, encoding inositol 1,4,5-trisphosphate 3 kinase-C on 19q13.2, that confers both increased risk of KD and CAL formation (5). This effect is likely mediated through upregulating of the Ca^{2+}/NFAT pathway in T cells, thus increasing IL-2 production. These findings supported the hypothesis that genetically determined modulation of the immune response is fundamental to KD pathogenesis and suggested that genes with immune regulatory function located in chromosomal regions with positive linkage signals should be considered potential candidates for KD susceptibility. In an attempt to identify a novel susceptibility gene, we performed a positional candidate gene study for 4q35 region. We found that there is a set of common variants in *caspase-3* (*CASP3*) gene significantly associated with KD in both Japanese and European American subjects. We also demonstrate a functional significance of one commonly associated SNP which affects binding of nuclear factor of activated T cells (NFAT) to the 5′ untranslated region (UTR) of the gene.

RESULTS

Identification of the variants of *CASP3* gene associated with KD susceptibility

The candidate region on 4q35 was attractive because several immune genes have been mapped around the peak of linkage, including the interferon regulatory factor 2 gene (*IRF2*), *CASP3* and toll-like receptor 3 gene (*TLR3*), which all lie within 1.7 Mb of the linkage peak. Previous reports describing delayed apoptosis of peripheral blood lymphocytes (6) and neutrophils (7) from KD patients led us to focus on *CASP3*, which is located at 185.8 Mb on chromosome 4 close to the linkage peak (184.9 Mb). Caspase-3 is a key molecule of activation-induced cell death (AICD) (8) and it has also been reported to cleave the inositol 1,4,5-triphosphate receptor, Type 1 (ITPR1) in apoptotic T cells. ITPR1 is a receptor for inositol 1,4,5-trisphosphate (IP3), a substrate for ITPKC in T cells (9).

Based on linkage disequilibrium (LD) data at the web site of the International HapMap project, we selected 12 tagging SNPs with minor allele frequency (MAF) greater than 5% from the 36 kb region containing the *CASP3* gene flanked by 10 kb upstream and 5 kb downstream (Supplementary Material, Fig. S1). Using Haploview 4.1, the tagging SNPs were classified into four SNP groups at a threshold of $r^2 > 0.8$. Four tagging SNPs (rs4647693, rs2696057, rs2720378 and rs2705881) were selected as representatives of each group (Supplementary Material, Fig. S1). For the first stage of screening, the genotype at these four locations was determined for 638 Japanese KD patients and 1031 healthy Japanese controls. Three SNPs showed significant association with KD ($P < 0.05$ after Bonferroni correction for four tests; Supplementary Material, Fig. S1) when comparing allele frequencies between cases and controls. We then resequenced the 36 kb region in 24 Japanese subjects (12 KD subjects and 12 controls) and genotyped the first case–control panel for 34 additional variants and compared allele frequencies (Supplementary Material, Table S1). Twenty-five of the 46 variants (12 tagging SNPs + 34 additional variants) showed *P*-values < 0.001 ($P < 0.05$ after a conservative Bonferroni correction for 46 tests) and most were clustered in the 5′ region of *CASP3* (Fig. 1). To validate the association and identify of the causative variant, these 25 loci were further examined in an independent Japanese case–control panel with 282 KD patients and 378 controls. In this case–control panel, all of the 25 variants showed the same trend of association and rs2720378 was the most significant in a meta-analysis by the Mantel–Haenszel method [odds ratio (OR) = 1.44, 95% confidence interval (CI) 1.27–1.62; $P = 3.5 \times 10^{-9}$; Table 1]. Most of the 25 significant variants except for rs4862399 and rs7693625 were in high linkage disequilibrium with rs2720378 ($r^2 > 0.69$) and showed the same trend of association. No increase of association due to haplotypic effect was seen for the combination of rs2720378 and any other variations including rs4862399 and rs7693625 in a haplotype association study and logistic regression analysis (Supplementary Material, Tables S2 and S3).

Screening of functionally significant variants

We next assessed the functional significance of the variants in *CASP3*. Because all of the 25 variants were in untranscribed or untranslated of *CASP3*, we postulated that the variant(s) might influence expression of *CASP3*. We screened for possible enhancer activity around the associated variants by a reporter gene assay. To facilitate the screening, we cloned four tandem copies of oligonucleotides corresponding to both alleles of the variants upstream of the SV40 promoter in the luciferase reporter vector, pGL3, and transfected them into Jurkat cells.