Current Review of the Etiology of Kawasaki Disease

Abstract: The etiology of Kawasaki disease remains unknown. Since Kawasaki disease shares many clinical features with scarlet fever and toxic shock syndrome, it was once suggested that superantigens, especially TSST-1, SPEA or SPEB, might be involved in the pathogenesis of Kawasaki disease. This paper focuses on the SPEC hypothesis of Kawasaki disease, which today appears to be the most persuasive.

Key words: Kawasaki disease; Superantigen; Streptococcal pyrogenic exotoxin C (SPEC)

Introduction

Kawasaki disease (KD) is an acute, febrile, exanthematous disease that generally occurs in children under the age of 5 years. Although it is usually benign, in 20–25% of the cases, it is complicated by coronary aneurysm or ectasia. Giant aneurysms may lead to thromboembolism or vascular stenosis, sometimes associated with cardiac infarction and sudden death. KD has now surpassed rheumatic fever as the leading cause of acquired cardiac diseases in Japan and the United States.

Changes in the Incidence of KD and the Current Morbidity

The incidence of KD is overwhelmingly high in Japan, but the reason still remains unclear. The National survey conducted every 2 years by the Kawasaki Disease Research Team constituted by the Ministry of Health and Welfare (now renamed Ministry of Health, Labor and Welfare) revealed that the total number of patients with KD in Japan was 153,803 in December 1998 (Fig. 1).1)

As shown in Fig. 1, the number of KD patients has been increasing yearly from 1968 onward throughout Japan, and reached its first peak in 1979, second peak in 1982, and third peak in 1986, which is indicative of an epidemic pattern every 3 to 4 years. Although no countrywide outbreak has occurred since 1987, about five thousand people developed the disease yearly from 1987 to 1993, and about six thousand developed the disease yearly from 1994 to 1998. The
Fig. 1 Yearly number of patients developing Kawasaki disease according to the National Survey of Kawasaki Disease (15 surveys, 1970 to 1998)\(^1\)

Table 1 Comparison of Clinical Features of Kawasaki Disease, Scarlet Fever, and Toxic Shock Syndrome\(^2\)

<table>
<thead>
<tr>
<th>Features of rash</th>
<th>Kawasaki disease</th>
<th>Scarlet fever</th>
<th>Toxic shock syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythema varying morphologically</td>
<td>Diffuse erythema with spotted papules</td>
<td>Diffuse erythoderma</td>
</tr>
<tr>
<td>Conjunctival injection</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Labial and oral hyperemia</td>
<td>Whole area +</td>
<td>Localized to pharynx, soft palate</td>
<td>Whole area +</td>
</tr>
<tr>
<td>Swollen cervical node</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Strawberry tongue</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythema of the palms and soles</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Palmar desquamation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Recurrence</td>
<td>3–4%</td>
<td>Rare</td>
<td>30%</td>
</tr>
<tr>
<td>Shock or hypotension</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coronary arteritis (aneurysm)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age at onset</td>
<td>(\leq 4) years of age</td>
<td>(\geq 3) years of age</td>
<td>90% comprising girls reaching the menstrual age</td>
</tr>
<tr>
<td>Epidemic</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cause</td>
<td>?</td>
<td>A streptococcal pyrogenic exotoxin (SPE-A, B, C)</td>
<td>Staphylococcus enterotoxin (TSST-1)</td>
</tr>
</tbody>
</table>
incidence of KD appears to be on the rise in parallel with the declining birth rate.

Recent epidemiological studies have shown that although no nationwide outbreak has occurred since 1987, small-scale outbreaks of KD have occurred across the country every year. These findings suggest that some infectious factors may be involved in the pathogenesis of KD. However, the precise cause of KD remains unknown despite extensive effort by scientists worldwide.

Clinical Features of KD and Hypotheses Regarding its Pathogenesis

It has been pointed out that KD shares many clinical features with scarlet fever and the toxic shock syndrome (TSS). Table 1 shows a comparison of the three conditions. Scarlet fever is caused by group A streptococcal pyrogenic exotoxins (SPEs), or SPEA, SPEB, and SPEC. As for TSS, the toxic shock syndrome toxin-1 (TSST-1) produced by exotoxin-producing strains of
Staphylococcus aureus was initially identified as the causative agent. Subsequently, *staphylococcal enterotoxin* (SE) A to H have also been demonstrated to be toxins that can cause TSS. Furthermore, SPEs were also found to cause TSS. All these toxins have the characteristics of superantigens (Table 2). As shown in Table 1, TSS and scarlet fever share many clinical features with KD, although there are a few differences. Therefore, it may not be unreasonable to suppose that superantigens may contribute to the pathogenesis of KD. Figure 2 shows a comparison of two antigen-binding complex models; one consisting of the superantigen-antigen-presenting cell (APC) MHC class II molecules and T cell antigen receptor (TCR), and the other consisting of a conventional antigen peptide, APC MHC class II molecules and TCR.

### Review of the Superantigen Hypothesis for the Etiology of Kawasaki Disease

Uchiyama et al. first demonstrated the superantigenicity of TSST-1, SPEA, and SPEB, using mouse T cells. Thereafter, it was suggested for the first time by Abe et al. that SPEA and SPEB might be involved in the pathogenesis of KD. Abe and his colleagues showed in 1991 that SPEA and SPEB are superantigens, based on the finding that the addition of SPEA to human peripheral blood lymphocytes in vitro could selectively activate TCR Vβ2+ , 12+ , 14+ T cells, and that SPEB could activate TCR Vβ2+ , 8+ T cells.

In 1992, they conducted a further analysis of the TCR Vβ repertoire of T cells using anti-Vβ monoclonal antibodies in peripheral blood samples obtained from KD patients, by PCR assay and cytofluorography. The results revealed a significant increase in the percentage of Vβ2+ and Vβ8.1+ T cells in the population of T cells in the peripheral blood, while the percentage of two Vβ subpopulation-positive T cells was markedly decreased in convalescent specimens of KD patients. In contrast, none of the other 20 TCR Vβ subpopulations showed any expansion.

Furthermore, Abe and his colleagues examined serum samples of patients with acute KD, and found that more than half of the samples were positive for anti-SPEA antibody and 15% were positive for anti-SPEB antibody. Based on these results, they suggested that the two SPEs might function as superantigens and contribute to the pathogenesis of KD. Subsequent studies performed to verify the hypotheses of Abe and his colleagues have remained inconclusive.

Five years after the proposition by Abe et al., Suzuki and his collaborators belonging to a cooperative research project of Wakayama Medical College and Shionogi Central Institute for Medical Science, Shionogi & Co., Ltd., presented a paper entitled “Analysis of TCR Vα and TCR Vβ repertoires in children with KD” at the 17th Japan Kawasaki Disease Research Meeting (Chairman: Professor Shunzo Chiba of Sapporo Medical University) held in Sapporo in October 1997. They found a significant expansion of Vβ2.1 and Vβ6.5 of the TCR Vβ repertoire in the peripheral blood T cells of KD patients in the acute phase as compared with that in the convalescent phase, based on an analysis of blood samples conducted from September 1994 to July 1995 using the adaptor-ligation PCR (AL-PCR) method and reverse dot-blotting (RDB). No marked change was found in the Vα populations during the observation period.

The collaborative research project team conducted further studies and presented two proposals at the 19th Japan Kawasaki Disease Research Meeting held in Hiroshima in November 1999. The first was a fundamental research entitled “Involvement of SPEC in the pathogenesis of Kawasaki disease” by Yoshioka et al. of Shionogi Institute for Medical Science, and the second was, “Anti-SPEC and anti-SPEA antibody titers in acute Kawasaki disease”, a study conducted from a clinical viewpoint by Suzuki and his coworkers of Wakayama Medi-
cal College. In the former proposal, Yoshioka and his collaborator, using a new method of assay, demonstrated a significant expansion of Vβ2+ and Vβ6.5+ T cells in the peripheral blood of acute KD patients in 1999 as compared with that in specimens obtained in the convalescent phase. These findings were consistent with the results of previous studies. They performed an additional stimulation test in vitro to confirm their findings, using purified recombinant SPEA (r-SPEA) and r-SPEC. The results showed that SPEC induced a selective expansion of TCR Vβ2 and TCR Vβ6.5, which led them to propose that SPEC probably plays an important role in the pathogenesis of KD.

The latter proposal presented by Suzuki et al.9) was based on a study of about 17 children and 207 age-matched healthy children, which revealed that the serum titers of antibodies to SPEA and SPEC tended to be higher in acute KD patients than in healthy controls, as measured by the enzyme-linked immunosorbent assay (ELISA), after the stimulation by r-SPEA and r-SPEC proteins. These proposals at the Hiroshima meeting were widely reported by newspaper and television media. Many people believed that SPEC had finally been identified as the cause of KD.

In April 2000, at the 103rd Japan Pediatric Society meeting, Professor Michio Koike of Wakayama Medical College, President of the meeting, presented a review entitled “Etiology of Kawasaki disease,”10) summarizing the collaborative study conducted by the Department of Pediatrics of Wakayama Medical College and the Shionogi Institute for Medical Science.11) Professor Koike of the Kawasaki Disease Research Team has contributed substantially to elucidation of the pathogenesis of KD. He and his collaborator, Professor Yorio Konuma, of the Shionogi Institute for Medical Science, along with the staff of the institute, assessed the validity of the superantigen hypotheses for KD, while rejecting many other hypotheses.

They focused on the superantigens, SPEA and SPEC, and transferred the genes encoding the two superantigens into E. coli to induce the production of the toxins. Then, they employed purified r-SPEA and r-SPEC in vitro to evaluate the role of these superantigens in the pathogenesis of KD by comparing blood samples between KD children and healthy adults using the adaptor-ligation PCR and microplate hybridization assay developed by Ryuji Suzuki and coworkers of the Shionogi Institute for Medical Science. This technique allowed Koike and his collaborators to measure 38 different TCR Vβ repertoires activated by superantigens in a single step.

The results showed that TCR Vβ2-positive cells were noted among peripheral blood T cells in 8/9 healthy subjects after stimulation with r-SPEA in vitro, and TCR Vβ6.5-positive cells were present in 4/9 patients. All healthy subjects (9/9) were TCR Vβ2- and/or TCR Vβ6.5-positive, however, none were TCR Vβ2- or TCR Vβ6.5-positive after the stimulation with r-SPEA; several subjects responded for TCR Vβ12, Vβ13.1, Vβ14, and Vβ15.

On the other hand, TCR Vβ2-positive peripheral blood T cells were detected without any exogenous stimulation in 41% of 22 acute KD children in 1995–1996, and in 38% of 16 acute KD children in 1999, while TCR Vβ6.5-positive cells were found in 59% of 22 acute KD patients in 1995–1996, and in 56% of 16 patients in 1999. TCR Vβ2- and/or TCR Vβ6.5-positive cells were found in 77% of acute KD children in 1995–1996, and in 81% in 1999. There were no marked differences in the positivity rates between the two periods (1995–1996 and 1999). The expansion of TCR Vβ2 and TCR Vβ6.5 in KD patients continued until the third week after the onset of the disease, and thereafter gradually declined in all patients during the convalescent phase, that is, 90 to 140 days after the onset. The measurement results of the two TCR Vβ segments in the convalescent KD phase were similar to those in age-matched healthy controls.

In addition, the serum tested positive for
antibodies to SPEA and SPEC in 24/28 and 25/28 children, respectively, revealing that the serum titers of the anti-SPEA and anti-SPEC antibodies markedly increased in patients with KD even earlier in the acute phase.

Professor Koike stated that these findings strongly suggested a relationship between group A streptococcal infection and KD. He concluded that the strong relationship between group A streptococci and KD might indicate the involvement of streptococcal pyrogenic exotoxin C (SPEC), a superantigen, in the pathogenesis of KD, based on analysis of the TCR Vβ repertoire.

If Professor Koike’s proposition were right, it will mark a milestone in the elucidation of the pathogenesis of KD. However, until now, many hypotheses for the pathogenesis of KD have made a promising debut but disappeared in the course of time. Further studies would be required to validate the SPEC hypothesis and to detect SPEC antigen peptides in the serum of patients with KD.

From a standpoint different from the Wakayama/Shionogi research team’s, the Kagoshima University Medical School Pediatric Research Team also suggested, in 1997, the involvement of SPEC in the pathogenesis of KD, and published a related paper in a journal in 1998. They stimulated peripheral blood T cells derived from 43 KD patients in different phases with SPEA, SPEC, and TSST-1. They observed that the peripheral blood T lymphocytes showed a transient lowering of response to SPEC up to 2 months after the onset of the disease, while no change in response of the peripheral blood T cells was observed after stimulation with SPEA and TSST-1. This transiently decreased response to SPEC by the peripheral blood T cells of KD patients recovered to normal within one year after the onset of the disease. The Kagoshima University investigators proposed that the decrease in response to SPEC might be attributable to anergy, or the absence of T cells responsive to SPEC as a result of migration of the cells to the sites of inflammation. No decrease in response was observed after stimulation with SPEA or TSST-1. They concluded that these findings suggested the involvement of SPEC in the pathogenesis of KD.

In summary, one study showed the activation of peripheral blood T cells by SPEC, and the other revealed a transient decrease in the peripheral blood T cell response to SPEC. It should be noted that both the different proposals for the pathogenesis of KD implicate SPEC, a group-A streptococcal pyrogenic exotoxin. Further studies will be required to validate this hypothesis.

REFERENCES

9) Suzuki, K., Koike, M., Uemura, S. et al.: Evi-


