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### THE INVOLVEMENT OF BACTERIAL PRODUCTS FROM THROAT FLORA IN THE PATHOGENESIS OF KAWASAKI DISEASE

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Kawasaki disease (KD) is an acute vasculitis of unknown etiology, however, some studies suggest that superantigens are involved in its pathogenesis. To clarify an association between KD and bacterial infection, we studied bacterial culture supernatants from throat flora of acute stage KD patients for mitogenic activity. Twenty-five individual bacterial strains (*Streptococcus sp.*, *Staphylococcus sp.*, *Neisseria sp.*, *Pseudomonas sp.* etc) were isolated from throat of nine acute-stage KD patients before treatments. Each of these bacterial strains was cultured overnight at 37°C in trypticase soy broth. Bacterial cells were removed by centrifugation and filtration with a 0.22 µm pore size filter. The mitogenic activity in the supernatants was determined by a three-day lymphocyte assay. Fresh peripheral blood mononuclear cells (PBMC) were obtained by ficoll-paque centrifugation of heparinized blood from healthy adult. PBMC were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum. The bacterial culture supernatant (10 µL) was added to the PBMC culture (10<sup>5</sup> cells in 0.1 mL of culture medium) and then cultured in each well of a 96-well microplate at 37°C in 5% CO<sub>2</sub>. Proliferation of PBMC was determined three days after by morphological observation and Cell Counting Kit-8 (Dojin Chemical). The mitogenic activity for human lymphocytes was observed in three of twenty-five supernatants. The three supernatants were contained two strains of *S. pyogenes* and one strain of methicillin-resistant *S. aureus*. The results indicate that at least three of nine acute-stage KD patients had bacteria producing mitogenic products in throat flora. These findings support the involvement of mitogenic products, such as superantigens, of group A streptococci and *S. aureus* in the pathogenesis of KD.

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### SUPERANTIGENIC ACTIVATION OF T LYMPHOCYTES AND ENDOTHELIAL CELLS: A MECHANISM FOR SUPERANTIGEN-INDUCED VASCULITIS

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Clinical and immunological similarities between Kawasaki Disease (KD) and classical superantigen (SAG) mediated diseases such as the toxic shock syndrome lend credence to the hypothesis that SAGs may be involved in the aetio-pathogenesis of the disease. There are only limited data available, however, examining how SAGs may cause vasculitis. We have, therefore, examined a possible mechanism of superantigen-induced T cell/endothelial cell activation. To confirm the ability of SAGs to stimulate T lymphocytes, peripheral blood mononuclear cells (PBMCs) were incubated for 4 hours with 10 ng/ml of TSST-1 or 100 ng/ml of SEB. This resulted in a Vb specific activation of T lymphocytes (Vb2 for TSST-1, Vb3 and 12 for SEB) as determined by expression of the early activation marker CD69 measured by flow cytometric analysis. Human umbilical vein endothelial cells (HUVECs), with or without pre-treatment with gamma interferon (g-INF) to induce MHC class II expression, and subsequently incubated for 4 or 24 hours with 100 ng/ml of the SAG SEB did not result in upregulation of the endothelial cell adhesion molecules ICAM-1, VCAM, E-selectin, or P-selectin. However, the addition of TSST-1 or SEB to a co-culture of purified CD3+ T lymphocytes (containing less than 0.8% HLA class II positive cells) and HUVECs resulted in up-regulation at 4 hours of ICAM-1, VCAM, and E-selectin (for TSST-1), and E-selectin (for SEB) on HUVECs pretreated with g-INF for 48 hours. At 24 hours, upregulation of ICAM-1, VCAM, and E-selectin occurred with both TSST-1 and SEB irrespective of HUVEC pre-treatment with g-INF. Conclusion: Our data underscore the ability of SAGs to cause T cell activation and, moreover, suggest a role for the HLA class II + endothelial cell as a superantigen presenting cell. The resultant T cell activation and subsequent upregulation of endothelial cell adhesion molecules could be one mechanism whereby SAGs cause vasculitis.

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### T CELL RECEPTOR V-BETA REPERTOIRES IN KAWASAKI DISEASE AND PRIMARY SYSTEMIC VASCULITIDES OF CHILDHOOD

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Introduction: Despite conflicting evidence and much debate, superantigenic stimulation of the immune system in Kawasaki disease (KD) remains an attractive hypothesis since there is considerable overlap between the clinical and immunological phenotypes of KD and classical superantigen-mediated diseases such as the toxic shock syndrome. Moreover, although there are limited data in adults suggesting that SAGs may be involved in the initiation of other primary systemic vasculitides, no such data exists for children. Methods: To investigate the possible aetiological role of SAGs, this study examined peripheral blood TCR Vb repertoires in children with KD (n=6), polyarteritis nodosa (PAN, n=23), Wegener's granulomatosis (WG, n=1), and microscopic polyangiitis (MPA, n=1). 20 normal children and 30 children with non-vasculitic inflammatory disease, or recipients of renal allografts served as controls and disease controls respectively. 3 colour FACS analysis of peripheral blood mononuclear cells stained with conjugated monoclonal antibodies to CD3, CD4, CD8, and 17 different Vb families was performed. Results: The mean % of CD4+ T cells bearing Vb2 was significantly increased in the KD group versus controls and disease controls (p=0.03 and p=0.01 respectively). Individual KD patients were also noted to have CD4+ T cell Vb expansions other than Vb2 (Vb5.1 n=2; Vb12 n=1). 60% of the primary systemic vasculitis patients had one or more TCR Vb expansions in the CD4+ lymphocyte population, compared with 30% of the controls (p=0.02-0.05), and 36% of the disease controls (p=0.05-0.1). Unlike KD, however, the pattern of Vb families expanded in individual patients was more diverse, perhaps indicative of the involvement of several different SAGs. Follow-up of 7 primary systemic vasculitis patients demonstrated a normalization of the CD4+ T cell Vb repertoire following induction of remission. Conclusion: Our preliminary data provide indirect evidence for an aetio-pathogenetic role for SAGs in KD and primary systemic vasculitides affecting children.

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### COLONIZATION OF THE SUPERANTIGEN-PRODUCING STAPHYLOCOCCUS AUREUS AMONG PATIENTS WITH KAWASAKI DISEASE

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**Objective.** We studied the distribution of the staphylococcal superantigen genes among Kawasaki Disease (KD) patients from Jan. 1997 to Dec. 2000. **Methods.** Rectal and throat swabs were obtained from 175 KD patients within 6 days after the onset of fever and throat swabs were obtained from 75 age-matched control children. More than 60% of the patients had received antibiotics before the cultures were obtained. The presence of *S. aureus* was determined by isolating the organism or by PCR of the *coagulase* and/or the *protein A* genes. The five prototypic enterotoxins (*sea, b, c, d, e*) as well as the three recently characterized enterotoxins (*seg, h, I*) and the *tsst-1* genes were amplified by PCR using the toxin-specific oligomer pairs. **Results.** *S. aureus* colonies were isolated from 32.0%, 20.7% of the throat/rectal swabs from KD, which was significantly higher than in controls (16.0%, p=0.009). By using PCR, the *coagulase/protein A* genes were amplified from 49.1%, 42.6% of the throat, rectal swabs from KD and 46.7% of the throat swabs from controls, respectively. The 7 enterotoxins (*sea, b, c, d, g, h, I*) and the *tsst-1* genes were detected from the throat and the rectal swabs with the comparable frequencies. However, the *seb* (13% vs. 4%, p=0.04) and the *sec* (14% vs. 4%, p=0.02) genes were detected more frequently from the throat swabs among KD patients than controls. **Conclusion.** These results indicate that *S. aureus*, which produce various combinations of superantigens, had colonized on the throat and/or rectal mucosa of KD patients more intensively than controls. This may predispose the young children who lack the protective anti-superantigen antibodies to develop symptoms of KD.

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### BACTEREMIA OF TOXIC SHOCK SYNDROME TOXIN-1 PRODUCING STAPHYLOCOCCUS AUREUS CAUSED SYMPTOMS OF KAWASAKI DISEASE IN A TWO-YEAR-OLD BOY

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Since the clinical feature of Kawasaki disease is similar to that of infective diseases caused by superantigen toxins, the relationship between Kawasaki disease and the superantigen has been investigated. We encountered a patient diagnosed as having Kawasaki disease, who was found to have bacteremia of toxic shock syndrome toxin-1 (TSST-1) producing *S. aureus*. This report may provide some clues for better understanding the cause of Kawasaki disease. A 2-year-old boy was admitted to our hospital because of prolonged high fever. White blood cell count was 15500/mm<sup>3</sup> and serum C-reactive protein level was 219 mg/l on admission (day 3). We started the treatment using antibiotics, PAMP/BP. Because polymorphous rash, erythema of palms and soles, swelling of hands and feet, strawberry tongue, fissured erythematous lips and cervical lymphadenopathy developed in addition to fever on day 4, we started the treatment using gamma-globulin and aspirin following the diagnosis of Kawasaki disease. Swelling and pain of the right knee joint also developed on day 5. All of the symptoms regressed until day 8. No cardiac complications other than mild pericardial effusion were shown in echocardiography during the course. *S. aureus* was detected in the blood culture taken on admission. PCR testing revealed that the strain had TSST-1, Staphylococcal enterotoxin(SE)C, SEG and SEI, but neither Exfoliative toxin A nor B. Western blotting revealed that IgM anti-TSST-1 was positive in the serum taken in his convalescent period.

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### IDENTIFICATION OF A NOVEL VIRUS-SPECIFIC CDNA DERIVED FROM PERIPHERAL BLOOD MONONUCLEAR CELLS OF ACUTE PHASE KAWASAKI DISEASE (KD) PATIENTS

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We have developed a three-phase screening procedure aimed at identifying KD-associated viral cDNA. In the first phase suppression subtractive hybridization (SSH) was carried out using cDNA which was reverse-transcribed from pooled RNA from PBMCs of 6 acute phase KD patients as the tester, and cDNA generated from pooled RNA from 6 non-KD febrile patients as the driver. In the second phase the subtracted cDNA sequences were inserted into a plasmid expression vector (pBAD), and an *E. coli* library was prepared. The expression library was screened using pooled convalescent KD sera as the primary antibody which was incubated with protein A or protein G alkaline phosphatase conjugates designed to identify the immunopositive clones. In the third phase the immunopositive clones were PCR amplified using primers based on flanking vector sequences and the recombinant inserts were sequenced. Molecular analysis of the immunopositive clones yielded 2 sequences: an 800 bp fragment identical to a portion of a known human gene and a 3400 bp PCR product which had no significant homology to known sequences using standard BLASTN or BLASTX database searches. However, using position specific iterated (PSI) BLAST search, the 3400 bp insert showed extensive but low-level homology (25-33% identities and 28-45% positives) throughout its entire length to gene pB602L of the African swine fever virus (ASFV), a lymphotropic virus which replicates in monocyte-macrophage lineage cells. Experiments are in progress to test the association of the novel virus with the course of KD by measuring the relative virus load in samples from acute, subacute and convalescent samples.