11

DOES STREPTOCOCCAL PYROGENIC EXOTOXIN-C TRIGGER THE ONSET OF KAWASAKI DISEASE? -ASSOCIATION BETWEEN SUPERANTIGENS AND AUTOANTIBODY AGAINST VASCULAR SMOOTH MUSCLE CELLS-

Hiroyuki Suzuki[1], Yasuteru Muragaki[2], Shigeru Uemura[1], Takashi Takeuchi[1], Takaomi Minami[1], Shoichi Shibuta[1], Akira Ooshima[2], Norishige Yoshikawa[1], Takeshi Yoshioka[3], Ryuji Suzuki[3] Department of Pediatrics, Wakayama Medical University, Wakayama, Japan[1], The First Department of Pathology, Wakayama Medical University, Wakayama, Japan[2], Department of Immunology, Shionogi Institute for Medical Science, Shionogi &Co. Ltd., Japan[3]

I) T cell receptor (TCR) usage We investigated TCR usage and antibody responses to streptococcal pyrogenic exotoxin-C (SPE-C) in order to determine whether an association exists between SPE-C and the onset of Kawasaki disease (KD). **Patients and Method** Fifty-four patients with KD were studied. Analysis of TCR usage was performed by adaptor-ligation PCR and microplate hybridization assay. Serum levels of antibody against SPE-C were assessed by ELISA using purified recombinant protein. **Results** Forty (74.1%) of the patients with KD showed significant polyclonal activation of Vβ2- and/or Vβ-6.5-bearing T cells. In 22 of 35 paired patients with KD, serum levels of anti-SPE-C antibodies (IgM) in the convalescent phase were lower than those in the acute phase. II) Detection of autoantibodies against vascular smooth muscle cells To investigate the pathogenesis of vasculitis in KD, we tested for the presence of autoantibodies against vascular smooth muscle cells in the patients. **Patients and Methods** Sera from 48 patients with KD were examined for reactivity with both coronary arterial wall tissues and cultured smooth muscle cells (CSMC) derived from human coronary artery, using immunofluorescence and Western immunoblotting. **Results** Sera from 16 of 48 patients with KD gave positive immunoreactions with the smooth muscle cells of coronary artery with anti-human IgA antibodies. Western immunoblotting revealed positive reactions of sera from 15 of 34 and 10 of 31 patients with KD against a 70-kDa protein from CSMC with IgA and IgM antibodies, respectively. Positive immunoreactivity of sera from KD patients was detected more frequently in patients with coronary arterial lesions (CAL) than in those without CAL ($p < 0.05$). **Conclusions** These results suggest that an association may exist between SPE-C and the onset of KD. In addition, autoantibody against vascular smooth muscle cells may cause systemic vasculitis in KD.

12

MATERNAL ANTIBODY FOR TSST-1 MAY INHIBIT THE KAWASAKI SYNDROME IN INFANTS UNDER 6 MONTH OF AGE

Yuichi Nomura[1], Kiminori Masuda[2], Syuji Takei[1], Koji Sameshima[2], Masao Yoshinaga[1], Koichiro Miyata[1] Department of Pediatrics, Faculty of Medicine, Kagoshima University, Kagoshima, Japan[1], Department of Pediatrics, Kagoshima City Medical Association Hospital, Kagoshima, Japan[2]

The etiology of Kawasaki syndrome (KS) is still unknown. The symptoms of KS suggest a possible relationship between KS and super-antigen(s) (SA). The infrequent occurrence of KS in early infants may be due to passive maternal antibody. So, we investigated the antibody titers for SAs in the early infants with KS. **Patients and Methods** : Fifteen KS patients under 6 months of age (before gammaglobulin therapy), 8 their mothers, and 2 mothers of other KS patients under 6 months of age were used for this study. Twenty-two infants under 6 months of age and 40 normal adult volunteers were also used as the controls. Antibody titers for SAs (SPEC, SPEA, TSST-1, SEB) were measured by enzyme-linked immunosorbent assay. A titer over the average value +2sd of the control was considered to be high. **Results** : The average titers for SPEC and SPEA were not different between KS and control. In the titers for TSST-1 and SEB, KS patients showed higher titers than did control (TSST-1; 0.482 ± 0.531 vs. 0.191 ± 0.244, SEB; 0.326 ± 0.525 vs. 0.252 ± 0.322), however, they were not significantly different. The ratio of high TSST-1 titer in KS was significantly higher than that in control (33% vs. 5%, $p = 0.031$). The average TSST-1 titer of the mothers was significantly lower than that of control adults (0.101 ± 0.165 vs. 0.447 ± 0.485, $p = 0.021$). In the 8 infant-mother pair, 6 infants showed a higher TSST-1 titer than that of their respective mothers. **Conclusions** : In KS patients under 6 months of age, TSST-1 may be related to KS, and maternal antibody for TSST-1 may inhibit the KS. Further examination is necessary to elucidate the etiology of KS.

13

IDENTIFICATION OF KAWASAKI DISEASE (KD) ANTIGENS USING SYNTHETIC KD ANTIBODIES

Anne H. Rowley[1], Susan C. Baker[2], Francesca Garcia[1], Stanford T Shulman[1] Department of Pediatrics, Children's Memorial Hospital, Northwestern University, Chicago IL, USA[1], Department of Microbiology/Immunology, Loyola University Medical Center, Maywood, IL, USA[2]

We reported the unique finding that IgA plasma cells are prominent in the inflammatory infiltrate in the vascular wall in acute fatal Kawasaki Disease (KD). Additionally, we sequenced IgA heavy chain genes in vascular tissue from three fatal acute KD cases and found evidence of an oligoclonal IgA response in all three KD patients, indicating an antigen-driven process. The presence of oligoclonal IgA-producing cells in the KD vascular wall provides a unique opportunity to identify the specific antigens being targeted by synthesizing the corresponding oligoclonal antibodies in vitro. Recent advances in molecular immunology enable the cloning of specific immunoglobulin heavy and light chain variable region genes into mammalian immunoglobulin expression vectors for the production of specific antibodies in tissue culture. We have prepared four synthetic KD antibodies from oligoclonal IgA genes prevalent in the KD vascular wall. In preliminary experiments, one of the synthetic antibodies binds to tissue sections from the spleen of a child who died of KD on day 13 of illness, but not to control spleen sections. This synthetic KD antibody also binds to upper respiratory tract sections from two other fatal KD cases, but not to control respiratory tract. Identification of the targeted antigen is underway by Western blot and cDNA library screening analysis. The use of synthetic KD antibodies provides a new approach for identifying antigens important in KD pathogenesis, as well for identification of the etiologic agent of KD and the development of a diagnostic test.

14

INCREASED PRODUCTION OF SERUM IGA-CLASS ANTIBODY TO LIPID A IN KAWASAKI DISEASE

Seiichiro Takeshita, Hiroko Kawase, Keigo Nakatani, Hiroshi Tsujimoto, Youichi Kawamura, Tomoharu Tokutomi, Isao Sekine Department of Pediatrics, National Defense of Medical College, Saitama, Japan

Objective: We recently reported that lipopolysaccharide (LPS) is bound to circulating neutrophils in the acute phase of Kawasaki disease (KD). The aim of the present study is to investigate the serum response to LPS in KD.Methods: We measured the serum levels of IgG-, IgM- and IgA-class antibodies (Abs) to lipid A, a toxic site of LPS, using ELISA in 20 patients with KD, 11 patients with gram-negative bacterial infection (GNBI), 27 healthy children, and 12 healthy adults. Results: The serum levels of anti-lipid A IgG, IgM and IgA tended to increase with advancing age in healthy children older than 6 months of age. The mean level of anti-lipid A IgM in the acute phase of GNBI and the mean levels of anti-lipid A IgM and IgA in the acute phase of KD were found to increase significantly, in comparison to the age-matched controls. Furthermore, the mean level of anti-lipid A IgA also showed a significant increase from the acute through the subacute phases of KD. Regarding the IgA-subclass response, higher titers of anti-lipid A specific Ab were seen in the IgA2 subclass than in the IgA1 subclass. Conclusion: These findings indicate that KD patients demonstrate an intense response to lipid A in the IgA, especially IgA2-subclass, thus suggesting that an unusual activation of the mucosal immune response to a ubiquitous antigen derived from gram-negative bacteria may be involved in the pathogenesis of KD.

15

ENHANCED B CELL SUPERANTIGEN-LIKE INTERACTION OF IVIG WITH IGG BUT NOT IGA CLONED FROM A KD PATIENT AFTER IVIG THERAPY

Peter Fischer, Stephan Leucht, Inka Pape, Martina M. Uttenreuther-Fischer, Gerhard Gaedicke Laboratory of Molecular Biology, Charite Children's Hospital, Humboldt-University, Berlin, Germany

The etiology of Kawasaki disease (KD) is still unknown, but an infectious origin and involvement of IgA B cells has been suggested. Coronary complications of KD can be reduced with IVIG therapy. It is not clear how IVIG interacts with the immune system. Previously, we selected a large number of IgG and IgM Fab specifically bound by IVIG molecules using phage display and antiidiotypic panning from three patients with autoimmune thrombocytopenia (Eur.J.Immunol. 1998; 28:4236-4247), a patient with lupus and from a healthy individual (Clin.Exp.Immunol. 2000; 121:37-46). The favoured VH germ-line gene segments of these IVIG-bound Fabs were 3-23 or 3-30/3-30.5, the most frequently rearranged VH genes among human B cells. The binding pattern suggested a B cell superantigen-like, specific interaction of an IVIG subset with B cells that present B cell receptors derived from these two germ-line genes (Arthritis Rheum. 2000, 43, 2722-2732). Here, to investigate whether or not treatment with IVIG influences this restricted interaction, we cloned and selected Fab fragments from a patient with KD before and after IVIG therapy. A favoured selection of IgG antibodies derived from both the 3-23 or 3-30/3-30.5 germ-line genes as before was observed. Importantly, the reactivity with IVIG was significantly higher for clones from the library prepared after the IVIG treatment, providing the first in vivo functional evidence that a subset of IVIG may selectively activate B cells of this germ-line origin (Clin. Immunol. 2001, 99, 18-29). This mechanism may add to the therapeutic effect of IVIG in the treatment of KD. In contrast, an IgA library from the same patient revealed only selection of 3-07 germ-line derived clones by IVIG. IVIG selected IgG clones of 3-07 origin showed a decrease after IVIG therapy, suggesting that those antibodies or their targets may be involved in the cause of KD.

16

ACTIVATION OF PERIPHERAL BLOOD MONOCYTES/MACROPHAGES IN KAWASAKI DISEASE

Susumu Furukawa Department of Pediatrics, Yamaguchi University School of Medicine, Yamaguchi, Japan

We have demonstrated that the activation of peripheral blood monocytes/macrophages plays a central role during acute Kawasaki disease(KD). Electron microscopy showed that peripheral blood $CD14^+$ monocytes/macrophages from patients with acute KD had nuclei with complex shapes, apparent nucleoli and abundant intracytoplasmic granules, some of which were positive for acid phosphatase. The quantity of intracytoplasmic granules was correlated with disease severity. Next, we examined peripheral blood $CD14^+$ monocytes/macrophages using a monoclonal antibody, PM-2K, which recognizes mature macrophages but not monocytes. Approximately 15-20% of peripheral blood $CD14^+$ monocytes/macrophages from KD patients were positive for PM-2K antibody as determined by immunoelectron microscopy. These results suggest that monocytes partly differentiate into macrophages in the peripheral circulation. Recently, it has been reported that the $CD14^+CD16^+$ monocyte/macrophage subpopulation plays a more important role in inflammation. We observed an increase in the number of peripheral blood $CD14^+CD16^+$monocytes/macrophages with acute KD, which was a positive correlation with C-reactive protein levels, and we observed only the patients with severe bacterial infections had increased this subpopulation during the acute stage among control diseases. We investigated the activation of transcription factor NF-κB for genes that encode the proinflammatory cytokines in $CD14^+$ monocytes/macrophages and $CD3^+$ T cells in peripheral blood by means of Western blot and flow cytometric analyses. NF-κB activation was more increased in $CD14^+$ monocytes/macrophages than in $CD3^+$ T cells in KD patients during the acute stage. The present findings suggest that peripheral blood $CD14^+$ monocytes/macrophages play an important role in cytokine production during acute KD.